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Invitro Anti-Proliferative Effect of Ruthenium(Ii)-Bipyridine-Phendione Complex on Sk-Mel-28 Cell Line

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Abstract
Melanoma is the most aggressive and chemoresistant form of skin cancer.SK-MEL-28 cell line is one of a series of melanoma cell lines.The objective of the present investigation is to study the anticancer activity of Ruthenium(II)-bipyridine-phendionecomplex{$[Ru(bpy)_2(phendione)]^{2+}$ (bpy = 2,2'-bipyridine and phendione = 1,10-phenanthroline-5,6-dione)} on SK-MEL-28 cell line. Themorphology of the SK-MEL-28 cell line at various concentrations of the $[Ru(bpy)_2(phendione)]^{2+}$complex is assessed by two-fold dilution method and the *invitro* anti-proliferative effect of the complex on the cell line is analysed by direct microscopic observation.The percentage viability of various concentrations of the complex in cancerous SK-MEL-28 cell line against the controlis calculated by MTT assay method. The IC_{50}value of this complex against the SK-MEL-28 cell is determined and it is found to be 28.600 µg/ml, which shows good anti-proliferative effect. The results revealed that the percentage of growth inhibition of the cell decreases with increase in the concentration of the complex and this is indicated by the formation of formazan crystal. Hence it is evident and clear that the synthesised $[Ru(bpy)_2(phendione)]^{2+}$ complexshows anti-skincanceractivity and shows late apotosis which is observed by double staining fluorescence microscopy.
Keywords:$[Ru(bpy)_2(phendione)]^{2+}$ complex, Anti-skin cancer activity, SK-MEL-28 cell line, Anti-proliferative effect,MTT assay, Fluorescence microscopy

1. INTRODUCTION

Metal complexes of nitrogen-substituted, phenanthrene-based ligands have shown significant potential as broad-spectrum agents capable of eliciting cytotoxicity toward diseases and infections manifested by cancer, viruses, bacteria and fungi[1].Metal complexes display unique properties in terms of tuneable geometry,electrochemical and photophysical properties and biological activity.In this context,ruthenium complexes have been the focus of much attention during the past decade as promising alternatives to platinum based anti-cancer agents.Ruthenium complexes have received increasing attention in the field of medicinal chemistry, especially in the development of chemotherapeutics that present minimal side

effects and immunity to the acquisition of drug resistance than platinum based complexes [2].Therefore, ruthenium based drugs may be delivered more efficiently to cancer cells, without exhibiting any specific interaction with the DNA [3].

Ruthenium (II) complexes with polypyridine ligands is of great interest due to their therapeutic values and pharmacological applications. Polypyridine ligand such as 1,10-Phenanthroline-5,6-dione (phendione), has a structure similar to 1,10-phenanthroline with the addition of two carbonyl groups attached at positions 5 and 6. The bi-functional character of phendione made it an extremely versatile ligand, with special reactivity arising from its quinonoid and diiminic sites, the quinonoid functionality of phendione confers redox capability, while the ideally suited diiminic sites coordinated to the metal ions. Phendioneis considered to have interesting biological properties, such as anticancer and antimicrobial activities [4].

Malignant melanoma is the most lethal form of skin cancer with a median overall survival of only 6 months once distant metastasis occurs [5]. Apoptosis resistance correlates with increased metastatic potential of melanoma, suggesting that acquisition of apoptosis resistance facilitates the vertical growth and deep invasion of melanoma. The development of agents capable of triggering cancer cell apoptosis may represent a novel therapeutic approach for melanoma treatment. The apoptosis resistance of melanoma is an area of intense investigation therefore,novel anticancer drugs that selectively counteract surviving expression are desirable not only to induce apoptosis but also to chemo-sensitize melanoma [6].Cellular death due to necrosis is different than apoptotic cell death, and result is loss of cell membrane integrity and uncontrolled release of products into the extracellular space. Apoptosis and necrosis can be induced by increased production of reactive oxygen species (ROS), including hydrogen peroxide, superoxide anion and nitric oxide, and decreased reduced glutathione levels [7]

Based on the literature survey, the present investigation focuses on*in-vitro*anti-proliferative effect of $[Ru(bpy)_2(phendione)]^{2+}$ (bpy = 2,2'-bipyridine) on SK-MEL-28 cell line. SK-MEL-28 cell line is one of a series of melanoma cell lines. The morphology of the SK-MEL-28 cell line and the percentage viability of cancerous SK-MEL-28 cell line against the control at various concentrations of the $[Ru(bpy)_2(phendione)]^{2+}$ complex is assessedby direct microscopic observation and by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl2H-tetrazoliumbromide) assay method.

2. Materials and Methods

2.1 Materials

$RuCl_3.3H_2O$, ligands (2,2'-bipyridine and 1,10-phenanthroline-5,6-dione) and ammonium hexafluorophosphate were procured from Sigma-Aldrich. HPLC grade solvents were used for the synthesis of the complexes.The complexes $[Ru(bpy)_2(phendione)]^{2+}$were synthesized by reacting the corresponding complex of $[Ru(bpy)_2Cl_2]2H_2O$ with phendione

according to the procedure previously described [8].SK-MEL 28 cell line were procured from National Centre for Cell Sciences.The *invitro* anti-proliferative studies on SK-MEL-28 cell line were carried out in Dulbecco's Modified Eagles Medium (DMEM,Himedia) supplemented with 10% FBS(Fetal Bovine Serum),L-glutamine, sodium bicarbonate and antibiotic solution containingPenicillin(100µg/ml),Streptomycin (100µg/ml) and Amphotericin B(2.5 µg/ml).

2.2 Equipment

The absorption spectrum of synthesized $[Ru(bpy)_2(phendione)]^{2+}$ complex was measured using SHIMADZU UV 1800 double beam spectrophotometer.SK-MEL-28 cell line was seeded in 96 well plate tissue culture plate and incubated at 37°C in a humidified 5 % CO_2 incubator (Galaxy® 170 Eppendorf, Germany).The anti-proliferative effect evaluation was carried out using inverted phase contrast tissue culture microscope(Labomed TCM-400 with MICAPS ^{TM} HD camera).All the absorbance values measured during MTT Assay were recorded by using microplate reader at a wavelength of 570 nm.Fluorescent microscopic image of the sample was analysed by Olympus CKX41 with OptikaPro5 camera.

2.3 Evaluation of Anti-proliferative Effect by MTT Assay Method

Sample solutions of $[Ru(bpy)_2(phendione)]^{2+}$ complex was freshly prepared for evaluation of *invitro* anti-proliferative effect.The freshly prepared samples in 5 % DMEM, was initially filtered to ensure the sterility [9].Two-fold dilution of the freshly prepared samples were five times serially diluted as 6.5,12.5,25,50,100 µg in 100 µl of 5% DMEM. The diluted samples were added in triplicates to the respective 96 cell well plates and incubated at 37°C in a humidified 5 % CO_2 incubator.The well tissue culture plate was observed at an interval of each 24 h, up to 72 h in an Inverted phase contrast tissue culture microscope and microscopic observation were recorded.Any changes in the morphology of the cells were considered as indicators of anti-proliferative effect.

For MTT assay, 15 mg of MTT was reconstituted in 3 ml PBS (Phosphate-buffered saline) and sterilized by filter sterilization. After 24 hof incubation period the MTT solution was added to all test and control wells,the plate was gently shaken well, then incubated at 37°C in a humidified 5 % CO_2 for 4 h.The supernatant solution was removed, add 100 µl of DMSOto solubilise the formazan crystals and the absorbance values were measured at 570 nm. The IC_{50} value of the complex on SK-MEL-28 cell line was determined from the percentage cellular viability.

$$\% \text{ of cellular viability} = \frac{\text{Mean OD samples}}{\text{Mean OD of control group}} \times 100$$

2.4 Determination of Apoptosis by AO/ EB Double Staining Method

The apoptosis was determined by double staining method after treating the test sample at IC_{50} concentration. The test sample along with the cells at IC_{50} concentration was incubated in a CO_2 incubator for 24 h and the cells were washed by cold PBS (Phosphate-buffered saline) to maintain the pH. The cells were stained with a mixture of aciridineorange (AO, 100 µg/ml) and ethidium bromide (EB, 100 µg/ml) at room temperature for 10min. The stained cells were washed twice with 1X PBS and observed by fluorescent microscope.

3. Results and Discussion

The structure of the synthesized complex used in the present study is shown in **Fig.1**.The absorption spectrum of $[Ru(bpy)_2(phendione)]^{2+}$ complex in aqueous medium shows a high energy absorption in the region 284 nm corresponding to the ligand centered π - π* transition and the low energy absorption at 438 nm assigned to the dπ - π* metal to ligand charge transfer transition (**Fig. 2**). These values are in accordance with the reported values [8].

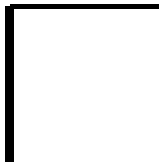

Fig. 1 Structure of $[Ru(bpy)_2(phendione)]^{2+}$ complex

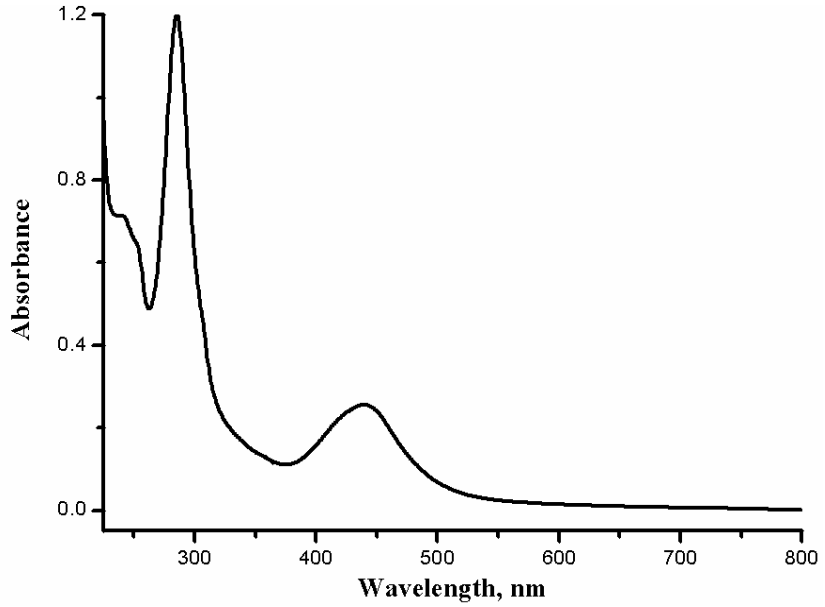

Fig. 2 UV spectrum of $[Ru(bpy)_2(phendione)]^{2+}$ complex

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The evaluation of anti-proliferative effect of $[Ru(bpy)_2(phendione)]^{2+}$ complex at various concentrations (6.5, 12.5,25, 50, 100 µg/ml) against the control on SK-MEL-28 cells is carried out by direct microscopic observation. The SK-MEL-28 cell line shows changes in the morphology of the cells and the number of cells gets decreased as the concentration of the complex increases (**Fig. 3**). This morphological changes in the cells are due to the production of reactive oxygen species (ROS) by the interaction of the phendione ligand present in the $[Ru(bpy)_2(phendione)]^{2+}$ complex. The recorded images of SK-MEL-28 cells against the control at various concentrations of $[Ru(bpy)_2(phendione)]^{2+}$ complex shows nuclear shrinkage, vacuole formation and chromatin condensation.

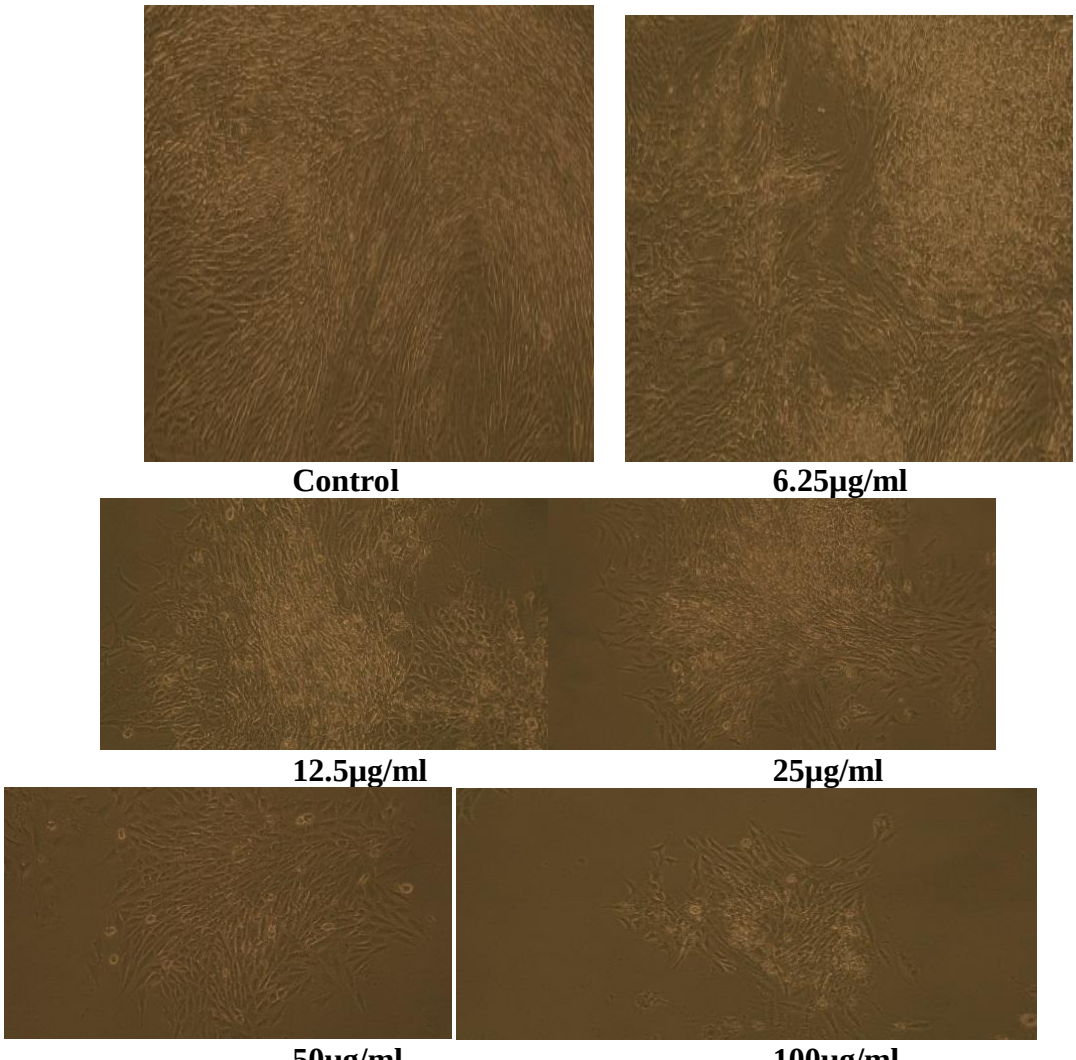


Fig. 3 Anti-proliferative effect of$[Ru(bpy)_2(phendione)]^{2+}$ complex on SK-MEL-28 cell line

The cultured cell line shows shrinkage of cells at complex concentrations 6.25 and 12.5 µg/ml, as the concentration of the complexes increases from 25 to 50 µg/ml the cells gets decreased and vacuolization in the cytoplasm of the cells appears. There is an enormous decrease in cells at 100 µg/ml which shows higher inhibition of the growth of cells and leads to lower cellular viability. The phendioneligand in the complex leads to cellular disruption,cell shrinkage and the formation of vacuoles which leads to cell death. Granato*et al*. have reported this type results in phendione based compounds in disturbing crucial physiological events of *Phialophoraverrucosa*[4].

The cell viability and the percentage cellular viability of the complex against the standard SK-MEL-28 cell line is assessed by MTT assay. The addition of MTT to the $[Ru(bpy)_2(phendione)]^{2+}$ complex makes the colour changes from blue topurple indicating the formation of formazan crystals which leads to cell death.The mitochondrial enzyme succinate dehydrogenase reduces MTT to formazan crystals. The MTT assay reaction is stopped by the addition of DMSO.The formed formazan crystals are subsequently solubilised using DMSO and the optical density is measured at 570 nm. The intensity of the purple formazan crystal indicates the percentage cellular viability (**Fig. 4**). The viable cells are quantified by the reaction of MTT with thesample on the cell line [10].In the present investigation, as the concentration of the complex increases the percentage viability decreases. This result is in accordance with the anticancer activity of Ru(II)-polypyridyl complexes having (2-(4-(diethoxymethyl)-1 H-imidazo[4,5-f][1,10] phenanthroline)) intercalative ligand [11].The concentration of 50 % cell death (IC_{50}) of the synthesized complex is calculated based on the measurement of cellular viability.The IC_{50} value of this complex against the SK-MEL-28 cell is 28.600 µg/ml and is found to be less than 100µg/ml.Hence the synthesized $[Ru(bpy)_2(phendione)]^{2+}$complex shows 50 % cellular viability at the concentration in between 25 and 50 µg/ml and shows good anti-proliferative effect.

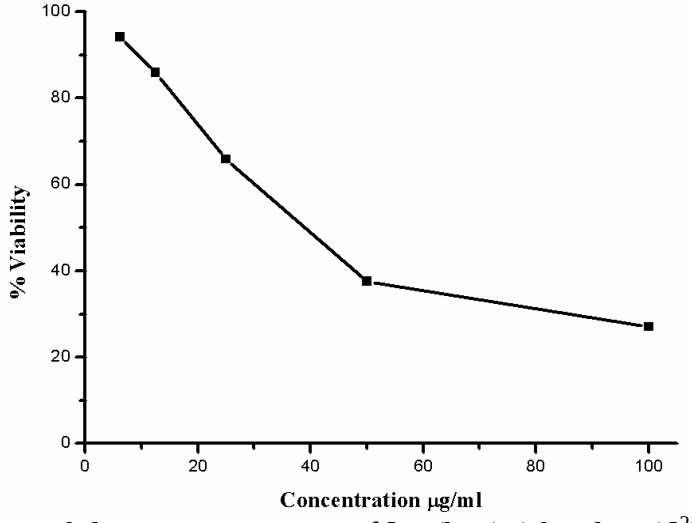
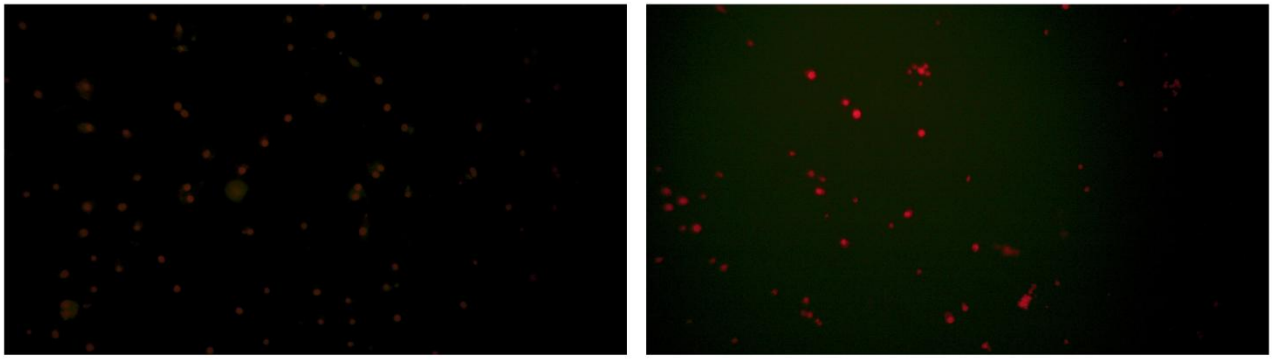


Fig. 4 Percentage cellular viability *vs*concentration of $[Ru(bpy)_2(phendione)]^{2+}$complex on SK-MEL-28 cell line

The cellular uptake and apoptosis effect of $[Ru(bpy)_2(phendione)]^{2+}$ complex at IC_{50} value, 28.600 µg/ml are studied by fluorescence microscopy using double staining method. In apoptosisstudy, AO dye emits green fluorescence by both viable and non-viable cells while EB emits red fluorescence by non-viable cells when gets intercalated into the DNA[12].The $[Ru(bpy)_2(phendione)]^{2+}$ complex on SK-MEL-28 cell line shows bright orange-stained nuclei with chromatin condensation indicating the inference of late apoptotic effect (**Fig. 5**). The late apoptotic inference on SK-MEL-28 cell line indicates that the synthesized $[Ru(bpy)_2(phendione)]^{2+}$ complex shows good *invitro* anti-proliferative effect and this is due to the damage in the DNA either by chromatin condensation or by the fragmentation of DNA.The bright-orange stained nucleus which indicates apoptotic cells of SK-MEL-28 cell line and green fluorescence indicates normal cells of SK-MEL-28 cell line [13].



(a)Control **(b) 28.600µg/ml**

Fig. 5 Fluorescence microscopic image of $[Ru(bpy)_2(phendione)]^{2+}$ complex on SK-MEL-28 cell line

The cell viability inhibition of the $[Ru(bpy)_2(phendione)]^{2+}$ complex may also be exerted by ROS which leads to mitochondria depletion, the quinone moiety of the phendione ligand present in the complex may also induce apoptosis in carcinoma cells due to endoplasmic reticulum stress and reactive oxygen species production [14].Hence the synthesized $[Ru(bpy)_2(phendione)]^{2+}$ complex shows late apoptotic effect on SK-MEL-28 cell line and shows excellent*invitro* anti-proliferative effect which inhibit the growth of cancerous cells and act as anti-skin cancer therapeutic agent.

CONCLUSION

The *invitro* anti-proliferative activity of the synthesized $[Ru(bpy)_2(phendione)]^{2+}$ complex on SK-MEL-28 cellshas been investigated. The SK-MEL-28 cell line shows changes in the morphology of the cells and the number of cells gets decreased as the concentration of the complex increases. The morphology of SK-MEL-28 cells at various concentrations of the complex under direct microscope shows decrease in the number of

cells, indicates the formation of vacuoles which leads to cell death. The percentage cellular viability of the $[Ru(bpy)_2(phendione)]^{2+}$complex revealed that the percentage of growth inhibition of the cell decreases with increase in the concentration of the complex. The IC_{50} value of the synthesized $[Ru(bpy)_2(phendione)]^{2+}$ complex on SK-MEL-28 cellsis found to be 28.600 µg/ml. The fluorescent microscopic image shows bright orange-stained nuclei with chromatin condensation indicating that the synthesized $[Ru(bpy)_2(phendione)]^{2+}$ complex shows late apoptotic effect on SK-MEL-28 cells. The phendione ligand in the complex leads to cellular disruption, cell shrinkage, formation of vacuoles which leads to cell death and it induce apoptosis. Hence the $[Ru(bpy)_2(phendione)]^{2+}$ complex shows good anti-proliferative effect and it is evident and clear that the synthesised $[Ru(bpy)_2(phendione)]^{2+}$ complex shows anti-skin cancer activity and shows late apotosis.

REFERENCES

1. McCann, M., Santos, A.L.S., daSilva, B.A., Romanos, M.T.V., Pyrrho, A.S., Devereux, M., Kellett, K., Fichtner, I., Kellett, A., (2012). *In vitro* and *in vivo* studies into the biological activities of 1,10-phenanthroline,1,10-phenanthroline-5,6-dione and its copper(II) and silver(I) complexes. *Toxic. Res.*, 1, 47 – 54.
2. Wana,D.,Laia, S.H.,Zenga, C.C.,Zhanga,C.,Tanga,B.,Liua, Y.J., (2017).Ruthenium(II)polypyridyl complexes:synthesis,characterization and anticancer activity studies on BEL-7402 cells.*J.of Inorg. Biochem.*,173, 1 – 11.
3. Mazuryk, O., Łomzik, M., Martineau, D., Beley, M., Brindell, M., Stochel, G., Gros, P.C. (2016). Anticancer activity of ruthenium(II) polypyridine complexes bearing pyrrolidine substituents. *Inorg. Chim. Acta*, 443, 86–90.
4. Granato, M.Q., Gonçalves, D.S., Seabra, S.H., McCann, M., Devereux, M., Santos, A.L.S., Kneipp, L.F. (2017). 1,10-Phenanthroline-5,6-dione–based compounds are effective in disturbing crucial physiological events of *Phialophoraverrucosa. Front.Micro*biol., 8, 1 – 9.
5. Parris, C.N.,Jezzard,S.,Silver,A.,MacKie,R.,McGregor, J.M.,Newbold, R.F., (1999). Telomerase activity in melanoma and non-melanoma skin cancer., *Br. J. Cancer*, 79, 47–53.
6. Yamanaka, K., Nakahara, T., Yamauchi, T., Kita, A., Takeuchi, M., Kiyonaga, F., Kaneko.N., Sasamata, M., (2011). Antitumor activity of YM155, a selective small-molecule survivinsuppressant, alone and in combination with docetaxel in human malignant melanoma models. *Clin. Cancer Res.*, 17, 5423–5431.
7. Zarić, R.Ž., Pirković, M.S., Hamzagić, N., (2019). Ruthenium(II) complexes as potential apoptosis inducers in cancer therapy. *Serbian Journal of Experimental and Clinical Research*,doi: 10.2478/sjecr-2019-0016.

8. Goss, C.A.,Abruña, H.D.,(1985). Spectral,electrochemical and electrocatalytic properties of 1,10-phenanthroline-5,6-dione complexes of transition metals.*Inorg. Chem*., 24, 4263 – 4267.
9. Sieroslawska,A., Rymuszka, A., (2019). Assessment of the cytotoxic impact of cyanotoxinbeta-N-methylamino-Lalanine on a fish immune cell line. *Aqua.Toxic*., 212, 214 – 221.
10. Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J., DeClercq, E., (1988). Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds.*J. Virol. Meth*.,20, 309 –321.
11. Vuradi, R.K., Dandu, K., Yata, P.K., Rani, M.V., Mallepally, R.R., Chintakuntla,N., Ravi C., Thakur, S.S., Rao, C.M., Satyanarayana,S., (2018). Studies on the DNA binding and anticancer activity of Ru(II) polypyridyl complexes by using a (2-(4-(diethoxymethyl)-1 H-imidazo[4,5-f][1,10] phenanthroline)) intercalative ligand. *NewJ.Chem*.,42,846–859.
12. Zhang, J.H., Yu, J., Li, W.X., Cheng, C.P., (1998). Evaluation of Mn^{2+} stimulated and Zn^{2+} inhibited apoptosis in rat corpus luteal cells by flow cytometry and fluorochromes staining. *Chin. J. Physiol*., 41, 121 – 126.
13. Xiao,P., Zheng,B.,Sun,J., Yang,J., (2017). Biochanin A induces anticancer effects in SK-Mel-28 human malignant melanoma cells *via* induction of apoptosis, inhibition of cell invasion and modulation of NF-κB and MAPK signaling pathways. *Oncl.Lett*., 14, 5989– 5993.
14. Hammouda, M.B.,Montenegro, M.F.,Campo, L.S.,Zakraoui, O., Aloui, Z., Chebbi,I.R., Karoui, H.,López,J.N.R., Benkhadir,K.E., (2016). Lebein, a snake venom disintegrin, induces apoptosis in human melanoma cells. *Toxins*, 8, 1 – 14.