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Developing A Novel Coumarone-Phenyl Amide Functionalized [Gd(III)-Pt(IV)] Complex as High T_1, T_2 Relaxive M-MRI Contrast Agent for Cancer Diagnosis

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ABSTRACT

Pt(IV) cored Gd(III) metal complex as a multimodal MRI contrast agent for cancer diagnosis is reported. The hetero nuclear complex [Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(II)] is highly soluble in water and stable at room temperature. The complex shows high longitudinal (r_{1p} = 24.43 mM^{-1} s^{-1}) and transversal (r_{2p} = 38.61 mM^{-1} s^{-1}) relaxivity values in neat aqueous solution at pH =7 and at 27 ^oC. The relaxivity value of the complex is greater than the low molecular weight, FDA approved MRI contrast agents. The presence of two water molecules in the first coordination sphere and a replaceable hydrogen atom in the linker enhances the proton relaxation rate and gives a huge relaxivity value. The r_{2p}/r_{1P} ratio of 1.58 confirms that the complex is a T_1-weighted contrast agent. The presence of high polar coumarone pendant arm and high rigid acridone moieties in the complex make the complex as better anticancer agent for ovarian cancer. The high polar nature of the coumarone, phenyl amide, acridone moiety will show better binding efficiency with ovarian cancer creating CA125 glycoprotein.

Keywords: Multimodal imaging agent, Ovarian cancer, Coumarone pendant arm, Gd-Pt complex, Acridone Pt(IV) complex.

1. Introduction

Magnetic resonance imaging (MR) is a test that uses a magnetic field and radio wave energy pulses to make 3D images of nearly all the organs and nervous systems in the body. Either way, MRI also provides different information on body structures, which cannot be seen with X-ray, ultrasound or computed tomography (CT). 3D images obtained from an MR imaging are digital images that will be saved on a computer for a more in-depth examination. To increase the colour contrast of the organ images, a paramagnetic complex called contrast agent administered before the MR scanning. The efficiency of a paramagnetic complex to enhance the relaxation of water proton nuclei characterized in vitro by its relaxivity, which normalizes the effect to a 1mM concentration of the paramagnetic ion.1−3. Gadolinium-based contrast agents are widely used as contrast agents by MRI because of their outstanding improved signal and ease of chemical modification. Gadolinium metal ions are very toxic; so many sects have been developing to bind gadolinium ions to prevent disease associated with free gadolinium. The design of new contrast agents needs to fulfil some requirements such as (1) thermodynamic and kinetic stability, a particularly important issue since the documentation of nephrogenic systemic fibrosis (NSF), an illness detected in patients with renal impairment after administration of certain contrast agents 4−6 (more recently, gadolinium deposition in bones and brains of patients with normal renal function has been described); 4,7 (2) high relaxivity, which allows the use of lower doses of the contrast agent and the detection of low concentration targets; 8,9 (3) good solubility, as contrast agents are injected in rather large doses due to the low sensitivity of the technique; 8 (4) rapid excretion to minimize the chances of complex dissociation and the consequent risk of deposition; and (5) low osmolality and viscosity to avoid adverse reactions and necrosis of the tissue [10,11]. However, it is increasingly recognised that information obtained from single modal molecular imaging cannot meet the higher requirements of efficiency and accuracy for clinical diagnosis and medical research, because it is limited and anchored by default in the single molecular imaging technique itself. To address the shortcomings of

single-function magnetic resonance contrast agents, the combination of multi-modality imaging has proven to be research over the past few years [12]. Mono to polynuclear Gd(III) complexes having high polar pendant arms shows multimodal behaviour has also been reported [13-17]. Some examples of thernostics Gd(III)-Pt(II) have come to light,18–20 to our knowledge, there are no examples of prodrugs Pt(IV) containing a Gd(III) complex for in vitro or in vivo contrast MR imaging.

Ovarian carcinoma is the most lethal female gynaecological malignancy in the world, characterized by significant morbidity and mortality [21]. Ovarian cancer is undetectable at an early stage and usually diagnosed at an advanced stage with malignant metastasis in the pelvis and abdomen [22]. Other reasons for the high mortality rate of ovarian cancer are limited and complicated treatments [23]. Antibody-based drugs that compete with ligand binding to the extracellular domain of cancer creating proteins have received regulatory approval for the treatment of cancer. Clinical studies have shown that the anti-tumour effectiveness has improved for agents that target both receptors simultaneously. The complication in treating ovarian cancer is due to the combination of immunogenic immune cells and a large number of immune cells infiltrating the target tissue. CA125 (MUC16), is surface glycoprotein like mucin, which is strongly overexpressed in stage III/IV epithelial ovarian cancer (EOCs) and in ovarian tumours. CA125 considered supporting tumour immune escape in the tumour microenvironment (TME). High expression of CA125 correlates with protecting against cytolytic destruction by natural killer cells (NK) , which has been linked to a reduction in immune activation synapses between NK and target cells and thereby a decrease in cell adhesion. CA125 thought to facilitate cell-cell pro-tumoral interactions in a glycane-dependent N-way. CA125 on the outside of ovarian tumour cells ties to glycoprotein mesothelium, communicated on epithelial cells, with a Kd raised from 5 to 10 nM. In the case of ovarian cancer, the CA125 overexpression has related to an increase in microvascular density, an advanced stage of the disease, a recurrence of the disease and a decrease in the survival rate of cancer patients. For example, monitoring serum CA125 levels in the ovaries is a promising target treatment for assessing response to conventional chemotherapy and surgical therapies.

2. Motivation

The article deals with the synthesis of CA125 targeting lipophilic phenyl substituted methyl coumarone, acridone fused biomolecules functionalized Gadolinium (III) –Platinum (IV) metal complex. The theranostic agents were synthesized by careful insertion of Platinum metal ion to the core of a dinuclear Gd(III) coumarone complex decorated over bis acredone core. The M-MRI CA is water-soluble and shows good binding towards CA125 protein. The complex is intended to improve the intracellular difference of ovarian disease cells, while ordinary Gd(III) contrast specialists are restricted to the extracellular space encompassing the tumors [24,25]. This Gd(III)–Pt(IV) stage has a second hub site on the suspended coumarone arm which can be utilized to couple target bunches for ovarian tumor explicitness, medications to battle fluorophores for multimodal imaging and approval.

3. Materials and Methods

Cyclen, 2-Chloro acetic acid, 7-(4-aminophenoxy)-4-methyl-2H-chromen-2-one, ethylenediamine, 1-chloro-1-phenylhexan-3-one, acridone, propanediol, bromoacetyl bromide, bromopyrogallol, HSA, celite,

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, lithium carbonate, Gadolinium (III) chloride hexahydrate, Platinum (III) chloride hydride, Amberlite IR-120 (H+, acidic), di-bromo propane, Sodium carbonate, Aniline, Resorcinol, Phenol, Sodium chloride, Xylenol orange indicator.

3.1 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 [26] 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.

3.2 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°) [27] 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. Procedures

4.1. SYNTHESIS OF 1,3-BIS((1-CHLORO-5-OXY)-ACRIDIN-9(10H)-ONE)PROPANE

A solution of 1,5-dichloroacridine-9(10H)-one (10.56 g, 20 mmol) and Na_2CO_3 (1.07 g, 10 mmol) in 100 mL of acetonitrile was taken in a round bottom flask, fitted with a double surface condenser. 1,3-propanediol (0.80 mL, 10 mmol), dissolved in 60 mL of acetonitrile was dropped in to the above mixture under stirring and refluxed for 4 h with vigorous stirring. The solution was cooled to room temperature and the solvent was removed and the colourless precipitate of 1,3-Bis(1-chloro-5-acridin-9(10H)-one)propanediol linker (1) was dried under vacuum. Yield 5.01 g (94.4 %), mp 197 ^oC. Anal. calcd. % for $C_{29}H_{20}Cl_2N_2O_4$ (Mr = 531): C, 65.55; H, 3.79; Cl, 13.34; N, 5.2; O, 12.04. Found: C, 64.28; H, 3.63; Cl, 13.01; N, 5.14; O, 12.16. IR (KBr, cm-1): 1102 ν(C-O-C) (ether), 2968 ν(C-H), 1615 ν(C=O), 1047 ν(C-N) (secondary amine), 549 ν(C-Cl) 3018 ν(C-H) (aromatic); 2984 ν_{as}(C-H) and 2929 ν_s(C-H) (aliphatic); 1569 and 1448 ν_s(C=C) (aromatic); 753 δ(C-H) (aromatic). MS (ESI): m/z 533 $[M+2H]^+$, 277 $[M-1]$-$C_{12}H_6O_2Cl_2]^+$.

4.2. SYNTHESIS OF 1,3-BIS(((1-CHLORO-5-OXY)-ACRIDIN-9-OXO-10(9H)-YL)ACETIC ACID)PROPANE

It has been synthesized by simple *N*-alkalization with 2-chloro acetic acid. About 60 ml of freshly filtered ethanol containing 1,3-Bis((1-chloro-5-oxy)-acridin-9(10H)-one)propane (5.31 g, 10 mmol) was taken in a 250 ml RB flask and allow to stir. To this pure Na_2CO_3 (2.22 g, 20.1 mmol) was added and heated slowly under stirring for 30 minutes, here Na_2CO_3 was acted as proton scavenger. When all the reactants gets dissolved a solution containing 2-chloroacetic acid (0.19 g, 20 mmol) in 50 mL of ethanol was dropped for about 360 mts with vigorous stirring. The solution was cooled to room temperature and concentrated to dryness to obtain the product

1,3-bis(((1-chloro-5-oxy)acridin-9-oxo-10(9H)-yl) acetic acid)propane as colourless compound at 92 % yield (5.95 g), mp 227 ^{o}C. Anal. calcd. % for $C_{33}H_{24}Cl_2N_2O_8$ (*M*r = 647): C, 61.22; H, 3.74; Cl, 10.95; N, 4.33; O, 19.77. Found: C, 61.04; H, 3.75; Cl, 10.88; N, 4.36; O, 19.56. IR (KBr, cm-1): 1107 ν(C-O-C) (ether), 2971 ν(C-H), 1628 ν(C=O), 1151 ν(C-N) (tertiary amine), 532 ν(C-Cl) 3024 ν(C-H) (aromatic); 2963 ν_{as}(C-H) and 2917 ν_s(C-H) (aliphatic); 1547 and 1463 ν_s(C=C) (aromatic); 781δ(C-H) (aromatic). MS (ESI): *m/z* 647 [M], 527 $[M-2]-C_4H_6O_4]^+$, 324 $[C_{18}H_{14}O_5N]^+$, 288 $[C_{15}H_{11}O_3NCl]^+$.

4.3. SYNTHESIS OF 2-CHLORO-N-(4-(4-METHYL-2-OXO-2H-CHROMEN-7-YLOXY)PHENYL) ACETAMIDE

A solution of 7-(4-aminophenoxy)-4-methyl-2H-chromen-2-one (8.01 g, 30 mmol) and Na_2CO_3 (3.18 g, 30 mmol) in 100 mL of acetonitrile was taken in a round bottom flask immersed in ice bath and stirred vigorously using magnetic stirrer for 30 minutes. Chloroacetylchloride (4.82 mL, 30 mmol), dissolved in 50 mL of acetonitrile was dropped in to the above mixture under stirring while maintaining the bath temperature between 0-5 ºC for 2 hrs. The reaction mixture was filtered and the solvent was removed under reduced pressure to collect 2-chloro-N-(4-(4-methyl-2-oxo-2H-chromen-7-yloxy)phenyl)acetamide as pale yellow powder. Yield 10.03g (97 %), mp (dec.) 318 ^{o}C. Anal. calcd. % for $C_{18}H_{14}ClNO_4$ (*M*r = 344): C, 62.89; H, 4.10; Cl, 10.31; N, 4.07; O, 18.62. Found: C, 63.19; H, 4.06; Cl, 10.28; N, 3.98; O, 18.51. IR (KBr, cm-1): 3290 ν(N-H) (amide), 2947 ν(C-H), 1642 ν(C=O) (amide), 1558 δ(N-H) (amide), 1271 ν(C-N), 1119 ν(C-O-C) (ether), 576 ν(C-Cl), 1566 ν(C=O), 3041 ν(C-H) (aromatic); 2912 νs(C-H) (aliphatic); 1651 and 1459 νs(C=C) (aromatic); 771 δ(C-H) (aromatic). MS (ESI): *m/z* 344 $[M]^+$, 315 $[(M+2)-CH_3O]^+$, 255 $[(M+1)-C_2H_3NCl]^+$, 236 $[(M+3)-C_3H_6NOCl]^+$, 197 $[(M+2)-C_7H_7O_3Cl]^+$.

4.4. SYNTHESIS OF 2-CHLORO-N-(4-(4-METHYL-2-OXO-2H-CHROMEN-7-YLOXY)PHENYL)-N- (3-OXO -1- PHENYLHEXYL)ACETAMIDE

A solution of 2-chloro-N-(4-(4-methyl-2-oxo-2H-chromen-7-yloxy)phenyl)acetamide (6.88 g, 20 mmol) and Na_2CO_3 (2.12 g, 20 mmol) in 75 mL of water was taken in a round bottom flask fitted with air condenser and heated for 20 minutes to get a clear solution. 1-Chloro-1-phenylhexan-3-one (4.21 g, 20 mmol), dissolved in 75 mL of ethanol was slowly dropped in to the above mixture for 1 hr. The reaction mixture was cooled to room temperature, filtered, and the solvent was removed under reduced pressure to give the compound as pale yellow solid. Yield 9.01g (87 %), mp (dec.) 247 ^{o}C. Anal. calcd. % for $C_{30}H_{28}ClNO_5$ (*M*r = 518): C, 69.56; H, 5.45; Cl, 6.84; N, 2.70; O, 15.44. Found : C, 70.16; H, 5.28; Cl, 6.31; N, 2.41; O, 16.04. IR (KBr, cm-1): 2951 ν(C-N) (3o amide), 1656 ν(C=O) (amide), 1093 ν(C-O-C) (ether), 545 ν(C-Cl), 1566 ν(C=O), 3021 ν(C-H) (aromatic); 2812 νs(C-H) (aliphatic); 1458 νs(C=C) (aromatic); 784 δ(C-H) (aromatic). MS (ESI): *m/z* 518 $[M]^+$, 491 $[(M+4)-Cl]^+$, 413 $[(M+4)-C_4H_5O_2Cl]^+$, 257 $[C_{15}H_6O_3Na]^+$, 197 $[C_{10}H_9ONCl]^+$.

4.5. SYNTHESIS OF 1,4,7,10-TETRAAZACYCLODODECANE-1,4,7-TRIS(CHLORO-N-(4-(4- METHYL-2-OXO-2H- CHROMEN-7-YLOXY)PHENYL)-N-(3-OXO-1-PHENYLHEXYL) ACETAMIDE

To a solution of 1,4,7,10-tetraazacyclododecane (3 gm, 17.5 mmol) in water was added 2-chloro-N-(4-(4-methyl-2 -oxo-2H-chromen-7-yloxy)phenyl)-N-(3-oxo-1-phenylhexyl)acetamide (26.93

gm, 52 mmol) and the pH of the solution was raised to 10 by using 1 M aqueous sodium carbonate. The reaction was carried out at 0 ºC for 2 h and the content was heated to 50 ºC and maintained for 3 h. The pH 10 was 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-Ch-Ph-Am] was separated using ion-exchange chromatography. The solution was concentrated and vacuum dried to yield DO3-Ch-Ph-Am as white solid with yield 23.77 g (84 %), mp 279 ^{o}C. Anal. calcd. % for $C_{98}H_{101}N_7O_{15}$ (*M*r = 1617): C, 72.80; H, 6.30; N, 6.06; O, 14.84. Found: C, 72.80; H, 6.30; N, 6.06; O, 14.84. IR (KBr, cm-1): 2917 ν(C-H), 1637 ν(C=O) (amide), 1139 ν(C-N) (tertiary amine), 1063 ν(C-N) (secondary amine), 2921 ν(C-N) (3^{o} amide), 1672 ν(C=O) (amide), 1018 ν(C-O-C) (ether), 2908 ν_{as}(C-H) and 2850 ν_s(C-H) (aliphatic). MS (ESI): *m/z* 1618 $[M+1]^+$, 1457 $[(M-1)C_{10}H_7O_2]^+$, 1259 $[M-C_{20}H_{22}O_6]^+$, 1134 $[(M-1)-C_{30}H_{28}NO_5]^+$.

4.6. SYNTHESIS OF 1,3-BIS(((1-CHLORO-5-OXY)-ACRIDIN-9-OXO-10(9H)-YL)ACETIC ACID) PROPANE PLATINUM (IV) COMPLEX

About 3.24 g (5 mmol) of 1,3-Bis(((1-chloro-5-oxy)-acridin-9-oxo-10(9H)-yl)acetic acid)propane and 1.33 g (5 mmol) $PtCl_4$ in 30 mL of a mixed solvent of H_2O and ethanol (v:v = 1:3), were heated at 120 °C under stirring for 10 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 colourless platinum complex obtained has been recrystallized from dilute ethanol, yield 4.12 g (98 %), mp (dec.) 197 ^{o}C. Anal. calcd. % for $C_{33}H_{22}Cl_2N_2O_8Pt$ (*M*r = 841): C, 47.16; H, 2.64; Cl, 8.44; N, 3.33; O, 15.23; Pt, 23.21. Found: C, 47.52; H, 2.58; Cl, 8.32; N, 3.14; O, 15.48; Pt, 23.01. IR (KBr, cm^{-1}): 2754 ν(C-H), 1661 ν(C=O), 3048 ν(C-H) (aromatic); 2937 ν_{as}(C-H) and 2875 ν_s(C-H) (aliphatic); 1523 and 1464 νs(C=C) (aromatic); 1198 ν_{as}(C-O-C); 653 ν_s(Pt-O) ; 731 δ(C-H) (aromatic). MS (ESI): *m/z* 841 $[M]^+$, 721 $[(M-H)-Cl_2O_3]^+$, 607 $[(M-3H)-PtCl]^+$, 371$[(M+H)-C_{14}H_8O_2PtCl]^+$, 315 $[C_{16}H_7O_4NCl]^+$, 255 $[C_{14}H_6O_2NCl]^+$.

4.7. SYNTHESIS OF 1,3-BIS(((5-OXY)-ACRIDIN-9-OXO-10(9H)-YL)ACETIC ACID)-1,1-DI((1,4,7,10-TETRAAZACYCLODODECANE-1,4,7-TRIS(N-(4-(4-METHYL-2-OXO-2H-CHROMEN-7-YL-OXY)PHENYL)-N-(3-OXO-1-PHENYLHEXYL)ACETAMIDE)) PROPANE PLATINUM (IV) COMPLEX

To a solution containing 16.17 g (10 mmol) of 1,4,7,10-tetraazacyclododecane-1,4,7-tris(chloro-N-(4-(4-methyl-2-oxo-2H-chromen-7-yloxy)phenyl)-N-(3-oxo-1-phenylhexyl) acetamide (DO3-Ch-Ph-Am) in 100 mL of water, pure Na_2CO_3 (0.6 g, 5 mmol) was added and stirred under heating for 30 minutes. About 4.205 gm, (5 mmol) of 1,3-Bis(((1-chloro-5-oxy)-acridin-9-oxo-10(9H)-yl) acetic acid)propane Platinum (IV) complex in 100 mL of water was added slowly in to the solution and heated under mild stirring for 2 hrs. The solution was concentrated and vacuum dried to yield [(Pr-(DO3-Ch-Ph-Am)$_2$Pt(IV)] complex: Pale yellow solid, yield 18.28 g (91.4%), Mp(dec.); 304 ^{o}C. Anal. calcd. for $C_{229}H_{222}N_{16}O_{38}Pt$ (*M*r = 4001): C, 68.74; H, 5.59; N, 5.60; O, 15.19; Pt, 4.88. Found: C, 66.94; H, 5.66; N, 5.13; O, 14.76; Pt, 4.71. IR (KBr, cm^{-1}): 2671 ν(C-H), 1651 ν(C=O), 2987 ν(C-H) (aromatic); 2910 ν_{as}(C-H) and 2842 ν_s(C-H) (aliphatic); 1501 and 1422 ν_s(C=C) (aromatic); 1074 ν_{as}(C-O-C); 669 ν_s(Pt-O) ; 724 δ(C-H) (aromatic), 1162 ν(C-N) (tertiary

amine), 2917 ν(C-N) (3° amide). MS (ESI): *m/z* 4001 $[M]^+$, 3958 $[(M+2)-3CH_3]^+$, 3442 $[(M+1)-C_{25}H_{33}O_2Pt]^+$, 2754 $[M-C_{72}H_{90}O_6Pt]^+$, 2582 $[M-C_{83}H_{100}O_8Pt]^+$, 2410 $[(M)-C_{58}H_{54}NO_5Pt]^+$.

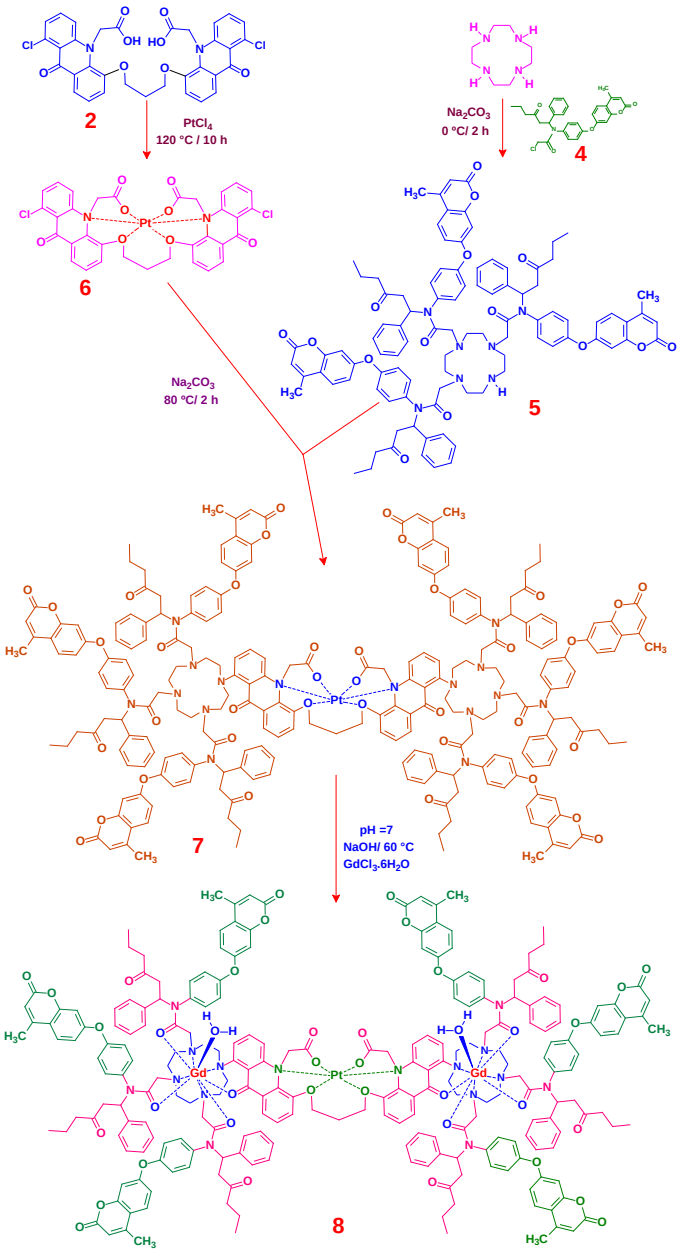

FIG.1. Scheme for the Synthesis of

$[Pr\text{-}(DO3\text{-}Ch\text{-}Ph\text{-}Am\text{-}Gd(III))_2Pt(IV)(H_2O)_2]$ COMPLEX

4.8. SYNTHESIS OF 1,3-BIS(((5-OXY)-ACRIDIN-9-OXO-10(9H)-YL)ACETIC ACID)-1,1-DI((1,4,7,10-TETRAAZACYCLODODECANE-1,4,7-TRIS(N-(4-(4-METHYL-2-OXO-2H-CHROMEN-7-YL-OXY)PHENYL)-N-(3-OXO-1-PHENYLHEXYL)ACETAMIDE-GADOLINIUM (III)) PROPANE PLATINUM (IV) COMPLEX

To a solution of the precursor $[(Pr\text{-}(DO3\text{-}Ch\text{-}Ph\text{-}Am)_2Pt(IV)]$ complex (12.00 g, 3 mmol) in 75 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 5 hrs. It was cooled to room temperature, filtered, and concentrated to dryness. The resulting colorless hygroscopic solid [Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(IV)]was recrystallized in water: yield (12.11 g, 93%). Anal. calcd. for $C_{229}H_{226}N_{16}O_{40}$ Gd_2Pt (*M*r = 4352): C, 63.20; H, 5.23; N, 5.15; O, 14.71; Gd, 7.23; Pt, 4.48. Found: C, 61.82; H, 4.99; N, 5.21; O, 14.49; Gd, 7.11; Pt, 4.51. IR (KBr, cm^{-1}): 1261 ν(C-N) (tertiary amine); 1599 ν(C=O), 2931 ν(C-H) (aromatic); 2932 ν_{as}(C-H) and 2847 ν_s(C-H) (aliphatic); 1628 and 1474 ν_s(C=C) (aromatic); 793 δ(C-H) (aromatic); 1185 ν_{as}(C-O-C); 625 ν_s(Pt-O); 441 ν(Gd-O). MS (ESI): *m/z* 4352 $[M]^+$, 4201 $[(M-3)-H_4O_9]^+$, 3810 $[(M+3)-2H_2OGd_2Pt]^+$, 3421 $[(M-3)-C_{24}H_{30}O_4Gd_2Pt]^+$, 3044 $[(M+1)-C_{48}H_{49}NO_6Gd_2Pt]^+$, 2641$[M-C_{64}H_{66}O_{12}Gd_2Pt]^+$.

5. Results and Discussions

The target complex has synthesized by systematic procedure that involves eight stages. The synthesis of the linker 1,3-bis((1-chloro-5-oxy)-acridin-9(10H)-one)propane was performed using *O*-alkylation process between 1,5-dichloroacridine-9(10*H*)-one with 1,3-propanediol in acetonitrile solvent. The acetic acid appended linker 1,3-Bis(((1-chloro-5-oxy)-acridin-9-oxo- 10(9H)-yl)acetic acid) propane was synthesized by the *N*-alkalization of 1,3-bis((1-chloro-5-oxy)- acridin-9(10H)-one) propane with 2-chloro acetic acid in ethanol. The coumarin substituted pendant arm 2-chloro-*N*-(4-(4-methyl-2-oxo-2H-chromen-7-yloxy)phenyl)acetamide was synthesized by reacting 7-(4-aminophenoxy)-4-methyl-2H-chromen-2-one with chloroacetyl- chloride in acetonitrile at -5 ^oC. The phenyl substituted coumarin pendant arm 2-chloro-*N*-(4-(4-methyl-2-oxo-2H-chromen-7-yloxy)phenyl)-*N*-(3-oxo-1-phenylhexyl)acetamide was synthesized by the *N*-alkylation reaction with 1-chloro-1-phenylhexan-3-one in ethanol. The tri *N*-substituted cyclen 1,4,7,10-tetraazacyclododecane-1,4,7-tris(chloro-*N*-(4-(4-methyl-2-oxo-2H-chromen-7-yloxy)phenyl)-*N*-(3-oxo-1-phenylhexyl) acetamide was synthesized by the reaction of 1,4,7,10-tetraazacyclododecane with **4** in 1:3 mole ratio in water under base pH followed by column separation of tetra substituted product. The Pt(IV) linker complex 1,3-bis(((1-chloro- 5-oxy)-acridin-9-oxo-10(9H)-yl)acetic acid)propane Platinum (IV) complex was synthesized by reacting the compound with $PtCl_4$ in H_2O and ethanol (v:v = 1:3) mixture. The precursor platinum complex [Pr-(DO3-Ch-Ph-Am)$_2$Pt(IV)] was prepared by the *N*-alkalization of **5** with **6** in water under mild stirring. Our hetero- tri- nuclear target complex [Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$ Pt(IV)$(H_2O)_2$] (**8**) was synthesized by the reaction of [Pr-(DO3-Ch-Ph-Am)$_2$Pt(IV)] with $GdCl_3.6H_2O$ in water at neutral pH. The difference in melting point, absorption property in IR-spectroscopy and fragmentation on ESI-Mass Spectrometry at every stage confirmed the formation of the product.

A) LONGITUDINAL RELAXIVITY OF [Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(IV)$(H_2O)_2$] COMPLEX

The longitudinal relaxation times of water protons for six concentrations of the complex have given in Table 1 and the plot of the concentration of the complex versus $1/T_1$ depicted in **Fig 2**. The hetero-tri-nuclear complex exhibits a relaxivity of 24.43 mM^{-1} s^{-1} that corresponds to 12.22 "per Gd". The "per Gd" r_{1p} value is 2.91, 2.55, and 3.21 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), [28] $[Gd(DO3A)(H_2O)_2]$ (r_{1p} = 4.8 mM^{-1} s^{-1}, 20 MHz, *q* =

2), [29] and $[Gd(DTPA)(H_2O)]^{2-}$ (r_{1p} = 3.8 mM^{-1} s^{-1}, 20 MHz, 25 °C), [30] respectively. The newly synthesized hetero-tri-nuclear complex exhibits a high relaxivity than other Gd(III) complexes such as, $[\{Gd(DO3A)\}_2L_6(H_2O)_4]$ (L_6 = 2,11-dihydroxy-4,9-dioxa-1,12-dodecane) (r_{1p} = 5.1 $mM^{-1}s^{-1}$, 20 MHz, 40 °C), [31] $[\{Gd(DO3A)\}_2\text{-}L_7(H_2O)_4]$ (L_7 = 1,5,6,- 10-tetrahydroxy-3,8- dioxa-dodecane) (r_{1p} = 6.4 $mM^{-1}s^{-1}$, 20 MHz, 40 °C),[32,33] $[\{Gd(DO3A)\}_2\text{-}L_8(H_2O)_4]$ (L_8 = 1,2,4,5-tetrol) (r_{1p} = 6.6 mM^{-1} s^{-1}, 20 MHz, 40 °C), $[\{Gd(DO3A)\}_2L_9(H_2O)_4]$ (L_9 = 3,6-bis((hydroxyl)methoxy) cyclohexane-1,2,4,5-tetrol) (r_{1p} = 5.4 $mM^{-1}s^{-1}$, 20 MHz, 40 °C), [34] $[pip\{Gd(DO3A)(H_2O)\}_2]$ (r_{1p} = 5.58 mM^{-1} s^{-1}, 40 MHz, 37 °C), [35] and $[bisoxa\{Gd(DO3A)(H_2O)\}_2]$ (r_{1p} = 4.43 $mM^{-1}s^{-1}$, 40 MHz, 37 °C).[36,37] The coordination sphere of each Gd(III) metal ion in $[Pr\text{-}(DO3\text{-}Ch\text{-}Ph\text{-}Am\text{-}Gd(III))_2\ Pt(IV)(H_2O)_2]$ is similar to that of $[Gd(DOTA)(H_2O)]$ complex. 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, hydrophilic and lipophylic groups on the periphery, and high rigid internal flexibility.

Table 1. Longitudinal relaxation times of

$[Pr\text{-}(DO3\text{-}Ch\text{-}Ph\text{-}Am\text{-}Gd(III))_2Pt(IV)(H_2O)_2]$

Concentration (mM)	T_1 x 10^{-3} s	$1/T_1$ x 10^3 s^{-1}
0.2	206.61	04.84
0.5	082.17	12.17
1.0	041.00	24.39
1.5	027.37	36.54
2.0	020.49	48.80
2.5	016.40	60.97

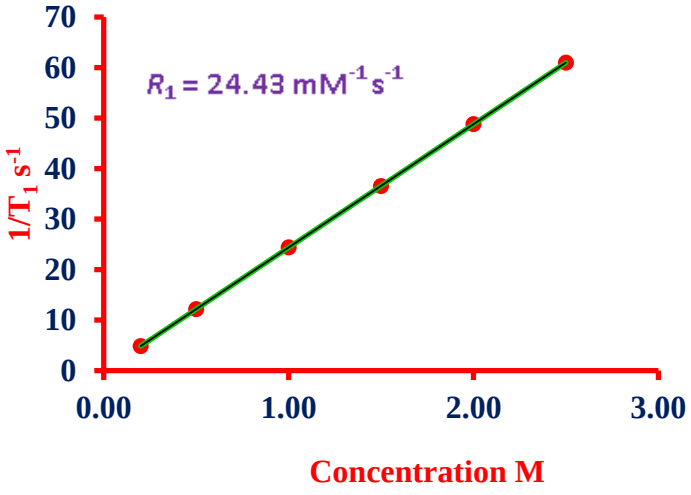

FIG.2. Plot of the Concentration of

$[Pr\text{-}(DO3\text{-}Ch\text{-}Ph\text{-}Am\text{-}Gd(III))_2Pt(IV)(H_2O)_2]$ versus $1/T_1$

B) TRANSVERSE RELAXIVITY OF [Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(IV)(H$_2$O)$_2$] COMPLEX

The transverse relaxation times for six concentrations of the complex are given in Table 2 and the plot of six different concentration of the complex from 0.2 to 2.5 Mol versus $1/T_2$ is shown in **Figure 3**. The transverse relaxivity value for the complex is found to be 38.61 mM^{-1} s^{-1} which corresponds to 19.30 "per Gd". The higher transverse relaxivity is due to the presence of six bulky 2-methyl coumarone and phenylhexan-3-one groups on the periphery of the complex. The ratio r_{2p}/r_{1P} is 1.58, which shows that the complex is a T_1-weighted contrast agent.

Table 2. Transverse relaxation times of

[Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(IV)(H$_2$O)$_2$]

Concentration (mM)	T_2 x 10^{-3} s	$1/T_2$ x 10^3 s^{-1}
0.2	129.60	07.72
0.5	051.73	19.33
1.0	025.92	38.58
1.5	017.28	57.87
2.0	012.96	77.16
2.5	010.37	96.43

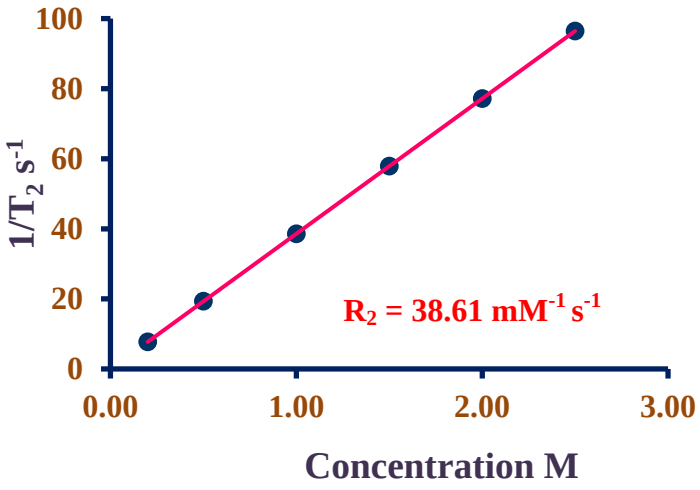

FIG.3. Plot of the Concentration of

[Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(IV)(H$_2$O)$_2$] versus $1/T_2$

6. Advantages

The "per Gd" r_{1p} value of the complex is 2.91, 2.55, and 3.21 units higher than that of the FDA approved small molecular Gd(III) complexes. The coordination sphere of each Gd(III) metal ion in [Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(IV)(H$_2$O)$_2$] is similar to that of [Gd(DOTA)(H$_2$O)] complex. Due to high water solubility, the

complex will bind over the target tissue (eg.CA125) and excrete out through kidney after MRI analysis. The high rigidity, support the relaxivity enhancement on the molecule and give superior relaxivity than other Gd(III), Mn(II), and Fe(II)/(III) metal based MRI contrast agents. The high kinetic and thermal stability of the complex at different pH shows that the complex has the potential to diagnose cancer tissues, because the cancer tissues will be more acidic (pH< 7) than normal one. The presence of coumarone and Pt(IV)-Acridone moieties on the complex will enhance the anticancer property, especially for ovarian cancer .

7. Conclusions

A novel hetero–tri-nuclear [Pr-(DO3-Ch-Ph-Am-Gd(III))$_2$Pt(IV)$(H_2O)_2$] complex have been synthesized, characterized and their relaxivity values in neat aqueous solution are reported. Our hetero-tri-nuclear complex is highly soluble in water and shows good thermal and kinetic stability. The xylenol orange test confirms the non-dissociation nature of toxic Gd(III) in our complex. The complex shows high longitudinal (r_{1p}) and transversal (r_{2p}) relaxivity values (r_{1p} = 24.43 mM^{-1} s^{-1} and r_{2p} = 38.61 mM^{-1} s^{-1} at pH 7 and 27 ^oC) in neat aqueous solution. The relaxivity values are quite higher with other mono and di nuclear Gd(III) complexes approved by FDA. The r_{2p}/r_{1P} ratio confirms that the complex is a T_1-weighted contrast agent. The presence of two water molecules in the inner coordination sphere and replaceable hydrogen atom in the linker enhances the proton relaxation rate and give huge relaxivity value. Since the complex has many high polar and rigid moieties like, acetic acid functionalized acridone linker and phenyl substituted methyl coumarone amide pendant arm the Gd(III) and Pt(IV) metals will experience the better binding with other proteins like Human serum albumin and CA125 ligands. Our hydrophilic Gd(III)-Pt(IV) complex will show promising results as M-MRA contrast agent for ovarian cancer. Since, coumarin and acridone has anticancer activity, our newly designed M-MRA CA will play triple role in identification of cancer cell, target protein binding, and killing drug for ovarian cancer.

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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