Volume 6, Issue 5, 2016, 1491 - 1496 ISSN 2069-5837
Biointerface Research in Applied Chemistry
www.BiointerfaceResearch.com
Original Research Article Open Access Journal
Received: 25.07.2016 / Revised: 28.09.2016 / Accepted: 10.10.2016 / Published on-line: 13.10.2016

Synthesis and biological evaluation of novel mononuclear Ru(II) compounds as potential antiviral and cytotoxic agents

Shyam Sunder Anchuri [1,*], Kiran Gangarapu [2], Sreekanth Thota [3], Subhas S. Karki [4], Erik De Clercq [5], Graciela Andrei [5], Robert Snoeck [5], Jan Balzarini [5]

[1]Department of Pharm. D, Balaji Institute of Pharmaceutical Sciences, Laknepally, Narsampet, Warangal, Telangana State (TS), India
[2]Department of Pharmacy, Anurag Group of Institutions, Venkatapur (V), Ghatkesar (M), Ranga Reddy (D), Telangana-501301, India
[3]CDTS, Fundação Oswaldo Cruz - Ministério da Saúde, Av. Brasil 4036 - Prédio da Expansão, 80Andar - Sala 814, Manguinhos, 21040-361 - Rio de Janeiro - RJ–Brasil
[4]Department of Pharmaceutical Chemistry, KLE University's College of Pharmacy, Bangalore-560010, Karnataka, India
[5]Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

*corresponding author e-mail address: ***shyamar9@gmail.com***

ABSTRACT
Ruthenium (II) complexes of the type $[Ru(S)_2(K)]^{2+}$, where S=1,10-phenanthroline and K=2-NO_2-phenyl thiosemicarbazone (Compound R_1) and 2-OH-phenyl thiosemicarbazone (Compound R_2) were evaluated for broad antiviral activity testing against a variety of viruses including Varicella Zoster Virus, Herpes Simplex Virus-1&2, Vaccinia Virus and Cytomegalovirus. Cytotoxic evaluation of these compounds were investigated against human embryonic lung cells (HEL), human epithelial cells (HeLa), African green monkey kidney cells (Vero cells), Crandell-Rees feline kidney cells (CRFK) and Madin Darby canine kidney cells (MDCK) using MTS assay. Among the compounds tested, R_1 showed inhibitory activity against cytomegalovirus (EC_{50}: 7.3-11 µM), while R_2 was found to show modest antiviral activity with EC_{50} values of 27 and 69 µM for feline corona virus and feline herpes virus, respectively. The ruthenium compound R_1 showed more cytotoxic activity against HeLa (MCC: 4µM) and MDCK (CC_{50}: 4.9 µM) cell lines than against HEL, Vero and CRFK cells (CC_{50} values of ≥ 100 µM). These results suggest that the test Ru(II) compounds might be the potential antiviral agents with good cytostatic potentiality to various cell cultures.
Keywords: *Ruthenium, Thiosemicarbazone, Antiviral, Cytotoxicity, MTS assay*.

1. INTRODUCTION
Viruses are the smallest infectious agents, consisting essentially of nucleic acid (either DNA or RNA) enclosed in a protein coat or capsid. They are obligate intracellular parasites and thus depend upon the host cell for their replication. Viral infections are very common and responsible for a variety of diseases ranging from the common cold to rabies and AIDS. In contrast to the enormous number of anti-bacterial drugs, much lesser effective antiviral drugs are available. One of the most important reasons for the lack of success in developing antiviral drugs is due to the nature of the viruses, which totally depend upon the host cell for their multiplication and survival. Accordingly many compounds that may cause the death of viruses are also very likely to injure the host cells that harbour them. Although several compounds have potent antiviral activity both in vitro and in vivo at present only a limited number of synthetic compounds and α-interferon have been approved by the FDA for antiviral therapy in humans [1].

However, none of these drugs are without toxicities and hence there is a demand for new antiviral agents, which needs all possible approaches towards the development of new antiviral drugs for the therapy of viral infections for which at present no clinically useful drugs or vaccines are available. Research on drugs based on ruthenium complexes is a fast developing field in medicine, especially in the development of chemotherapeutic agents with minimal side effects and immunity to acquisition of drug resistance [2].

One particular group of Schiff bases as chelating agents that have been investigated over the past decade are the thiosemicarbazones (TSCs). TSCs are potent inhibitors of the enzyme ribonucleotide reductase and are capable of impairing the DNA synthesis and repair [3]. Thiosemicarbazone derivatives have a great importance in chemistry and biology due to their antiprotozoal [4], antibacterial [5], antiviral [6], antifungal [7] and antineoplastic [8] activities. The more electrophilic ruthenium complexes are always less toxic than their corresponding ligands [9]. It has been observed that the presence of certain bulky groups at position N^4 of the thiosemicarbazone moiety greatly enhances the activity [10]. The synthesis of ruthenium complexes with thiosemicarbazone ligands has been receiving considerable attention due to the pharmacological properties of both complexes and ligands [11]. The thiosemicarbazone ligands usually coordinate to ruthenium through oxygen, nitrogen and sulfur donor atoms in their (N, S) bidentate form [12].

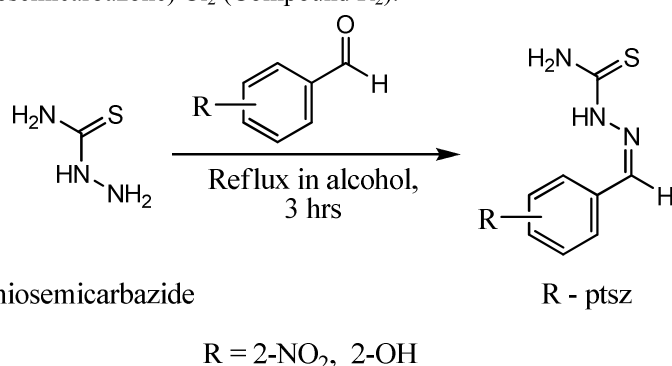
Many of the biological properties have been attributed to ruthenium complexes including anticancer activity [13-18], antinociceptive [19, 20], antioxidant [21, 22], antitubercular [23], antimalarial [24, 25] and antimicrobial activities [26, 27]. Ruthenium-based anticancer chemotherapies are making significant advances in clinical trials. For example, the two ruthenium (III) compounds namely, imidazolium [trans-tetrachloro [1H-imidazole) (S-dimethylsulfoxide) ruthenate (III)] (NAMI-A) [28] and indazolium [*trans*-tetrachlorobis (1H-

indazole) ruthenate (III)] (KP1019 or FFC14A) [29] as antimetastatic drugs have successfully completed Phase 1 clinical trials and are scheduled to enter Phase 2 trials in the near future.

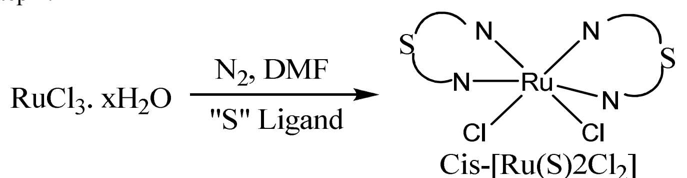
The two ruthenium complexes of the type thiosemicarbazones (TSCs) with the general formula $[Ru(S)_2(K)]^{2+}$, where S=1,10-phenanthroline and K=2-NO_2-phenyl thiosemicarbazone (ptsz) and 2-OH-ptsz, have been studied for in vitro antiviral activity against various viruses including varicella zoster virus (VZV), herpes simplex virus-1 and-2 (HSV-1&2), vaccinia virus and cytomegalovirus (CMV) and cytotoxic activity against HEL, HeLa, Vero Cells, CRFK and MDCK cell lines using the MTT assay.

2. EXPERIMENTAL SECTION

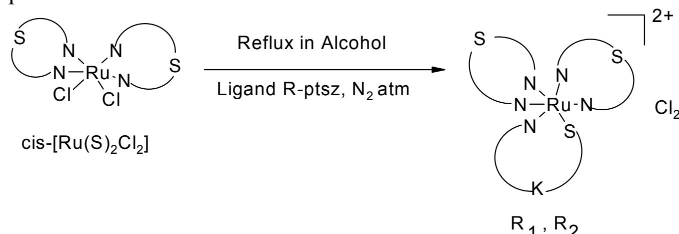
The ruthenium complexes R_1 & R_2 were prepared using the synthetic strategy described in Schemes 1-2. The synthesis began by preparation of ptsz ligands [2]]. The next step was performed by commercially available ruthenium trichloride with 1,10-phenanthrolene. The final ruthenium complexes were synthesized by treating $[Ru(S)_2Cl_2]$ with phenyl thiosemicarbazone ligands to provide the corresponded ruthenium complexes Ru (1,10-phenanthroline)$_2$ (2-nitro phenyl thiosemicarbazone) Cl_2 (Compound R_1) and Ru (1,10-phenanthroline)$_2$ (2-hydroxy phenyl thiosemicarbazone) Cl_2 (Compound R_2).



Scheme 1. Synthesis of Nitrogen & Sulphur bearing ligands. (34)
Step 1:



Step 2:



Scheme 2. Synthesis of Ruthenium Complexes (R_1&R_2), where S= 1, 10 phenanthroline, K=2-NO_2-ptsz/2-OH-ptsz, **R_1**=Ru (1,10-phenanthroline)$_2$ (2-nitro phenyl thiosemicarbazone) Cl_2, **R_2**=Ru (1,10-phenanthroline)$_2$ (2-hydroxy phenyl thiosemicarbazone) Cl_2

2.1. Chemistry.

2.1.1. Preparation of Complexes.

The solvents of AR grade were obtained from S.D Fine Chem., Mumbai, and E. Merck, Mumbai. The reagents (puriss grade) were obtained from Fluka and E. Merck.

Hydrated ruthenium trichloride was purchased from Loba Chemie, Mumbai, and used as received. UV-visible spectra were on a Jasco spectrophotometer. FTIR spectra were recorded in KBr powder on a Jasco V410 FTIR spectrometer by the diffuse reflectance technique. ^{1}H-NMR spectra were measured in $CDCl_3$ and DMSO-d6 on a Bruker Ultraspec 500 MHz/AMX 400 MHz/300 MHz spectrometer. FAB mass spectra were recorded on a JEOL JMS600 spectrum with mNBA matrix.

2.1.2. Preparation of substituted phenyl thiosemicarbazones (R-ptsz) [3]] (Scheme-1).

A mixture of substituted benzaldehyde (1 mmol) and thiosemicarbazide (1 mmol) in 100 mL of ethanol was refluxed for 3hrs and left Cytotoxicity, MTS assay, overnight. The solid was filtered, dried and purified by recrystallization from alcohol.

2-NO_2-phenyl thiosemicarbazone (2-NO_2-ptsz):

Yield 84%, M.P. 256-258ºC (lit.,256ºC). IR (KBr) cm^{-1}: 3417-3380 (NH_2& N-H), 3136 (C-H), 2901 (C-H), 1370 (C=S). Calcd. for $C_8H_8O_2N_4S$: C, 42.85; H, 3.57; N, 25.00. Found C, 42.69; H, 3.52; N, 24.94%.λmax nm (MeOH): 230, 307, 375. ^{1}H-NMR (DMSO-d6): δ= 10.36 (1H, s), 9.02 (1H, s), 8.04 (2H, d), 7.83 (2H, d), 7.24 (2H, d, J= 8.6 Hz).

2-OH-phenyl thiosemicarbazone (2-OH-ptsz):

Yield 56%, M.P. 234-235ºC (lit.,235ºC). IR (KBr) cm^{-1}: 3500-3200 (O-H), 3469-3320 (NH_2 & N-H), 3133 (C-H), 1610 (N-H), 1328 (C=S). Calcd. for $C_8H_9N_3OS$: C, 49.21; H, 4.64; N, 21.52. Found C, 49.20; H, 4.62; N, 21.28%.λmax nm (MeOH): 242, 321, 398. ^{1}H-NMR (DMSO-d6): δ= 12.6 (1H, s), 11.24 (1H, s), 8.07 (1H, s), 7.99 (1H, s), 7.89 (1H, s, -OH), 7.73 (2H, d, J=8.6 Hz), 6.95 (2H, d, J=8.6 Hz).

2.1.3. Preparation of cis-[bis(S)dichlororuthenium(II)] cis-$[Ru(S)_2Cl_2]$ [4]] (Step-1).

$RuCl_3.H_2O$, 1g (2.5 mmol) and Ligand S (5 mmol) were refluxed in 50 mL DMF for 3hrs under nitrogen atmosphere. The reddish brown solution slowly turned purple and the product precipitated in the reaction mixture. The solution was cooled overnight at 0^oC. A fine microcrystalline mass was filtered off. The residue was repeatedly washed with 30% LiCl solution and finally recrystallized. The product was dried and stored in a vacuum desiccator over P_2O_5 for further use (Yield 75%).

2.1.4. Preparation of $[Ru(S)_2(K)Cl_2]$ (Where S=1,10-phenanthroline; K=2-NO_2-ptsz, 2-OH-ptsz) (Step-2).

To the black microcrystalline *cis*-[*bis*(S)dichloroRu(II)] *cis*-$[Ru(S)_2Cl_2]$ (2 mmol) excess of ligand (2-NO_2-ptsz, 2-OH-ptsz) (2.5 mmol) was added and refluxed in ethanol under nitrogen atmosphere. The initial colored solution slowly changed to

brownish orange at the end of the reaction, which was verified by TLC on silica plates. Then the excess of ethanol was distilled off and this solution was added onto silica gel (60-120 mesh). The product was purified by column chromatography by using silica gel as stationary phase and chloroform-methanol as mobile phase.

Compound R_1 [Ru(phen)$_2$(2-NO_2-ptsz)Cl_2]:

Yield 44%, black crystals, IR (KBr) cm^{-1}: 3417-3380 (NH_2&N-H), 3136 (C-H) 2958 (C-H), 1370 (C=S). Calcd. for $C_{32}H_{24}Cl_2N_8O_2Ru_1S_1$: C, 50.79; H, 3.17; N, 14.81. Found C, 50.65; H, 3.15; N, 14.73%. ^{1}H-NMR (DMSO-d6): δ ppm: 12.49 (1H, s), 9.04 (1H, s), 8.58 (3H, t), 8.36 (d, J=4.9 Hz, 2H), 8.26 (d, J=8.4 Hz, 2H), 8.06 (3H, t), 8.01 (d, J=5.0 Hz, 2H), 7.96 (3H, m), 7.74 (3H, t), 7.62 (d, 2H, NH_2), 6.23 (2H, d). FAB-MS (m-NBA): 756 $[Ru(phen)_2\ (2\text{-}NO_2\text{-ptsz})]^{2+}(Cl_2)^-$; 685 $[Ru(phen)_2\ (2\text{-}NO_2\text{-ptsz})]^{2+}$; 505 $[Ru(phen)\ (2\text{-}NO_2\text{-ptsz})]^{2+}$; 462 $[Ru(phen)_2]$

Compound R_2 [Ru(phen)$_2$(2-OH-ptsz)Cl_2]:

Yield 46%, black crystals, IR (KBr) cm^{-1}: 3510-3200 (O-H), 3402-3329 (NH_2&N-H), 3036 (C-H), 1611 (N-H), 1328 (C=S). Calcd. for $C_{32}H_{25}Cl_2N_7ORu_1S_1$: C, 52.81; H, 3.43; N, 13.48. Found C, 52.26; H, 3.39; N, 13.32%. ^{1}H-NMR (DMSO-d6): δ ppm: 10.02 (d, J=5.1 Hz, 1H), 9.03 (1H, s), 8.91 (d, J=4.9 Hz, 1H), 8.84 (t, J=8.6 Hz, 2H), 8.63 (d, J=8.4 Hz, 1H), 8.49 (d, J=8.4 Hz, 1H), 8.34-8.20 (6H, m), 8.15-8.08 (2H, m), 7.91 (d, J=5.0 Hz, 1H), 7.81-7.75 (2H, m), 7.68-7.64 (s, 1H, O-H), 7.49-7.45 (1H, m), 6.91 (s, 2H, br, NH_2), 6.73 (d, J=14.6 Hz, 2H), 6.13 (1H, s). FAB-MS (m-NBA): 727 $[Ru(phen)_2\ (2\text{-OH-ptsz})]^{2+}(Cl_2)$; 656 $[Ru(phen)_2\ (2\text{-OH-ptsz})]^{2+}$; 475 $[Ru(phen)\ (2\text{-OH-ptsz})]^{2+}$; 462 $[Ru(phen)_2]$.

2.2. Biological Activities.

Ruthenium compounds (R_1&R_2) were evaluated for their antiviral and cytostatic activity in HEL, HeLa, Vero, CRFK and MDCK cells according to well-established procedures [5]].

2.2.1. Antiviral assay.

Different cell types were seeded at a density of 5x10³ cells per well in 96-well cell culture plates in suitable medium. Following 24 hrs incubation at 37°C and 5% CO_2, medium was removed and 5-fold serial dilutions of the test ruthenium compounds were added in a total volume of 100 µL, after which the virus inoculum was added to each well. This inoculum resulted in a greater than 90% destruction of the cell monolayer after 3-5 days of incubation at 37°C depending on the nature of the virus. The cytopathicity was determined microscopically or in the presence of a MTS solution that was added to each well. Following two hours of incubation at 37°C, the optical density of each well was then read at 498 nm in a microplate reader to measure the reduction of the MTS dye by cellular dehydrogenases (20 µL MTS for 3hrs at 37ºC) into a water soluble colored formazan product. The 50% effective concentration (EC_{50}) was defined as the concentration of compound of which 50% cell viability was protected from the virus-induced cytopathic effect (CPE). The results are shown in Tables 2-4.

Table 2. Antiviral activity of ruthenium compounds against CMV, VZV, HSV, VV and VSV in HEL cell cultures.

Compounds	Effective concentration EC_{50} (µM)[a] HEL cell cultures								
	CMV		VZV						
	AD-169	Davis	TK+VZV	TK-VZV	HSV-1 (KOS)	HSV-2 (G)	HSV-1 KOS ACV^r(TK$^-$)	Vaccinia virus	Vesicular stomatitis
R_1	11	7.3	>20	>20	>20	>20	>20	>20	>20
R_2	>20	>100	>20	>100	>100	>100	>100	>100	>100
Ganciclovir	7.9	7.9	-	-	0.02	0.01	4	>100	>100
Cidofovir	0.95	1.3	-	-	2	1	0.9	22	>250
Acyclovir	-	-	2.1	143	0.4	0.2	22	>250	>250
Brivudin	-	-	0.019	104	0.04	112	50	4	>250

[a]Effective concentration required to reduce virus-induced cytopathicity by 50%.
CMV: Cytomegalovirus, VZV: Varicella-Zoster virus, HSV: Herpes simplex virus

Table 3. Antiviral activity of ruthenium compounds in HeLa and Vero cell cultures.

Compounds	Effective concentration EC_{50} (µM)[a]							
	HeLa cell culture			Verocell culture				
	Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus	Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
R_1	>0.8	>0.8	>0.8	>20	>20	>20	>20	>20
R_2	>20	>20	>20	>20	>20	>20	>20	>20
DS-5000	1	>100	0.8	>100	>100	>100	>100	>100
(S)-DHPA	>250	>250	>250	>250	>250	>250	>250	>250
Ribavirin	4	112	4	85	146	>250	250	25

[a]Effective concentration required to reduce virus-induced cytopathicity by 50%.

Table 4. Antiviral activity of ruthenium compounds against feline corona and feline herpes virus, and influenza viruses.

Compounds	Effective concentration EC_{50} (µM)[a]							
	CRFK cell culture		MDCK cell culture					
	Feline Corona Virus	Feline Herpes Virus	Influenza A virus (H_1N_1 subtype)		Influenza A virus (H_3N_2 subtype)		Influenza B virus	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
R_1	>100	>100	>4	>4	>4	>4	>4	>4
R_2	27	69	>100	>100	>100	>100	>100	>100
HHA	4.2	11	-	-	-	-	-	-
UDA	1.6	1.2	-	-	-	-	-	-
Ganciclovir	>100	1.4	-	-	-	-	-	-
Ribavirin	-	-	2	3.1	2	2.2	0.8	2.7
Amantadine	-	-	5	3.2	0.9	0.8	>200	>200
Rimantadine	-	-	>200	>200	0.4	0.6	>200	>200

2.2.2. Cytotoxic assay.

MDCK cells were seeded at a density of $5x10^3$ cells per well of a 96-well plate in suitable media. Twenty four hours later, serial dilutions of the test ruthenium compounds were added. Cells were allowed to proliferate for 3 days at 37°C, after which the cell number was determined by means of the MTS method. The 50% cytostatic concentration (CC_{50}) was defined as the compound concentration that inhibited the proliferation of exponentially growing cells by 50%. Results are shown in Table1.

Table 1. Cytotoxicity of Ruthenium compounds.

Compounds	MCC(µM)[a]			CC (µM)[b]	
	HEL	HeLa	Vero	CRFK	MDCK
R_1	100	4	100	>100	4.9
R_2	≥100	100	100	>100	>100
Ganciclovir	>350	-	-	>100	-
Cidofovir	>300	-	-	-	-
Brivudin	>300	-	-	-	-
DS-5000	-	>100	>100	-	-
(S)-DHPA	-	>250	>250	-	-
Ribavirin	-	>250	>250	-	-
HHA	-	-	-	>100	-
UDA	-	-	-	26.5	-
Amantadine	-	-	-	-	>200
Oseltamivircarb oxylate	-	-	-	-	>100

[a]Minimum cytotoxic concentration (MCC) that causes a microscopically detectable alteration of cell morphology.
[b]Cytostatic concentration (CC) required to reduce cell growth by 50%.
HEL: Human Embryonic Lung cells, HeLa: Human epithelial cervix carcinoma cells,
Vero: African green monkey kidney cells, CRFK: Crandell-Rees Feline Kidney cells,
MDCK: Madin Darby Canine Kidney cells

3. RESULTS SECTION

The compounds of the newly synthesized ruthenium complexes were confirmed by UV, FT-IR, ^1H NMR, Mass spectroscopy and C, H, N analysis. In the UV spectra all the ruthenium complexes showed broad and intense visible bands between 340 and 510 nm due to metal to ligand charge transfer transition (MLCT). In the UV region the bands at 280 and 310 nm were assigned to 1,10-phenanthroline ligand $\pi-\pi^*$ charge transfer transitions. The IR spectras contained the absorption bands revealing the existence of the NH_2, C=N, C=S gps. The ^1H-NMR spectra of the complex, $[Ru(phen)_2(2\text{-}NO_2\text{-}ptsz]Cl_2$ showed 24 resonance peaks (δ 10.03-6.13). The mass spectra of the Complex R_1 gave the anticipated molecular ion peak and main fragmentation peaks, which were in accordance with the title complexes.

Both antiviral activity and cytotoxicity were determined by means of microscopical reading or the MTS method. For each

compound, the 50% effective concentration (EC_{50}) and the minimum cytotoxic concentration (MCC) or the 50% cytostatic concentration (CC_{50}) were determined. Among the tested ruthenium compounds, R_1 afforded more cytotoxic activity against HeLa (MCC of 4μM) and MDCK (CC_{50} of 4.9μM) cell lines than against HEL, Vero and CRFK cells, whereas compound R_2 was found to show less toxicity to the above cells even at concentrations up to 100 μM.

The compounds were also tested for *in vitro* antiviral activity against CMV, VZV, (HSV-1and -2), influenza virus (A and B), feline corona virus and feline herpes virus, vesicular stomatitis, vaccinia virus, Coxsackie virus B4, respiratory syncytial virus, reovirus-1, Sindbis virus and Punta Toro virus in different cell cultures. From the obtained results it was found that the compound R_1 afforded antiviral activity against both CMV strains in HEL cell cultures (EC_{50}: 2.3-11 μM). In these assays, the anti-CMV activity of the established cidofovir (EC_{50}: 0.95-1.3 μM) and ganciclovir (EC_{50}: 7.9 μM) was also determined. Thus, the R_1 compound was about equally active against CMV as ganciclovir. The other viruses were not affected (minimal antiviral effective concentration ≤5-fold lower than the minimal cytotoxic concentration) by the R_1 and R_2 compounds, except compound R_2 that was also found to show a modest antiviral activity against feline corona and feline herpes virus with EC_{50} values of 27 and 69 μM, respectively. It should, however, be noticed that compound R_1 is more cytotoxic than ganciclovir and cidofovir, but may be the rational basis for further exploration of novel related derivatives. The results suggest that the test Ru(II) compounds might be the potential antiviral agents with moderate cytostatic potentiality to various cell cultures.

4. CONCLUSIONS

The present study involves the synthesis of two Ruthenium complexes by reaction with $RuCl_3$ with thiosemicarbazones. The complexes were purified by column chromatography and characterized by FT-IR, NMR and Mass spectra. The complexes were screened for antiviral activity against various stains of DNA and RNA viruses and exhibited moderate antiviral activity and compared with reference compounds. Among the two complexes, R_1 could be a potential antiviral agent with good cytostatic potentiality to various cell cultures.

5. REFERENCES

[1] Babu S.N., Rangappa K., Design, synthesis, antibacterial and antitubercular activity of cationic antimicrobial peptide, ovine bactenecin5, *Indian journal of chemistry Section B, Organic including medicinal*, 47, 297, **2008.**

[2] Muggia F., Platinum compounds 30 years after the introduction of cisplatin: implications for the treatment of ovarian cancer, *Gynecologic oncology*, 112, 275-281, **2009.**

[3] Stacy, A. E., Palanimuthu, D., Bernhardt, P. V., Kalinowski, D. S., Jansson, P. J., & Richardson, D. R.. Zinc (II)–Thiosemicarbazone Complexes Are Localized to the Lysosomal Compartment Where They Transmetallate with Copper Ions to Induce Cytotoxicity. *Journal of medicinal chemistry.* 59 (10), 4965–4984, **2016.**

[4] Sanchez-Sanchez, M., Rivera, G., A Garcia, E. and Bocanegra-Garcia, V., Therapeutic Targets for the Development of Anti-Trypanosoma Cruzi Drugs: A Brief Review. *Mini-Reviews in Organic Chemistry*, 13(3), 227-243, **2016.**

[5] Ammar, Y.A., El-Sharief, M.A.S., Ghorab, M.M., Mohamed, Y.A., Ragab, A. and Abbas, S.Y., New Imidazolidineiminothione, Imidazolidin-2-one and Imidazoquinoxaline Derivatives: Synthesis and Evaluation of Antibacterial and Antifungal Activities. *Current Organic Synthesis*, 13(3), 466, **2016.**

[6] Haribabu, J., Subhashree, G. R., Saranya, S., Gomathi, K., Karvembu, R., & Gayathri, D. Isatin based thiosemicarbazone derivatives as potential bioactive agents: Anti-oxidant and molecular docking studies. *Journal of Molecular Structure*, 1110, 185-195, **2016..**

[7] Altıntop, Mehlika Dilek, et al. "Synthesis and biological evaluation of new naphthalene substituted thiosemicarbazone derivatives as potent antifungal and anticancer agents." *European journal of medicinal chemistry* 108, 406-414, **2016.**

[8] Altintop, M. D., Sever, B., Özdemir, A., Kuş, G., Oztopcu-Vatan, P., Kabadere, S., & Kaplancikli, Z. A.. Synthesis and evaluation of naphthalene-based thiosemicarbazone derivatives as new anticancer agents against LNCaP prostate cancer cells. *Journal of enzyme inhibition and medicinal chemistry*, 31(3), 410-416, **2016.**

[9] Liu W, Gust R. Update on metal N-heterocyclic carbene complexes as potential anti-tumor metallodrugs. *Coordination Chemistry Reviews*. 15;329:191-213, **2016.**

[10] Wani, W. A., Baig, U., Shreaz, S., Shiekh, R. A., Iqbal, P. F., Jameel, E., ... & Hun, L. T. Recent advances in iron complexes as potential anticancer agents. *New Journal of Chemistry*, 40(2), 1063-1090, **2016.**

[11] Thota, S., Vallala, S., Yerra, R., Rodrigues, D. A., & Barreiro, E. J. Design, synthesis, structural characterization and in vitro cytotoxic activity of mononuclear Ru (II) complexes. *Medicinal Chemistry Research*, 1-6, **2016**.

[12] Zafar, M. N., Nazar, M. F., Sumrra, S. H., Yasmin, A., & Atif, A. H. Development of some important nitrogen donor ligands for transition metal homogeneous catalysis. Russian Journal of Coordination Chemistry, 42(4), 225-251, **2016**

[13] Dougan S.J., Sadler P.J., The design of organometallic ruthenium arene anticancer agents, CHIMIA *International Journal for Chemistry*, 61, 704, **2007.**

[14] Bruijnincx P.C., Sadler P.J., Controlling platinum, ruthenium, and osmium reactivity for anticancer drug design, *Advances in inorganic chemistry,* 61, 1, **2009.**

[15] Süss-Fink G., Arene ruthenium complexes as anticancer agents, *Dalton Transactions*, 39, 1673, **2010.**

[16] Govender P., Renfrew A.K., Clavel C.M., Dyson P.J., Therrien B., Smith GS., Antiproliferative activity of chelating N, O-and N, N-ruthenium (II) arene functionalised poly (propyleneimine) dendrimer scaffolds, *Dalton Transactions*, 40, 1158, **2011.**

[17] Thota S., Imran M., Udugula M., Karki S.S., Kanjarla N., Yerra R., Balzarini J., De Clercq E., Synthesis, spectroscopic characterization, antineoplastic, in vitro-cytotoxic, and antibacterial activities of mononuclear ruthenium (II) complexes, *Journal of Coordination Chemistr,*.65, 823, **2012.**

[18] David S., Perkins R.S., Fronczek F.R., Kasiri S., Mandal S.S., Srivastava R.S., Synthesis, characterization, and anticancer activity of ruthenium-pyrazole complexes*, Journal of inorganic biochemistry,* 111, 33, **2012.**

[19] Cristiano M., Cardoso D., da Silva Paula M., Costa-Campos L., Antinociceptive effect of a ruthenium complex in mice, *Autonomic and Autacoid Pharmacology,* 28, 103, **2008.**

[20] Shyam Sunder A., Dhulipala S., Thota S., Yerra R., Balzarini J., De Clercq E., Antinociceptive and antitumor activity of novel synthetic mononuclear Ruthenium (II) compounds, *Journal of research in medical sciences,* 18, 215, **2013.**

[21] Marques da Silva Paula M., Pich C.T., Petronilho F., Drei L.B., Rudnicki M., Roberto de Oliveira M., Moreira J.C.F., Henriques J.A.P., Franco C.V., Dal Pizzol F., Antioxidant activity of new ruthenium compounds, *Redox Report,* 10, 139, **2005.**

[22] Sunder Anchuri S., Thota S., Yerra R., Dhulipala S., *In vitro* antioxidant activity of some novel synthetic mononuclear ruthenium (II) compounds, *Letters in Drug Design & Discovery,* 9, 421, **2012.**
[23] Hadda T.B., Akkurt M., Baba M.F., Daoudi M., Bennani B., Kerbal A., Chohan Z.H., Anti-tubercular activity of ruthenium (II) complexes with polypyridines, *Journal of enzyme inhibition and medicinal chemistry*, 24, 457, **2012.**
[24] Glans L., Ehnbom A., de Kock C., Martínez A., Estrada J., Smith P.J., Haukka M., Sánchez-Delgado R.A., Nordlander E., Ruthenium (II) arene complexes with chelating chloroquine analogue ligands: synthesis, characterization and in vitro antimalarial activity, *Dalton Transactions*. 41, 2764, **2012.**
[25] Anchuri S.S., Thota S., Bongoni R.N., Yerra R., Reddy R.N., Dhulipala S., Antimicrobial and Antimalarial Activity of Novel Synthetic Mononuclear Ruthenium (II) Compounds, *Journal of the Chinese Chemical Society*, 60, 153, **2013.**
[26] Bolhuis A., Hand L., Marshall J.E., Richards A.D., Rodger A., Aldrich-Wright J., Antimicrobial activity of ruthenium-based intercalators, *European Journal of Pharmaceutical Sciences*, 42, 313, **2011.**
[27] I Ramos A., M Braga T., S Braga S., Ru (II)-based antimicrobials: looking beyond organic drugs, *Mini reviews in medicinal chemistry,* 12, 227, **2012.**
[28] Bratsos I., Jedner S., Gianferrara T., Alessio E., Ruthenium anticancer compounds: challenges and expectations, *CHIMIA International Journal for Chemistry*, 61, 692, **2007.**
[29] Lentz F., Drescher A., Lindauer A., Henke M., Hilger R.A., Hartinger C.G., Scheulen M.E., Dittrich C., Keppler B.K., Jaehde U., Pharmacokinetics of a novel anticancer ruthenium complex (KP1019, FFC14A) in a phase I dose-escalation study, *Anti-cancer drugs,* 20, 97, **2009.**
[30] Cun-De W., Jun L., Xin-zhong S., Yun-hua F., Synthesis of thiosemicarbazones under microwave irradiation, *Synthetic communications*, 29, 3057, **1999.**
[31] de Oliveira R.B., de Souza-Fagundes E.M., Soares R.P., Andrade A.A., Krettli A.U., Zani C.L., Synthesis and antimalarial activity of semicarbazone and thiosemicarbazone derivatives, *European journal of medicinal chemistry*, 43, 1983, **2008.**
[32] Karki S.S., Thota S., Darj S.Y., Balzarini J., De Clercq E., Synthesis, anticancer, and cytotoxic activities of some mononuclear Ru (II) compounds, *Bioorganic & medicinal chemistry,* 15, 6632, **2007.**
[33] Mazumder U.K., Gupta M., Karki S.S., Bhattacharya S., Rathinasamy S., Sivakumar T., Synthesis and pharmacological activities of some mononuclear Ru (II) complexes, *Bioorganic & medicinal chemistry*, 13, 5766, **2005.**
[34] De Clercq E., Holý A., Rosenberg I., Sakuma T., Balzarini J., Maudgal P.C., A novel selective broad-spectrum anti-DNA virus agent, *Nature*, 323, 464, **1986.**
[35] Balzarini J., Naesens L., Slachmuylders J., Niphuis H., Rosenberg I., Hol A., Schellekens H., De Clercq E., 9-(2-Phosphonylmethoxyethyl) adenine (PMEA) effectively inhibits retrovirus replication *in vitro* and simian immunodeficiency virus infection in rhesus monkeys, *Aids,* 5, 21, **1991.**

6. ACKNOWLEDGEMENTS

The authors acknowledge the kind help of Mr. A. Madhukar Reddy, Secretary and Correspondent, S.R. College of Pharmacy, Warangal, India for providing laboratory facilities for the present research work and the technical assistance of Mrs. Leentje Persoons, Mrs. Lies Van den Heurck, Mr. Steven Carmans and Mrs. Anita Camps was greatly appreciated. The antiviral/cytostatic assays were supported by the KU Leuven (GOA 10/14).

© 2016 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).