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Full Paper

Samarium- 5-fluorouracil complex induces prominently the anticancer activity of human colon cancer cell line

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Abstract

In the present study samarium - 5-fluorouracil (5-FU) complex was prepared to enhance the effectiveness of the 5-FU drug. This complex was characterized by UV/VIS spectrometry high performance liquid chromatography and differential scanning calorimetry. Furthermore, the antitumor effect of the prepared complex was explored on the human colon cancer cell Caco2 via evaluation of the cytotoxic activity of this complex through trypan blue cell viability. Apoptosis was also assessed through morphological changes, by Annexin V=PI flow cytometric analysis. The results revealed that the trivalent Sm enhance the 5-FU effect against the chemo-resistant colorectal carcinoma cell line.

Keywords: Samarium 5-fluorouracil complex, HPLC fluorescence, UV-visible, thermodynamic parameters, anticancer activity, flow cytometric.

1.Introduction

The colorectal cancer (CRC) is considered as the third largest common cancer in the world and is the second leading causes of cancer related deaths. In 2012, new cases were diagnosed (nearly about1.4 million) [1, 2]. 5-FU is the most common drug used for the treatment of a wide range of

cancers including colorectal, colon, breast, ovarian, liver, gastrointestinal, head and neck cancers [3-5]. It was the primary chemotherapeutic drug successfully applied for treatment of colorectal cancer for more than 60 years [6]. 5-FU is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen (Figure 1). It rapidly enters the cell using principally the same transport mechanism as uracil [7]. 5-FU is converted intracellular to several active metabolites acting during the S-phase (Synthesis Phase) of the cell cycle that block DNA synthesis by the inhibition of thymidylate synthase activity, and induces DNA and RNA strand breaks by direct incorporation into these molecules[4]. More than 80% of administered 5-FU is normally catabolized primarily in the liver, approximately 15-20% is eliminated in the urine and only a small fraction remains available to exert its anti-tumor action [4-8] to increase the response rate. 5-FU is often given with drugs such as folinic acid which increases efficacy, oxaliplatin or irinotecan, as well as immunotherapy [4]. The interaction of metal ions with drugs administered for therapeutic purposes is currently a subject of considerable interest in the field of inorganic biochemistry [9-13]. Owing to the fact that some drugs work by chelation or inhibiting the formation of metalloenzymes, metal ions might play a crucial role during the biological processes of drug utilization in the body.

Trivalent lanthanides attracted considerable attention in the past 40 years [14-16]. They possess variable properties such as magnetic and physical, photophysical, biological activity including antimicrobial, antitumor, anti-virus and anti-clotting, as well as its ability to prevent atherosclerosis [17-19]. The redox stability of Ln^{3+} ions- in general- makes them highly suitable for cellular applications in the presence of biological reducing agents like ascorbate and thiols, with their interesting luminescent properties due to 4f-5d, charge-transfer, and f-f transitions [20]. There appear to be only few reports on rare earth complexes of 5-fluorouracil and its derivatives [21-23]. The Sm(III) complex with 5-fluorouracil is synthesized for the first time in this work and its anticancer activity was tested.

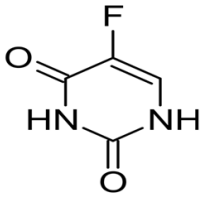

Figure 1: Molecular Structure of 5-forouracil

Therefore, the aim of the present work was to prepare and characterize the complexes resulting from the interaction of trivalent Sm(III) with 5-FU and to evaluate their antitumor effect against the human colon cancer cell Caco2 via their cytotoxic activity

2.Materials and methods

2.1Materials

5-Flourouracil, samarium(III) chloride, methanol and all other reagents were obtained from Sigma-Aldrich. Water was purified using the Milli-Q reverse osmosis system (EMD Millipore, Billerica, MA) and was stored at room temperature. All experiments were performed using dilutions of the stock solutions with the culture medium. Human colon cancer cell line (Caco-2) was obtained from Serum and Vaccine Institute, Cairo, Egypt. Cell line was cultured in humidified, 5% CO_2 at

37 °C incubator. RPMI-1600 media were purchased from Sigma (St Louis, MO, USA) and were supplemented with 10% heat-inactivated FBS (Hyclone Laboratories, Logan, UT, USA) and 1% antibiotic–antimycotic solution (Gibco Invitrogen, Carlsbad, CA, USA). Caco-2 cell line was cultured on a 6-well plate for 24 hours. This was followed by separate treatment with the anti-cancer drugs, 5-flourouracil alone, samarium (III) and 5-flourouracil metal complex, (Sm(III) -5-FU) with 1:1 molar ratio.

2.2 Methods

2.2.1 UV/VIS Spectroscopy measurements were recorded using Spectro UV-VIS double beam PC scanning spectrophotometer UVD-2950 from Lambomed, Inc. A computer data system of a UVWin5 software v 5.0.5 was used for measuring wavelength and absorbance. The spectrophotometer range was 200-400 nm using 1cm matched stoppard quartz cells.

2.2.2 Calorimetric measurements were carried out on a Shimadzu DSC-60 with TA-60ws model FC-60A, SSC-30 sensitive differential scanning calorimeter (DSC) using the standard TA-60ws software package for data acquisition and Origin software for DSC data analysis. All scans were performed in the temperature range from ambient temperature to 600°C at a scan rate of 0.1 °C/min or 0.1 °C/hr.

2.2.3 High performance liquid chromatography analysis was carried out using an Agilent HPLC 1200 Series (Agilent Technologies, USA) system consisting of degasser, quaternary pump, autosampler, thermostated column department and fluorescence detector which were all controlled by Agilent ChemStations. For the binding confirmation of Sm(III) with 5-flurouacil (5-FU), the HPLC mobile phase was 70% methanol and 30% Milli-Q was set at a flow rate of 1.5 ml/min. A Zorbax Extended C18 column (150×4.6 mm I.D ×5µm particle size) was used for the confirmation of binding. Injection volumes were 100 µL at 30°C. Emission was observed through an emission monochromator set at 335 nm with an excitation wavelength at 280 nm

2.2.4 Cell viability assay using trypan blue (TB) exclusion assay method

For cell viability assay, cells were seeded into 12-well culture plates at a concentration of 5×10^3 cells/well that were suspended in 1.5 ml culture medium and incubated for 24 hours. The washed cells were cultured in a medium containing 5-FU (47 µM), Sm (III) (47 µM) and other with Sm (III) – 5-FU with ratio of 1:1. The Trypan blue (TB) exclusion assay method was used for determination of living or dead cells, based on a total cell sample. Viability measurements were used to evaluate died or viable cancerous cells.

2.2.5 Apoptotic analysis using flow cytometry

Flow cytometry was performed with the FACSCalibur system (BD, San Jose, CA) in South Egypt Cancer Institute, Assiut University. Data were analyzed with CELL QUEST software (BD, San Jose, CA), Caco2 cell lines were plated at a density of 5×10^4 cells/well on 6-well plates and grown overnight. The cells were incubated at 37 °C for 24 hr with different

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concentrations of 5-FU alone, Samarium salt and the 5-FU / Samarium complex that were dissolved in DMSO. After treatment, the cells were rinsed, trypsinized, and washed with medium RPMI and the cells were washed twice in PBS (Phosphate-buffered saline). Next, 100 µL of cell suspension were transferred to a FACS tube and then mixed with 5 µL annexin V-FITC and 5 µL propidium iodide to each 100µl of cell suspension. The tubes were gently vortexed and incubated for 15 min at room temperature in the dark. Binding buffer (400 µL) was added to the FACS tube and the tubes were run through a FACS machine within 1 hr. Each experiment was done in duplicate. The cell was analyzed for the expression of annexin V and PI to identify apoptosis.

3. Results and discussion

3.1 UV-VIS spectrophotometric characterization of Sm(III) -5-FU complex

The absorption spectra of Sm(III) solution, 5-FU and Sm(III)-5-FU were measured. An increase in the UV light absorption (hyperchromic) and red shift (bathochromic) of the 5-FU is observed leading to a complex formation reaction. The three-dimensional absorption spectra of Sm(III)-5-FU are depicted in (Figure 2).

3.2 HPLC fluorescence characterization of Sm(III)- 5-FU complex

In order to confirm the presence of this complex, fluorescence detection of this complex was measured (Figure 3). The fluorescence intensity of 5-FU and its complex were excited at 280 nm. It is increases -based on the peak area measurements- compared to the fluorescence of 5-FU alone as cited in Table 1. This indicates that 5-FU interacts with Sm(III) effectively. The increase in the fluorescence accompanying the complex formation compared to 5-FU is probably due to the increased rigidity of the 5-FU by coordination to Sm(III). Coordination reduces the thermal vibrations and as a result the nonradioactive decay of the intra-ligand excited state decreases [24].

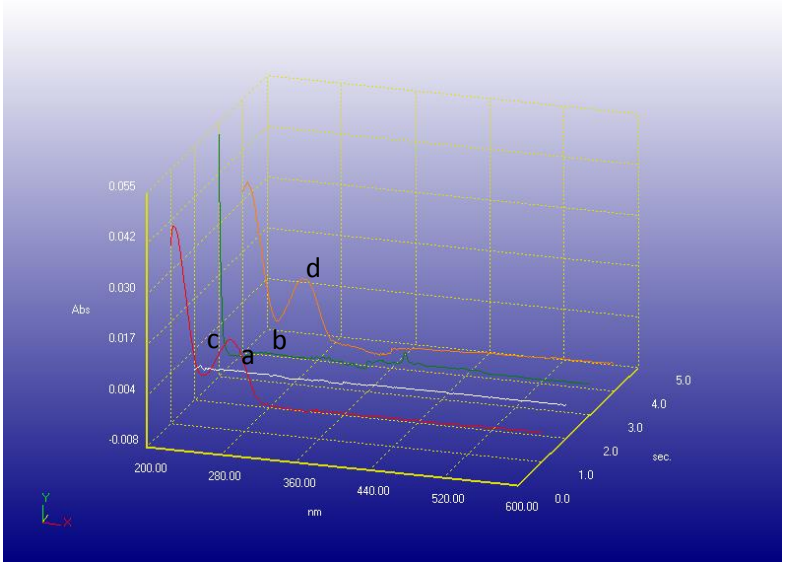

Figure 2: The three-dimensional UV/VIS spectra of (a) Blank ethanol:milli-Q , (b) Sm alone, (c) 5-FU alone, and (d) Sm (III)- 5FU

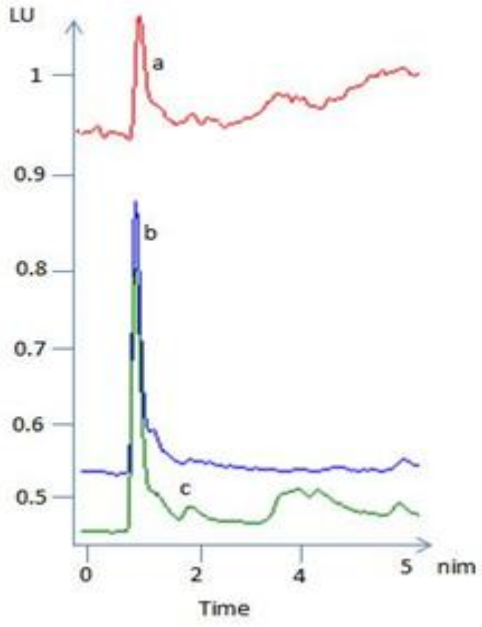

Figure 3: Fluorescence chromatogram of Sm(III)-5-FU complex (a) Sm (III) alone, (b) 5-FU alone, and (c) Sm (III)-5-FU

Table 1: Peak area values of 5-FU with Samarium

Parameter	Peak area
5-FU	5.4
Sm(III)	1.8
Sm(III)-5-FU	8.0

3.3 Differential Scanning Calorimetry (DSC) for thermodynamic parameters calculation

Differential scanning calorimetry was applied on 5-FU and its complex and the DSC thermogram of the Sm(III) complex is depicted in (Figure 4). The DSC thermogram of 5-FU exhibits an exothermic peak at about 113°C. Enthalpy (ΔH°) change upon addition of the ligand to the Sm(III) ion is shown in (Table 2), value is positive for the Sm(III) complex which indicates the hydrophobic interaction of the ligand.

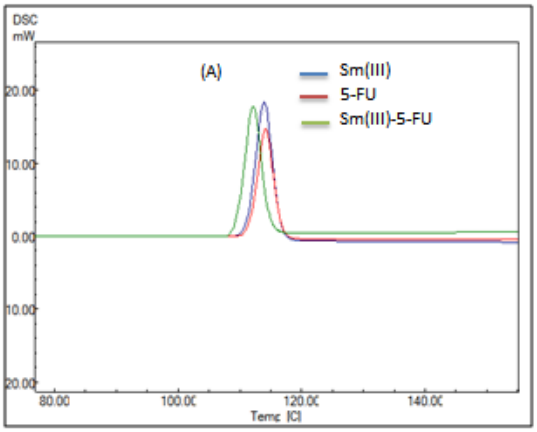

Figure 4: DSC thermogram of Sm (III)-5-FU Complex.

Table 2: (ΔH°) (mJ) values of 5-FU with samarium

Parameter	(ΔH°) (mJ)
5-FU	192.40
Sm(III)	239.81
Sm(III)-5-FU	228.24

3.4 Trypan blue (TB) exclusion assay method

Trypan blue dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, whereas dead cells do not. Cell suspension was simply mixed with dye and then visually examined to determine whether cells exclude dye or not. Viable cells will have a clear cytoplasm whereas nonviable cells will have a blue cytoplasm according to Warren Ströber [25]. Regarding the examination in the biological system, the concentration of drug that reduced cell proliferation by 50 % (LC50) was taken to be 47 µM according to Tolba et al [26]. The cancer colon cell line Caco2 that was exposed to the Sm(III)-5-FU complex (1:1) is photography illustrated in Figure 5, the result shows that the trivalent samarium

with 5-FU entered the Caco2 cell line very efficiently, and was used, decrees the viability in cancer cell compared with that observed for cell line treated with 5-FU only. Control Caco2 cell line had 10, 000 number of dead cells whereas cells treated with free 5-FU and Sm (III) at 47 µM individually had 40,000 and 50,000 number of dead cells respectively on the other hand the cell line treated with trivalent samarium complex Sm (III) with 5-FU have 74, 285.71 number of dead cells.

3.5 Cell apoptosis

To determine whether the inhibition of proliferation resulting from the complex on the cell line Caco2 is attributed to the induction of apoptosis or not, Annexin V/PI staining and flow cytometry were applied to measure the percentage of apoptosis as shown in (Figure 6). Interestingly, cellular treatment with Sm (III)-5FU individually at 1:1 ratio was significantly more biologically effective they give apoptosis percent 85.5% than any of the free 5-FU which has 50.76% apoptosis and free metal ion Sm (III) which give 55.49% or even untreated control that give 13%.

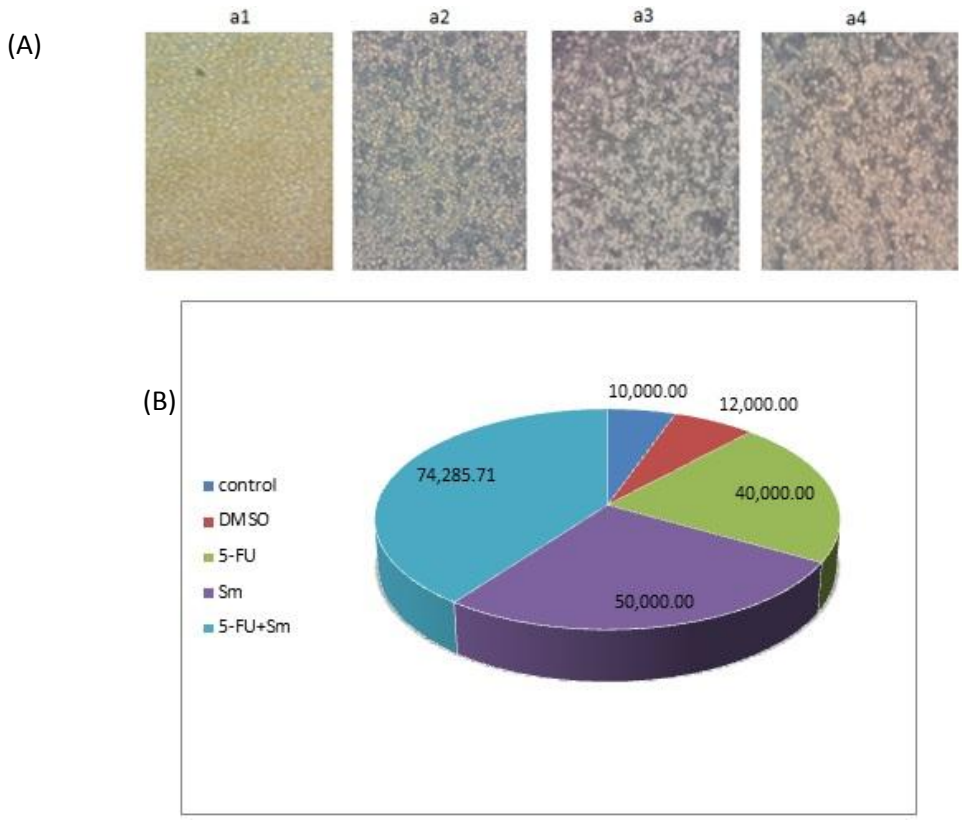

Figure 5: Images and corresponding growth curves for the Caco2 cell line. Legend A: morphology of Caco2 cell line by inverted microscope at original magnification ×10. (a1) Control Caco2, (a2) Caco2 treatment with 5-FU 47 µM only, (a3) Caco2 treatment with Sm (III) 47 µM and (a4) Caco2 treatment with Sm (III)-5-FU. Legend B: The cell growth curves of the colon cancer cell lines, and trivalent samarium with 5-FU. Cell counts for cell line were made in 24 h, using trypan blue exclusion to assess viable cells

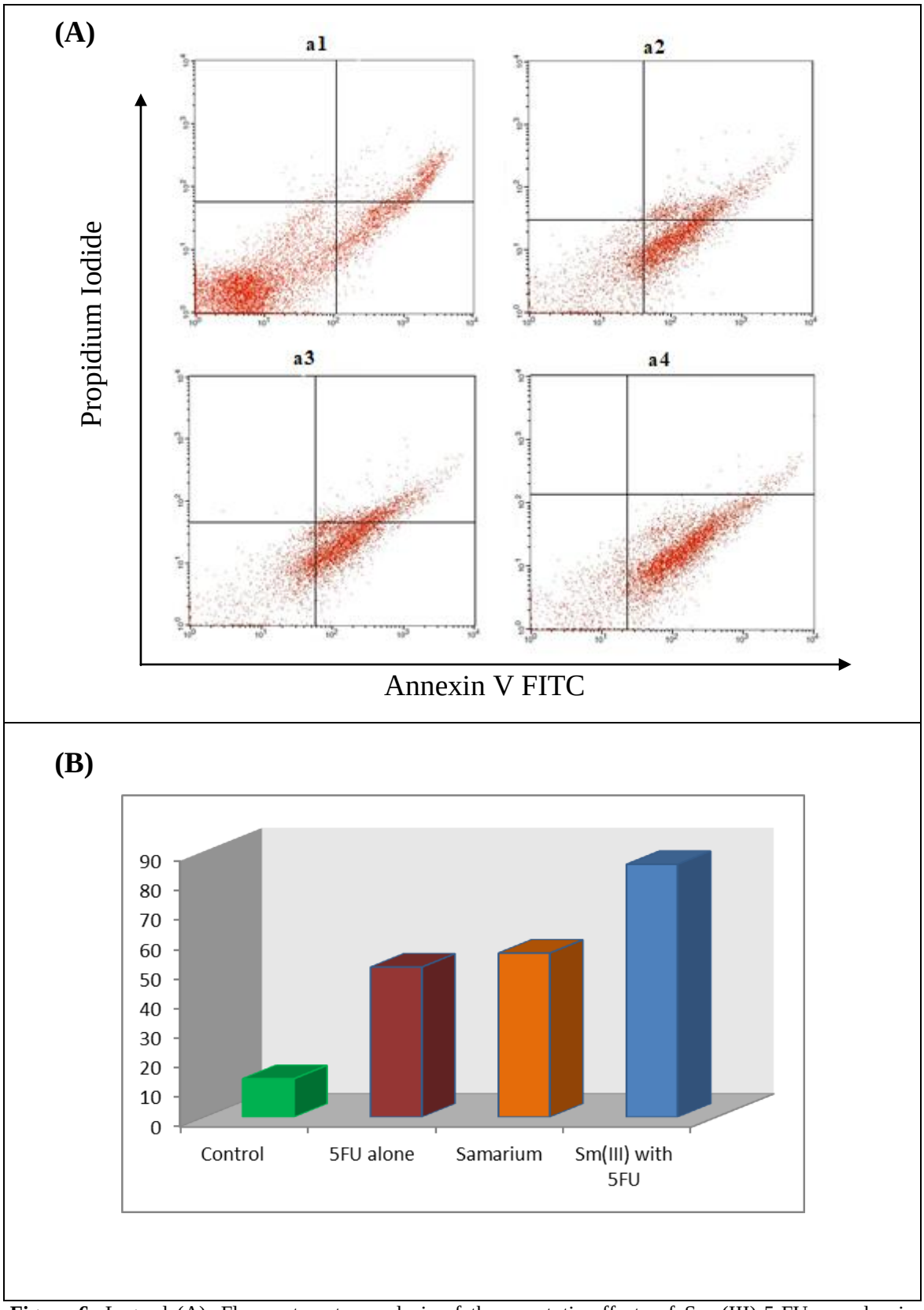

Figure 6: Legend (A): Flow cytometry analysis of the apoptotic effects of Sm (III)-5-FU complex in Caco2 cell line. Increase in Annexin V signal indicates apoptotic changes in cell membranes: (a1) Control Caco2, (a2) Caco2 treatment with 5-FU 47 µM only, (a3) Caco2 treatment with Sm(III) 47 µM, (a4) Caco2 treatment with Sm(III)-5-FU complex. Legend (B): The percent of apoptotic curves of trivalent samarium with 5-FU

4. Conclusions

A 1:1 complex formed between Sm(III) and 5-FU was prepared and characterized by spectrophotometry as well as with HPLC fluorescent detection. The complex exhibits a detectable enhancement as an antagonist to the human cell line colon cancer Caco-2 relative to the 5-FU alone

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5. References

1. R. Siegel, D. Naishadham and A. Jemal, CA Cancer J. Clin., 62 (2012) 283.
2. J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman and F. Bray, Int. J. Cancer, 136 (2015) E359.
3. D. K. Jangir, S. Charak, R. Mehrotra and S. Kundu, J. Photochem. Photobiol. B: Biol., 105 (2011) 143.
4. D. B. Longley, D. P. Harkin and P. G. Johnston, Nature Reviews Cancer, 3 (2003) 330.
5. U. Lönn, S. Lönn, U. Nylen and G. Winblad, Cancer Res., 49 (1989) 1085.
6. Y. Fu, G. Yang, F. Zhu, C. Peng, W. Li, H. Li, H.-G. Kim, A. M. Bode and Z. Dong, Cell Death Dis., 5 (2014) e983.
7. R. M. Wohlhueter, R. S. McIvor and P. G. Plagemann, J. Cell. Physiol., 104 (1980) 309.
8. R. B. Diasio and B. E. Harris, Clin. Pharmacokinet., 16 (1989) 215.
9. L. Yang, D. Tao, X. Yang, Y. Li and Y. Guo, Chem. Pharm. Bull., 51 (2003) 494.
10. A. Albert, Chapman and Hall, London (1979).
11. M. Hughes and J. Wiley, FEBS Lett. (1981).
12. J. Liu, E. Wang, J. Peng and Y. Zhou, JOURNAL OF RARE EARTHS, 17 (1999) 139.
13. S. Chakrabarti, D. Dasgupta and D. Bhattacharyya, JBP, 26 (2000) 203.
14. M. Albrecht, O. Osetska and R. Fröhlich, DTr (2005) 3757.
15. D. Parker, R. S. Dickins, H. Puschmann, C. Crossland and J. A. Howard, Chem. Rev., 102 (2002) 1977.
16. R. Hunter and W. Walker, Nature, 178 (1956) 47.
17. D. M. Kramsch, A. J. Aspen and L. J. Rozler, Sci, 213 (1981) 1511.
18. E. M. Hodnett and W. J. Dunn III, J. Med. Chem., 15 (1972) 339.
19. Y. Wang and Z.-Y. Yang, Transition Met. Chem., 30 (2005) 902.
20. J.-C. G. Buenzli, Coord. Chem. Rev., 293 (2015) 19.
21. W. Dawei, Journal of Lanzhou University Medical Sciences (Quarterly) (1991) 2.

22. Y. Liu, J. Kang, Z. Wang, L. Wang, L. Gao, C. Xia and J. Cui, J. Coord. Chem., 52 (2000) 1.
23. M.-L. Hu, J.-X. Yuan and A. Morsali, Solid state sciences, 8 (2006) 981.
24. J. Ji, Y. Zhang, J.-D. Fu, Z.-L. Liu and Y.-H. Wen, Journal of Inorganic and Organometallic Polymers and Materials, 23 (2013) 1347.
25. W. Strober, Curr. Protoc. Immunol., 21 (1997) A. 3B. 1.
26. M. F. Tolba and S. Z. Abdel-Rahman, Sci. Rep., 5 (2015) 15239.