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# Novel Designing of Ir(III) (Tris-Coumarin) Cored Gd(III) Complex as Targeted MRI CAs for Ovarian Cancer Treatment

T.Lurthu Pushparaj[1]* & S.Sathya[2]

[1]*Assistant Professor, PG Department of Chemistry, Tirunelveli Dakshina Mara Nadar Sangam College, India.*
[2]*PG Scholar, PG Department of Chemistry, Tirunelveli Dakshina Mara Nadar Sangam College, India.*

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ABSTRACT

*Iridium metal based compounds find their application in selective killing of cancer cells without harming good tissues/cells. We have reported a high water soluble coumarin substituted Gd(III)-Ir(III) complex to target ovarian cancer cells. The complex shows high longitudinal ($r_{1p}$) and transversal ($r_{2p}$) relaxivity values of 26.19 and 37.21 $mM^{-1} s^{-1}$, respectively, at 25 $^{o}C$ and pH 7. The $r_{2p}/r_{1P}$ ratio of 1.42 confirms that the complex is a $T_1$-weighted contrast agent. The significant increase in relaxivity values are due to the presence of high polar and rigid moieties like, acridone linker, 2-coumarin compound, and octadecanyl amide pendant arms which are strongly binded on Gd(III)-Ir(III) complex. The presence of two water molecules in the inner coordination sphere and replaceable hydrogen atom in the acridone linker enhances the proton relaxation rate ($\tau_R$) and give huge relaxivity value. The high polar interaction behavior of our complex will give detail understanding of mechanisms of immune modulation in ovarian carcinoma and dramatically improve the clinical outcomes of ovarian cancer patients.*

***Keywords:*** *Cancer imaging agent, Ovarian cancer cell, Tris-coumarin, Gd-Ir complex, Bis-Acridone Ir(III) complex.*

## 1. Introduction

Early detection of most cancers is imperative for cure and the survival rate of sufferers [1, 2]. Magnetic resonance imaging (MRI) is the most frequently used imaging method for the detection and diagnosis of cancer. However, it is still hard to distinguish between tumor regions and normal regions. Many contrast agents (CAs) have been developed to enhance contrast intensity and contrast effect on the region of interest.[3-7] In MRI paramagnetic metals, Gadolinium-based contrast agents are widely used as a contrast agent. The agents that have entered the clinical practice and affect the relaxation rates of tissue water protons in the regions where they distribute [8,9]. Since gadolinium ions are toxic, many ligands are developed to bind gadolinium ions to prevent free gadolinium-associated disease. However, many reports indicated that linear chelator-based contrast agents are associated with nephrogenic systemic fibrosis (NSF) in patients with low kidney function [10-14]. Moreover, there have been various attempts to improve the signal intensity and sensitivity of Gd3 +-based CAs to the region of interest [15–22]. One of the methods is the accumulation of CAs using conjugation with bioactive moieties to increase the concentration of CAs in specific regions, tumor specific antibody, and stimuli-responsive polymer [23–27]. The use of low molecular weight exogenously administered contrast agents allows compartment-specific enhancement of tumors, enabling imaging of functional blood and interstitial volumes [28,29]. Current efforts are directed at enhancing the capabilities of MRI in oncology by adding contrast agents with molecular specificities to the growing armamentarium of diagnostic probes that produce a signal by changing local proton relaxation times because of specific contrast agents binding to cell surface receptors or extracellular matrix components [30]. MRI methods such as DCE have proven effective in detecting tumors as small as 1 to 2 mm and conventional MR methods are still improving for more accurate cancer detection and prognosis. In addition, conventional MRI methods frequently lack specificity for typing cancers for the purpose of differential diagnosis. Tumor extracellular matrix has a profusion of cancer-related proteins that can use as biomarkers for most cancers molecular

imaging. Innovative layout and enhancement of harmless and top notch targeted contrast agent to difference entrepreneurs to these biomarkers would allow tremendous MR most cancers molecular imaging with immoderate spatial choice. Iridium is the second densest metal in the world as well as the most resistant to corrosion, belonging to the same family of metals as platinum that is also widely used in chemotherapy treatments. Iridium has used to kill cancer cells without harming healthy tissue by damaging the proteins for heat shock stresses and glucose metabolism, which have identified as key biochemical pathways in the development of cancers. An unprecedented view of the individual proteins inside the cancer cells that were binding to the iridium compound can be identified using ultra-high mass spectrometry facilities [31]. The purpose of this study was to design a new coumarone substituted Ir(III) cored molecule containing lipophilic group functionalized cyclen macrocyclic Gd(III) complex appended at the periphery as a high relaxivity contrast agent for MR imaging of tumors.

## 2. Motivation

Antibody therapeutics towards special goal antigens are extensively used in the cure of one-of-a-kind malignancies, consisting of ovarian carcinomas, however, this ailment nonetheless requires greater dealers that are effective. Research in developing novel chemotherapeutic regimes targeted and other therapies does not show any significant improvements in diagnosing ovarian cancer. The challenge for any cancer researcher is in the identification of overexpressed protein, gene, etc. We created a compound of iridium and organic material that can directly target cancer cells. These compounds then transfer energy into the cancer cells, which turn the diatomic oxygen molecules inside the cells into singlet oxygen, a high-energy form of the element, which is toxic to cells and leads to their destruction.

## 3. Materials and Methods

### *3.1 Materials*

7-Hydroxy coumarin, Octadecanoyl chloride, Cyclen, 1,2-diaminoethane, 1,5-dichloroacridine-9(10H)-one, 7-(3-bromopropoxy)-4-methyl-2H-chromen-2-one, toluene-4-sulfonyl chloride, bromopyrogallol, celite, Sodium hydroxide, Analine, di-bromopropane, Sodium chloride salt, Sodium carbonate, Resorcinol, Phenol, Xylenol orange indicator, bromine, Gadolinium (III) chloride hexa hydrate, Iridium (III) chloride hydride, Deuterium oxide (99.9 atom % pure), DMSO-d6 (99.99 atom % pure), $CDCl_3$ (100 atom % D), Dowex 50W X 8 ion exchange resin, Dowex 1 X 8-200 ion exchange resin, Amberlite IR-120 (H+, acidic), molecular sieves (4Å) and lithium aluminium hydride.

### *3.2 Spectral* Methods

An infrared spectrum was recorded on a Perkin-Elmer Spectrum RX-I FT IR Spectrometer in the range of 4000-400 $cm^{-1}$. Spectra of solid samples were recorded by making transparent KBr pellets. Potassium bromide (FT IR grade, Aldrich) was used to make the pellets. The electrospray ionization mass spectra (ESIMS) were recorded using a Micromass Quattro-II Triple Quatrapole Mass Spectrometer. The sample was dissolved in a suitable solvent and introduced into the ESI capillary using a 5 μL syringe pump. The ESI capillary was set at 3.5 kV with the cone

voltage of 40 V. The averaged spectra of 6-8 scans were printed. EI mass spectra were recorded in FINNINGAN MAT 8230 mass spectrometer with accelerating voltage of 70 eV at room temperature. CHNS & O microanalyses were carried out using a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer, interfaced with a Perkin-Elmer AD 6 Autobalance. Helium was used as the carrier gas. *Caution!* All hygroscopic compounds were dried in desiccator over silica gel for 24 h prior to the analysis.

### *3.3 Longitudinal and Transverse Relaxivity ($R_{1P}$ & $R_{2P}$)*

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, $T_1$. The $T_1$ measurements will be made using the standard inversion recovery pulse sequence (180°-τ-90°) with phase sensitive detection [32] with $\tau$ values ranging from 50 ms to 6s for six different concentration of the complex. The slope of the plot $1/T_1$ vs concentration of the complex gives the longitudinal relaxivity.

The pH will be maintained by adding the TRIS or MES buffer. The transverse relaxivity will be determined from the transverse relaxation time $T_2$. A standard CPMG pulse sequence (90°-$\tau$-180°) [33] 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. Synthetic Procedures

### *4.1. Synthesis of 1,2-BIS (5-Acridin-9(10h)-One-1-Chloro) Amino Ethane Linker*

A solution of 1,5-dichloroacridine-9(10*H*)-one (10.56 g, 20 mmol) and $Na_2CO_3$ (1.06 g, 10 mmol) in 150 mL of acetonitrile was taken in a round bottom flask, fitted with a double surface condenser. 1,2-Diaminoethane (0.60 mL, 10 mmol), dissolved in 50 mL of acetonitrile was dropped in to the above mixture under stirring and refluxed for 6 h with vigorous stirring. The solution was cooled to room temperature and the solvent was removed and the pale yellow precipitate of 1,2-bis(5-acridin-9(10H)-one-1-chloro)amino ethane (**1**) was dried under vacuum. Yield 4.72 g (92 %), mp 226 $^oC$. Anal. calcd. for $C_{28}H_{20}Cl_2N_4O_2$ ($M_r$ = 515): C, 65.25; H, 3.91; Cl, 13.76; N, 10.87; O, 6.21. Found: C, 64.75; H, 3.73; Cl, 14.01; N, 10.78; O, 6.48. IR (KBr, $cm^{-1}$): 3234 ν(N-H) (amine), 2971 ν(C-H), 1618 ν(C=O), 1049 ν(C-N) (secondary amine), 546 ν(C-Cl) 3032.5 ν(C-H) (aromatic); 2994.9 $\nu_{as}$(C-H) and 2920.0 $\nu_s$(C-H) (aliphatic); 1566.4 and 1446.3 $\nu_s$(C=C) (aromatic); 751.3 δ(C-H) (aromatic). MS (ESI): *m/z* 517 $[M+2]^+$, 288 $[M-C_{13}H_7NOCl]^+$, 227 $[M-C_{13}H_7NOCl]^+$.

### *4.2. Synthesis of 1,4,7,10-Tetraazacyclododecane-1,4,7-Tri-Octadecamide (DO3-SA-AM)*

To a solution of 1,4,7,10-tetraazacyclododecane (5 gm, 29 mmol) in water was added octadecanoyl chloride (26.4 gm, 87 mmol ) and the pH of the solution was raised to 10 by using 1 M aqueous sodium hydroxide. The reaction has carried out at -4 $^oC$ for 6 h and the content has heated to 50 $^oC$ and maintained for 4 h. The pH 10 has maintained throughout the reaction. The reaction afforded major amount of tri-*N*-substituted compound and trace of tetra-*N*-substituted compounds. The tri-*N*-substituted (DO3-Sa-Am) was separated using ion-exchange chromatography. The solution was concentrated and vacuum dried to yield DO3-Sa-Am: white solid, yield 22.56 g (80 %), mp 283 $^oC$. Anal. calcd. for $C_{62}H_{122}N_4O_3$ ($M_r$ = 972): C, 76.64; H, 12.66; N, 5.77; O, 4.94. Found: C,

75.94; H, 12.67; N, 5.59; O, 4.96. IR (KBr, $cm^{-1}$): 2924 ν(C-H), 1638 ν(C=O) (amide), 1124 ν(C-N) (tertiary amine), 2922.8 $\nu_{as}$(C-H) and 2866.0 $\nu_s$(C-H) (aliphatic). MS (ESI): *m/z* 971 $[M-H]^+$, 699 $[M-C_{18}H_{41}O]^+$, 443 $[M-C_{36}H_{65}O_2]^+$, 361 $[M-C_{40}H_{85}NO_2]^+$.

### *4.3. Synthesis of 1,2,3-Trioxy(7-(3-Propoxy)-4-Methyl-2h-Chromen-2-One)-5-Bromobenzene (Tris-Coumarin Wedge)*

It has been synthesized by simple *O*-alkalization reaction. About 50 ml of freshly prepared ethanol was taken in a 250 ml RB flask, to this bromopyrogallol (2.04 g, 10 mmol) and solid $Na_2CO_3$ (1.59 g, 15 mmol) was added and heated slowly under stirring for 30 minutes, here $Na_2CO_3$ has been acted as proton scavenger. When all the reactants gets dissolved a solution containing 7-(3-bromopropoxy)-4-methyl-2H-chromen-2-one (9.80 g, 33 mmol) in 100 mL of ethanol was dropped for about 1 hr with vigorous stirring. The solution was cooled to room temperature and concentrated to dryness to obtain the product 1,2,3-trioxy(7-(3-propoxy)-4-methyl-2H-chromen-2-one)-5-bromobenzene as colorless compound in yield 7.40 g (87 %), mp 158 °C. Anal. calcd. for $C_{45}H_{41}BrO_{12}$ ($M_r$ = 854): C, 63.31; H, 4.84; Br, 9.36; O, 22.49. Found: C, 62.91; H, 4.38; Br, 8.96; O, 22.08. IR (KBr, $cm^{-1}$): 2988 ν(C-H), 1632 ν(C=O), 529 ν(C-Br) 3045 ν(C-H) (aromatic); 2958 $\nu_{as}$(C-H) and 2880.0 $\nu_s$(C-H) (aliphatic); 1564 and 1463 $\nu_s$(C=C) (aromatic); 1240.3 $\nu_{as}$(C-O-C); 753 δ(C-H) (aromatic). MS (ESI): *m/z* 854 $[M]^+$, 674 $[M-C_{10}H_{12}NO_3]^+$, 289 $[M-C_{33}H_{25}O_9]^+$, 173 $[C_{10}H_7O_3]^+$.

### *4.4. Synthesis of 1,2-BIS((5-Acridin-9(10h)-One)-1,1-Di(1,4,7,10-Tetraazacyclo- Dodecane-1,4,7-Tri-Octadecamide))Amino Ethane*

A solution of 1,4,7,10-tetraazacyclododecane-1,4,7-tri-octadecamide (19.43 g, 20 mmol) and $Na_2CO_3$ (1.06 g, 10 mmol) in 150 mL of ethanaol was taken in a round bottom flask, fitted with a double surface condenser. N,N-bis(5-acridin-9(10H)-one-1-chloro) amino ethane (5.514 g, 10 mmol), dissolved in 50 mL of water was dropped in to the above mixture under stirring and refluxed for 1 h with vigorous stirring. The solution was cooled to room temperature and the solvent was removed and the pale yellow precipitate of 1,2-bis((5-acridin-9(10H)-one)-1,1-di(1,4,7,10-tetraazacyclododecane-1,4,7-tri-octadecamide)) amino ethane was dried under vacuum. Yield 21.65g (90.7 %), mp 257 °C. Anal. calcd. for $C_{152}H_{262}N_{12}O_8$ ($M_r$ = 2386): C, 76.52; H, 11.09; N, 7.04; O, 5.36. Found: C, 76.19; H, 10.94; N, 6.83; O, 5.39. IR (KBr, $cm^{-1}$): 3185 ν(N-H) (2° amine), 2918 ν(C-H), 1598 ν(C=O), 1026 ν(C-N) (secondary amine), 3025 ν(C-H) (aromatic); 2949 $\nu_{as}$(C-H) and 2900 $\nu_s$(C-H) (aliphatic); 1124 ν(C-N) (tertiary amine); 1664 and 1463 $\nu_s$(C=C) (aromatic); 765 δ(C-H) (aromatic). MS (ESI): *m/z* 2388 $[M+2]^+$, 1511 $[M-C_{58}H_{117}NO_3]^+$, 1327 $[(M+1)-C_{68}H_{125}NO_3]^+$, 1143 $[(M-6H)\ C_{80}H_{157}N_4O_4]^+$, 775 $[C_{108}H_{219}O_6]^+$.

### *4.5. Synthesis of 1,2,3-Trioxy(7-(3-Propoxy)-4-Methyl-2h-Chromen-2-One)-5- Bromobenzene Iridium Complex*

About 4.27 g (5 mmol) of 1,2,3-trioxy(7-(3-propoxy)-4-methyl-2H-chromen-2-one)-5- bromobenzene iridium derivative and 1.49 g (5 mmol) $IrCl_3 \cdot 3H_2O$ in 60 mL of a mixed solvent of $H_2O$ and ethanol (v:v = 1:3), were refluxed at 120 °C under stirring for 24 hours. After the reaction was completed, the mixture was cooled to room temperature. The solid was collected by suction filtration, washed with distilled water, gathering the solid product.

The yellow colour product obtained has been recrystallized from dilute ethanol, yield 5.08 g (97 %), mp 243 $^{o}$C. Anal. calcd. for $C_{45}H_{41}BrIrO_{12}$ ($M_r$ = 1046): C, 51.68; H, 3.95; Br, 7.64; Ir, 18.38; O, 18.36. Found: C, 50.86; H, 3.89; Br, 6.87; Ir, 18.43; O, 19.66. IR (KBr, cm$^{-1}$): 2897 ν(C-H), 1611 ν(C=O), 3018 ν(C-H) (aromatic); 2927 $\nu_{as}$(C-H) and 2857 $\nu_s$(C-H) (aliphatic); 1514 and 1483 $\nu_s$(C=C) (aromatic); 1243 $\nu_{as}$(C-O-C); $\nu_s$(Ir-O) 403; 753 δ(C-H) (aromatic); 515 ν(C-Br). MS (ESI): *m/z* 1048 $[M+2H]^+$, 828 $[(M-H)-C_{13}H_{13}O_3]^+$, 814 $[(M+H)-C_{13}H_{13}O_4]^+$, 772 $[(M-2H)-BrIr]^+$,690 $[(M-5H)-C_{10}H_7O_2Ir]^+$, 634 $[(M-3H)-C_{13}H_{13}O_3Ir]^+$.

### *4.6. Synthesis of 1,2-BIS((5-Acridin-9(10h)-One)-1,1-Di(1,4,7,10-Tetraazacyclodo- Decane-1,4,7-Tri-Octadecamide))-1',2'-Bis((1,2,3-Trioxy(7-(3-Propoxy)-4-Methyl-2h-Chromen-2-One)-5-Benzene)Iridium)Amio Ethane Complex*

To a solution of 1,2-bis((5-acridin-9(10H)-one)-1,1-di(1,4,7,10-tetraazacyclododecane-1,4,7-tri-octadec-amide))amino ethane (11.93 gm, 5 mmol) in 100 mL of water, pure $Na_2CO_3$ (0.6 g, 5 mmol) was added and stirred under heating for 30 minutes. About 10.46 g (10 mmol) of 1,2,3-trioxy(7-(3-propoxy)-4-methyl-2H- chromen-2-one)-5-bromobenzene Iridium complex in 100 mL of water was added slowly in to the solution and heated under mild stirring for 1 hr.

The solution was concentrated and vacuum dried to yield 1,2-bis((5-acridin-9(10H)-one)-1,1-di(1,4,7,10-tetraazacyclododecane-1,4,7-tri-octadecamide))-1',2'-bis-((1,2,3-trioxy(7-(3-propoxy)-4-methyl-2H-chromen-2-one) -5-benzene) Iridium)amino ethane complex: white solid, yield 19.38 g (89 %), dec. 313 $^{o}$C. Anal. calcd. for $C_{242}H_{342}Ir_2N_{12}O_{32}$ ($M_r$ = 4316): C, 67.35; H, 7.99; Ir, 8.91; N, 3.89; O, 11.86. Found: C, 66.73; H, 7.43; Ir, 8.52; N, 3.21; O, 11.27. IR (KBr, cm$^{-1}$): 3118 ν(N-H) (2$^{o}$ amine), 1124 ν(C-N) (tertiary amine); 1563 ν(C=O), 1068 ν(C-N) (secondary amine), 3051 ν(C-H) (aromatic); 2937 $\nu_{as}$(C-H) and 2912 $\nu_s$(C-H) (aliphatic); 1647 and 1429 $\nu_s$(C=C) (aromatic); 788 δ(C-H) (aromatic); 1254 $\nu_{as}$(C-O-C); $\nu_s$(Ir-O) 401. MS (ESI): *m/z* 4316 $[M]^+$, 3932 $[M-Ir_2]^+$, 3292 $[(M+1)-C_{36}H_{33}Ir_2O_{11}]^+$, 2754 $[(M+2H)-C_{66}H_{68}Ir_2O_4]^+$, 2410 $[(M-3H)-C_{99}H_{195}N_4Ir_2O_5]^+$.

### *4.7. Synthesis of 1,2-BIS((5-Acridin-9(10h)-One)-1,1-Di(1,4,7,10-Tetraazacyclo- Dodecane-1,4,7-Tri-Octadecamide-Gadolinium(Iii)))-1',2'-Bis((1,2,3-Trioxy(7-(3-Propoxy)-4-Methyl-2h-Chromen-2-One)-5-Benzene)Iridium) Amino Ethane Complex*

To a solution of the precursor iridium complex (12.95 g, 3 mmol) in 80 mL of water, $GdCl_3.6H_2O$ (2.23 g, 6 mmol) in 50 mL of was added. The pH of the solution was maintained at 7 throughout the reaction by adding an aqueous solution of NaOH and heated to 60 °C under argon atmosphere for about 15 h.

It was cooled to room temperature, filtered, and flash evaporated to dryness. The resulting colorless hygroscopic solid was and recrystallized in water: yield (12.76 g, 91%). Anal. calcd. for $C_{242}H_{346}Gd_2Ir_2N_{12}O_{34}$ ($M_r$ = 4666): C, 62.29; H, 7.47; Gd, 6.74; Ir, 8.24; N, 3.60; O, 11.66. Found: C, 61.54; H, 7.14; Gd, 6.27; Ir, 8.58; N, 3.34; O, 10.98. IR (KBr, cm$^{-1}$): 3021 ν(N-H) (2$^{o}$ amine), 1184 ν(C-N) (tertiary amine); 1546 ν(C=O), 1082 ν(C-N) (2$^{o}$ amine), 2984 ν(C-H) (aromatic); 2903 $\nu_{as}$(C-H) and 2874 $\nu_s$(C-H) (aliphatic); 1679 and 1492 $\nu_s$(C=C) (aromatic); 791 δ(C-H) (aromatic); 1245 $\nu_{as}$(C-O-C); 409 $\nu_s$(Ir-O); 438 ν(Gd-O). MS (ESI): *m/z* 4666 $[M]^+$, 4076 $[(M+2)-(CH_7O_2Gd_2Ir_2)]^+$, 4049 $[M-C_{18}H_{39}O_3Gd_2Ir_2]^+$, 3887 $[M-C_3H_{13}O_2Gd_2Ir_2]^+$, 3837 $[M-C_6H_{27}O_2Gd_2Ir_2]^+$, 3670 $[M-C_{16}H_{26}O_5Gd_2Ir_2]^+$.

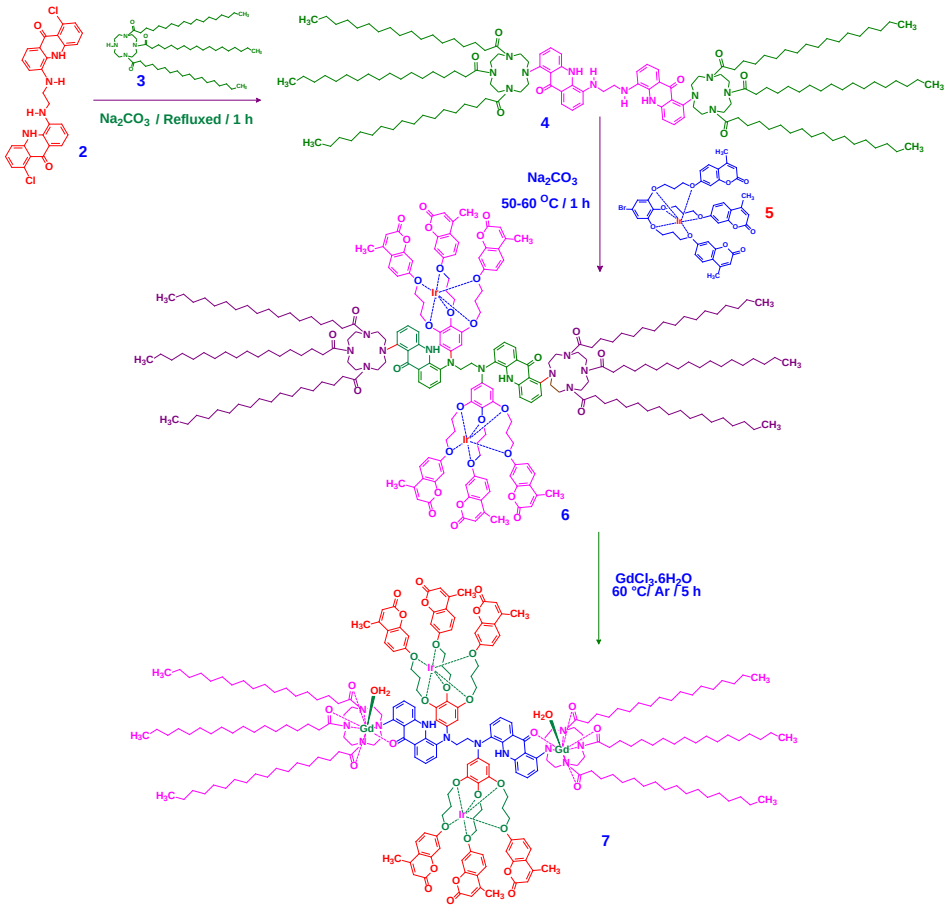


**FIG.1.** SCHEME FOR THE SYNTHESIS OF $[GD_2\{BIS\text{-}ACDN(DO3ODA)_2(H_2O)_2\}\{IR_2\{BIS\text{-}PHENYL(TRIS(2\text{-}CHROMONE)\}]$

## 5. Results and Discussions

The bis-acridone linker (**1**) was synthesized by simple *N*-alkylation of 1,5-dichloroacridine-9(10*H*)-one with 1,2-diaminoethane in acetonitrile. Tri substituted cyclen (**2**) was synthesized by *N*-alkylation of cyclen with octadecanoyl chloride in triple distilled water. Tris-coumarin wedge (**3**) was synthesized by *O*-alkalization reaction of bromopyrogallol with 7-(3-bromopropoxy)-4-methyl- 2H-chromen-2-one. The compound (**4**) was synthesized by the reaction of **2** with **1** in water : ethanol mixture. The coumarone functionalized Ir(III) metal wedge (**5**) was synthesized by the reaction of **3** with $IrCl_3 \cdot 3H_2O$ in 1:1 ratio in a mixed solvent of $H_2O$ and ethanol (v:v = 1:3), at room temperature. The precursor complex (**6**) was synthesized by reacting **4** with **5** in water. The target complex (**7**) was synthesized by the reaction of the precursor Ir(III) complex **6** with $GdCl_3.6H_2O$ in water at pH 7. All the ligands and the complexes reported here has characterized by using melting point apparatus, IR, and ESI mass spectroscopy. The results confirmed the formation of products.

### *5.1 Relaxivity Measurements*

#### *5.1.1 Longitudinal Relaxivity of the Target Complex (7)*

The longitudinal relaxation times of water protons for six concentrations of [**7**] have given in Table 1 and the plot of the concentration of the complex versus $1/T_1$ depicted in **Fig. 2**. The heterotetranuclear complex

exhibits a relaxivity of 26.19 $mM^{-1} s^{-1}$ which corresponds to 13.09 "per Gd". The "per Gd" $r_{1p}$ value is 3.11, 2.73, and 3.44 units higher than that of FDA approved mononuclear Gd(III) complexes like $[Gd(DOTA)(H_2O)]^-$ ($r_{1p}$ = 4.2 $mM^{-1} s^{-1}$, 20 MHz), $[Gd(DO3A)(H_2O)_2]$ ($r_{1p}$ = 4.8 $mM^{-1} s^{-1}$, 20 MHz, $q$ = 2), and $[Gd(DTPA)(H_2O)]^{2-}$ ($r_{1p}$ = 3.8 $mM^{-1} s^{-1}$, 20 MHz, 25 °C), respectively. The $r_{1p}$ value of the complex is 2.63 and 2.73 units higher than that of the trinuclear complexes $[\{Gd(DO3A)\}_3L_{12}(H_2O)_3]$ ($r_{1p}$ = 8.8 $mM^{-1} s^{-1}$, 20 MHz, 40 °C) and $[\{Gd(DO3A)\}_3$-$L_{13}(H_2O)_3]$ ($L_{13}$ = tris(*N*-(4-ethanethioamidophenyl)acetamido)-2- aminoethylamine) ($r_{1p}$ = 8.5 $mM^{-1} s^{-1}$, 20 MHz, 40 °C), respectively[34-36]. The coordination sphere of each Gd (III) metal ion in the complex is similar to that of $[Gd(DOTA)(H_2O)]$. The higher $r_{1p}$ value is due to the presence of two inner-sphere water molecules coordinated to the two Gd(III) ions, higher molecular weight, and limited internal flexibility.

**Table 1.** Longitudinal relaxation times of the complex at six different concentrations

| Concentration (mM) | $T_1$ x $10^{-3}$ s | $1/T_1$ x $10^3$ $s^{-1}$ |
|---|---|---|
| **0.2** | 190.48 | 05.25 |
| **0.5** | 076.39 | 13.09 |
| **1.0** | 038.21 | 26.17 |
| **1.5** | 041.24 | 39.78 |
| **2.0** | 018.64 | 53.65 |
| **2.5** | 013.65 | 73.28 |

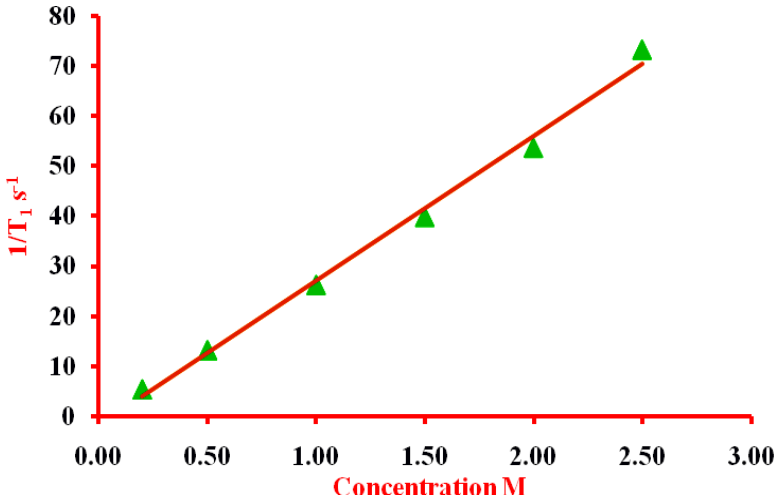


**FIG.2.** PLOT OF THE CONCENTRATION OF THE COMPLEX VERSUS $1/T_1$

***5.1.2 Transverse relaxivity of [Gd$_2${Bis-Acdn(DO3ODA)$_2$(H$_2$O)$_2$}{Ir$_2${Bis-phenyl(Tris(2-Chromone)}]***

The transverse relaxation times for six concentrations of [**7**] are given in Table 2 and the plot of the concentration of the complex versus $1/T_2$ is shown in **Fig. 3**. The transverse relaxivity value for **7** is found to be 37.21 $mM^{-1} s^{-1}$ which corresponds to 18.60 "per Gd". The "per Gd" $r_{2p}$ value is higher than that of the

complexes [$Gd_2${pr-(DO3VA)$_2$}$(H_2O)_4$] ($r_{2p}$ = 4.75 $mM^{-1}s^{-1}$, 20 MHz) and [$Gd_2${acamido-et(DO3VA)$_2$} $(H_2O)_2$] ($r_{2p}$ = 7.17 $mM^{-1}s^{-1}$ "per Gd"). The higher transverse relaxivity is due to the presence of six bulky 2-octadecane amide and 2-Chromone groups on the periphery of the complex. The ratio $r_{2p}/r_{1P}$ is 1.42 which shows that the complex is a $T_1$-weighted contrast agent.

**Table 2.** Transverse relaxation times of the complex (**7**) at six different concentrations

| Concentration (mM) | $T_2$ x $10^{-3}$ s | $1/T_2$ x $10^3$ $s^{-1}$ |
|---|---|---|
| **0.2** | 134.23 | 07.45 |
| **0.5** | 165.29 | 18.62 |
| **1.0** | 026.85 | 37.25 |
| **1.5** | 017.58 | 56.87 |
| **2.0** | 013.40 | 74.63 |
| **2.5** | 010.64 | 94.01 |

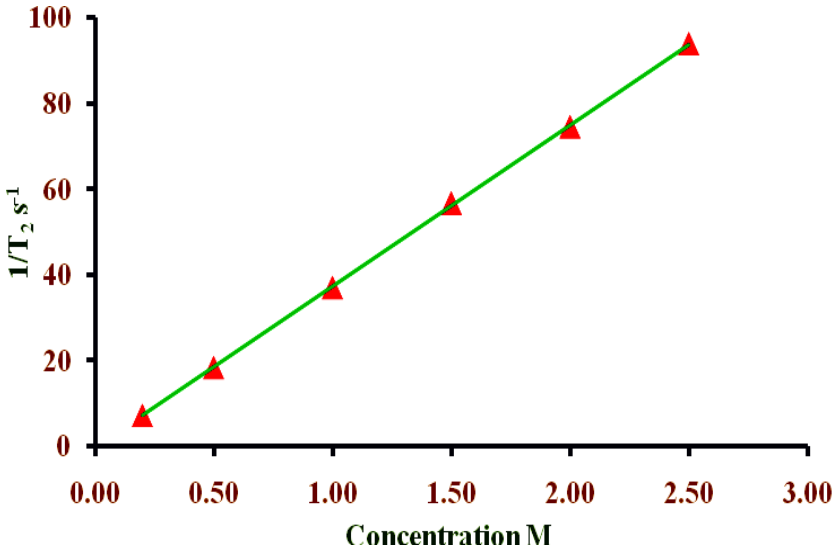


**FIG.3.** PLOT OF THE CONCENTRATION OF THE COMPLEX (**7**) VERSUS $1/T_2$

## 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

A new coumarone based bulky group substituted Gd(III)-Ir(III) complex has been synthesized and their $T_1$ and $T_2$ values in neat aqueous solution are reported. The target complex $[Gd_2\{Bis\text{-}Acdn(DO3ODA)_2(H_2O)_2\}\text{-}\{Ir_2\{Bis\text{-}phenyl(Tris(2\text{-}Chromone)\}]$ is highly soluble in water. The complex shows high longitudinal ($r_{1p}$) and transversal ($r_{2p}$) relaxivity values of 26.19 $mM^{-1}$ $s^{-1}$ and 37.21 $mM^{-1}$ $s^{-1}$, respectively. The $r_{2p}/r_{1P}$ ratio of 1.42 confirms that the complex is a $T_1$-weighted contrast agent. Since the complex has six high polar coumarone moiety and two rigid acridone moieties the Gd(III) - Ir(III) metal complex shall experience the better binding with almost all kinds of internal organs, typical proteins, and enzymes. Our lipophilic Iridium-gadolinium complex can serve as an effective contrast agent for imaging many cancer cells and their detailed anticancer study can reveal suitability to improve clinical outcomes of ovarian cancer patients.

## 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.

***Code availability***

The programming code that we have used for this research is available and authors are willing to share when it is required.

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