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# MONONUCLEAR RUTHENIUM (II) COMPOUNDS AS NOVEL CYTOTOXIC AGENTS

**Shyam Sunder A[1*], Satyavati D[2], Sreekanth T[3], Ramakrishna S[4] Lakshma Nayak V[4], Vishnuvardhan MVPS[4]**

[1]Department of Pharm.D, Balaji Institute of Pharmaceutical Sciences, Laknepally, Narsampet, Warangal, Telangana State, India.

[2]Director, Brilliant Grammar School Educational Society's Group of Institutions, Integrated Campus, Engineering and Pharmacy, Abdullapurmet, Rangareddy, Hyderabad, Telangana State, India

[3]CDTS, Fundação Oswaldo Cruz - Ministério da Saúde, Av. Brasil 4036 - Prédio da Expansão, 8[0]Andar - Sala 814, Manguinhos, 21040-361 - Rio de Janeiro - RJ – Brasil.

[4]Division of Pharmacology, Indian Institute of Chemical Technology, Hyderabad, Telangana State, India.

*Corresponding Author Email: shyamar9@gmail.com

## ABSTRACT

*The present study was aimed to evaluate in vitro anticancer activity of newly synthesized two mononuclear Ru(II) compounds of the type $[Ru(A)_2(B)]Cl_2^-$ where A=1,10-phenanthroline B=2-$NO_2$-PTSZ (Compound $R_1$), 2-OH-PTSZ (Compound $R_2$) against various cancer cell lines using MTT assay. A panel of cancer cell lines: A549 (lung cancer), HT-29 (colon cancer), PC-3 (prostate cancer) and B-16 (mouse melanoma) were studied for cytotoxicity using MTT assay. The results revealed that the test Ru(II) compounds were able to inhibit the cancer cell growth in a dose dependent manner. The compound $R_2$ showed significant cytotoxic activity with $IC_{50}$ values ranging from 16 to 38µg/ml against all the above cell lines, whereas the compound $R_1$ displayed the $IC_{50}$ values ranging from 74 to 127µg/ml and was found to be inactive against HT29 cell lines. The results concluded that the compound $R_2$ appears to be better cytotoxic agent than $R_1$.*

## KEY WORDS

*Carcinogenicity, Cytotoxicity, MTT, PTSZ, Ruthenium compounds.*

## 1. INTRODUCTION

Cancer is a generic term in which abnormal cells divide mitotically without control and it includes a large group of diseases that can affect any part of the body. In today's world cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012. It is expected that annual cancer cases will rise from 14 million in 2012 to 2022 within the next 2 decades (World Cancer Report-2014)

In recent years, metal-based therapeutic drugs have been used as anticancer agents [1] for example, cisplatin is considered to be one of the most effective and widely used metal based anticancer drugs [2]. However, the efficacy of cisplatin is reduced by increasing tumour resistance and high toxicity [3,4]. These drawbacks have started interest towards the design and evaluation of transition metallic complexes, especially the ruthenium compounds for their reduced toxicity to host cells at therapeutic doses [5].

One particular set of Schiff bases as chelating agents that have been studied over the past decade is the thiosemicarbazones (TSCs). TSCs are

potent inhibitors of the enzyme ribonucleotide reductase and are capable of impairing the DNA synthesis and repair [6]. The biological properties of the ligands can be modified and mostly enhanced by linkage to metal ions [7]. In this view, we synthesized some mononuclear Ruthenium(II) compounds of the type $[Ru(A)_2(B)]Cl_2^-$ where A=1,10-phenanthroline B=2-$NO_2$-phenyl thiosemicarbazone (compound $R_1$), 2-OH-phenyl thiosemicarbazone (compound $R_2$) and were evaluated for cytotoxicity against various cancer cell lines.

Several studies have shown that tumor growth can cause antioxidant disturbances in certain tissues of the tumor hosts [8]. Cancer cells can generate large amounts of hydrogen peroxide free radicals, which may have the ability to mutate, damage normal tissues and invade other tissues. This suggests that there was a direct correlation between changes in the rate of cancer cell proliferation and the antioxidant defensive machinery [9]. In view, recently we have reported the above mononuclear Ru(II) complexes for *in vitro* free radical scavenging activity and the compounds possess an excellent antioxidant activity toward selected free radical donors [10]. On this basis we have evaluated the above test compounds for cytotoxic activity using different cancer cell lines, A549 (lung cancer), HT-29 (colon cancer), PC-3 (prostate cancer) and B-16 (mouse melanoma) and the results concluded that the compounds possess significant inhibition of proliferation of cancer cells. By inhibiting the proliferation, we can able to suppress the free radical generation by tumor cells.

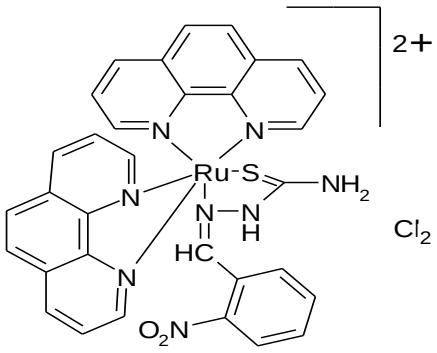
## 2. MATERIALS AND METHODS

### 2.1. Chemicals:

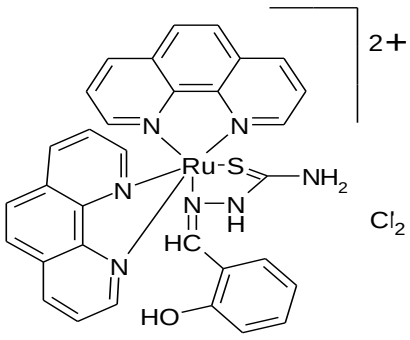
Dulbecco's modified eagle's medium (DMEM), Fetal bovine serum (FBS), penicillin, amphotericin B, and streptomycin were purchase from Himedia (Mumbai, India). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) purchased from Sigma Chemical Company (St. Louis, MO, USA).

### 2.2. Structures of test compounds:

The structures of test ruthenium compounds ($R_1$ and $R_2$) were shown below:




**Compound $R_1$**
Ru(1,10-phenanthroline)$_2$(2-nitro-Phenyl thiosemicarbazone) $Cl_2$




**Compound $R_2$**
Ru(1,10-phenanthroline)$_2$(2-hydroxy-phenyl thiosemicarbazone) $Cl_2$

### 2.3. *In vitro* cytotoxicity assay:

Cytotoxicity of the ruthenium compounds were determined using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) reduction assay [11] and is suitable for measuring cell proliferation, cell viability or cytotoxicity. $1\times10^4$ cells/well were seeded in 100 µl DMEM supplemented with 10% FBS in each well of 96-well micro culture plates and incubated for 24 h at 37°C in a $CO_2$ incubator. Ruthenium

compounds diluted to the desired concentrations in culture medium and incubated with the cells. After 48 h of incubation, media were removed and to each well 10 µl of MTT (5 mg/mL) was added and the plates were further incubated for 4 h. Supernatant from each well was carefully removed, formazan crystals were dissolved in 100 µl of DMSO and absorbance at 540 nm wavelength was recorded. The absorbance of the test was compared with that of DMSO control to get the %inhibition and then $IC_{50}$ values were calculated and compared with standard, Doxorubicin.

## 3. RESULTS

The synthesized compounds, $R_1$ and $R_2$ were evaluated for their *in vitro* anti cancer activity against a panel of four cancer cell lines, A549 (Lung cancer), HT-29 (Colon cancer), PC-3 (Prostate cancer) and B-16 (Mouse melanoma) along with standard compound, Doxorubicin by employing MTT assay. *In vitro* cytotoxic activity of ruthenium compounds revealed that these compounds inhibited the growth of various cell lines in dose dependent manner. In this assay, compound $R_2$ showed a significant anti cancer activity with the $IC_{50}$ values, 38.01, 16.21, 20.89 and 32.35 µg in lung, colon, prostate and melanoma cancer cell lines respectively. However, compound $R_1$ showed a moderate activity with $IC_{50}$ values of 79.4 against A549, 127.9 against PC-3, 74.6 µg against B-16 and was found to be inactive against HT-29 cell lines (**Table 1 & Figures 1-4**).

**Table 1. Inhibitory effect of $R_1$ & $R_2$ complexes on proliferation of A549, HT-29, PC-3 &B-16 cell lines**

| Compounds | $IC_{50}$ (µg/ml) | | | |
|---|---|---|---|---|
| | A549 | HT-29 | PC-3 | B-16 |
| $R_1$ | 79.4±1.05 | Not active | 127.9±3.54 | 74.6±1.98 |
| $R_2$ | 38.01±0.97 | 16.21±1.08 | 20.89±2.89 | 32.35±3.16 |
| Doxorubicin | 1.81±1.56 | 1.23±0.99 | 1.31±0.09 | 1.94±2.99 |

**Figure 1. Cytotoxicity of ruthenium complexes on A549 cell lines**

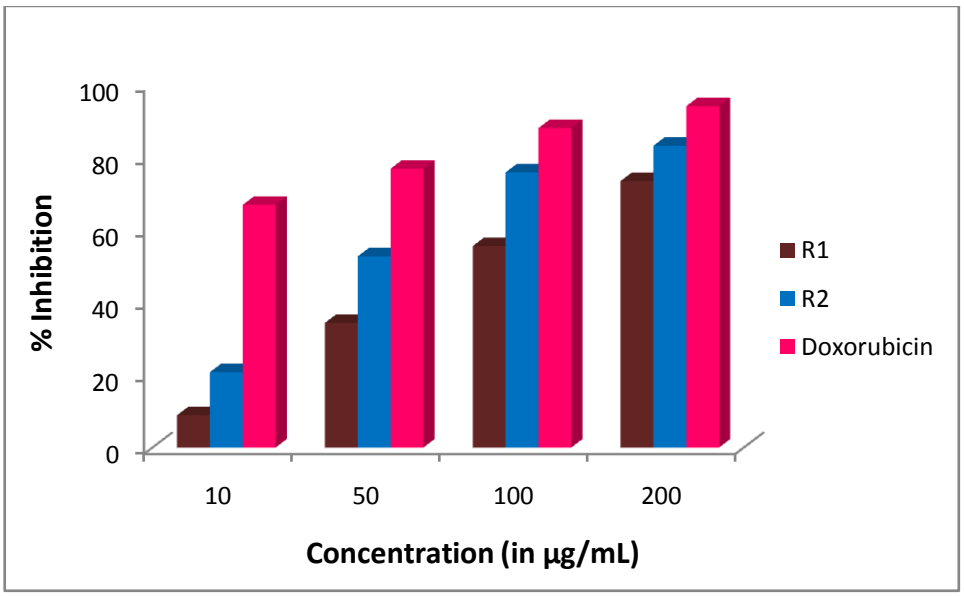


**Figure 2. Cytotoxicity of ruthenium complexes on HT-29 cell lines**

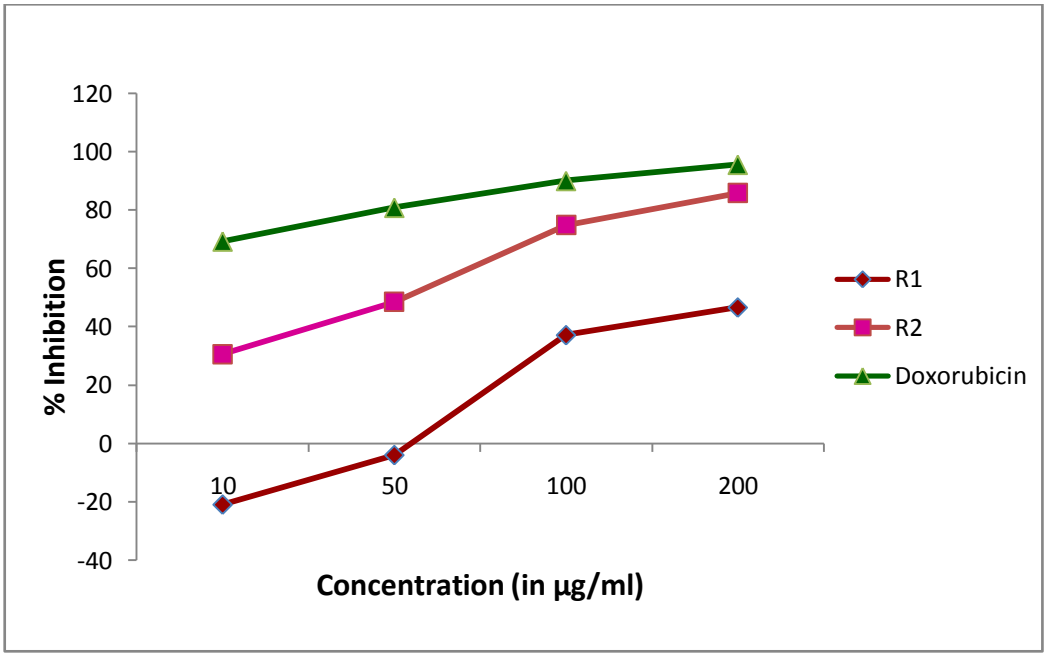



**Figure 3. Cytotoxicity of ruthenium complexes on PC-3 cell lines**

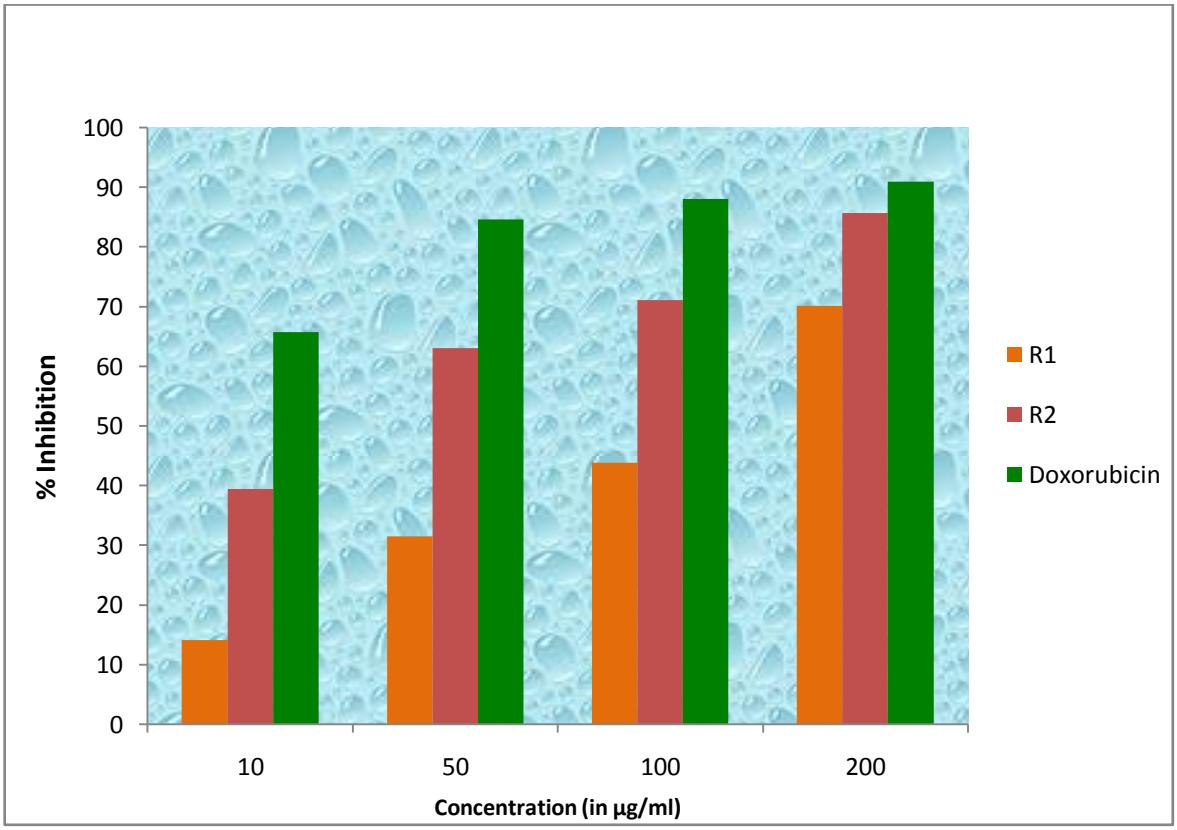
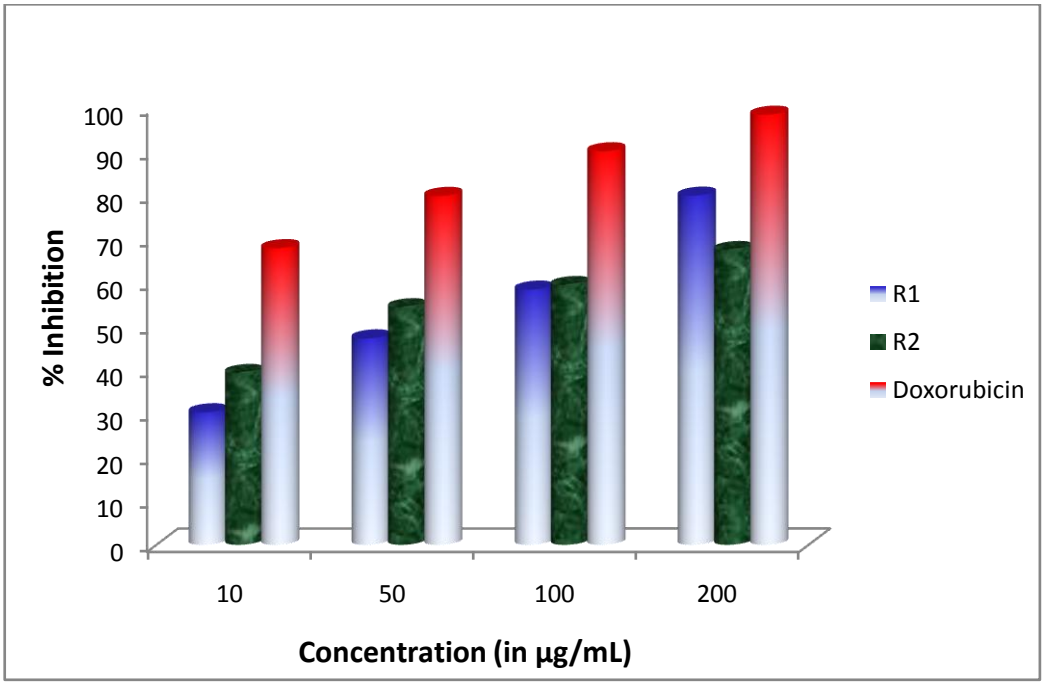


**Figure 4. Cytotoxicity of ruthenium complexes on B-16 cell lines**




## 4. DISCUSSION

Metal and metal ions are showing a good impact on cellular processes [12], such as they influence not only natural processes like cell division and gene expression but also toxicity, carcinogenicity and antitumor chemistry [13]. Among the various transition metallic complexes that have been investigated, ruthenium compounds have placed as promising one [14] because of proposed mechanisms such as "activation by reduction" and "transferrin-targeted delivery" accounted for the excellent cytotoxicity and low general toxicity of ruthenium complexes [15].

Research on drugs based on ruthenium complexes is a fast developing field in medicine, especially in development of chemotherapeutic agents with minimal side effects and immunity to acquisition of drug resistance [16]. Many of biological properties have been attributed to ruthenium complexes, for example, antitumor activity [17-24], antioxidant activity [25], antinociceptive [26,27], antitubercular activity [28], antimicrobial & antimalarial activity [29] and immunomodulatory activity [30].

Successful completion of phase 1 trials of two ruthenium(III)-based compounds, namely Imidazolium [trans-tetrachloro (1H-imidazole) (S-dimethylsulfoxide) ruthenate(III)] (NAMI-A) [31,32] and Indazolium [trans-tetrachlorobis (1H-indazole) ruthenate(III)] (KP1019) [33,34] as antimetastatic agents can encouraged for the synthesis of above ruthenium compounds and were evaluated for anticancer activity in vitro. The test ruthenium compounds have shown a dose dependant inhibition of cancer cell growth, it may be due to the antioxidant activity of test compounds. Of these two ruthenium compounds, $R_2$ was found to be better cytotoxic properties than $R_1$. By comparing the $IC_{50}$ values of ruthenium complexes with the doxorubicin, it was found that the test compounds were less potent than the standard drug. It may be due to lack of selectivity or insufficient dose to that particular cell line. Hence, further *in vitro* work is needed to know the selectivity of ruthenium compounds against other cell lines.

**Conflict of interest:** we have no conflict of interest.

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***Corresponding Author:**
*shyamar9@gmail.com*