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# Synthesis, characterisation, DFT computation and DNA binding study of Ir(III)-dichloro bis[1-alkyl-2-(tolylazo)]imidazoles complexes

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Ir(III) complexes of 1-alkyl-2-(tolylazo)imidazoles (TaiR, **1**; R = Me (**a**), Et (**b**), $CH_2Ph$ (**c**)), $[Ir(TaiR)_2Cl_2](ClO_4)$ (**2**) have been synthesized. All the complexes have been characterized by elemental and spectral (IR, UV-Vis, Mass and $^1H$ NMR) analysis. The DNA binding property of the complexes has been studied by spectroscopic methods. Density functional theory (DFT) computation technique has been performed to interpret the electronic structures and spectral properties of the complexes.

Keywords: 1-Alkyl-2-(tolylazo)imidazoles, DFT calculation, DNA binding study, Ir(III) complex, synthesis and characterisation.

## Introduction

The study of interaction of transition metal complexes with DNA has received much attention over the past few decades because of its subsequent utility in the field of cancer chemotherapy research[1–16]. DNA is the primary target of the synthetic anti-tumor complexes[17–19]. The interaction of metal complexes with DNA affects both replication and transcription of DNA which ultimately leads to the cell death[16]. After the discovery of anticancer activity of cisplatin, a large number of platinum metal complexes have been synthesized and used against different tumor cells[21–25]. Narrow range of activity, rapid development of drug resistance and nephrotoxicity of platinum complexes limit their widespread use[26,27] and enforce to explore safer complexes. Over the past few years, an intensive effort has been dedicated to develop metal-based drugs with improved clinical effectiveness, reduced toxicity and broader spectrum of activity[28–35]. Many of them are very promising, and have already reached for clinical trials[31,32].

We have been engaged for the last several years to synthesize arylazoheterocycles belong to azoimine function, -N=N-C=N-, and this is isoelectronic to diimine (-N=C-C=N-) function. The transition and nontransition metal coordination chemistry of this ligand is studied by us[36–43]. These ligands are pH-responsive, photochromic, redox active and exhibit photo-electron communication and non-linear optical property[41–43]. Recently we have become interested to study the DNA-binding property of metal complexes[44,45] and the present work is the continuation of that. In this article, the synthesis and the spectral characterization of iridium(III) complexes of 1-alkyl-2-(tolylazo)imidazoles are described. The DNA binding ability of complexes is established by absorption and fluorescence spectroscopic studies and the electronic properties are correlated with DFT calculation.

## Experimental

*Materials and physical measurements:*

$IrCl_3.3H_2O$ was procured from Arora Matthey, India. The required solvents and $NaClO_4$ were purchased from E. Merck, India. The ligands, 1-alkyl-2-(tolylazo)imidazoles (TaiR, **1**) used in this work were prepared by reported procedure[46]. Chemicals used for syntheses were of analytical grade. Spectroscopic grade solvents obtained from Lancester, UK were utilized for spectral studies.

*Caution! Perchorate salts are generally explosive. Although no detonation tendencies have been observed, care is advised and handling of only small quantities recommended.*

Microanalyses (C, H, N) were carried out with Perkin-Elmer 2400 CHN elemental analyzer. Spectroscopic measurements were studied using the following instruments: UV-Vis spectra, Lambda 25 Perkin-Elmer; the IR spectra, Perkin-

Elmer L120-00A FTIR spectrophotometer (KBr disk); $^1$H NMR spectra in $CDCl_3$, Bruker 300 MHz FT-NMR spectrometers in presence of TMS as internal standard.

*Synthesis:*

The following common procedure was used to synthesis all the complexes. Yield varied 60–70%.

*Synthesis of $[Ir(TaiMe)_2Cl_2](ClO_4)$ (**2a**):*

The mixture $IrCl_3.3H_2O$ (150 mg, 0.425 mmol) and 1-alkyl-2-(tolylazo)imidazoles (TaiMe) (175 mg, 0.85 mmol) in methanol was refluxed for 8 h under stirring condition and was allowed to cool to room temperature. Then addition of saturated aqueous solution of $NaClO_4$ afforded a brown precipitate. The product was filtered, washed with methanol and ether, and dried under vacuum. The dried product was dissolved in dichloromethane and was subjected to chromatographed. A brown band was eluted with acetonitrile-toluene (1:3, v/v). The evaporation of the solution afforded analytically pure product **2a**. Yield was 242 mg (61%).

Anal. Calcd. for $[Ir(TaiMe)_2Cl_2](ClO_4)$ (**2a**): C, 34.62; H, 3.15; N, 14.69. Found: C, 34.56; H, 3.19; N, 14.62%. TOF-MS: *m/z* 663.88 $(M\text{-}ClO_4^-)^+$, 628.5 $(M\text{-}ClO_4^-\text{-}Cl)$, 592 $(M\text{-}ClO_4^-\text{-}2Cl)$. IR (KBr disk): $\nu_{N=N}$, 1368 $cm^{-1}$; $\nu_{C=N}$, 1596 $cm^{-1}$; $\nu_{ClO_4^-}$, 1100 $cm^{-1}$; $\nu_{Ir\text{-}Cl}$, 319. UV ($\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$), $CH_3CN$): 500 (4340), 421 (15034), 415 (15007), 272 (9900), 264 (11024).

Anal. Calcd. for $[Ir(TaiEt)_2Cl_2](ClO_4)$ (**2b**): C, 36.43; H, 3.54; N, 14.17. Found: C, 36.52; H, 3.47; N, 14.11%. TOF-MS: *m/z* 690.86 $(M\text{-}ClO_4^-)^+$, 655.27 $(M\text{-}ClO_4^-\text{-}Cl)$. IR (KBr disk): $\nu_{N=N}$, 1361 $cm^{-1}$; $\nu_{C=N}$, 1597 $cm^{-1}$; $\nu_{ClO_4^-}$, 1101 $cm^{-1}$; $\nu_{Ir\text{-}Cl}$, 318. UV ($\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$), $CH_3CN$): 500 (5332), 421 (16368), 416 (16338), 272 (14084), 264 (15736).

Anal. Calcd. for $[Ir(TaiCH_2Ph)_2Cl_2](ClO_4)$ (**2c**): C, 44.61; H, 3.50; N, 12.25. Found: C, 44.55; H, 3.56; N, 12.17%. TOF-MS: *m/z* 816 $(M\text{-}ClO_4^-)^+$, 781 $(M\text{-}ClO_4^-\text{-}Cl)$, 745 $(M\text{-}ClO_4^-\text{-}2Cl)$. IR (KBr disk): $\nu_{N=N}$, 1362 $cm^{-1}$; $\nu_{C=N}$, 1584 $cm^{-1}$; $\nu_{ClO_4^-}$, 1100 $cm^{-1}$; $\nu_{Ir\text{-}Cl}$, 322. UV ($\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$), $CH_3CN$): 503 (6090), 408 (15764), 275 (10442), 267 (12174).

*Computational methods:*

All computations were performed using the Gaussian 09 program package[45]. The Becke's three-parameter hybrid exchange functional and the Lee-Yang-Parr nonlocal correlation functional (B3LYP) was used throughout this computation[48]. Elements except iridium were assigned a 6-31G basis set in our calculations. For iridium the Los Alamos effective core potential plus double zeta (LanL2DZ) basis set were employed[49]. The geometric structures of the ligand and the complexes in the ground state ($S_0$) were fully optimized at the B3LYP level. In all cases, vibrational frequencies were calculated to ensure that optimized geometries represented local minima. Using the respective optimized $S_0$ geometries we employed time dependent density functional theory (TD-DFT) at the B3LYP level to predict their absorptions characteristics[50].

*DNA binding study:*

*Preparation of the complex solutions for DNA binding study:*

The stock solutions of complexes (2 mM) were prerpared in acetone free methanol and diluted with Tris-HCl buffer to get required concentration before each set of experiments.

*Preparation of calf thymus and pUC19 plasmid DNA:*

The solution of calf thymus (CT) DNA (Bangalore Genei, India) was prepared in 5 mM Tris-HCl/50 mM NaCl buffer, pH 7.2 using deionised and sonicated HPLC grade water (Merck). The CT-DNA used in the experiments was sufficiently free from protein (UV absorption ratio $A_{260nm}/A_{280nm}$ ~1.9). The DNA concentration was determined with the help of its extinction coefficient, $\varepsilon$ of 6600 $M^{-1}$ $cm^{-1}$ at 260 nm. The DNA stock solution was stored at 4°C and used within 4 days after preparation.

*Ethidium bromide (EB) stock solution preparation:*

Ethidium bromide (EB) dust (Sigma-Aldrich, USA) was dissolved in double distilled water at a concentration of 1 mM. Stored stocks (at 4°C in dark) were diluted freshly before each experiment.

*Absorption spectroscopic studies of the complexes in presence of CT-DNA:*

Absorption spectroscopic studies were done on a spectrophotometer (Perkin-Elmer, Lambda-25). The interaction between the metal complexes and CT-DNA was observed by adding increasing concentrations of CT-DNA (2 μM to 20 μM) to a fixed concentration of complex (40 μM) and increasing concentrations of complex (2 μM to 20 μM) to fixed concentration of CT-DNA (100 μM). After each addition, the

DNA and complex mixtures were incubated at room temperature for 15 min and scanned from 290 nm to 700 nm. The self-absorption of DNA was eliminated in each set of experiments. Each sample was scanned for a cycle number of 2, cycle time of 5 s at a scan speed of 100 nm/min. Modified Benesi-Hildebrand[51] plot was used for the determination of ground state binding constant between the complexes and CT-DNA. The binding constant "*K*" was determined by using the following relation:

$$A_0/\Delta A = A_0/\Delta A_{max} + (A_0/\Delta A_{max})\times 1/K\times 1/L_t$$

where $\Delta A = A_0 - A$, $\Delta A_{max}$ = maximum change in reduced absorbance,

$A_0$ = maximum absorbance of receptor molecules (without any ligand),

$A$ = reduced absorbances of the receptor molecules (in presence of ligand),

$L_t$ = ligand concentration.

*Fluorescence spectroscopic studies of the complexes with EB bound DNA:*

Fluorescence spectroscopic studies of EB bound CT-DNA with varing concentrations of the complexes (0–100 μM) were done by using by LS 55 Perkin-Elmer spectrofluorimeter at room temperature (298 K). The EB bound CT-DNA was prepared freshly before each experiment by treating with $10^{-5}$ *M* DNA solution with $10^{-5}$ *M* of EB solution and it was incubated for 30 min.

The experiments was carried out with gradual addition of the complexes (10 μM) into EB bound DNA mixture, incubated for 15 min and the fluorescence spectra were taken. The excitation wavelength was 500 nm and the emission spectra were scanned from 510 nm to 750 nm, spectral response of 2 s, along with a scanning speed of 60 nm/min[52].

The study is based on the competitive binding of the complex to DNA by replacing EB from EB bound DNA and this is observed by the quenching of the fluorescence intensity. The fluorescence quenching of EB bound DNA is expressed by the Stern-Volmer equation[53,54]

$$I_0/I = 1 + K_{SV}[Q] = 1 + kq\tau_0[Q]$$

where $I_0$ and $I$ are the fluorescence intensities of BSA in the absence and in the presence of the quencher (i.e. the metal complex), respectively, $K_{SV}$ is the Stern-Volmer quenching constant, [*Q*] is the concentration of the quencher, $kq$ is the quenching rate constant of the biomolecule and $\tau_0$ is the average lifetime of the molecule in the absence of the quencher. A linear $I_0/I$ vs [*Q*] plot indicates that a single type of quenching mechanism is involved, either static or dynamic, while a deviation from linearity suggests a mixed quenching mechanism[55].

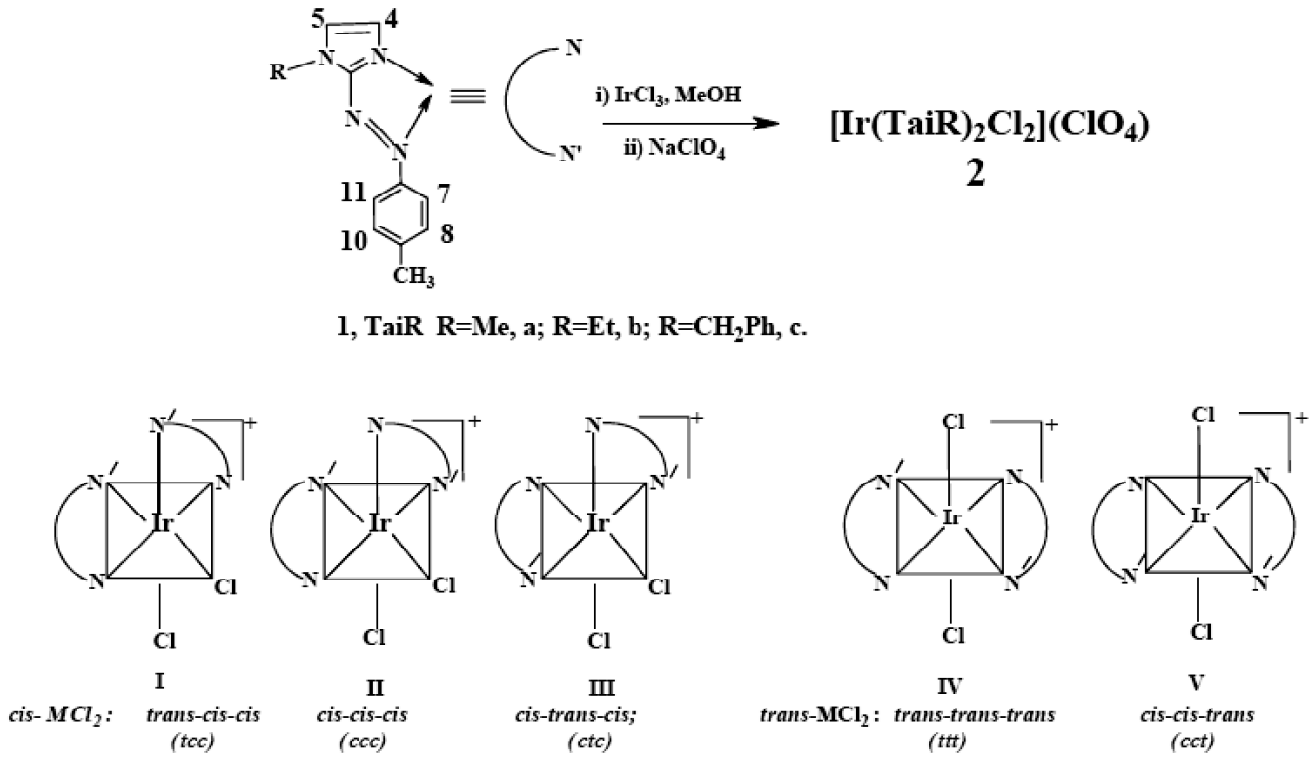
## Results and discussion

*Synthesis and formulation:*

1-Alkyl-2-(tolylazo)imidazoles (TaiR, **1**; R = Me (**a**) (Scheme 1: TaiR, **1**; R = $CH_3$ (**a**), $CH_2$-$CH_3$ (**b**), $CH_2Ph$ (**c**)) are N,N′-bidentate chelator where N and N′ refer to N(imidazole) and N(azo) donor centers respectively. The ligand, TaiR reacts with $IrCl_3.3H_2O$ in 2:1 mole ratio in methanol under refluxing condition to result a red colour solution which upon treatment with a saturated aqueous solution of $NaClO_4$ isolates a brown precipitate of $[Ir(TaiR)_2Cl_2]ClO_4$ (**2**). The composition has been supported by elemental analysis and FAB mass data. The conductance of the complexes in acetonitrile ($\Lambda_M$, 115–125 $\Omega^{-1}$ cm$^2$ mol$^{-1}$) proposes 1:1 electrolyte nature for them. $[Ir(TaiR)_2Cl_2]^+$ can exist in five isomeric forms out of them three are *cis*-$MCl_2$ (I-III, Scheme 1) and two are *trans*-$IrCl_2$ (IV and V, Scheme 1) configuration (Scheme 1). Because of unavailability of good quality crystals of **2** the spectral (UV-Vis, IR and $^1$H NMR, Mass) techniques have been used to characterize the structure of the compounds. The spectral characterization shows that the configuration of the isolated complex **2** is *cis-cis-trans* (*cct*) fashion with respect to N(imidazole), N(azo) and Cl respectively.

*Spectral characterization:*

The FT-IR spectra of the complexes have been studied in KBr disc. The azo ($\nu_{N=N}$) and imine ($\nu_{C=N}$) stretching frequencies of the complexes are significantly shifted to lower frequencies compared to free ligand values and appear at 1361–1368 cm$^{-1}$ and 1584–1597 cm$^{-1}$ respectively[46]. The lowering of these frequencies establishes the coordination of ligand to the metal centre through azo (-N=N-) and imine (-C=N-) nitrogens. The IR spectra of the complexes also show one $\nu_{Ir\text{-}Cl}$ stretch at 318–322 cm$^{-1}$ (Supplementary Material, Fig. S1). This fact implies that two Ir-Cl bonds are similar and proposes the *trans*-$MCl_2$ type configuration for the complexes. The presence of $ClO_4^-$ as a counter ion is observed




**Scheme 1.** The ligands and the complexes.

at 1100 $cm^{-1}$ along with a comparatively weak band at 625 $cm^{-1}$.

The mass fragmentations by ESI-MS (QTOF) of the complexes show expected fragmentation pattern. The spectra of the complexes recorded $(M\text{-}ClO_4^-)^+$ ion peak as base peak at (Supplementary Material, Fig. S2 ) *m/z* 664.88 for **2a**, 690.86 for **2b** and 816.12 for **2c**.

The $^1H$ NMR spectra of the complexes in $CDCl_3$ recorded the downfield shifting of proton signals compared to free ligand data[46] which may be the outcome of the electron withdrawing effect of the coordinated iridium(III) (Table 1). The imidazole proton, 4-H suffers maximum downfield shifting by 0.5–0.6 ppm and appear as a broad singlet at ~7.6–7.8 ppm while 5-H suffers downfield shifting by 0.2–0.3 ppm and appear at 7.26 ppm. These observations support the coordination of imine-N to the metal centre. The $^1H$ NMR spectra also recorded the appearance of every different type of protons of both the coordinated ligands of a complex at a same δ value. For example, the N(1)-$CH_3$ protons of **2a** appear as singlet at 4.06 ppm and N(1)-$CH_2$- protons of **2b** and **2c** appear as quartets at 4.52 ppm and 5.7 ppm respectively (Table 1 and Supplemetary Material, Fig. S3). This fact strongly indicates that the two coordinated ligands of the each complex are equivalent. Therefore, the expected geometry of isolated isomer should be *cis-cis-trans* (*cct*) with respect to N(imidazole), N(azo) and Cl respectively[56–58].

**Table 1.** $^1H$ NMR spectral data of Ir(III)-TaiR complexes in $CDCl_3$ at 300 K

| Compd. | δ (ppm) (*J* (Hz)) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4-H[a] | 5-H[a] | 7, 11-H[b] | 8,10-H[b] | 9-R[c] | 1- $CH_3$[c] | 1-$CH_2$ | (1-$CH_2$)$CH_3$[d] | Ph-H |
| Ir-TaiMe | 7.59 | 7.26 | 7.83(7.2) | 7.34(7.0) | 2.43 | 4.06 | | | |
| Ir-TaiEt | 7.63 | 7.26 | 7.89(7.2) | 7.36(7.2) | 2.47 | 4.52[e](7.5) | 1.53(7.0) | | |
| Ir-TaiBz | 7.75 | 7.26 | 7.91(7.4) | 7.39(7.2) | 2.51 | | 5.7[c] | | 7.26–7.61 |

[a]Broad singlet, [b]doublet [c]singlet, [d]triplet, [e]quartet.

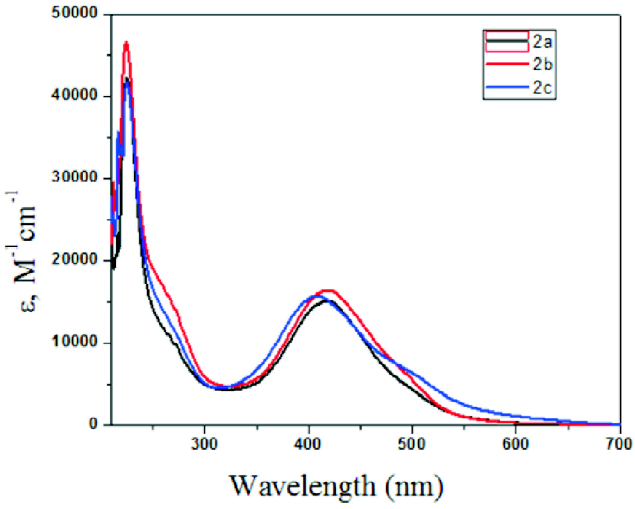



**Fig. 1.** UV-Visible spectra of the complexes (**2a, 2b and 2c**) in acetonitrile.

The absorption spectra of the complexes in acetonitrile solution (Fig. 1) shows one high intense transitions (ε ~$10^4$ $M^{-1}$ $cm^{-1}$) at ~420 nm along with a weak at 500 nm (ε ~$10^3$ $M^{-1}$ $cm^{-1}$) in the visible region and two moderately intense transitions (ε ~103 $M^{-1}$ $cm^{-1}$) within 265–275 nm in the UV region. The high energy transitions (< 400 nm) region may be assigned ligand centred transitions and those in the visible region (> 400 nm) may be regarded as metal-to-ligand charge transfer transitions as these are not present in free ligands. These assignments of absorption bands are further discussed by TDDFT calculations.

*DFT calculation and electronic structure:*

As we have failed to determine the single crystal structure, a theoretical structure of **2b** has been optimized in gas phase to get information about the structural parameters. The optimized structure is shown in Fig. 2 and the figure showing that iridium(III) is surrounded by two Cl (*trans* to each other) and four N (two azo-N and two imine-N from two coordinated ligands) forming a distorted-octahedral coordination

**Table 2.** Selected bond lengths (Å) and angles (°) for the $[Ir(TaiEt)_2Cl_2]ClO_4$ (**2b**) (from DFT calculation)

| Bond length (Å) | Theoretical value | Bond angle (°) | Theoretical value |
|---|---|---|---|
| Ir(55)-N(1) | 2.049 | N(1)-Ir(55)-N(9) | 76.10083 |
| Ir(55)-N(16) | 2.049 | N(16)-Ir(55)-N(27) | 76.10097 |
| Ir(55)-N(9) | 2.099 | N(1)-Ir(55)-N(27) | 176.20552 |
| Ir(55)-N(27) | 2.099 | N(16)-Ir(65)-N(9) | 176.20463 |
| Ir(55)-Cl(22) | 2.421 | N(1)-Ir(55)-N(16) | 101.75566 |
| Ir(55)-Cl(23) | 2.421 | N(16)-Ir(65)-N(9) | 106.21215 |
| N(3)-N(9) | 1.283 | Cl(22)-Ir(55)-Cl(23) | 176.84603 |
| N(26)-N(27) | 1.283 | | |

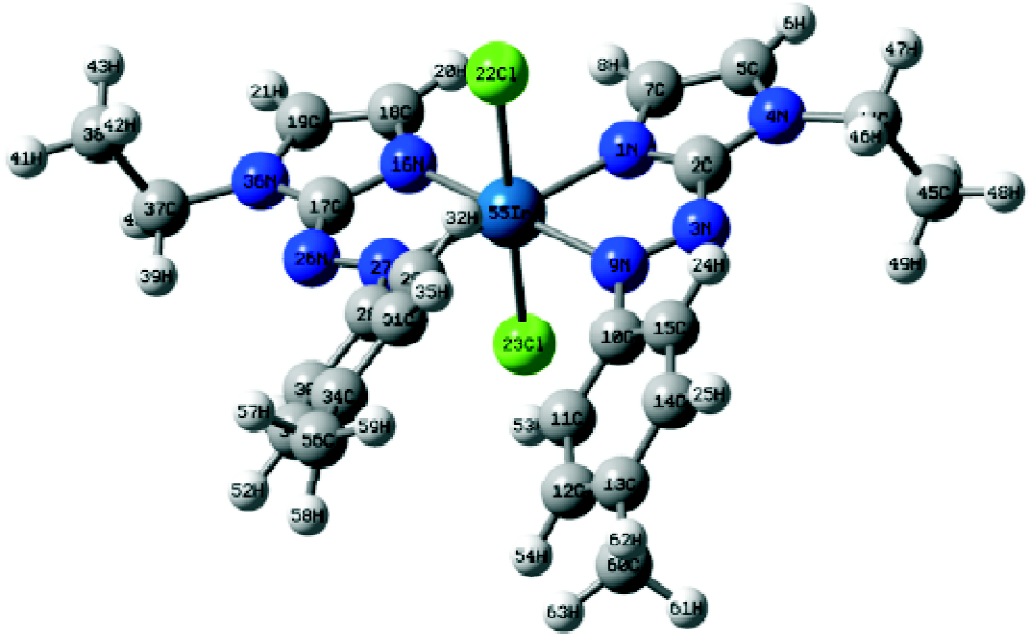



**Fig. 2.** Optimised structure of $[Ir(TaiEt)_2Cl_2]ClO_4$ (**2b**).

environment. The relevant bond parameters are given in Table 2. These theoretical bond parameters show good agreement with the reported values[44,59].

The energy and the composition of some selected molecular orbitals are given in the Supplementary Material, Table S1 and surface plots of some frontier orbitals are shown in Fig. 3.

The energies of HOMO and LUMO are –8.65 eV and –6.07 eV respectively. The highest occupied molecular orbital, HOMO is composed of 41% ligand, 31% Cl and 28% metal. The occupied frontier orbitals H-1, H-3, H-4 and H-5 are mainly constituted (65–95%) with ligand pπ orbitals while in case of H-2, 68% contribution come from Cl and 25% contribution metal d-orbitals. The LUMO and L+1 are mostly delocalized on ligand π* orbitals (95%, and 91% respectively). The LUMO+2 is constituted by metal (Ir, 50% ) and Cl (39%) while LUMO+3 is carrying 52% metal and 39% Cl contribution.

To gain an insight about the nature of electronic transitions and to explain the electronic spectra, the TD-DFT calculations were performed. The experimental spectrum of **2a** correlates well with the theoretical spectrum (Supplementary Material, Fig. S4). The experimental spectrum shows a transition at 501 nm which appears at 510 in theoretical spectrum is mainly due to the transitions HOMO-1→LUMO and can be regarded as admixture of intraligand charge transfer

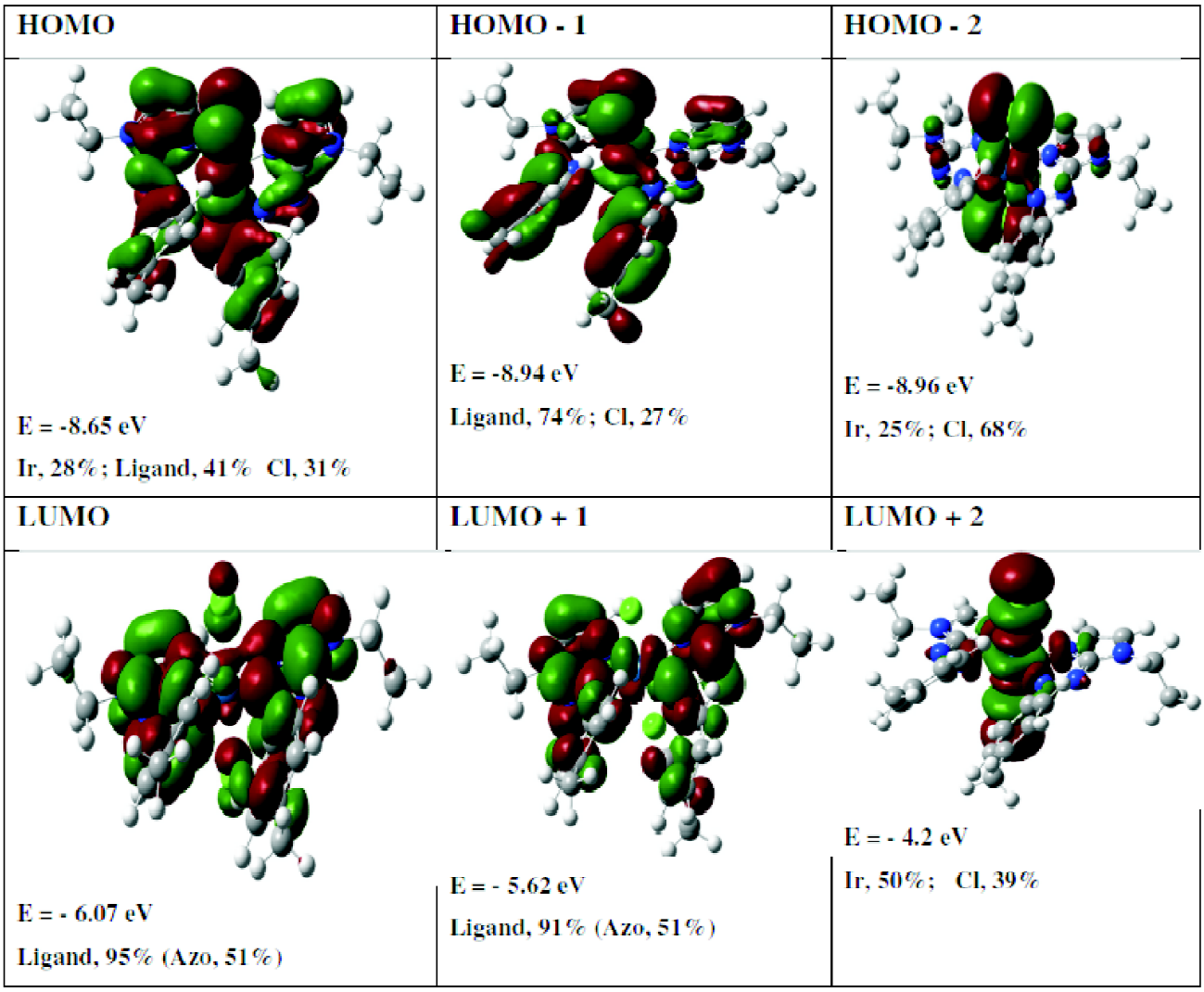



**Fig. 3.** Surface plots of some frontier orbitals of $[Ir(TaiEt)_2Cl_2]ClO_4$ (**2b**).

transition, ILCT [Im, Ph→Azo] and chloride-to-ligand, (XLCT: Cl →Azo, Im) and metal-to ligand (MLCT: Ir→Azo) charge transfer transitions (Supplementary Material, Table S2). The transition in the experimental spectrum of **2b** at ~420 nm is result of the transitions HOMO-3, HOMO-6→LUMO+1 and is assigned to the transition mainly originated from ILCT [Im, Ph→Azo]. The other transitions at shorter wavelengths are mixture of ILCT [Im, Ph→Azo], XLCT [Cl →Azo, Im], MLCT [Ir→Azo] transitions.

*Interaction of complexes with DNA:*

*Absorption spectroscopic studies of the complexes in presence of CT-DNA:*

The study of interaction of the complexes with CT-DNA was observed by recording the changes in UV-Vis absorption spectra. Any change in absorption spectra due to the mixing of DNA and complexes is the one of indication of interaction between them[60]. Upon addition of increasing concentrations of complexes to 100 μM CT-DNA (100 μM), the absorption of DNA at 260 nm was decreasing successively and while the addition of increasing concentrations of CT-DNA to fixed concentration of complexes recorded a steady decrease in absorption with a slight red shift (Fig. 4 and Supplementary Material, Figs. S5-6). Such changes in absorbance indicate the specific interaction between the DNA and the complex molecules. In order to compare the binding ability of the complexes, we have calculated the ground state binding constant ($K_b$) between the complexes and DNA at the absorption maximum of DNA by using modified Benesi-Hildebrand (BH) plot (Fig. 4 and Supplementary Material, Figs. S5-6). The calculated binding constants are $7.8\times10^4$ $M^{-1}$ (**2a**), $5.296\times10^4$ (**2b**), and $3.44\times10^5$ (**2c**). These observed values reveal a decreasing tendency of the binding constant with the increasing size of the complexes which may be due to the better interchalating ability of the smaller positive ions into the adjacent base pairs of DNA. Thus the complex **2a** binds strongly with CT-DNA than the other complexes.

*Fluorescence spectroscopic studies of the complexes with ethidium bromide (EB) bound DNA:*

To further verify the interaction of the complexes with DNA, we studied the ability of the complexes to displace ethidium bromide (EB) from EB bound DNA by fluorescence quenching method. Either DNA or EB do not have fluorescence property alone but being a fluorescence probe, EB emits intense fluorescent light in presence of DNA due to its strong intercalation between adjacent base pairs. The studies showed that the gradual addition of complexes to EB bound DNA solution caused quenching in fluorescence intensity. The fluorescence quenching of EB bound DNA upon gradual addition of complexes follow the classic linear Stern-Volmer equation (Fig. 5 and Supplementary Material, Figs. S7-8). The Stern-Volmer quenching constants ($K_{sv}$) obtained from the slope of the plot [*Q*] vs $I_0/I$ are $1.2935\times10^4$ $M^{-1}$ (**2a**), $1.1\times10^4$ $M^{-1}$ (**2b**) and $4.086\times10^3$ $M^{-1}$ (**2c**). These values indicate the strong interaction of the complexes with the DNA[60,61]. The

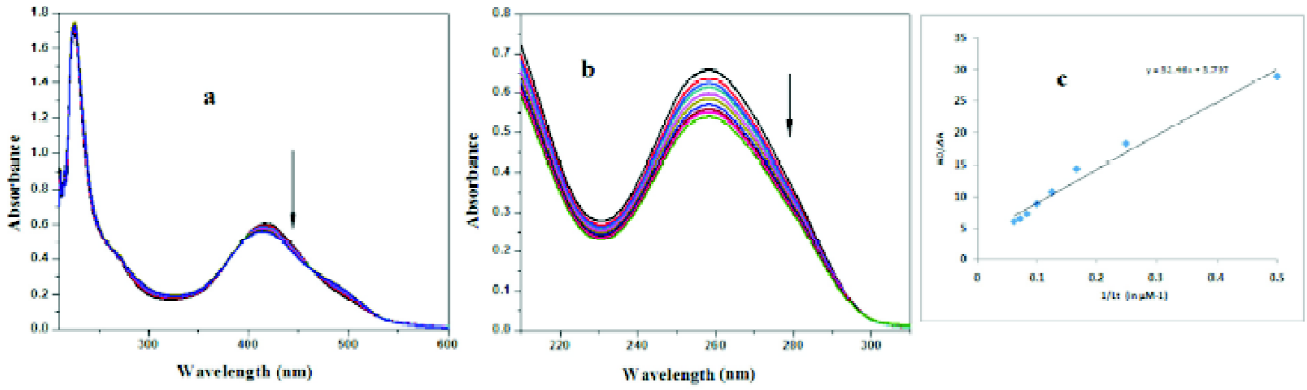



**Fig. 4.** (a) Absorption spectroscopic study of 40 μM complex **2a** with increasing concentrations of CT-DNA (0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μM) respectively; (b) absorption spectroscopic study of CT-DNA (100 μM) with increasing concentrations of complex **2a** (0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μM) respectively; (c) modified Benesi-Hildebrand plot for the determination of ground state binding constant between CT-DNA and rhodium complex **2a**.

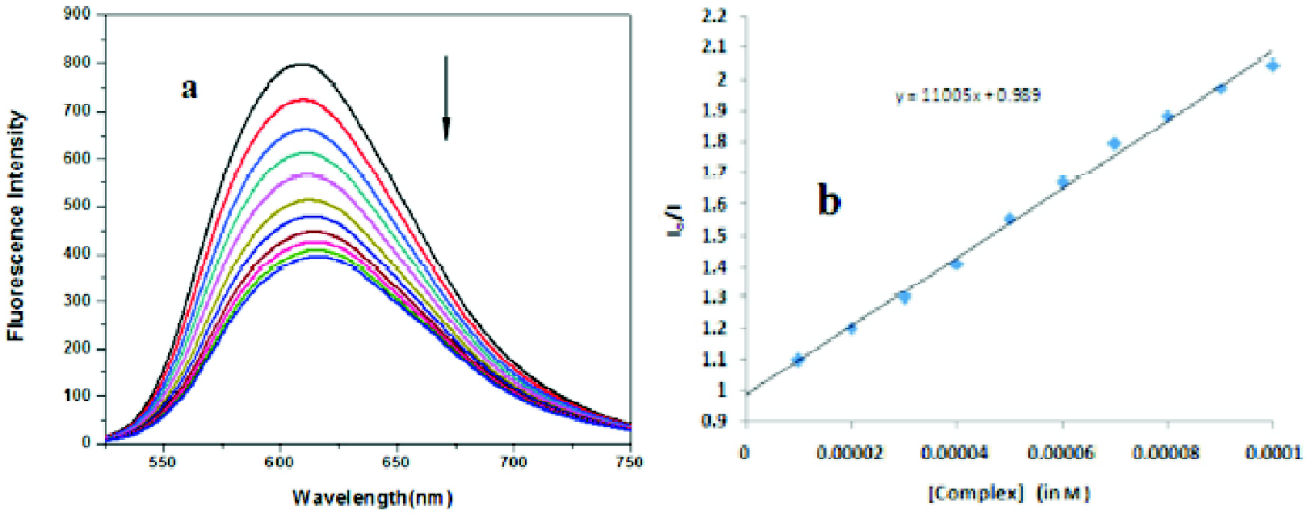



**Fig. 5.** (a) Fluorescence spectroscopic study of EB bound DNA with increasing concentrations of complex **2b** (0, 10, 20, 30 40, 50, 60, 70, 80, 90 and 100 μM) respectively; (b) Stern-Volmer plot for complex **2b**.

apparent binding constant ($K_{app}$) was also calculated from the equation $K_{EB}$[EB] = $K_{app}$[complex], where $K_{EB}$ = 1.0×10$^7$ M$^{-1}$, [EB] = 50 μM; and [complex] is the concentration that causes a 50% quenching of the initial EB fluorescence[62]. The $K_{app}$ values of the complexes are 6.4035×10$^5$ M$^{-1}$ (**2a**), 5.4426×10$^5$ M$^{-1}$ (**2b**) and 2.11×10$^5$ M$^{-1}$ (**2c**) which also suggest the strong interaction between DNA and complex molecules[60–62].

## Conclusion

The Ir(III) complexes of 1-alkyl-2-(tolylazo)imidazoles have been synthesized and characterized by elemental and spectroscopic techniques. The spectral characterization suggest octahedral geometry for the Ir(III) complexes with *cis-cis-trans* (*cct*) configuration respect to N(imidazole), N(azo) and Cl respectively. Density functional theory (DFT) study interprets the electronic structures and their spectral properties. The DNA binding study by absorption and fluorescence spectroscopic methods show the DNA binding ability of the complexes. The complex **2a** binds most strongly while the least binding ability was observed in case of complex **2c**.

## Supplemetary material

Supplemetary Material includes the energy and composition of the selected frontier molecular orbitals calculated by DFT, electronic transition data from TD-DFT calculation, $^1$H NMR and IR spectra, and some figures regarding DNA binding study.

## Acknowledgements

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