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Therapeutic Properties of Gd(III)-Ir(III) Complex for Non-invasive Detection of Ovarian Cancer through M-MR Imaging

T. Lurthu Pushparaj[1*], E. Francy Irudaya Rani[2] & S. Sathya[3]

[1]*Assistant Professor, PG Department of Chemistry, Tirunelveli Dakshina Mara Nadar Sangam College, India.*
[2]*Department of Electronics and Communication Engineering, Francis Xavier Engineering College, Tirunelveli, India.*
[3]*PG Scholar, PG Department of Chemistry, Tirunelveli Dakshina Mara Nadar Sangam College, India.*
Corresponding Author Email: chemlurthu2006@gmail.com[*]

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ABSTRACT

Tris-Coumarin Ir(III) with Gd–complexes (Gd–Com) can serve each for imaging and remedy of a number most cancers cells. Here, we evaluated the anti-cancer ability of lipophilic Gd–Ir complicated thru on and offline mode. We detected no cytotoxicity of Gd–Ir in human serum albumin protein. Furthermore, splendid anticancer undertaking in opposition to HeLa cells and excellent binding closer to CA-125 protein (Ovarian Cancer Creating Protein) have been located the use of MTT-Assay find out about and Molecular Docking procedure. Moreover, Gd–Ir complicated exhibit more desirable relaxivity in the presence of 4.5 % HSA in PBS buffer. Also, the complicated well-known shows pH accountable relaxivity modifications when the answer pH understudy used to be modified from 2.6 to 10.6, this helps the suitability of the complicated for early analysis of most cancers tissue at acidic environment. The excessive polar interplay conduct of the complicated with HSA and CA-125 proteins confirms the suitability of the complicated for the prognosis and therapy of deadliest ovarian cancer.

Keywords: *Ovarian cancer imaging, CA-125 binding, Coumarin Gd-Ir complex, HSA binding.*

1. Introduction

Cancer is implicated in the pinnacle lists of disease-specific mortality fees in developed and growing countries. This disease effects from a genetic mutation in cells, inflicting an uncontrolled boom of mobile phone clones successful in circumventing all the preventive regulatory mechanisms due to the privileges furnished through the mutation [1]. Although one of a kind mechanisms are worried in the etiology of cancer; however, most of the effect is attributed to the presence of reactive oxygen species, over-expressing enzymes, interior the cells main to the DNA mutation with its deleterious penalties [2-4]. Moreover, the interactions between molecular oxygen, overexpressed proteins, and biomolecules in the organic structures produce metabolic perturbations [5-7]. Intravenously administered paramagnetic MRI distinction marketers are a hallmark of the radiological practice. These MRI distinction dealers are supposed to preferentially accumulate in metabolic perturbed tissue enabling expanded disorder detection via transformations in the magnetic houses of the nearby tissue [8]. Specifically, paramagnetic MRI distinction marketers (e.g., gadolinium chelates [9]) shorten the T1 and T2 magnetic rest time constants of the neighborhood tissue.

While these paramagnetic MRI distinction sellers supply elevated disorder detection individually, the mixture of two or greater distinction retailers in a single MRI scan may want to furnish extra diagnostic and prognostic data [10-14]. As an example, two one of a kind MRI distinction sellers ought to be co-administered to concurrently determine a tumor's vasculature (e.g., a large, macromolecular blood-pool MRI distinction agent [15]) as nicely as the tumor's vascular permeability (e.g., a smaller extravascular distinction agent [16]) to grant a greater complete evaluation of the tumor's vascular network. The endured growth of the portfolio of highly-specific molecular MRI distinction marketers will supply several additional multi-agent imaging opportunities. In this situation, a molecular "theranostic" MRI distinction agent should be used to reveal the transport of a therapeutic molecule to the ailment website [17], whilst a 2d molecular MRI distinction agent

ought to be used to check therapeutic efficacy [18] offering a mixed simultaneous voxel-wise evaluation of therapeutic transport and response. Alternatively, two molecular MRI distinction retailers ought to be mixed to determine each gene expression (e.g., reporter genes [19,20]) and the downstream results of the gene's feature such as neurotransmitter launch [21], ion attention [22], protein manufacturing [23], or enzymatic undertaking [24]. In this way, a "two-color" MRI approach should be used in a comparable trend to how multi-agent imaging research is automatically carried out in simple science fluorescence imaging experiments [25]. Gadolinium (Gd) –based distinction marketers are drastically used for magnetic resonance imaging (MRI). Gd+3 types insoluble phosphate salt in organic fluids and cultivation media.

Therefore, Gd+3 is used in complicated types of soluble chelates [26]. These complexes are believed to be steady and non–toxic due to the fact of excessive balance regular [27]. Recently, the accumulation of Gd–based distinction marketers has been tested in more than a few organs like the kidney, liver, and frightening system. It is supposed that poisonous results can be prompted by means of the dissociation of Gd ions from chelated complexes [28,29]. Case reviews pointed to the induction of nephrotoxicity, hepatotoxicity, and neurotoxicity, and uncommon acute destructive reactions to Gd–based distinction sellers had been additionally located in sufferers [30]. Iridium has been recognized as having the viable to serve as some other approach for most cancers treatment, which would contain the use of photo-imaging and goal unique cancer-killing therapy. Albumin is a blood protein ample in the blood serum and binds to a large vary of biologically and clinically vital molecules. A distinction agent with an anti-cancer drug was once allowed to bind to the blood protein via polar –polar team interaction.

The ensuing compound additionally glowed brightly as phosphorescence of the iridium complicated was once more advantageous upon conjugation with the human serum albumin (HSA) so its passage into cancer cells may want to be tracked.

2. Motivation

Multimodal MRI (MRI-M) would offer the greatest benefits of oversized spatial resolution, 3D imaging capabilities, and boundless imaging depth that are essential for non-invasive human imaging studies. The improvement of this "multimodal" MRI distinction agent cum drug opens a plethora of plausible purposes for most cancers treatment. The capability to reuse the compound inside cells should end result in smaller, greater environment friendly doses of chemotherapeutic drugs.

This would assist to decrease the disagreeable aspect outcomes most cancers sufferers go through via the route of their treatment. Also, the specificity of the coumarin and octadecane crew should be an imperative enchantment to different metal-based pills that assault non-cancerous cells. This may want to radically limit the hair loss and different facet results related with present day drugs.

3. Materials

HeLa cell lines, Xylenol orange indicator, Human Serum Albumin, Phosphate Buffer Solution, 3-(4,5-dimethyl thiazol-2-yl) 2, 5-diphenyltetrazolium bromide, Triple distilled water, Petri dish.

4. Procedures

4.1 Longitudinal Relaxivity (R_{1P})

The longitudinal relaxivity of the target complex at 20 MHz (the frequency at which MRI scanning is carried out) will be determined from the spin lattice relaxation time, T1. The T1 measurements will be made using the standard inversion recovery pulse sequence (180°-τ-90°) with phase sensitive detection [31] with τ values ranging from 50 ms to 6s for six different concentration of the complex.

The slope of the plot 1/T1 vs concentration of the complex gives the longitudinal relaxivity. The pH will be maintained by adding the TRIS or MES buffer.

4.2 Transverse Relaxivity (R_{2P})

The transverse relaxivity will be determined from the transverse relaxation time T_2. A standard CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence (90°-τ-180°) [32] with a τ value of 50 ms will be used to determine T_2.

The transverse relaxivity is calculated from the slope of the regression line, obtained by the plot of $1/T_2$ vs concentration of the complex by least squares fitting method.

4.3 Human Serum Albumin Binding Studies

The longitudinal and transverse relaxivities of the complexes in the presence of human serum albumin will be determined by treating the complexes with 4.5% human serum albumin in PBS buffer (the content of albumin in blood) till equilibrium is attained and by measuring the T_1 and T_2 for the different time intervals of 30 minutes to 2 days.

All relaxivity measurements carried out in a physiological background in the presence of carbonate, lactate, and/or malonate [33].

4.4 PH-Responsive Relaxivity Studies

The relaxivity of the complex at ten different pH, say 2.6, 3.6, 4.6, 5.6, 7.2, 7.5, 8, 8.5, 9.6, and 10.6 will be prepared by using standard buffer solutions. The r_{1p} and r_{2p} values for each pH will be measure using the standard pulse sequences.

Throughout the study the pH will be maintained using the standard buffer solutions.

4.5 Molecular Docking Study with CA125 Protein

Molecular-docking studies on the complexes has been performed using HEX 5.0 software and Q-site finder, which is an interactive molecular graphics program for the interaction, docking calculations, and to identify possible binding sites of the biomolecules. The coordinates of the metal complex has been taken from the optimized structure as a .mol file and has be converted to .pdb format using PYMOL software. The crystal structure of CA125 has been retrieved from the protein data bank (http://www.rcsb.org/.pdb). Visualization of the docked systems has been performed using PYMOL Tool. Default parameters will be used for the docking

calculations with correlation type shape and FFT mode at 3D level, grid dimension of 6 with receptor range 180, ligand range 180 with twist range 360, and distance range 40.

5. Results and Discussions

A) T_1, T_2 STUDY OF [Gd_2{Bis-Acdn(DO3ODA)$_2$(H_2O)$_2$}{Ir_2{Bis-phenyl(Tris(2-Chromone)}] IN 4.5 % HSA

The longitudinal and transverse relaxation times for six concentrations of [Gd_2{Bis-Acdn(DO3ODA)$_2$(H_2O)$_2$}{Ir_2{Bis- phenyl(Tris(2-Chromone)}] (**Fig. 1**) in presence of 0.1 ml 4.5 % HSA in PBS buffer are given in Table 1 and the plot of the concentration of the complex versus relaxivity (R) is shown in **Fig. 2**. The complex exhibits r_{1p} and r_{2p} values of 81.21and 112.46 mM^{-1} s^{-1}, respectively, in the presence of 4.5 % HSA. The "per Gd" value of the complex is found to be r_{1p} = 40.6 and r_{2p} = 56.23 $mM^{-1}s^{-1}$, respectively, in the presence of 4.5 % HSA. The "per Gd" r_{1p} and r_{2p} values are 3.1 and 3.02 times higher than that in neat solution. The higher relaxivity of the complex is due to the remarkable binding of the highly rigid bis acridone linker, two DO3ODA groups, and six 2-cumarone moiety with the serum protein which results in a higher rotational correlation time for the albumin-bound macromolecular adduct. HSA, which constitutes about 4.5% of plasma (0.67 mM), is a large globular protein (67 kDa) that binds and transports many exogenous and endogenous chemicals including fatty acids, metabolites, metal ions, and pharmaceuticals. The hydrophobic pockets formed by the amino acid residues are also responsible for ligand binding. Binding of gadolinium(III) chelates to albumin also reduces the fraction of free chelate available for glomerular filtration in the kidneys, thus slowing the renal excretion rate and contributing to an extended blood half-life. The result of this is a longer period for imaging, which allows the radiologist to perform multiple imaging experiments and to image under steady-state conditions. Since the complexes display higher relaxivity in the presence of serum protein they can be used as blood pool agents for magnetic resonance angiography (MRA).

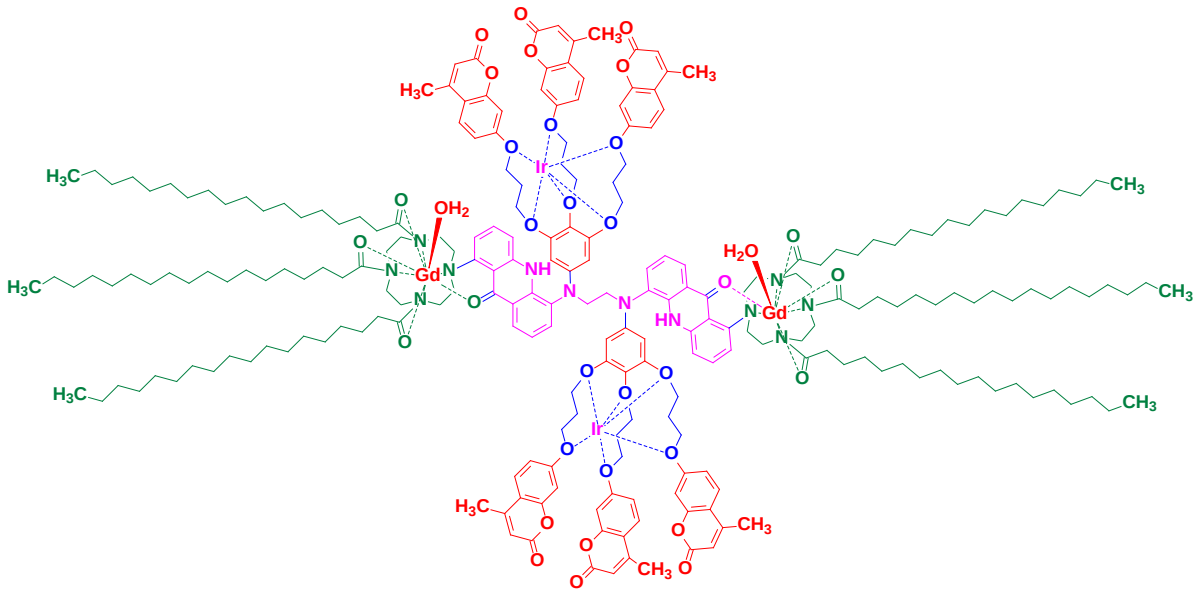

FIG.1. STRUCTURE OF [Gd_2{Bis-Acdn(DO3ODA)$_2$(H_2O)$_2$}{Ir_2{Bis-phenyl (Tris(2-Chromone)}] UNDER STUDY

Table 1. Longitudinal and Transverse relaxation times of the complex

in presence of 0.1 ml 4.5% HSA

Concentration M	$1/T_1 \times 10^3\ s^{-1}$	$1/T_2 \times 10^3\ s^{-1}$
0.20	14.7	21.53
0.50	37.96	55.67
1.00	81.13	112.2
1.50	127.3	177.49
2.00	176.51	247.02
2.50	245.38	320.61

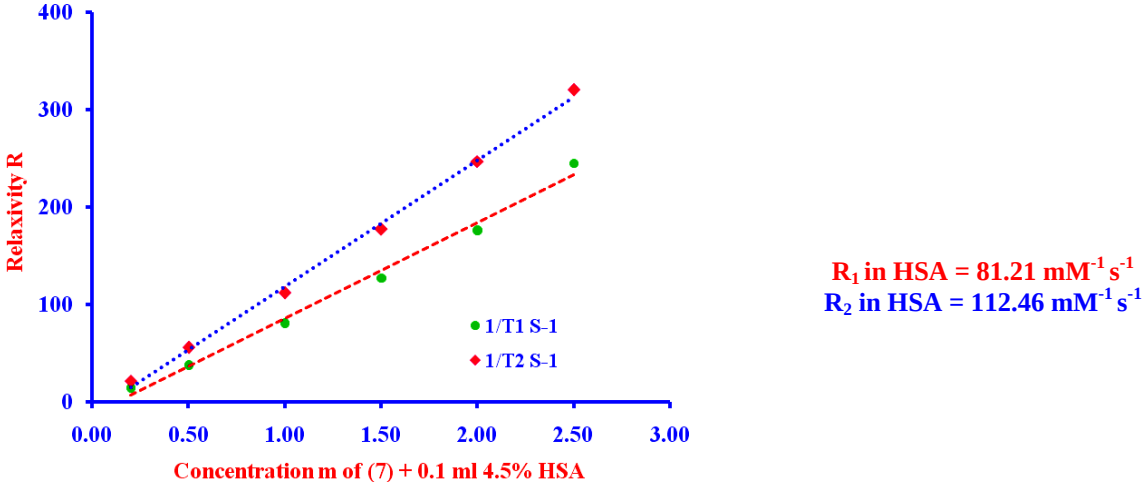

FIG.2. Plot of the concentration of complex + 0.1 ml 4.5 %

HSA in PBS buffer versus $1/T_1$ and $1/T_2$

B) P^H DEPENDENT RELAXIVITY OF THE COMPLEX

The pH dependent relaxivity of our complex has been fit according to standard SBM theory [34]

$$R_{1P} = (N/55.5)q\ (T_{1M} + \tau_M)^{-1} + R_{lp}^{os} \qquad (1)$$

where, N, is the molar concentration of the complex, q, is the quantity of first sphere water atoms T_{1M}, is the longitudinal relaxation time of the coordinated water protons and R_{lp}^{os} is the second sphere water proton relaxation rate. The complex shows relaxivity changes when the solution pH is changed **Fig.3**. The r_{1p} values of the complex are 73.64, 64.29, 53.33, 42.89, 31.37, 27.36, 21.36, 17.32, 15.08, and 13.28 $mM^{-1}\ s^{-1}$ at pH 2.6, 3.6, 4.6, 5.6, 7.2, 7.5, 8, 8.5, 9.6, and 10.6, respectively. The obtained altered relaxivity values are higher than that of the complex in aqueous solution (r_{1p} = 24.43 $mM^{-1}\ s^{-1}$, pH = 7). The r_{2p} values the complex are of 114.34, 99.85, 87.22, 73.24, 58.59, 50.21, 36.81, 30.94, 26.25, and 24.54 $mM^{-1}\ s^{-1}$ at pH 2.6, 3.6, 4.6, 5.6, 7.2, 7.5, 8, 8.5, 9.6, 10.6, respectively. The relaxivity steadily diminishes on expanding the pH and arrives at the base of

13.28 and 24.54 mM^{-1} s^{-1} at pH 10.6, respectively. The higher relaxivity at low pH might be brought about by the protonation of the carboxylate oxygen, the nitrogen group of the ligand, and the water hydrogen attached to the second coordination sphere of the six coumarone –phenyl amide functionalized pendant arm.

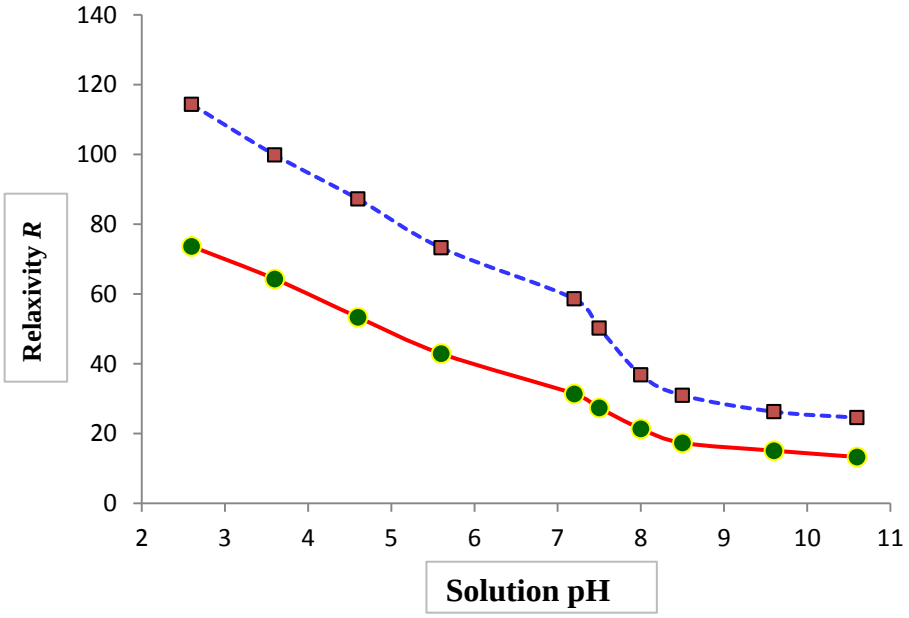

FIG.3. T_1 AND T_2 RELAXIVITIES OF THE COMPLEX

AT VARIOUS P^H (2.6–10.6)

C) CELL VIABILITY AND CYTOTOXIC STUDY BY MTT ANALYSE

The cell culture and cell killing quality of our bio-molecule functionalized complex was performed utilizing (MTT =3-(4,5-dimethyl thiazol-2-yl) 2, 5-diphenyltetrazolium bromide) colorimetric examine created by Mosmann.[35] It is a high-throughput screening approaches in 96-well plates. Since MTT has lipophilic side groups and positive net charge it will pass the cell membrane and convert to needlelike formazan crystals which destroy the cell's integrity and thus leads to cell death. This method detects living, but not dead cells and the absorbance of the colour generated (optical density) is dependent on the degree of activation of the cells and can, therefore, be used to measure cytotoxicity, proliferation, or activation. The level of development inhibitory pace of the treated cells was determined utilizing the following relationship ($A_{control}$ - $A_{compound}/A_{control}$ - $A_{cell\ free}$) x 100 (***A***, *is the mean worth determined by utilizing the information from the triplicate tests*). The MTT examine was performed to weigh up the cytotoxic impacts of the complex against the HeLa cell lines. The HeLa cell lines were treated with 1,2,5,10,15,25,50,65,85, and 100 µM solution of our complex for 50 h at 37 °C in PBS. The examination yield shows about 63.64, 55.29, 46.33, 37.89, 28.37, 14.36, 7.36, 4.32, 1.08, and 0.7 % cytotoxic movement, separately, though at 50 µM fixation the cell reasonability is diminished to 7.36 % (Maximum cell death had occurred).

$$Y = -3.44+ (63.2896-3.44) / (1+(x/13.0457)^{1.2955}) \quad (2)$$

$IC_{50} = 13.0457$

The IC_{50} estimation of the complex (*calculated by four parameter regression model Eq.2*) has displayed at 13.05 μM, **Fig.4**. These outcomes demonstrates that the complex give indications of unrivalled cytotoxic action against the HeLa cell lines and have the credible to be examine as anticancer operators.

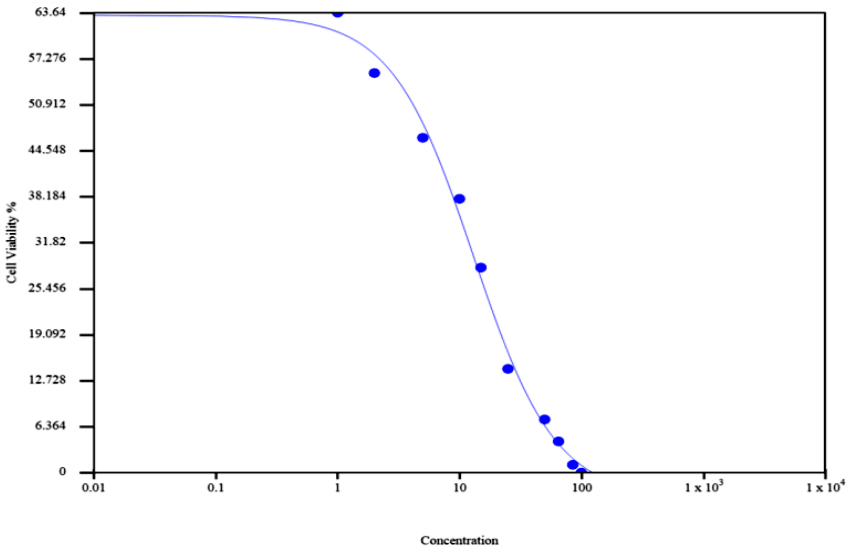

FIG.4. SCHEMATIC REPRESENTATION OF KEY DOSE-INHIBITER PARAMETERS IC_{50}, OF COMPLEX, CALCULATED BY CURVE FITTING TO THE CELL SURVIVAL DATA

D) MOLECULAR DOCKING STUDY WITH CA125 PROTEIN

A molecular docking study on the complex has performed using HEX 5.0 software and Q-site finder to identify the possible binding sites on the biomolecules [36].

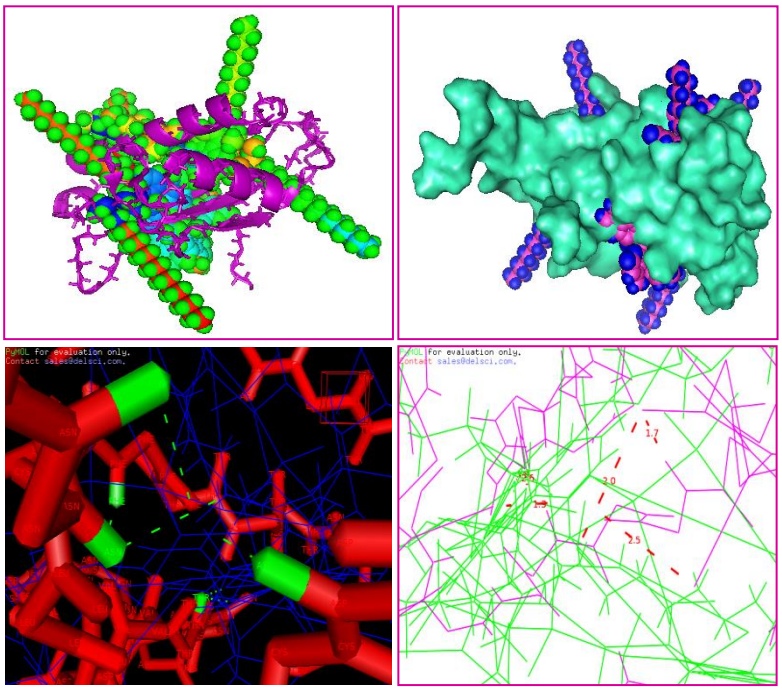

FIG.5. Molecular Docking Image of $[Gd_2\{Bis\text{-}Acdn(DO3ODA)_2(H_2O)_2\}\{Ir_2\{Bis\text{-}phenyl(Tris(2\text{-}Chromone)\}]$ with CA125 Protein (PDB No. : 1ivz)

It is a worldwide search among researcher to find a molecule which can image the presence of CA125 protein in human tissues in early stage itself. CA125 contains GLY, SER, GLN, HIS, PHE, ASN, LEU, THR, ILE, PRO, TYR, ASP, ALA, LYS, ARG, GLU, CYS, VAL, and MET types of amino acid links in their skeleton which makes the molecule high polar. Here we examine the binding efficiency of the complex with the amino acid sequence in CA125 protein. As it is expect our highly polar [Gd_2{Bis-Acdn(DO3ODA)$_2$(H_2O)$_2$}{Ir_2{Bis-phenyl(Tris (2-Chromone)}] binds through π – interaction with key amino acids like ASP 63, ASN 86, ASN 76, ARG 42, GLN 38, PHE 18, and THR 79 groups in CA125 protein with a coupling energy of -282.14 kcal mol^{-1}. The docking structure confirms that our complex easily bind with the main chain of the cancer creating CA125 protein and gives information of the site where it presents. The conceivable hydrogen bonding cooperation between the edifices with the receptor are given in **Fig. 5**.

6. Advantages

The coordination sphere of each Gd(III) metal ion in [Gd_2{Bis-Acdn(DO3ODA)$_2$(H_2O)$_2$}{Ir_2{Bis-phenyl-(Tris(2-Chromone)}] is similar to that of [Gd(III)(DOTA)(H_2O)] complex. The presence of two water molecules in the inner coordination sphere, Extra water molecules binded on the II coordination sphere, and replaceable hydrogen atom in the acridone linker enhances the proton relaxation rate and give huge relaxivity value. The polar group in the complex supports binding over many macromolecular compounds like polymers, colestrol, fat, vitamins, and proteins, behaves like a one single macromolecule, and gives remarkable relaxivity at room temperature itself. The lipophilic groups like coumarin, octadecanyl group, and acridone provide affinity towards CA125 protein, prevent its accumulation in liver, and increase its plasma half-life.

7. Conclusion

Our Gd(III)-Pt(IV) complex shows remarkable relaxivity enhancement in the presence HSA (r_{1p} = 77.68 mM^{-1} s^{-1} and r_{2p} = 127.36 mM^{-1} s^{-1}) and at different pH solutions (2.6-10.6). The r_{2p}/r_{1p} ratio confirms that the complex is a T_1-weighted contrast agent this is similar with that of the complex in neat aqueous solution. The high polar coumarone derivative at the periphery of the complex supports the binding with HSA macro molecule and helps in reducing the rotation correlation time and enhanced the relaxivity. The complex shows pH dependence T_1 and T_2 values (r_{1p}= 73.64 to 13.28, r_{2p} = 114.34 to 25.54) which confirms the suitability of the complex for imaging cancer tissue. The MTT assay study confirms the anticancer property of the complex against HeLa cells. Further, the IC_{50} estimation of the complex gives an unexpected value of 13.05 μM, which shows that the complex give indications of unrivalled cytotoxic action against the HeLa cell lines. Since, coumarin and acridone has anticancer activity, our newly designed M-MRI CA will play triple role in identification of cancer cell, target protein binding, and killing drug for ovarian cancer.

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Declarations

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Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Consent for publication

We declare that we consented for the publication of this research work.

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