Bioanalytical and Theoretical Studies of the Spectrophotometrically Investigated Iridium (III)-3-Hydroxy-2-(4-Methoxyphenyl)-4*H*-Chromen-4-one Complex

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ABSTRACT: *A pioneering approach for the spectrophotometric inquisition of microscale amounts of iridium (III) under aqueous conditions has been explored using a novel benzopyran derivative, 3-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (HMPC) as a ligand, hence, employing complexation as a basis of reaction between the two. The spontaneous complexation between iridium (III) and HMPC is manifested by the expeditious formation of a pale yellow complex at pH 4.87 in which the metal to ligand ratio [M:L] is estimated as 1:2. The complex absorbs paramountly at 423-430 nm and is markedly stable. For ensuring the formation of a stable complex, optimal conditions have been fixed with reference to the parameters regulating its formation. Accordingly, the system shows coherence to linearity between 0.0-1.7 µg/mL of iridium (III). The molar attenuation coefficient and Sandell's sensitivity are 6.824×10^4 L/mol cm and 0.00281µg Ir (III) cm^{-2}, respectively at 425 nm. Statistical*

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parameters including RSD, correlation coefficient (r), and detection limit are respectively, 0.2169%, 0.9999, and 0.0123 µg/mL. As a check for flexibility and usability of the method, intervention concerning complexing agents and cations of prime analytical importance has been carried out indicating the majority of these do not induce any interference during determination. The accomplished studies serve as proof of the versatility, flexibility, and sensitivity of the method. Keeping in view the aforesaid characteristics of the complex, it was subjected to numerous biological investigations and has been satisfyingly found to possess anti-cancerous, bactericidal, and antioxidant properties thus expanding its novel utility domain in the therapeutic world. Quantum chemical parameters (DFT and MEP), based on analysis of electronic properties of the complex in its most stable least energy conformation, are used to better understand the chemistry of the produced complex.

KEYWORDS*: Iridium; Spectrophotometric determination; DFT; Anticancer; Antibacterial; Antioxidant.*

INTRODUCTION

In the present-day industrial society, Platinum Group Metals (PGMs) because of their vast range of applications have become imperative and their demand has profoundly increased. Iridium, a third-row group 9 transition element, an analogue of cobalt and rhodium, is one of the members of PGMs. It is comparatively precious having less natural abundance (^{193}Ir 62.7%, ^{191}Ir 37%) and chemically inert material. Iridium metal is one of the most corrosion resistant and second densest (22.56 g cm^{-3}) element in the periodic table possessing high electrical conductivity. In spite of its chemically inert nature and rarity, iridium forms expansive domain of stable and substantial range of compounds [1-6].

On account of the aforesaid characteristic properties, iridium and its compounds find applications in technology and have attracted much attention in the wide range of areas, especially medicine and catalysis. Owing to its high melting point, high electrical conductivity and extreme corrosion resistance, iridium metal is highly practical for electronics and industrial applications as well [7]. Iridium complexes being substitutionally inert and kinetically labile possess tunable reactivity and have the ability to undergo ligand exchange reactions inside the biological systems. In recent years, developments in medicinal chemistry have led a keen and deep attention towards iridium-based complexes and it has been observed that the complexes can act as promising anticancer, antimicrobial, and antioxidant scaffolds. Besides this iridium complexes exhibit good cell permeability and show marvelous photo stability extending their application as bio-sensing and bio-imaging agents [8-10].

Taking into consideration inert and green nature potential to form diverse range of compounds, tunable and flexible reactivity and promising medicinal applications of iridium complexes, a substantial and deep cognizance is essential for the establishment of techniques which can be employed for their investigation and exploration. In this context, a vent ought to be given to discerning and exquisite methodologies which are capable enough to aid in the effectual and prolific determination of iridium complexes covering the application aspects as well. From time to time numerous techniques [11-16] have been put forth for the purpose of analysis of iridium complexes. However, these techniques are still in the stage of infancy as per their effectiveness and sensitivity. To fulfill the existing gaps, spectrophotometry [17-22] owing to its simple, sensitive, selective, versatile and economic nature has evolved as a promising technique curbing all the shortcomings and inadequacies of the previous methodologies. In particular, among the spectrophotometric methods, non-extractive aqueous phase methods are more effective and sensitive in contrast to the extractive ones involving inefficient procedures and solvent-assisted drawbacks.

In the present communication, 3-hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one has been for the first time unraveled as a chromogenic reagent for the determination of iridium in its trivalent state under aqueous phase and non- extractive spectrophotometric conditions. The proposed method in addition of being simple, sensitive and economical, is non-extractive thus

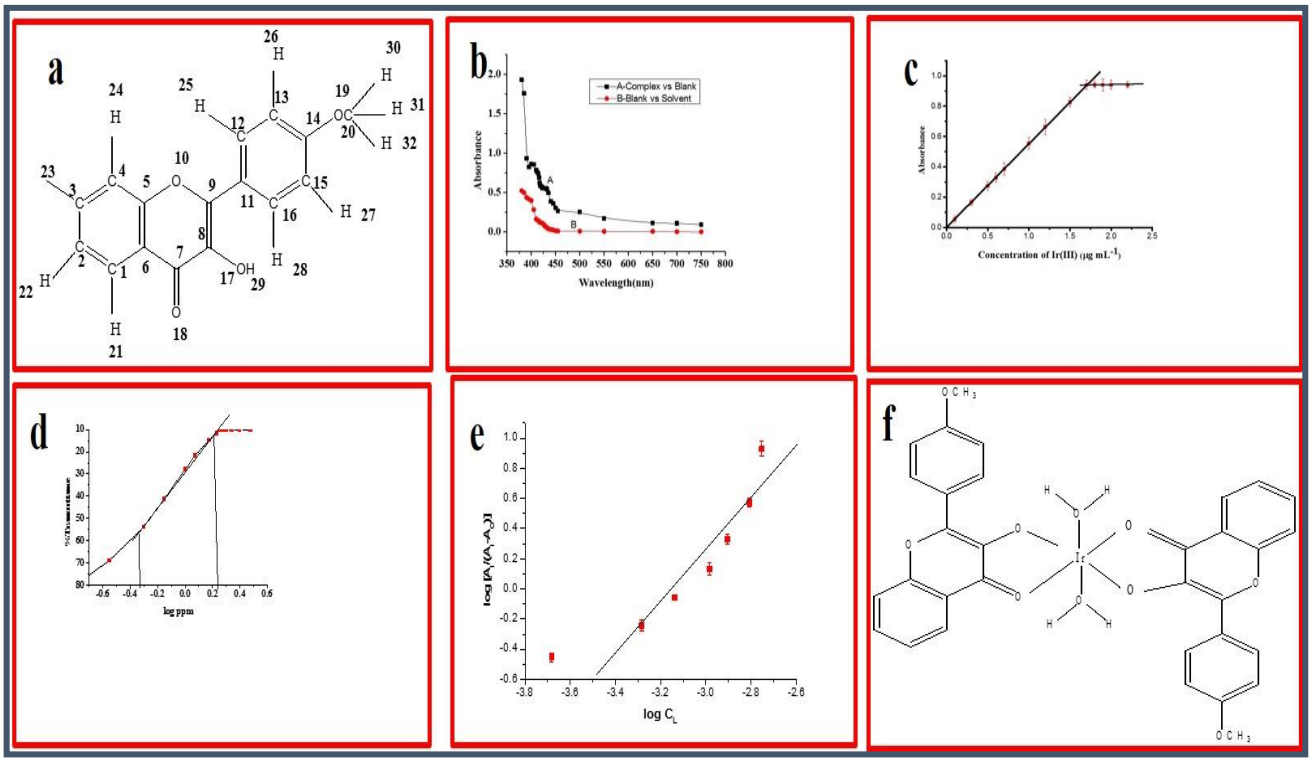


Fig. 1: Spectrophotometric Analysis, a) Structure of* 3-hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one *(HMPC); b) Absorption spectra of Ir (III)-HMPC complex, A-Complex against reagent blank,B- Reagent blank against pure water 1.0 µg Ir (III) mL^{-1}, other conditions as cited in the procedure; c) Beer's law of Ir(III)-HMPC complex (425nm); d) Ringbom plot of Ir (III)-HMPC complex (425nm); e) Determination of composition of the complex by Equilibrium shift method, Concentration of the metal ion fixed =1.040×10^{-3}M, Slope = 1.905 λ_{max} = 425 nm; f) Proposed structure of Ir (III)-HMPC complex

avoiding the hazardous and deleterious effects associated with the use of organic solvents.

Thorough literature survey revealed that iridium-based complexes have emerged as promising and competent agents in the field of medicine possessing intriguing *in-vitro/in-vivo* antimicrobial, antioxidant, antiproliferative, and versatile antitumor properties [17, 23-27]. Keeping in view the aforesaid properties of iridium-based complexes; the present complex was screened for anticancer, antibacterial, and antioxidant properties. Ir (III)-HMPC complex successfully qualified its role as an antitumor, antioxidant, and antibacterial agent. The antitumor activity of the complex was estimated by implementing MTT assay which being the highly responsive and infallible index of the metabolic activity at the cellular level, is favorable over other methods. The potency of the complex as a bactericidal and an antioxidant agent has been divulged by using Agar well diffusion assay and RSA towards DPPH, respectively. In order to achieve understanding and apprehension towards plausible electronic structural details and various reactivity parameters, Density Functional Theory (DFT) has been employed to confirm and authenticate structural properties of the studied complex. The computational studies have been performed in correlation to those previously applied to the analogous metal complexes [17, 28, 29].

EXPERIMENTAL SECTION

Spectrophotometric analysis

Instrument, Chemicals, Reagents and Solutions

All the chemicals used during the course of experimentation were highly pure and of the AR grade. Double-deionized water was used throughout for solutions. Absorbance measurements and spectral studies were carried out on a double-beam UV–Vis spectrophotometer (Electronics India; EI-2375) with 10 mm matched quartz cuvettes. Electronic balance and pH meter (Electronics India; EI-101) of high sensitivity were used for weighing and noting pH of the medium, respectively. The stock solution of Ir (III) containing 1 mg/mL of the metal

ion was prepared by dissolving 0.155 g of iridium trichloride, $IrCl_3$ obtained from Central Drug House Ltd. (CDH), New Delhi, in 100 mL of 6 mol L^{-1}HCl. Working solutions of the metal ion were prepared by dilution therefrom. Solutions at mg mL^{-1} level of the other cations and anions for experimental examination were prepared by dissolving suitably weighed quantities of their respective sodium or potassium salts either in double distilled water or in mineral acid. [17].

The benzopyran-derived bidentate chromone, 3-hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (Fig. 1a) used as a ligand during the current study was synthesized by the procedure as followed in AFO reaction [30]. The process of complexation was best effective by using the ligand solution of 0.1% concentration in ethyl alcohol. It was prepared fresh by dissolving 0.025g of HMPC in 25 mL ethyl alcohol.

Spectrophotometric determination

The accurate, precise, and faultless results during the spectrophotometric analysis were obtained by carrying out experiments in the most refined and impetus manner. By proper dilution of the standard stock solution (1000 µg/ mL), a 10 µg/mL working solution of Ir (III) was prepared. The complexation reaction between Ir (III) and HMPC was executed by taking 1 mL of the working solution in a standard 10 mL volumetric flask, 1 mL of the reagent (HMPC) solution (0.1% w/v) under the acidic conditions provided by 0.7 M phosphoric acid medium at an optimal pH of 4.87 and ultimately making up the aqueous volume using double-deionized water. It was observed that upon the addition of 1 mL of HMPC, a light-yellow complex was instantaneously formed. The optical density of the formed complex was noted at 425 nm against the similarly treated reagent blank.

Computational details

The computation of quantum chemical parameters along with the optimized configuration of HMPC and Ir (III)-HMPC complex was accomplished using the B3LYP at LANL2DZ (Becke's three-parameter hybrid functional employing the LYP correlation functional) basis set [31]. The energies, HOMO-LUMO [31] and the band gap (E_{gap}=E_{HOMO}–E_{LUMO}) [32], had been estimated out so as to be used for electronic properties determination of the ligand and the complex molecules including the electron affinity (A) [33], ionization energy (I), chemical potential (μ), electronegativity (χ), absolute hardness (η), global softness (σ) and electrophilicity index (ω) utilizing the following corresponding equations [34]:

$$\text{Electronegativity } (\chi) = I+A/2 \quad \text{(i)}$$
$$\text{Chemical potential } (\mu) = -(I+A)/2 \quad \text{(ii)}$$
$$\text{Chemical hardness } (\eta) = I-A/2 \quad \text{(iii)}$$
$$\text{Chemical softness } (\sigma) = 1/2\eta \quad \text{(iv)}$$
$$\text{Electrophilicity index } (\omega) = \mu^2/2\eta \quad \text{(v)}$$

The reactivity of a molecule could be explained with consideration of chemical hardness, which is directly proportional to the HOMO–LUMO energy gap [35, 36]. Higher chemical hardness or higher E_{gap} indicates lower reactivity of molecules.

Evaluation of In-vitro cytotoxic potency

Evaluation of the anticancer activity of the complex was achieved by the MTT assay. The MTT method is a preferred sensitive and reliable indicator of cellular metabolic activity and it helps to examine how quickly cells divide and conversely, how much cell viability is lost when metabolic processes result in apoptosis. The assay helps in measuring changes in color to detect cellular proliferation (cell growth) [37, 38]. This assay is established on the enzymatic reduction of the MTT molecule to formazan when exposed to viable cells.

Cell lines

A breast cancer cell line (T-27D) was obtained from the National Centre for Cell Sciences (NCCS, Pune, India) and cultured by providing Dulbecco's modified Eagle's medium supplemented with penicillin (100 U/mL), streptomycin (100 mg/mL) and fetal calf serum (10% heat-inactivated). The selected cell lines were grown at 37°C in 95% air with the addition of 5% CO_2 . Cell lines were tested and confirmed to be free of contamination.

Cell viability assessment

Cell viability of the breast cancer cell line (T-27D) was measured with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [39]. Approximately 5×10^3 cells were seeded into 96-well culture plates and incubated overnight for attachment. Only medium was used for the blank control. Ir (III)-HMPC complex concentrations in ten-fold dilutions

(100 µM, 10 µM and 1 µM) were added in triplicates and incubated for 24 and 48 hours at 5% CO_2 at 37°C. Thereafter, the cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. A microplate reader was used to quantify the visible absorbance of each well at 540 nm. The results obtained were presented as a percentage of viable cells in comparison to the cells alone.

In-vitro antibacterial assay

The efficacy of the newly reported Ir (III)-HMPC complex as a bactericidal agent was explored by selecting four bacterial strains for their clinical significance in producing diseases in humans. For bactericidal studies, two gram-positive strains [*S. aureus* (MTCC 96) and *B. subtilis* (MTCC 121)] and two of gram-negative bacteria [*E. coli* (MTCC 1652) and *P. aeruginosa* (MTCC 741)] were taken as standard culture. The microbial cultures used were procured from the Microbial Type Culture Collection (MTCC) situated in the Institute of Microbial Technology (IMTech), Chandigarh, India. The strains were further sub-cultured on the Nutrient Agar (NA) plates or slants at a temperature of 37°C for suspension preparation. An incubation period of 24 hours was given to the bacterial cultures after the addition of the complex in wells created in the agar media. After the completion of the incubation period, the zone of inhibition was measured for the detection of the bactericidal potential of the prepared complex.

Agar well diffusion assay

Agar well diffusion assay was employed for the inspection of the bactericidal potential of the Ir (III)-HMPC complex. The suspension of the bacterial strains was prepared by following the same methodology as used in the earlier study [17, 40-42]. The bactericidal action of the complex has been determined by measuring the area of growth of indirection (together with the diameter of the well) counter to the selected strains of bacteria (as test organisms) by the aid of a zone reader (Hi Antibiotic Zone Scale). A broad-spectrum antibiotic, ciprofloxacin (5 mg/mL) was used as a positive control for bacteria growth inhibition. As the complex showed remarkable bactericidal action, it was further tested for minimum inhibitory concentration studies.

The approach followed for ascertaining the Minimum Inhibitory Concentration (MIC) of Ir (III)-HMPC complex

The minimum concentration of an antimicrobial compound inhibiting the growth of microorganisms after overnight incubation is termed Minimum Inhibitory Concentration (MIC). An agar well diffusion assay was used for the purpose of testing MIC of the Ir (III)-CHMTB complex against bacterial strains. In order to achieve decreasing concentration ranges of 250 to 3.91 µg per 100 µL, two-fold serial dilutions of the complex were prepared by dissolving the complex in dimethylsulfoxide (DMSO). This was followed by the introduction of 100 µL volume of each dilution (in triplicate) in the agar plates already seeded with 100µL of standardized inoculum (10^6 cfu/mL) of the bacterial strains. All seeded plates were incubated aerobically at 37^0C and observed for the inhibition zones for each concentration. The MIC was recorded for the complex against each selected bacterial strain. MIC, taken as the lowest concentration of the complex that completely inhibited the growth of microbes, was indicated in the form of a clear zone of inhibition. Ciprofloxacin was used as a positive control against pathogens while solvent DMSO as a negative control.

In-vitro antioxidant assay

The antioxidant activity of HMPC and the prepared Ir (III)-HMPC complex was examined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay (RSA) and employing UV-Visible spectrophotometer.

Assessment of antioxidant activity of HMPC and Ir (III)-HMPC complex using DPPH radical scavenging

Among the most regularly used assays, scavenging of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) is the most frequently used assay for investigation of anti-radical activity. DPPH, a stable and readily accessible free radical, confers a purple color when dissolved in methyl alcohol and upon reaction with an antioxidant species changes to a yellow colour [27]. The property of a compound to act as an antioxidant is based upon its ability to donate hydrogen to $DPPH^{\cdot}$. The potency of a compound to act as an anti-radical (i.e. capability to reduce DPPH radical) is examined by measuring the decrease in its absorbance at the wavelength of 517 nm. A 100 mL of 1.0 mM standard stock solution of DPPH was prepared by dissolving precisely weighed, 0.394 g of it in methyl alcohol. Stock solution of the complex having strength

Table 1: Variation of optical density of Ir (III)-HMPC complex with respect to the physical parameters

H_3PO_4[a]/M	0.001	0. 003	0.004-0.011	0.012	0.015	0.017	0.020			
pH	5.43	5.39	5.32-4.83	4.81	4.79	4.73	4.68			
Absorbance	0.427	0.461	0.555	0.472	0.439	0.379	0.353			
HMPC[b]/mL	0.1	0.2	0.3	0.5	0.7	0.9-1.3	1.4	1.5	1.7	2.0
Absorbance	0.046	0.103	0.159	0.273	0.382	0.555	0.470	0.392	0.346	0.333

Conditions:

[a]Ir (III) = 10 µg; HMPC [0.1% solution in ethyl alcohol] = 1mL; Solvent = Water; water phase volume = 10 mL ; λ_{max} = 425 nm; pH = Variable

[b]pH = 4.87; remaining conditions the same as in (a) excluding variation in HMPC strength; HMPC = 3-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one

1000 µg/mL was prepared by dissolving 5 mg of it in 5 mL of methanol. A negative control solution was prepared by pipetting out 5 ml from the DPPH stock solution in volumetric flask (25 mL) and making up the final volume by adding methanol. A series of solutions of HMPC and Ir (III)-HMPC complex having concentrations in the range of 500 µg/mL to 31.25 µg/mL were prepared from 1000 µg/mL stock solution. Solutions of the similar concentrations of the positive control i.e. gallic acid were also prepared simultaneously. All the prepared solutions were allowed to react in dark for half an hour at a temperature of 37°C. The absorbance of all the solutions was measured spectrophotometrically at 517 nm. The potency of selected compounds to scavenge DPPH radical was evaluated using the equation given below:

$$\text{DPPH RSA}(\%) = [(A_{\text{negative control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Where $A_{\text{negative control}}$ stands for absorbance of negative control containing all reagents except the test compounds and A_{sample} is the absorbance of the compounds put for antioxidant study (Gallic acid, HMPC, Ir (III)-HMPC complex).

RESULTS AND DISCUSSION

Spectrophotometric analysis

With 0.008 M phosphoric acid medium exhibiting pH 4.87, iridium (III) instantly reacted with HMPC to form a yellow-coloured complex. The iridium (III)-HMPC complex thus formed maintained its robustness for 5 days. The complex absorbed maximum in the wavelength range 423-430 nm whereas the reagent blank under a similar set of conditions manifested imperceptible absorbance (Fig. 1b). Taking into consideration the maximum absorbance, a wavelength of 425 nm was chosen for the spectrophotometric examination of Ir (III)-HMPC complex against an analogously prepared reagent blank.

Optimum set of conditions for Ir (III)-HMPC complex with respect to various parameters

Choice of solvent

Various organic solvents, both polar and non-polar were explored for appraisal of the extraction behavior of Ir (III)-HMPC complex but none of these solvents was found efficacious for its extraction. However, the process of complete complexation was found to be highly efficient in aqueous phase conditions making the aforesaid condition as the choice for further exploration.

Variation of pH

Among various acids (H_2SO_4, HCl, $HClO_4$, H_3PO_4 and CH_3COOH) and bases ($NaHCO_3$, Na_2CO_3 and NaOH) explored, phosphoric acid medium was found to be the most effectual for the purpose of analysis of the metal in form of its complex as could be inferred from the maximum absorbance value in this medium at 425 nm. Exploring and monitoring complexation in the phosphoric acid medium , the absorbance was found maximum at 0.004-0.011 M H_3PO_4 and pH 5.32-4.83 (Table 1) and therefore, set as the ideal complexation condition.

Variation of reagent concentration

While varying the volume of reagent added to the metal ion, complete complexation and hence the maximum optical density was observed between of 0.9-1.3 mL of the ethanolic HMPC solution and was hence set as the optimum condition for Ir (III)-HMPC complex (Table 1).

Effect of intervening ions

In the current study, a total of 24 anions/complexing agents and 33 cations were analyzed for their intervention with the suggested method so as to evaluate selectivity and hence the commercial adaptability of the investigated

Table 2: Influence of diverse ions (anions/complexing agents) on colour intensity of Ir (III)- HMPC complex

Anion/ Complexing agent	Salt used	Tolerance limit mg/10mL	Anion/Complexing agent	Salt used	Tolerance limit mg/10mL
Chloride	NaCl	100	Thiocyanate	KSCN	90
Bromide	KBr	100	Dithionite	$Na_2S_2O_4$	50
Iodide	KI	100	Hydrazinium ion	$N_2H_6SO_4$	50
Sulphate	Na_2SO_4	100	Acetate	CH_3COONa	50
Sulphite	Na_2SO_3	100	Ascorbic acid	$C_6H_8O_6$	50
Nitrate	$NaNO_3$	100	Oxalate	$K_2C_2O_4$	50
Sulfosalicylic acid	$C_7H_6O_6S$	100	EDTA´disodium'	$C_{10}H_{14}N_2Na_2O_8$	40
Carbonate	Na_2CO_3	100	Tartarate	$KNaC_4H_4O_6$	40
Bicarbonate	$NaHCO_3$	100	Citrate	$Na_3C_6H_5O_7$	40
Phosphate	Na_3PO_4	100	Fluoride	NaF	10
Nitrite	$NaNO_2$	90	Glycerol	$C_3H_8O_3$	1mL
Thiourea	C_2H_5NS	90	Hydrogen peroxide	H_2O_2 (100 vol, 30%)	1mL

**Conditions as in procedure of determination.*

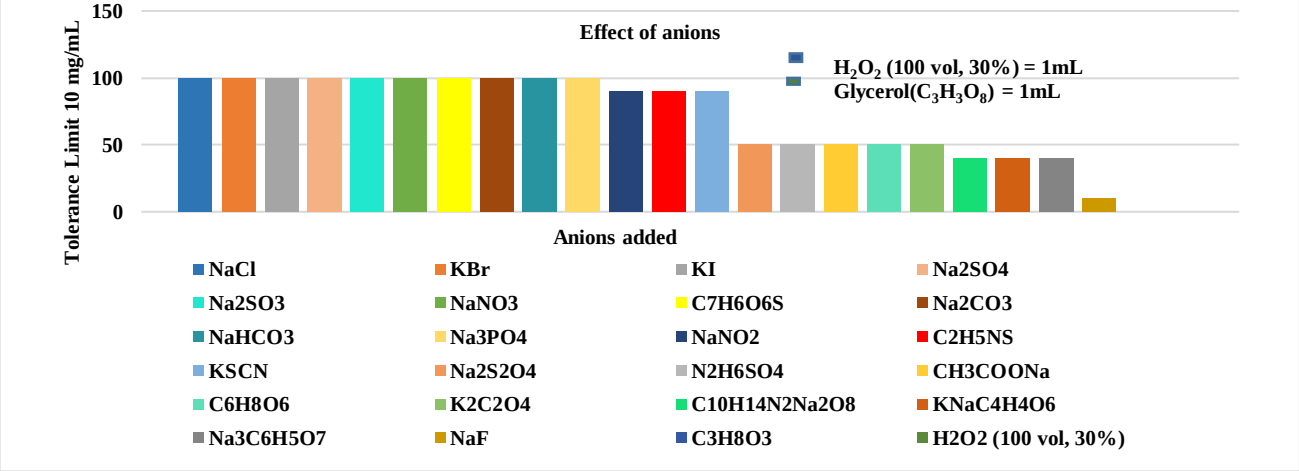
Table 3: Influence of diverse ions (cations) on colour intensity of Ir (III)-HMPC complex

Cation[x]	Salt used	Tolerance limit, mg/10mL	Cation[x]	Salt used	Tolerance limit, mg /10mL
Sr (II)	$SrSO_4$	10.0	As(V)	Na_2HAsO_4	5.0
Mn (II)	$MnCl_2.4H_2O$	10.0	Ti(IV)	TiO_2	1.0
Zn (II)	$ZnCl_2$	10.0	Sn(II)	$SnCl_2$	1.0
Co (II)	$CoCl_2.6H_2O$	10.0	W(VI)	$Na_2WO_4.2H_2O$	1.0
Ni (II)	$NiSO_4$	10.0	Pd(II)	$PdCl_2$	1.0
Mg (II)	$MgCl_2$	10.0	Ru(III)	$RuCl_3$	1.0
Pb (II)	$PbNO_3$	10.0	Os(VIII)	OsO_4	1.0
Ba (II)	$BaCl_2.H_2O$	10.0	Au(III)	$AuCl_3$	1.0
Hg(II)	$HgSO_4$	10.0	Pt (IV)	H_2PtCl_2	1.0
Ca (II)	$CaCl_2$	10.0	Zr(IV)	$ZrOCl_2.8H_2O$	1.0
Ag (I)	$AgNO_3$	10.0	Fe(II)[y]	$FeSO_4.7H_2O$	1.0
Cu (II)	$CuSO_4.5H_2O$	10.0	Mo(VI)	$(NH_4)_2MoO_4$	0.8
Al(III)	$AlCl_3$	10.0	Nb(V)	Nb_2O_5	0.8
Cd (II)	$CdCl_2$	10.0	Fe(III)[y]	$FeCl_3$	0.5
Se(IV)	SeO_2	5.0	V(V)[z]	$NaVO_3$	0.5
Cr(III)	$CrCl_3$	5.0	Ce(IV)	$NH_3[Ce(NO_3)_6]$	0.5
Cr(VI)	$K_2Cr_2O_7$	5.0			

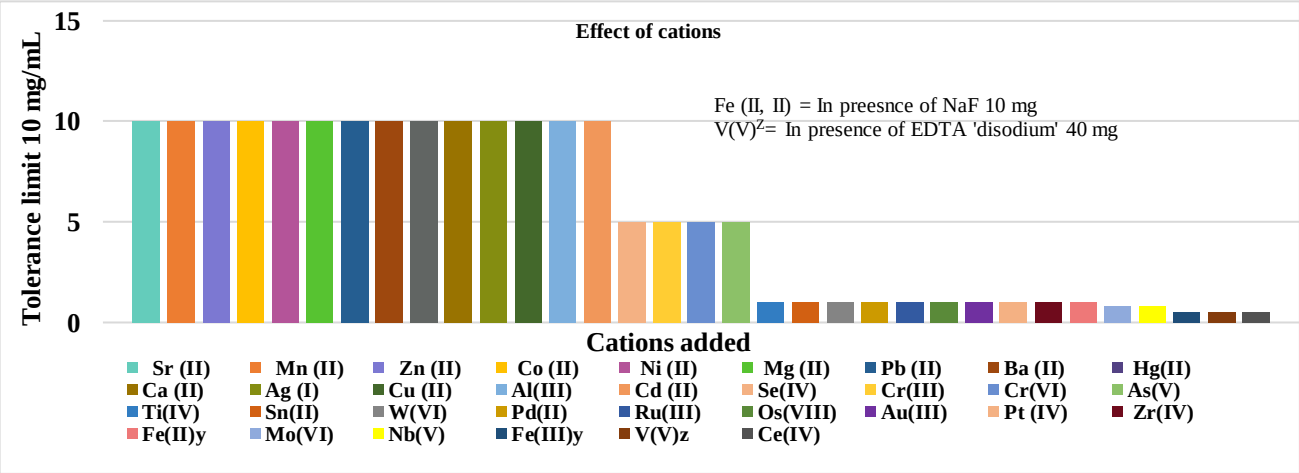
[x]Initial oxidation state shown in parentheses; [y]In the presence of Fluoride (10 mg) as maskingagent,[Z]In the presence of EDTA (40 mg) as masking agent.

Ir (III)-HMPC complex. The effect of diverse ions was studied by taking 10 µg Ir(III) per 10 mL aqueous volume under the proposed conditions. The various anions/complexing agents (Table 2; Bar Chart 1) and cations (Table 3; Bar Chart 2) were added before the addition

of the reagent. This was observed that the ions did not affect the absorbance of Ir (III)-HMPC complex. However, iron in both of its common oxidation states (II and III) and vanadium in the pentavalent state did not interfere with the presence of sodium fluoride (10 mg/10 mL) and EDTA, the disodium salt (40 mg/10 mL), respectively added as masking agents. The masking agents were added to the aqueous phase before addition of the reagent (HMPC).



Bar Chart 1: Influence of anions/complexing agents on colour intensity of Ir (III)-HMPC complex



Bar Chart 2: Influence of cations on colour intensity of Ir(III)- HMPC complex

Optical parameters and analytical figures of merit

The effect of concentration of the colored constituents in solution upon absorption was studied by employing Beer's law which followed a linear relationship between the two parameters up to a concentration 1.7 µg Ir(III) per mL as is indicated by a straight line plot between absorbance and concentration of the metal ion and thereafter deviated positively from linearity attributable to increased concentration of the solution (Fig. 1c). However, the accuracy of analysis further was confirmed by the Ringbom's plot [43] exhibiting an optimum range of determination as 0.459 to1.624 ppm of Ir (III) as is shown in Fig. 1d. The parameters defining the sensitivity of the 1:2 (M:L) stable system (stability-5 days) i.e. molar extinction coefficient, sensitivity (Sandell's) and detection limit were calculated, respectively to be 6.824×10^4 L/(mol·cm), 0.00281 µg Ir (III) cm^{-2} and 0.0064 µM at 425 nm. From Beer's law, equation of linearity was worked out as Y = 0.5543X-0.001 and the linear relationship between the variables (absorbance and concentration) is confirmed by the coefficient of determination (r) being equal to 0.9999. The reproducibility of the method is tested by performing ten sets of experiments taking 1 µg Ir(III) per mL each time. The results obtained are highly reproducible with the standard deviation of ± 0.00077 absorbance unit with %RSD as equal to 0.2169%. Various optical and statistical parameters obtained after the optimization of conditions are given in the Table 4 below.

Composition of Ir (III)-HMPC complex

A 1:2 (M:L) stoichiometry was deduced and confirmed for the studied complex by Job's continuous variations

Table 4: Optical parameters and analytical figures of merit of the complex

S.No.	Criterion	Stipulation
1	Wavelength of maximum absorption (λ_{max})	423-430 nm
2	Molar extinction coefficient (Ɛ)	6.824×10^4 L mol^{-1} cm^{-1}
3	Sandell's sensitivity (S)	0.00281 µg cm^{-2}
4	Linearity range (Beer's law)	0-1.7 µg mL^{-1}
5	Ringbom's range	0.459-1.624 ppm
6	Regression equation	Y = 0.554 X - 0.001
7	Regression coefficient (r)	0.9999
8	Detection limit	0.0123 µg/mL /0.0064 µM
9	Standard deviation (SD)	± 0.00077
10	Relative standard deviation (RSD)	0.2169 %
12	Composition	1:2 (M:L)
13.	Stability	5 days

Table 5: Quantum chemical descriptors

S.No.	Molecule	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE_{gap} (eV)
1.	HMPC	-6.1007	-1.9841	-4.1166
2.	Ir (III)-HMPC complex	-5.3932	-2.8521	-2.5411

method [44] (Supplementary Table 1 and Fig. S_1) as is improved by Vosburgh and Cooper [45], mole Ratio [46] Supplementary Table 2 and Fig. S_2) and equilibrium shift [47] methods**.** At a fixed metal ion concentration of 1.040×10^{-3} mol/L, the equilibrium shift method was evaluated with respect to variable concentrations of the ligand (C_L) ranging from 2.08×10^{-4} mol/L to 2.288×10^{-3} mol/L. The absorbance (A_I) corresponding to different reagent concentrations was measured at 425 nm. The plot of $\log/(A_0-A_I)$ versus $\log C_L$ (A_0 being the maximum value attained during complete complex formation) yielded a straight line with a slope of 1.905 (Fig. 1e) confirming the 1:2 metal-to-ligand ratio in the formed species.

Proposed structure of Ir (III)-HMPC complex

The analysis completed as above led to the proposed structure of the studied Ir (III) complex as depicted in Fig. 1f.

Theoretical analysis employing DFT

Validity and associated investigation regarding electronic and structural details of the proposed structure of Ir (III)-HMPC complex as carried out by DFT calculation is explained as under.

Frontier Molecular Orbitals

The frontier molecular orbitals provide a precise picture of a molecule's intended positions in the donor–acceptor relationship between the ligand and its metal complex [48-50]. Fig. 2a shows the optimized geometry of both the molecules (ligand and its complex), as well as HOMO and LUMO electron density distributions. The major orbitals involved in chemical stability are the HOMOs and LUMOs. HOMO and LUMO energy gaps inside the molecules explained charge transfer interaction; the electronic absorption being the excitation of one electron from HOMO to LUMO, hence relating to the transition from the ground to the first excited state. The energy gap for ligands between the transition from HOMO (-6.10 eV) to LUMO (-1.98 eV) of the molecule is -4.12 eV, according to the B3LYP/6-311++G(d,p) calculation. On the other hand, the energy gap for the complex is about -2.54 eV. In ligands, HOMO is found above the rings, as well as the C=O group, however, LUMO is partially delocalized over the oxygen atom (Table 5). Additionally, other chemical descriptors such as hardness (chemical /global; η) which is proportional to HOMO–LUMO energy gap is higher for the complex; the large value indicates the chemical stability of the substance. Similarly, the attraction of electrons in a covalent bond is estimated by electronegativity value. The low reactivity of the stable complex is further represented by global softness (σ), the reciprocal of global hardness. The electrophilicity index (ω), further supports high stability of the complex which indicates that it will not decompose into its constituents (Table 6).

Table 6 : Global chemical reactivity indicators of HMPC and Ir (III)-HMPC complex

S.No.	Molecule	Electronegativity, χ (eV)	Chemical hardenss, η (eV)	Chemical softness, σ(eV)	Electrophilicity index, ω (eV)
1	HMPC	4.0424	2.0583	0.4858	3.9695
2	Ir(III)-HMPC complex	4.1226	2.5411	0.3935	3.3441

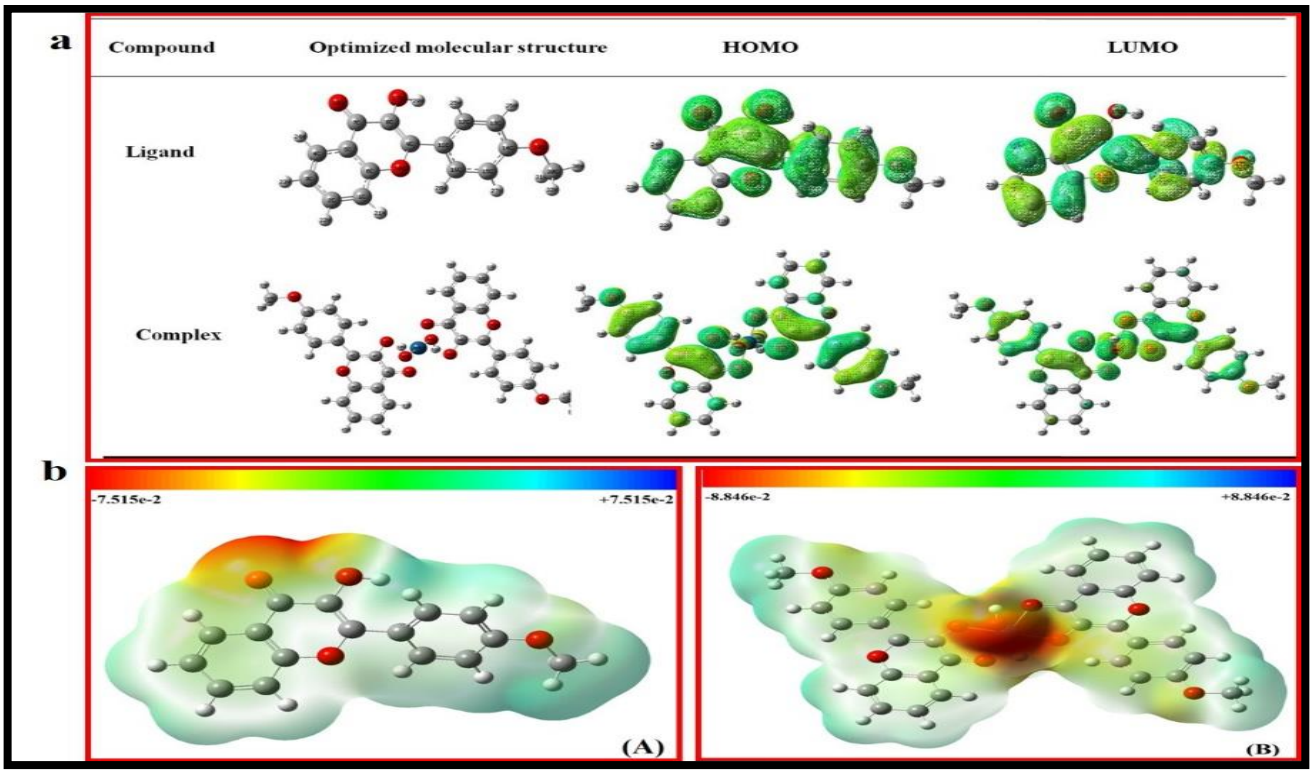


Fig: 2: Computational Analysis, a) Optimized molecular structure and HOMO-LUMO plot of ligand and the complex ; b) Molecular electrostatic potential maps (A) HMPC (B) Ir (III)-HMPC complex

Molecular Electrostatic Potential (MEP)

The link between molecular structures and their physiochemical property relationships, including biomolecules and medicines, has been discovered to be a highly effective tool in the analysis of molecular electrostatic potential [35, 51]. It generally contains information on the molecular chemical reactivity. The MEPs for ligand and complex were plotted using the B3LYP/6-311++G(d,p) and LANL2DZ basis sets, as shown in Fig. 2b. Electrophilic or nucleophilic properties were explained by the electrostatic potential generated around a molecule by charge distribution [36]. The different colors are indicative of variable values of the electrostatic potential. Red, blue, and green, respectively, represent negative, positive, and zero electrostatic potentials. The MEP map shows that the negative potential regions are around oxygen and iridium atoms, while the positive potential areas are around H-atoms, based on the calculated findings. These negative spots clearly illustrate the compound's biological action. The ligand's negative and positive regions are between -7.515e-2 and +7.515e-2 while negative and positive regions of the complex are -8.846e-2 and +8.846e-2. The oxygen atoms in the ligand and complex, which are donor atoms, have a negative charge. Based on these findings, ligands and complexes are ready for both electrophilic and nucleophilic reactions.

Analytical applications

An attempt was made to check the versatility and appositeness of the proposed method by employing it in a large number of analytical synthetic mixtures of varying compositions (Table 7). All the miscellaneous combinations of the mixtures yielded satisfactory results as can be deduced from the amount of Ir(III) found in various samples, thus, confirming the applicability and sensitivity of the method. Furthermore, the method was successfully

Table 7: Investigation of miscellaneous mixtures and alloys as per suggested methodology

S.No.	Matrix Composition[a]	Ir (III) taken (µg /10 mL)	Ir (III) found(µg /10 mL)[b]
1	Ca (5), V* (0.1), Se (0.05)	4	4.0±0.08
2	Sr (0.5), Mg(2), Hg(2)	5	4.95±0.12
3	Pd(0.1), Ni(2), Mo(0.1)	7	6.98±0.09
4	Cu(3), Zn (2), Cr^{III}(3),Mg(2)	15	14.91±0.16
5	Ti(0.5),Fe^{III**}(0.3),Ag(2), Zn(2)	12	12.01±0.03
6	Co (2), Sr(0.5), W(0.1)	7	7.07±0.06
7	Pt(0.1), Nb(0.1), Zr(0.1)	16	16.04±0.09
8	Pb(2),Mn(3),W(0.05),Ce(0.1)	5	5.01±0.07
9	Os (0.1), Cu(5), Ag(0.1)	6	5.96±0.01
10	Ru(0.1), Mo(0.1), Al(3)S	5	4.93±0.01
11	Mg(5),Sn(0.5),W(0.05)	5	5.00±0.02
12	Cu(2), Co(2), Fe^{II**}(0.1)	7	6.95±0.06
13	Cr^{III}(2), Pd(0.5) , Ti(0.5)	5	4.95±0.02
14	Os(0.4)[c]	0.6	0.59±0.01
15	Os(0.8)[c]	0.2	0.19±0.01

*[a]Figure in brackets indicates mg/10 mL quantity of metal ion. [b]Mean of three replicates ± SD. [c]Composition analogous to iridosmine and osmiridium, respectively. *In the presence of 40 mg EDTA 'disodium salt', **In the presence of 10 mg fluoride.*

employed to the most important alloys of iridium, iridosmine and osmiridium, further adding to its versatility.

Evaluation of in-vitro cytotoxic potential

The anti-cancerous activity of the reported compounds was measured in the form of percent cell viability after 24 and 48 hours of incubation at different concentrations (Fig. 3a). The viability of cancerous cells was assessed by performing the MTT assay which indicates a significant decrease in the viability of breast cancer cell line (T-27D). Dimethyl sulfoxide (DMSO) was used as a solvent to dissolve the complex and ligand. The control value obtained due to DMSO had been considered equal to 100% cell viability. The percentage of cell viability was calculated for the complex and ligand in comparison to control. The graph was plotted to compare the relative viability percentage of the complex [Ir(III)-HMPC), ligand (HMPC) and solvent (DMSO)] at different concentrations and incubation time. The deviation of results due to experimental repetitions was reflected in the form of error values (STDs).

The complex [Ir (III)-HMPC] and free ligand (HMPC) exhibit a marked drop in the percent cell viability with the increase in concentration (Fig. 3a). However, the viability of cancer cells did not decrease as significantly with the ligand as it did for the complex. Because the free ligand hardly inhibits cell growth by 50%, this ensures that the complex's strong anticancer effect is due to the metal core. Ir (III)-HMPC complex has been showing a significant increase in cell death as compared with control (61% and 65% of cell death at 100 µM after 24 h and 48 h of incubation respectively; $p < 0.05$; Fig. 3a). The reported maximal cell growth inhibition (%) demonstrated that the Ir (III)-HMPC complex exhibited significant anticancer effects in breast cancer cell line.

Evaluation of in-vitro antibacterial activity

As discussed in the methodology, two gram-positive (*B. subtilis* and *S.aureus*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*) strains were selected to check the antibacterial potential of the newly

synthesized Ir (III)-HMPC complex by determining their respective MICs. The tested complex showed highly significant zone of growth inhibition against all the bacterial strains as mentioned in Table 8. The studied complex inhibited the selected bacterial cultures in the range of 40 mm to 48 mm (zone of inhibition). Ciprofloxacin had been used as the positive control to compare the results. Ciprofloxacin was showing zone of inhibition in the range of 33-35 mm which was less in comparison to the tested complex (Fig. 3b).

Table 8: Antibacterial activity of the complex displaying diameter of growth inhibition in mm

Solutions	Diameter of growth inhibition (mm)[a]			
	Bacillus subtilis	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
Ir (III)-HMPC Complex	45 ± 0.75	42 ± 0.45	40 ± 0.57	48 ± 0.75
Ciprofloxacin	34 ± 0.86	33 ± 0.70	35 ± 0.75	34 ± 0.75

[a]*Values, including the diameter of the well (8 mm), are the mean of triplicates ± SD.*

Table 9: Minimum inhibitory concentration (MIC) (µg/100µL) of Ir (III)-HMPC complex

Solutions	Gram-positive bacteria		Gram-negative bacteria	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Ir(III)-HMPC Complex	3.91	3.91	3.91	3.91
Ciprofloxacin	7.81	7.81	7.81	15.62

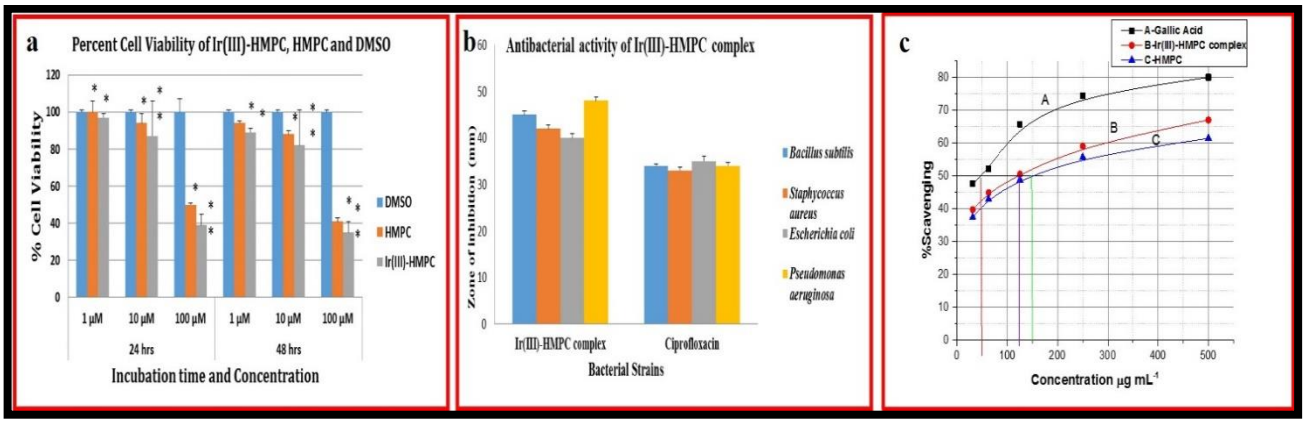


Fig. 3: Bioanalytical Studies, a) Percent Cell Viability of Ir (III)-HMPC complex, HMPC ligand and DMSO at different incubation times and concentrations.*p < 0.05 ; **p < 0.01; b) Graphical representation of In- vitro antibacterial activity of Ir (III)-HMPC Complex; c) % RSA of A-Gallic acid, B- Ir (III)-HMPC Complex, C- HMPC

In order to compare the bactericidal potential of Ir (III)-HMPC complex with the standard antibiotic, a comparison of their Minimum Inhibitory Concentration (MIC) values was done as depicted in Table 9. It was observed that the antibacterial activity against the screened strains was more remarkable for the complex showing MIC values up to 3.91 µg/100µL. Ciprofloxacin used as a standard antibiotic exhibited MIC values in the range of 7.81 to 15.62 µg/100 µL (Supplementary Fig. S_3). It can thus be inferred that Ir (III)-HMPC complex possesses potent bactericidal action. Furthermore, the bactericidal action of the complex is higher than that exhibited by ciprofloxacin and is hence a promising bactericidal agent.

DPPH· radical scavenging activity

The free radicals generated by various metabolic processes or UV radiations cause the deterioration of DNA, proteins and lipids. Under such circumstances, for the protection of biological systems, the administration of antioxidants from outside is recommended, as the latter play a vital role in their protection. Antioxidants work by scavenging free radicals and stopping chain reactions by giving the free radical an electron or hydrogen. As a result, there's been a surge in interest in creating new, safer antioxidants with fewer adverse effects.

The antioxidant activity of HMPC ligand and its Ir (III) complex was determined at various concentrations by measuring the de-colorization of DPPH. The percentage RSA increased with the increase in concentration of compounds. HMPC and Ir (III)-HMPC complex exhibited the highest scavenging activity of 61.39% and 67%, respectively at 500 µg/mL concentration. Under experimental conditions, IC_{50} of Ir (III)-HMPC complex is 124.5 µg/mL. The RSA can be compared in the following order: gallic acid > Ir (III)-HMPC complex > HMPC (Table 10; Fig. 3c). Thus, the Ir (III)-HMPC complex

Table 10: Antioxidant Activity of the studied ligand and its complex in terms of % scavenging activity

Concentration (µg/mL)	% Scavenging		
	HMPC*	Ir (III)-HMPC**	Gallic Acid***
31.25	37.37	39.7	47.6
62.5	42.91	44.9	52.2
125	48.76	50.5	65.7
250	55.64	59	74.3
500	61.39	67	80

*IC_{50} values=*153µg/mL ,**124.5 µg/mL and ***50 µg/mL*

displays greater scavenging activity than the free ligand, hence can be used as an effective antioxidant.

CONCLUSIONS

In the present investigation, a phosphoric acid mediated aqueous phase spectrophotometric analysis for the micro determination of iridium (III) following its complexation with 3-hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (HMPC) resulting in the production of a robust yellow colored complex, has been fortuitously carried out. The composition of Ir (III)-HMPC has been validated as 1:2 [Ir (III):HMPC] by employing Job's method, mole-ratio method, and equilibrium-shift method. The coherence to linearity is shown up to 1.7 µg Ir (III) mL^{-1}. The selectivity, sensitivity, ingeniousity, reproducibility and cost-effectiveness have been amply depicted and emphasized by the respective values of the parameters and other studies performed during the course of conduction of the present work including its intervention studies, application in synthetic mixtures, molar extinction coefficient and Sandell's sensitivity and their comparison with the previously reported methods [17-22]. The optimized geometry of the proposed structure and structural and electronic details have been examined by employing DFT and MEP studies as has been successfully employed by several authors for analogous complexes [17, 28, 29]. Apart from being an ultra-refined spectrophotometric method, it is noteworthy to mention that the current study successfully elucidates the Ir (III)-HMPC complex as a therapeutic agent possessing bactericidal, antiradical and anticarcinogenic potential. In nutshell, the present study is highly a novel addition of the bioanalytical applications and computational studies to the ongoing spectrophotometric investigation, hence anticipating that it will pave the way for the synthesis of novel antibacterial, antioxidant and anticancer metallodrugs by the incorporation of biologically active ligands to the trivalent state of the studied platinum group metal, iridium.

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REFERENCES

[1] ElGuindy M., Platinum Group Metals: Alloying, Properties, and Applications, *Encycl. Mater. Sci. Tech., Oxford*, 7117-21 (2001).

[2] Blaser H. U., Iridium Complexes in Organic Synthesis-Application of Iridium Catalysts in the Fine Chemicals Industry (2008).

[3] Livingstone S.E., "The Chemistry of Ruthenium, Rhodium, Palladium, Osmium, Iridium and Platinum in Comprehensive Inorganic Chemistry", Pergamon Press Ltd., Headington Hill Hall, Oxford, OX3 OBW, England (1975).

[4] Wang G., Zhou M., Goettel J. T., Schrobilgen G. J., Su J., Li J., Schloder T., Riedel S., Identification of an Iridium-Containing Compound with a Formal Oxidation State of IX, Nature, **514**: 475-477 (2014).

[5] Singh S.B., Iridium Chemistry and its Catalytic Applications: A Brief Review, *Green Chem. Tech Let.*, **2**: 206-210 (2016).

[6] Liu Z., Sadler P.J., Organoiridium Complexes: Anticancer Agents and Catalysts, *Acc. Chem. Res.* **47**:1174-1185 (2014).

[7] Lu L., Liu L., Chao W., Zhong H., Wang M., Chen X., Lu J., Li R., Dik-Lun M.A., Chung-Hang L., identification of an Iridium (III) Complex with Anti-Bacterial and Anti-Cancer Activity, *Sci. Rep.*, **5**: 14544 (2015).

[8] Konkankit C., Marker S., Knopf K., Wilson J., Anticancer Activity of the Third Row Transition Metals, Rhenium, Osmium and Iridium, *Dalton Trans* **47**: 9934-9974 (2018).

[9] Ko C., Li G., Leung C., Ma D., Dual Function Luminescent Transition Metal Complexes for Cancer Theranostics: the Combination of Diagnosis and Therapy, *Coord. Chem. Rev*., **381**: 79-103 (2019).

[10] Lo K., Zhang K., Iridium (III) Complexes as Therapeutic and Bioimaging Reagents for Cellular Applications, *RSC Adv.* **2**: 12069-12083 (2012).

[11] Tsutomu M., Yuto L., Shun S., Precise Determination of Iridium by Neutron Activation Analysis coupled with Internal Standard Method, *J. Radioanal. Nucl. Chem.*, 324: 1007-1012 (2020).

[12] Druskovic V., V. Vojkovic, Miko S., Spectrofluorimetric Determination of Iridium (IV) Traces Using 4-Pyridone Derivatives, *Talanta*, **62**:489-495 (2004).

[13] Ingo O., Michael S., Heike S., William S.S., Gust R., Atomic Absorption Spectrometric Determination of Iridium Content in Tumor Cells Exposed to an Iridium Metallodrug, *J. Pharm. Biomed. Anal*. **47**: 938-942 (2008).

[14] Locatelli C., Iridium and Lead as Vehicle Emission Pollutants: Their Sequential Voltammetric Determination in Vegetable Environmental Bio-Monitors, *Microchem. J*., **106**: 282-288 (2013).

[15] Zhang G., Tian M., A Rapid and Practical Strategy for the Determination of Platinum, Palladium, Ruthenium, Rhodium, Iridium and Gold in Large Amounts of Ultrabasic Rock by Inductively Coupled Plasma Optical Emission Spectrometry Combined with Ultrasound Extraction, *Opt. Spectrosc*., **118**: 513-518 (2015).

[16] Khomutova E.G., Zagorodnikova S.A., Ostanina O.I., Individual Catalytic Determination of Iridium and Rhodium Using the Oxidation of Sulfarsazene by Potassium Periodate as an Indicator Reaction in a Flow-Injection System *J. Anal. Chem*., **68**: 611-615 (2013).

[17] Mohmad M., Agnihotri N., Kumar V., Kumar R., Kaviani S., Iridium (III)-3-Hydroxy-2-(3'-Methyl-2'-Thienyl)-4-oxo-4*H*-1-Benzopyran Complex: The Analytical, *In-vitro* Antibacterial and DFT Studies, *Inorg. Chem. Commun.*, **139**: 109333 (2022).

[18] Borgave S.S., Barhate V.D., Extractive and Spectrophotometric Determination of Iridium (III) Using 2-(5- Bromo-2-Oxoindolin-3-Ylidene) Hydrazinecarbothioamide as an Analytical Reagent, *J. Chem. Pharm. Res.*, **8**: 584-589 (2016).

[19] Kuchekar S.R., Pulate S.D., Shelar Y.S., Han S.H., Spectrophotometric Study of Interaction of o-Methylphenylthiourea with Iridium(III) and Development of a Precise Determination Method from Hydrochloric Acid Media, *Indian J. Chem. Technol.*, **21**: 120-126 (2014).

[20] Kuchekar S., Bhumkar S., Aher H., Han S. H., Solvent Extraction, Spectrophotometric Determination of Iridium (III) Using P-Methylphenylthiourea as a chelating Agent: Sequential Separation of Iridium (III), Ruthenium (III) and Platinum (IV), *J. Mater. Environ. Sci.*, **10**: 1200-1213 (2019).

[21] Druskovic V., Vojkovic V., Spectrophotometric Determination of Microamounts of Iridium (IV) with 3-Hydroxy-2-Methyl-1-Phenyl-4-Pyridone or 3-Hydroxy-2-Methyl-1-(4-Tolyl)-4-Pyridone, *Croat. Chem. Acta.*, **76**: 49-54 (2003).

[22] Long W.R., Ding Z.T., Cao Q.E., Catalytic Spectrophotometric Determination of Trace Iridium (IV) Using Iridium (IV)-KIO4-Chlorophosphonazo-mA System, *Trans. Metal Chem.*, **27**: 40-42 (2007).

[23] Lapasam A., Shadap L., Tripathi D.K., Poluri K.M., Kaminsky W., Kollipara M.R., Arene Ruthenium, Rhodium and Iridium Complexes Containing Benzamide Derivative Ligands; Study of Interesting Bonding Modes, Antibacterial, Antioxidant and DNA Binding Studies, *J. Organomet. Chem*., **937**: 121731 (2021).

[24] Lapasam A. , Mawnai I.L., Banothu B., Kaminsky W., Kollipara M.R., Ruthenium, Rhodium and Iridium Complexes Containing Pyrimidine Based Thienyl Pyrazoles: Synthesis and Antibacterial Studies, *J. Organomet. Chem.*, **911**: 121155 (2020).

[25] Quan H.A.N., Yanyan H. U. O., Hong C., Anticancer and Antibacterial Activity in-Vitro Evaluation of Iridium (III) Polypyridyl Complexes, *J. Bio. Inorg. Chem.*, **24**: 151-169 (2019).

[26] Kollipara M.R ,.Shadap L., Banothu V., Agarwal N., Fluorenone Schiff Base Derivative Complexes of Ruthenium, Rhodium and Iridium Exhibiting Efficient Antibacterial Activity and DNA-Binding Affinity, *J. Organomet. Chem*., **915**: 121246 (2020).

[26] Tabrizi L., Nguyen T., Duy D., Experimental and Theoretical Investigation of Cyclometalated Phenyl Pyridine Iridium (III) Complex Based on Flavanol and Ibuprofen Ligands as Potent Antioxidants, *Royal Soc. Chem.*, **9**: 17220-17237 (2019).

[27] Dhonchak C., Agnihotri N., Kumar A., Zirconium (IV)-3-Hydroxy-2-Tolyl-4*H*-Chromen-4-One Complex- The Analytical and DFT Studies, *J. Mol. Model*., **27**: 336 (2021).

[28] Kataria R., Patra R., Sharma H.K., Singh G., Kumar G., Sharma S.K., Spectrophotometric Determination of Tin (II) with 6-Chloro-2-(2'furyl)-3-Hydroxy-7-Methyl-4-Oxo-4*H*-1-Benzopyran and its Solution State Structure Study by DFT, *Sens. Lett*. **15**: 25-31 (2017).

[29] Algar J., Flynn J. P., A New Method for the Synthesis of Flavones, *Proc. R. Ir. Acad.*, **42B**: 1-8 (1934); Oyamada T., A New General Method for the Synthesis of the Derivatives of Flavonol, *Bull. Chem. Soc. Jpn*., **10**: 182- 186 (1935);
Wera, M., Serdiuk, I., Roshal, A., Jerzy, B., 3-Hydroxy-2-(4-Methoxyphenyl)-4H-Chromen-4-One, *Acta Cryst*., **E67**: 0440 (2011).

[30] Kumar A., Trivedi M., Bhaskaran S.R.K., Singh G., Synthetic, Spectral and Structural Studies of a Schiff Base and its Anticorrosive Activity on Mild Steel in $_{H2}S_{O4}$, *New J. Chem.*, **41**: 8459–8468 (2017).

[31] Muscat J., Wander A., Harrison N.M., On the Prediction of Band Gaps from Hybrid Functional Theory, *Chem. Phys.*, **342**: 397–401 (2001).

[32] Rienstra-Kiracofe J.C., Barden C.J., Brown S.T., Schaefer H.F., Electron Affinities of Polycyclic Aromatic Hydrocarbons, *J. Phys. Chem.*, **105**: 524–528 (2001).

[35] Vektariene A., Vektaris H.F., Svoboda J., A Theoretical Approach to the Nucleophilic Behavior of Benzofusedthieno [3,2-b] Furans Using DFT and HF Based Reactivity Descriptors, *ARKIVOC*., **7**: 311–329 (2009).

[36] Noureddine O., Gatfaoui S., Brandan S.A., Sagaama A., Marouani H., Issaoui N., Experimental and DFT Studies on the Molecular Structure, Spectroscopic Properties and Molecular Docking of 4-Phenylpiperazine-1-Ium Dihydrogen Phosphate, *J. Mol. St.*, **1207**: 127762 (2020).

[37] Scrocco E., Tomasi J., Electronic Molecular Structure, Reactivity and Intermolecular Forces: An Euristic Interpretation by Means of Electrostatic Molecular Potentials, *Adv. Quantum Chem*., **11**: 115–121 (1978).

[38] Ulukaya E., Colakogullari M., Wood E., Interference by Anti-Cancer Chemotherapeutic Agents in the MTT-Tumor Assay, *Chemotherapy*, **50**: 43–50 (2004).

[39] Florento L., Matias R. , Tuano E., Santiago K., Dela Cruz F., Tuazon A., Comparison of Cytotoxic Activity of Anticancer Drugs against Various Human Tumor Cell Lines Using In Vitro Cell-Based Approach, *Int. J. Biomed*., **8**: 76–80 (2012).

[40] Lee M. M.L., Chan B.D., Wong W.Y., Leung T. W., Qu Z., Huang J., Zhu L., Lee C.S., Chen S., Tai W.C.S., Synthesis and Evaluation of Novel Anticancer Compounds Derived from the Natural Product Brevilin A, *ACS omega*, **5**: 14586-14596 (2020).

[41] Kaur K., Kumar K., Beniwal V., Kumar V., Kumar N., Sharma V., Jaglan S., Synthesis of Some Novel Oxazolidinone-Thiazole Hybrids as Potential Antimicrobial, Antioxidant and UV Mediated DNA Damage Protecting Agents, *Med. Chem. Res*., **25**: 2237-2249 (2016).

[42] Kamal R., Kumar R. , Kumar V., Kumar V., Bansal K.K., Sharma P.C., Synthesis, Anthelmintic and Antimicrobial Evaluation of New 2-Arylidene-1-(4-Methyl-6-Phenylpyrimidin-2-yl)Hydrazines, *Chem. Select* **4**: 713-717 (2019).

[43] Singh S., Agnihotri N., Rathi P., Agnihotri R., Kumar V., Molecular Dynamics, Biological Study and extractive Spectrophotometric Determination of Vanadium (V)-2-Methyl-8-Quinolinol Complex, *Iran. J. Chem. Chem. Eng. (IJCCE)*. **40(1)**: 207-214 (2021).

[44] Karthikeyan J., Naik P.P., Shetty N., A Rapid Extractive Spectrophotometric Determination of Copper (II) in Environmental Samples, Alloys, Complexes and Pharmaceutical Samples Using 4-N, N (Dimethyl) Amino] Benzaldehyde Thiosemicarbazone, *Environ. Monit. Assess.*, **176:** 419-426 (2011).

[45] Joseph S.R, Laura L.T., Evan H.T., David B.C., Method of Continuous Variations: Applications of Job Plots to the Study of Molecular Associations in Organometallic Chemistry, *Angewantde Rev*., **52**: (2013) 11998-12013.

[46] Vosburgh W.C., Cooper G.R., Complex Ions I., The Identification of Complex Ions in Solution by Spectrophotometric Measurements, *J. Am. Chem. Soc*., **63**: 437-442 (1941).

[47] Yoe J.H., Jones A. L., Colorimetric Determination of Iron with Disodium-1,2-Dihydroxybenzene-3,5-Disulfonate, *Ind. Eng. Chem.*, (*Anal. Ed.*) **16**: 111-115 (1944).

[48] Tarasiewicz H.P., Grudiniewska A., Tarasiewicz M., An Examination of Chlorpromazine Hydrochloride as Indicator and Spectrophotometric Reagent for the Determination of Molybdenum (V), *Anal. Chem. Acta.*, **94**: 435-442 (1977).

[49] Allah M.A.A.H., Balakit A.A., Salman H.I., Abdulridha A.A., Sert Y., New Heterocyclic Compound as Carbon Steel Corrosion Inhibitor in 1 M H_2SO_4, High Efficiency at Low Concentration: Experimental and Theoretical Studies, *J. Adhes. Sci. Technol.*, 1-23: (2022) .

[50] Abdulridha A.A., Allah M.A.A.H., Makki S.Q., Sert Y., Salman H.E., Balakit A.A., Corrosion Inhibition of Carbon Steel in 1 M H_2SO_4 Using New Azo Schiff Compound: Electrochemical, Gravimetric, Adsorption, Surface and DFT Studies, *J. Mol. Liq.*, **315**: 113690 (2020).

[51] Kansız S. , Tolan A. , Azam M. , Dege N., Alam M. , Sert Y. , Al-Resayes S.I. , İçbudak H., Acesulfame Based Co(II) Complex: Synthesis, Structural Investigations, Solvatochromism, Hirshfeld Surface Analysis and Molecular Docking Studies, *Polyhedron*, **218**: 115762 (2022).

[52] Dege N., Gökce H., Doğan O. E., Alpaslan G. , Ağar T., Muthu S., Sert Y. , Quantum Computational, Spectroscopic Investigations on N-(2-((2-chloro-4,5-Dicyanophenyl)amino)Ethyl)-4-Methylbenzenesulfonamide by DFT/TD-DFT with Different Solvents, *Molecular Docking and Drug-Likeness Researches, Colloids Surf. A Physicochem. Eng. Asp*., **638**: 128311 (2022).