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Theranostic Agent Study for Cancer Treatment by the TDDFT Method: Case of Some Ruthenium Azopyridine Complexes

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Abstract: The interest of ruthenium azopyridine complexes lies in the diversity of their properties. The use of these complexes in this work is part of the dynamics to fight against cancer. The main objective is to show by Density Functional Theory (DFT) method the possibility of using these complexes in the diagnosis and treatment of cancers. Optimization, frequency calculation and properties of the β and δ isomers of these azopyridine complexes were determined using DFT and TDDFT methods at the B3LYP/Lanl2DZ level. The results of this analysis show on the one hand that the most cytotoxic isomers by mode of intercalation between DNA base pairs are the δ-$RuCl_2$ $(Azpy)_2$ and δ-$RuCl_2$ $(Nazpy)_2$. The free enthalpy of reaction values indicate that the substitution of the phenyl group by the naphthol group changes the stability of these azopyridine complexes. In terms of reactivity, it can be said that the substitution decreases the nucleophilicity and increases the electrophilicity of these ruthenium azopyridine complexes. The Nazpy isomers are the most soluble in organic solvents. On the other hand, Nazpy isomers were discovered the best complexes suitable for diagnostic and deep penetration treatments. Furthermore, the substitution of the phenyl group by the naphthol group improves the cytotoxicity and fluorescence properties of these complexes. Therefore, for the subsequent work, we would like to extend this study to the elucidation of the mechanism of photodynamic therapy regarding these Nazpy ruthenium complexes.

Keywords: Azopyridine, Cancer, TDDFT, Theranostic

1. Introduction

Theranostics is a neologism that is used in the medical field to designate chemical systems that combine both the therapeutic and diagnostic aspects. It combines therapy and diagnosis in a single agent in order to establish more specific and individualized therapies for various pathologies [1]. This combination leads to a promising therapeutic synergy involving diagnosis, drug delivery and monitoring of treatment response [1]. This precision therapy allows for efficient and cost-effective clinical management for individuals with reduced time and cost. Significant advances in chemistry, biology, biotechnology, medicine and imaging technologies are contributing significantly to the progress of theranostics [2]. The unprecedented success of theranostics in nuclear oncology in the 1940s was due to the beneficial effects of these image-guided therapies for patients with radioiodine-avid thyroid cancer [3, 4]. In addition, the encouraging results of recently published major trials for the medical imaging and treatment of neuroendocrine neoplasms (NEN), which targets the somatostatin receptor (SSTR) [3, 4] or reports of beneficial effects of this concept for the treatment of prostate cancer (PC), as evidenced by the prospective LuPSMA or TheraP trials, have made this therapeutic option attractive in various clinical scenarios in oncology [3, 5, 6]. Theranostic

nanoparticles generally used emit both β-particles for the therapeutic aspect and γ-rays for the diagnostic aspect [1]. This is a classic example of a theranostic agent. In the treatment of thyroid cancer, these particles primarily target the thyroid cells, so that the radiation can destroy the thyroid glands and any other thyroid cells (including cancer cells) that absorb iodine with little effect on the rest of the body [1]. These theranostic nanoparticles offer an exciting new way for diagnostic potential, as traditional imaging strategies have limitations, such as being able to identify tumours smaller than 0.5 cm and being able to distinguish benign from malignant tumours non-invasively [7]. Thus, an ideal theranostic NP for cancer must have several properties such as rapid and selective accumulation in the tumour target, the ability to report biochemical and morphological characteristics of the cancer, the efficient release of an amount of drug(s) on demand without damaging healthy tissue, and the ability of the NP to be eliminated from the body within a few hours or its biodegradation into non-toxic by-products [7]. Theranostic nanoparticles can have organic or inorganic properties. Various types of particles or agents can be exploited for theranostic purposes. These include radioisotopes, genetic materials (DNA sequences, RNA and proteins), quantum dots, dendrimers, liposomes, magnetic nanoparticles, fullerenes and nanotubes, gold nanoparticles, microbubbles, porous nanoparticles, micelles, conjugated polymer-based drugs, antibodies [2]. Although a large number of theranostic nanoparticles have been developed with the aim of achieving dual theranostic functionality, some limitations remain [2]. In this work, we propose to identify a new family of theranostic particles based on azopyridine organometallic complexes of ruthenium. Metal complexes offer therapeutic and luminescent advantages, with various synthetic structures, as well as easily tunable oxidation states, allowing modulation of pharmacokinetic properties without impairing the therapeutic effect [8, 1, 9]. Drugs based on these compounds have shown high efficacy against solid tumour metastasis both in experimental tumours and in human tumours transplanted into nude mice [10, 11]. In contrast to organic compounds, inorganic complexes can be deployed as a stable inactive prodrug in the system, until activated in situ by dissociation of labile ligands or by an external stimulus such as light [12]. Transition metals offer long fluorescent lifetimes, photostability, and long Stokes shifts, allowing them to compete with autofluorescence, resist irradiation for long periods, and avoid self-extinction [12, 13]. The photophysical properties of polypyridyl ruthenium (II) complexes make them good candidates for luminescence probes. Recent studies have shown that the luminescence of these complexes is enhanced under hypoxic conditions. A class of ruthenium complexes is the subject of intense research. These are azopyridine complexes. Azopyridines are organic compounds consisting of a pyridine group and an aromatic ring, linked together by an azo N=N bond. The electron-rich azo group (-N=N-) gives the azopyridine ligand a certain rigidity. The ruthenium (II) arylazopyridine complexes, $RuCl_2(Azpy)_2$ (where Azpy = 2-phenylazopyridine), represent a well-characterised class of anti-cancer compounds. There are five different isomers of these complexes, three of which have an unequivocal structure established by X-ray crystallography. Their activity has a strong structural dependence [14]. The structural characteristics of these compounds have a significant impact on the efficacy of cytotoxic compounds. This is why we want to evaluate, using theoretical tools, the effect of substitution of the phenyl group of the Azpy ligand by a naphthol group on the therapeutic and fluorescence properties of these ruthenium azopyridine complexes $RuCl_2(Azpy)_2$ [15]. One of the particularities of this naphthol group lies in its structure. This group has two benzene rings bonded that allow for extended conjugation and an auxochrome group (the OH group) that is crucial for a fluorophore. Figure 1 shows the structure of the two ligands Azpy and Nazpy.

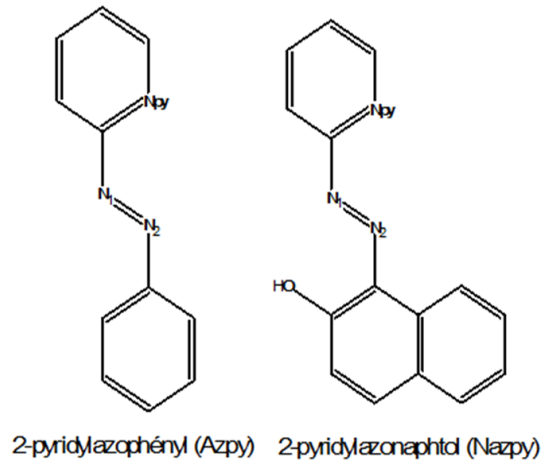

Figure 1. *Structures of azopyridine ligands.*

Five types of isomers are likely to be formed with each type of ligand. This work will focus on two of them, namely β-$RuCl_2L_2$ or (β-Cl) and δ-$RuCl_2L_2$ (δ-Cl) with L= Azpy or Nazpy. Figure 2 shows these two isomers.

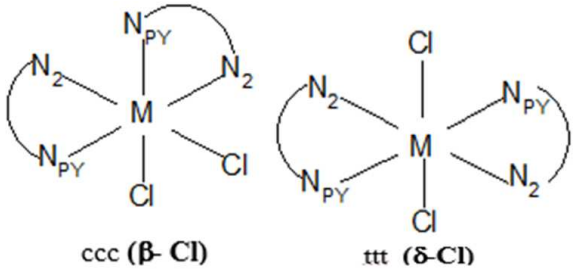

Figure 2. *Structures of two isomers.*

The aim of the structural modification of these complexes is to provide improved therapeutic and fluorescence properties. We will focus on these properties in order to highlight the theranostic characteristics of these ruthenium azopyridine complexes.

2. Calculation Methods

2.1. DFT Calculations

The optimization of the molecules was performed in vacuum using the software GAUSSIAN 09 Rev. A.02 [16] with its graphical interface Gauss View 5.0.8. The minimum energy structure was performed using density functional theory (DFT). All DFT calculations were performed using Becke's 3-parameter hybrid function B3LYP [17-20] and the basis set containing the double zeta pseudopotential Lanl2DZ [21, 22]. Today, the DFT method is used to clarify many chemical phenomena in a wide range of fields including energy production (photovoltaics, fuel cells, nuclear), geochemistry (minerals, earth core) and biology (enzymes, proteins, DNA). In this last field, for example, the study of enzymatic catalysis has made it possible to rationalize the experimental data and to elucidate the reaction mechanisms of many enzymes [16]. The emergence of DFT has led to a dramatic change in the use of computational chemistry techniques, and it is now possible to perform routine calculations on desktop computers in a matter of days. Compared to ab initio methods, DFT uses electron density-based methods rather than wave function-based methods, and produces accurate results in short periods. Later, time-dependent density (TD-DFT) was developed [23] (Runge and Gross, 1984) as an extension of DFT to describe the behavior of molecules in excited states. These methods have several advantages due to their simplicity and system independence. The geometric optimizations were performed at the ground states of these complexes and they were assumed to be in a singlet state [24]. Furthermore, the stable configuration of the isomers was confirmed by frequency analysis in which no imaginary data were observed for all minimum energy configurations. Therefore, the DFT methods with the B3LYP and Lanl2dz basis set are assumed so far to be consistent with the experimental data performed on the ruthenium complexes. Several quantities have been evaluated. The energy of the boundary molecular orbitals (HOMO and LUMO) has been analyzed to predict the overall reactivity of these ruthenium complexes. Analysis of the population of natural NPA orbitals was also performed to predict the affinity of these complexes to DNA molecules. The standard free enthalpy was calculated to predict the spontaneity and stability of these complexes. The determination of the standard free enthalpy of formation was performed according to the following reaction.

$$(RuCl_3, 3H_2O) + 2L \rightarrow RuCl_2L_2 + 1/2Cl_2 + 3H_2O \text{ où } L= \text{Azpy ou Nazpy.}$$

The thermodynamic quantities were calculated from the formation quantities according to the following equation:

$$\Delta_f \Phi^0(298K) = \sum_{produits} \Phi^0(298K) - \sum_{réactifs} \Phi^0(298K)$$

2.2. TDDFT Calculations

The absorption spectra of the complexes were obtained with the TDDFT method by calculating the roots of the first 20 singlets and triplets in vacuum. TDDFT calculations in vacuum are currently known to be accurate to about 0.2 eV (5 kcal/mol) [25]. These calculations were performed on the optimized geometries of the complex in vacuum. The B3LYP function was chosen for the TDDFT calculations for its ability to quantitatively reproduce the experimental results [25]. The energy calculations were performed in a single point. The excited state corresponds to a temporary electron shift from the fundamental layer to a higher level after absorption of a photon by the atom or molecule.

To determine the ability of the $RuCl_2$ $(Azpy)_2$ and $RuCl_2$ $(Nazpy)_2$ complexes to be engaged in a fluorescence process, we evaluated several parameters. First, the absorption wavelength λ_{Abs} that determines the absorption band of the molecule. Second, the stokes shift (Ss) [26] which assesses the ability of the molecule to distinguish between absorption and emission light. The detection of the fluorescence signal depends on the value of the stokes shift. The larger it is, the better the detection of the fluorescence signal is. Furthermore, the stokes shift is defined as the difference between the maximum emission and absorption wavelengths according to the following formula: $S_s = \lambda_{max}(émission) - \lambda_{max}(absorption)$. Third, we determined the transition lifetime (τ) [27]. A short excited state lifetime is favorable for the fluorescence phenomenon while a long lifetime could generate competition between fluorescence and other photophysical processes. The lifetime of the excited state is measured according to the following formula [28]: $\tau = \frac{1,499}{f\sigma^2}$ With f and σ representing the oscillation strength of the transition and the wavenumber in cm^{-1} respectively. Fourth, the attachment energy of an exciton E_b [26] which is obtained by making the difference between the energy gap and the optical gap. An exciton is a pair comprising an electron and a hole linked by a coulombic (electrostatic) force and located in the same region of space (overlapping orbitals). The exciton is formed when an electron moves from the HOMO band to the LUMO band and remains bound to the hole it left behind. It is a neutral quasi-particle that is treated as a hydrogen system and determines several optical and optoelectronic properties of materials [29]. The attachment energy of an exciton E_b is also defined as the energy difference between the energy gap E_{L-H} and the fluorescence energy E_{flu}. According to the following relationship:

$E_b = \Delta E_{L-H} - E_{Flu}$. The fluorescence energy is determined according to the formula: $E_{Flu} = \frac{1240}{\lambda_{max}(nm)}$. Fifth, the extinction coefficient which is proportional to the intensity of the emitted light. In the absence of phenomena that can compete with the fluorescence process, the higher the fluorescence intensity will induce a great molar absorption coefficient for a given incident light intensity [26]. Sixth, the light harvesting efficiency LHE = $1 - 10^{-A} = 1 - 10^{-f}$ [30] Where A (or f) is the oscillatory force of the electronic transition of greatest absorbance.

3. Results

3.1 Study of the Reactivity of $RuCl_2L_2$ Complexes at the Ground State with DNA or RNA, L= Azpy or Nazpy

3.1.1. Global Descriptors of the Reactivity of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy at Ground State

Table 1 shows the energies of the boundary orbitals such as the HOMO (Highest occupied molecular orbital) and the LUMO (Lowest unoccupied molecular orbital), the energy gaps and the free enthalpies of reaction of $RuCl_2L_2$ complexes with L= Azpy or Nazpy in the ground state in vacuum. These parameters are global indicators of the reactivity of these molecules.

Table 1. *Global descriptors of the reactivity of $RuCl_2L_2$ complexes with L= Azpy or Nazpy ruthenium in the ground state (in Kcal.mol^{-1}).*

Isomers	E_{HOMO}	E_{LUMO}	ΔE_{L-H}	$\Delta G°$
β -$RuCl_2(Azpy)_2$	-5,53	-3,22	2,31	-13,33
δ-$RuCl_2(Azpy)_2$	-5,23	-3,43	1,80	-9,64
β-$RuCl_2(Nazpy)_2$	-5,61	-3,35	2,26	-35,98
δ-$RuCl_2(Nazpy)_2$	-5,90	-3,95	1,95	-45,49

The values of ΔG° are all negative for all isomers. It can be said that the reaction to form these isomers is spontaneous. This spontaneity is much more accentuated with the substitution of the phenyl group by the naphthol group. Furthermore, the ΔG° values also indicate the relative stability of these complexes. The ΔG° values of the Azpy isomers are -13.33 and -9.64 for the β and δ complexes respectively while their Nazpy analogues have ΔG° values that are -34.98 and -45.49 respectively. In general, the following order of free enthalpy of reaction can be established: ΔG° (β-Cl) < ΔG° (δ-Cl) for Azpy complexes. This ranking indicates that the β-$RuCl_2$ $(Azpy)_2$ complex is the most stable with an estimated ΔG° (β-Cl) value of -13.33. This ranking undergoes a change with the substitution of the phenyl group with the naphthol group. Thus, the order of stability of the Nazpy complexes is as follows: ΔG° (δ-Cl) < ΔG° (β-Cl). This ranking shows that the δ-$RuCl_2(Nazpy)_2$ complex is the most stable complex of the Nazpy isomers with a value of ΔG° (δ-Cl) equal to -45.49 Kcal.mol^{-1}. Thus, it can be concluded that the substitution of the phenyl group by the naphthol group changes the stability of these azopyridine complexes. The most stable isomer of all these complexes is δ-$RuCl_2$ $(Nazpy)_2$. As for the energy gaps, they indicate the overall reactivity of these molecules in the presence of other substrates. The molecules with the lowest energy gap values are the most reactive. Thus, the most chemically stable isomers are the δ isomers for these ruthenium complexes. These isomers are also the most kinetically stable. Furthermore, the electronic interaction of these complexes with other molecules will be of two forms: either these complexes will act as nucleophiles or they will act as electrophiles. In the case of a nucleophilic reaction, the reactivity of these complexes will be assessed from the HOMO energies of these complexes and the most active will be the one with the highest HOMO energy. In the case of an electrophilic reaction, the reactivity of these complexes will be evaluated from the energies of the LUMO orbitals of these complexes and the most active will be the one with the lowest LUMO energy. In the context of interactions of these complexes with DNA, these complexes can behave as electrophiles. Indeed, Bamba et al [31] have shown that the most preferred association for these ruthenium complexes is the intercalation mode. During this association, the complexes will be able to intercalate between the DNA bases allowing the ligands to accept electrons or donate electrons. However, the most likely interaction is the former, i.e. the ligands of these complexes will be able to receive electrons from the DNA. The elucidation of this assertion is done by comparing the energies of the HOMO orbitals of the complexes and the LUMO orbital of DNA on the one hand and the energies of the LUMOs of the complexes and the HOMO orbital of DNA on the other. The energies of the boundary orbitals of the stacked CG/CG base pairs of the DNA macromolecule were predicted by Kurita and Kobayashi [32]. They showed that the HOMO energy of the GC/CG base pair is -1.27 eV and that of the LUMO orbital is 1.14 eV. Regarding Azpy and Nazpy complexes we notice:

The energies of the HOMO orbitals of the Azpy complexes are -5.53 eV and -5.23 eV respectively for the β and δ Azpy isomers. However, the HOMO energies of the Nazpy complexes are -5.61 eV and -5.90 eV respectively for the β and δ Nazpy isomers. Furthermore, the energies of the LUMO orbitals of the Azpy complexes are -3.22 eV and -3.43 eV respectively for the β and δ Azpy isomers while those of the Nazpy complexes are -3.35eV and -3.95 eV respectively for the β and δ Nazpy isomers.

Considering, on the one hand, the values of the energies of the HOMO and LUMO boundary orbitals of these DNA base pairs and, on the other hand, the energies of the HOMO and LUMO orbitals of the azopyridine complexes, we can define the following overlap energies according to each approach. The first approach, i.e. an overlap between the HOMO orbitals of the complexes and the LUMO orbital of the DNA, the energies required are 6.67eV and 6.37eV for the β and δ Azpy complexes and between 6.75eV and 7.04eV for Nazpy complexes. Considering the second approach, i.e. an overlap between the LUMO orbitals of the complexes and the HOMO orbital of the DNA, the energies required are -1.95 eV and -2.16 eV for the Azpy complexes and -2.35 eV and -2.68 eV for the Nazpy complexes.

From the above statement, the most favorable recovery is that of the second approach [31]. LUMO electronic gap of these GC/CG base pairs equal to 2.41 eV resulting that the complexes will be electron acceptors. Consequently, the ligands would play a key role in the interactions between these complexes and DNA. That is, the complex with the lowest LUMO energy will be able to bind easily to DNA [33]. Thus the order of the energies of the LUMO orbitals of Azpy complexes is as follows: E_{LUMO} (β-Cl)> E_{LUMO} (δ-Cl). This ranking indicates that the δ-$RuCl_2(Azpy)_2$ complex has a high electronic affinity to bind to the DNA. This ranking is modified by the substitution of the phenyl group with the naphthol group in these complexes. It should also be noted

that the δ-$RuCl_2(Nazpy)_2$ complex is the most active complex. The order obtained with the substitution is as follows: E_{LUMO} (β-Cl)> E_{LUMO} (δ-Cl). Similarly the least active isomer for both sets of complexes is the β-isomer. We can assume that the low reactivity of this isomer is due to steric hindrances within this isomer. Thus, in a nucleophilic reaction, Azpy complexes are the most active. However, in an electrophilic reaction, Nazpy complexes are the most active. The δ-isomer of Azpy complexes is the most nucleophilic and the δ-isomer of Nazpy complexes is the most electrophilic. From these values we can say that substitution decreases the nucleophilicity and increases the electrophilicity of these ruthenium azopyridine complexes.

3.1.2. Dipole Moments of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy at the Ground State

The measure of the ability of a molecule in chemistry to interact or not with water molecules (hydrophobicity) is determined by the value of log P. Indeed, the value of Log P expresses the solubility of the compound in an organic solvent or in water. This notion of hydrophobicity can be evaluated by the dipole moment. In fact, the dipole moment indicates the strength of solubility of a molecule in water. Consequently, a high value of this dipole moment implies low solubility in an organic solvent and high solubility in water. In fact, the most effective drugs are fat-soluble because many anti-metastatic drugs carry out their activity in organic solvents [34]. Table 2 highlights the solubility of the isomers of the $RuCl_2L_2$ complex by comparing their calculated dipole moment.

Table 2. *Dipole moments of $RuCl_2L_2$ complexes with L= Azpy or Nazpy in Debye at ground state.*

	μ			
	X	**y**	**Z**	**Total**
β- $RuCl_2(Azpy)_2$	-1,74	0,97	8,60	8,83
δ- $RuCl_2(Azpy)_2$	0,00	0,00	-1,33	1,33
β-$RuCl_2(Nazpy)_2$	-7,70	3,51	2,30	8,77
δ-$RuCl_2(Nazpy)_2$	0,00	0,00	0,00	0,00

We can observe in Table 2 the following order: μ (β-Cl)> μ (δ-Cl) for both types of complexes. We can say that the substitution of the ligands does not affect the solubility order of these complexes. This ranking also indicates that the δ-Cl isomer here represents the most active compound with the largest Log P value. It has a dipole moment equal to 1.33 D in the Azpy isomers and 0 D in the Nazpy isomers. This reflects its high solubility in organic solvents. Consequently, it will be able to display the highest value of logP.

3.1.3. Structure-Activity Relationships (SAR) of Azpy and Nazpy Complexes

A SAR study on the series of these complexes will consist on looking for a relationship between the electronic properties of the ruthenium complexes and their cytotoxic activities. Velders et al. [35] have already experimentally tested in vitro three α-Cl, β-Cl and γ-Cl isomers of $RuCl_2(Azpy)_2$ on seven types of human cancers which are: breast cancer (MCF-7, EVSA-T), colon cancer (WIDR), ovarian cancer (IGROV), melanoma cancers (M19-MEL), renal cancer (A498) and tongue cancer (H226). The results were compared to the activities of Cisplatin (CPT) and 5-fluorouracil (5-FU), which were considered the most active compounds ever discovered. As a result of this comparison, they established the following order of activity for these three complexes: A (α-Cl)> A (β-Cl)> A (γ-Cl). Since the most important mode of DNA binding remains the intercalation mode where the drugs are supposed to insert and stack between the base pairs of DNA strands [36], it becomes necessary to consider this mode of association and then compare the activity of all isomers of $RuCl_2(Azpy)_2$ or $RuCl_2(Nazpy)_2$. Therefore, the parameters affecting this mode of association of these complexes with DNA should be in this case the flatness of the ligand structure, the hydrophobicity factor; the charge of the azopyridine ligand; the energy and population of the lowest unoccupied molecular orbital (LUMO) of the complex [37, 31]. Table 3 summarizes the abovementioned parameters.

Table 3. *Summary of the parameters affecting the mode of association of the complexes with DNA: dipole moment, the charge of the azopyridine ligand, the energy of the lowest unoccupied molecular orbital (LUMO) of the azopyridine complex in the vacuum at B3LYP/ Lanl2DZ level.*

	Q_{ligand}	E_{LUMO}	μ	**Geometry molecular**
β- $RuCl_2(Azpy)_2$	0,71	-3,22	8,83	ccc
δ- $RuCl_2(Azpy)_2$	0,79	-3,43	1,33	ttt
β-$RuCl_2(Nazpy)_2$	0,72	-3,35	8,77	ccc
δ-$RuCl_2(Nazpy)_2$	0,78	-3,95	0,00	ttt

Considering the geometrical structure of the complexes, the structure-activity relationship may be better if both Azopyridine ligands (Azpy or Nazpy) of the complex are in the same plane. Therefore, only the trans (δ-Cl) isomers correspond to this structure [33]. Concerning the cis isomers (β-Cl) the two azopyridine ligands are not in the same plane. Therefore, the intercalation of these complexes between DNA base pairs is hindered. Thus, the most convenient and competitive structures that favors binding to DNA are the δ-Cl isomers. Considering the energy of the boundary molecular orbital, the reaction is most efficient between these two molecules when the HOMO of the first molecule (electron donor) is close to the LUMO of the second (electron acceptor). Knowing that the HOMO is carried by the DNA base pairs and the LUMO by the ruthenium complex then the more reactive complex must have the lowest LUMO energy. Knowing the HOMO energy of DNA fixed at -1.27 eV according to Kurita and Kobayashi, the energy of the LUMO orbital of the Azpy and Nazpy complexes becomes decisive in the evaluation of the structure-activity relationship of these complexes. Thus, the order of the energies of these complexes was established as follows: E_{LUMO} (β-Cl)> E_{LUMO} (δ-Cl) for the isomers of Azpy and Nazpy complexes. From these two rankings, it appears that the δ-Cl isomers are the most available to readily accept electrons from the HOMO of the DNA base pairs. This analysis is confirmed by that obtained with the geometric structures. Regarding the hydrophobic parameter expressed by log P, it expresses the absorption capacity of the therapeutic molecule. It is in fact a main

parameter in studies of the quantitative structure-activity relationship QSAR of biological molecules. Theoretically, the parameter that describes the hydrophobic factor is the dipole moment [13]. In fact, a high dipole moment means high absorption and solubility in water. However, low dipole moment value implies efficient absorption and solubility in fat. Thus, the calculated dipole moment ranking of the isomers of the two series of complexes indicates the following order: μ (β-Cl)> μ (δ-Cl). Through this ranking, the δ-Cl isomers show the highest cytotoxicity. Finally, the atomic net charge of the ligands in the complexes influences their ability to intercalate to DNA base pairs. As DNA base pairs have HOMO molecular orbitals, they are negatively charged. Therefore, for the base pairs to bind to the ligands, the ligands must carry a high positive charge. Thus, Table 1 shows the net charge QL of ligands in these isomers. The charge classification of the ligands in these complexes is as follows QL (δ-Cl)> QL (β-Cl). This ranking indicates that the Azpy and Nazpy ligands of the δ-Cl isomers have the highest affinity to accept the electron from DNA. Given the above properties, we can say that the most cytotoxic isomers by mode of intercalation between DNA base pairs are the δ-$RuCl_2(Azpy)_2$ and δ-$RuCl_2(Nazpy)_2$ isomers. Furthermore, the substitution of the phenyl group by the naphthol group improves the cytotoxic properties of these complexes. These results are in agreement with those obtained by Bamba et al [13].

3.2. Florescence

3.2.1. Study of the Absorption Properties of the β-isomers of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy

(i). Electronic Transitions of the β-isomers of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy

The structures of the β isomers of the Azpy and Nazpy complexes are given in Figure 2. Among the five isomers of $RuCl_2L_2$ complexes with L= Azpy or Nazpy, this is the only one that does not have a C2 axis. This β-isomer has all its substituents in the cis-position, i.e. the Cl chlorine, N_{py} pyridine nitrogen and N_2 nitrogen atoms of the azo group are all in the cis-position. Hence, this isomer is named ccc or β-Cl.

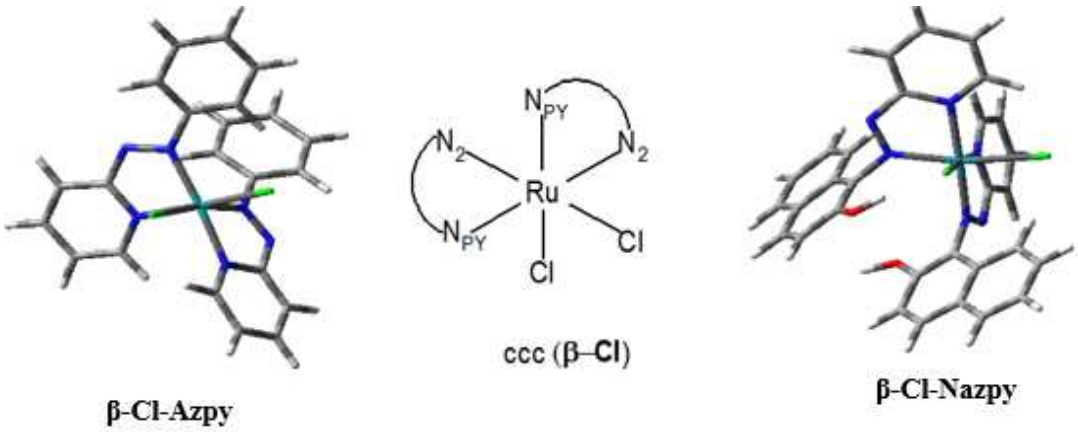

Figure 3. *Geometric structures of the β or ccc isomers of $RuCl_2L_2$ complexes with L= Azpy or Nazpy.*

Table 4 shows the spectral characteristics of the β-Cl complexes. In the UV-visible absorption spectrum of these β-isomers in vacuum, β-Azpy isomer has eight characteristic bands and β-Nazpy isomer has three bands with oscillation strengths greater than 0.03. In terms of the emission spectrum of these isomers, we have four bands and two emission bands for the Azpy isomer and the Nazpy isomer respectively. The values of the λ_{max} wavelengths, extinction coefficients of the transitions of these isomers also are recorded.

Table 4. *Spectral characteristic of the β-isomers in the vacuum of $RuCl_2L_2$ complexes with L= Azpy or Nazpy at the B3LYP/LANL2DZ level.*

	β-$RuCl_2(Azpy)_2$				**β-$RuCl_2(Nazpy)_2$**			
	Absorption / Emission bands		**Wavelength**	**ε (L/mol.cm)**	**Absorption / Emission bands**		**Wavelength**	**ε (L/mol.cm)**
Absorption	Soret Band		388	11300	Soret Band		559	5400
	Q-bands	VII	382	6800	Q-bands	VII	601	3800
		VI	394	3800		VI	708	5300
		V	414	4300		V	-	-
		IV	421	4200		IV	-	-
		III	474	3900		III	-	-
		II	597	4200		II	-	-
		I	671	4300		I	-	-
Emission	Soret Band		888	1800	Soret Band		691	5500
	Q-Bands	II	545	1000	Q-Bands	II	657	1000
		I	507	1700		I	613	2000

These indicative values of the spectrum of the β-Azpy complex in vacuum show a Soret band which is located at 388 nm with a high extinction coefficient value of 11300 $M^{-1}cm^{-1}$. Experimentally, this Soret band is located at 576

nm with an extinction coefficient of 6200 $M^{-1}cm^{-1}$in chloroform [38]. In addition to this band, we also note the presence of seven Q bands located at 382 nm, 394 nm, 414 nm, 421 nm, 474 nm, 597 nm and 671 nm respectively. The absorption band at 671nm has an extinction coefficient of 4300 $M^{-1}cm^{-1}$. This makes the isomer suitable for use in PDT [39]. On the other hand, the β-Nazpy isomer has less absorption band. Its absorption spectrum is composed of a Soret band located at 559 nm with an intensity of the absorption band of the order of 5400 $M^{-1}cm^{-1}$ and two other bands of which the most intense is located at 708nm. In the case of emission, the Soret band is found at 691nm with an intensity of 5500 $M^{-1}cm^{-1}$. The substitution of the phenyl group with the naphthol group improves the PDT and fluorescence properties of the β-isomer. Figures 3 show the spectra of these β-isomers.

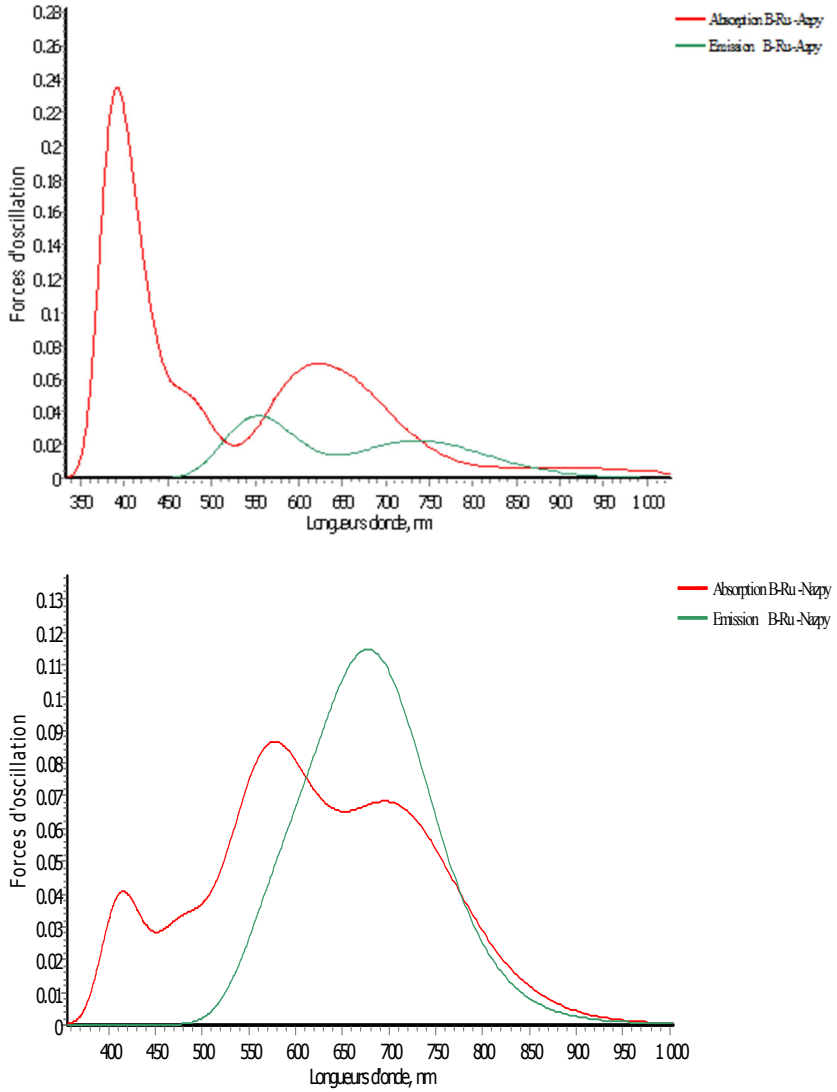

Figure 4. *Red absorption and green emission spectra of the β-isomer of the $RuCl_2L_2$ complex with L= Azpy or Nazpy of ruthenium in vacuum at the B3LYP/LAND2Z level.*

In order to determine the element responsible for these changes, we were interested in the nature of the bands observed in these complexes. The results obtained are shown in Table 5.

(ii). Nature of the Electronic Transitions of the β-isomers of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy

The β-isomer transition bands of $RuCl_2L_2$ complexes with L= Azpy or Nazpy which are between 300 nm and 500 nm are strongly dependent on the contribution of ligands. These ligand contributions can be electrons from a π-bond or electrons from a free doublet. Thus, these bands will be assigned to n →π* and π→π* transitions related to the presence of azo groups and heteroatoms within the ligands. The transitions beyond 500 nm, are predominantly of MLCT transitions precisely of the t_{2g} (Ru) →π*(L) type. Here, the absorption band of major therapeutic interest for this isomer is that occurring at 671nm. It occurs from HOMO-1 to LUMO. Furthermore, concerning the substitution of the phenyl group by the naphthol group, the most significantly observed transitions are the following at 559 nm, 601 nm and 708 nm. They are all of the MLCT type and contribute to the improvement of the therapeutic

properties.

Table 5. *Electronic transitions, transition coefficients, transition percentage, nature of transitions, wavelength, excitation energy and oscillation strengths of $RuCl_2L_2$ complexes with L= Azpy or Nazpy.*

complexes	S	TE	CET	Pt	NT	OM_i		OM_j		λ_{cal} (nm)	E (eV)	f> 0,030
						%M	%L	%M	%L			
β-$RuCl_2(Azpy)_2$	5	H-1→ L	0,257	84	MLCT	32	68	14	86	671	1,85	0,038
	7	H-3→ L+1	-0,112	70	MLCT	6	94	17	83	597	2,08	0.037
	10	H-4→ L	0,149	85	LLCT/ILCT	24	76	14	86	474	2.62	0.030
	13	H-1→ L	-0,121	68	LLCT/ILCT	11	89	14	86	421	2,94	0,034
	14	H-6→ L+1	-0,113	65	LLCT/ILCT	11	89	17	83	412	3,00	0,038
	17	H→ L+2	0,212	51	LLCT/ILCT	45	55	3	97	394	3,15	0,032
	19	H-8→ L	-0,112	53	LLCT/ILCT	23	77	14	86	388	3,20	0,097
	20	H-8→ L+1	-0,193	56	LLCT/ILCT	23	77	14	86	382	3,25	0,055
β-$RuCl_2(Nazpy)_2$	4	H→L	-0,182	76	MLCT	8	92	14	86	708	1,75	0,041
	7	H-3→L+1	-0,378	87	MLCT	40	60	14	86	601	2,06	0,030
	9	H-4→L	0.493	61	MLCT	33	67	14	86	559	2,22	0,041

3.2.2. Study of the Absorption Properties of the δ-isomers of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy

(i). Electronic Transitions of the δ-isomers of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy

The structures of the δ isomers of the Azpy and Nazpy complexes are given in Figure 4. In this isomer, all groups are in the trans position. That is, the chlorine Cl atoms, the nitrogen N_{py} atoms of the pyridine groups and the N_2 atoms of the azo groups are all diametrically opposite. Hence, they are named ttt or δ-Cl isomers. This configuration of the molecule generates great stability to the molecule as it minimizes steric hindrance within it.

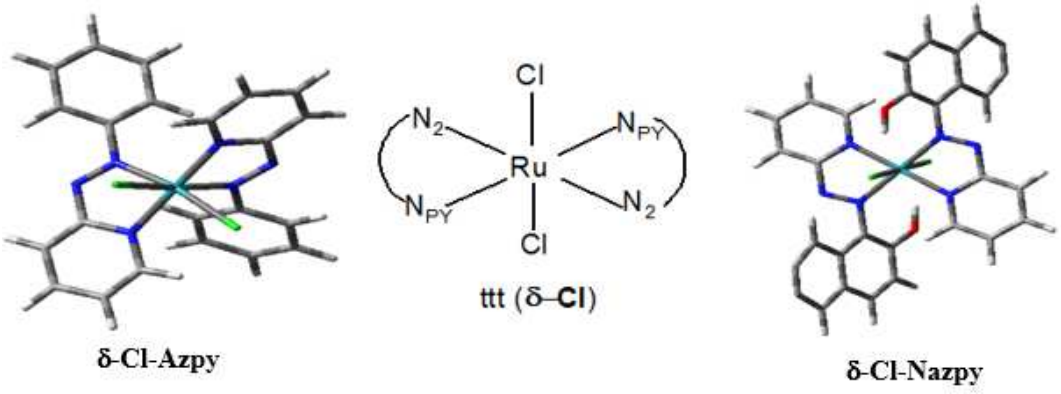

Figure 5. *Geometric structures of the δ or ttt isomers of $RuCl_2L_2$ complexes with L= Azpy or Nazpy.*

Table 6 shows the spectral characteristics of the δ-Cl complexes.

In the UV-visible spectrum of the δ-Azpy isomer and the δ-Nazpy isomer in vacuum, three characteristic bands with oscillation strengths greater than 0.03 for the δ-Azpy isomer and four for δ-Nazpy are observed. The characteristic Soret band values and Q-bands are summarized in Table 6. The fluorescence spectrum of these isomers determined at the B3LYP/LANL2DZ level also indicates three emission bands with oscillation strengths greater than 0.01.

Table 6. *Spectral characteristics of the δ isomer in the vacuum of $RuCl_2L_2$ complexes with L= Azpy or Nazpy at the B3LYP/LAND2Z level.*

	***δ*-$RuCl_2(Azpy)_2$**				***δ*-$RuCl_2$ (Nazpy)$_2$**			
	Absorption / Emission bands		**Wavelength**	**ε (L/mol.cm)**	**Absorption / Emission bands**		**Wavelength**	**ε (L/mol.cm)**
Absorption	Soret Band		393	9000	Soret Band		810	6200
	Q-bands	III	821	3200	Q-bands	III	752	3100
		II	418	3100		II	621	4800
		I	-	-		I	535	3600
Emission	Soret Band		518	4200	Soret Band		584	3600
	Q-Bands	II	613	600	Q-Bands	II	737	500
		I	466	500		I	562	1600

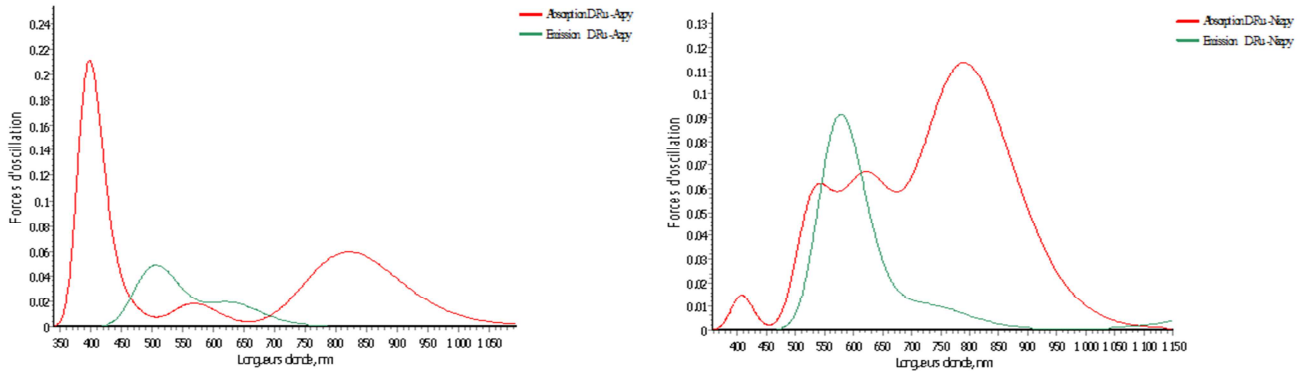

Figure 6. *Red absorption and green emission spectrum of the δ-isomer of the $RuCl_2L_2$ complex with L= Azpy or Nazpy of ruthenium in vacuum at the B3LYP/LAND2Z level.*

The Soret band of the δ-Azpy isomer is located at 393 nm with an extinction coefficient of 9000 L/mol.cm. Experimentally, this absorption band is located at 694 nm in chloroform [39]. This absorption band will be shifted towards longer wavelengths with a 31.11% decrease in intensity following the substitution of the phenyl group by the naphthol group. Thus this band will be located at 810 nm in the Nazpy isomer. This ability to absorb at 821 nm and 810 nm confers an important role for these δ isomers in the development of photodynamic therapy or PDT. Indeed, the absorption in this region allows a strong penetration into the cells (beyond 3mm) [39]. The δ-Nazpy isomer has a wavelength of the Soret band at the absorption level that is longer than the wavelength of the emission band. Hence the presence of an anti-stokes shift which will be evaluated in the following work. As for fluorescence, the δ-Azpy isomer has three emission bands located at 613 nm, 518 nm and 466 nm respectively while δ-Nazpy has its bands located at 737 nm, 584 nm and 562 nm respectively. In general, it can be seen that the substitution shifts the emission bands to longer wavelengths accompanied by a decrease in the emission intensity except for the band at 562 nm of the δ-Nazpy complex. Figures 5 show the spectra of these δ-isomers.

The characteristics of these absorption bands are summarized in Table 7.

(ii). Nature of the Electronic Transitions of the δ-isomers of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy

The results in Table 7 indicate that the δ-isomers of $RuCl_2L_2$ complexes with L=Azpy and Nazpy absorb between 300 nm and 900 nm. The Soret band of this δ-Azpy isomer is located at 393 nm and is of LLCT or ILCT type. This transition is from HOMO-7 to LUMO with a percentage of 70% and an oscillation strength of 0.161. This transition receives a contribution of 23% d-orbitals and 77% π-orbitals. The d-orbitals are derived from the metal atom and the π-orbitals are derived from azo group, phenyl and chlorine atoms during this transition. It is directed towards the LUMO which is 98% composed of π orbitals from pyridine groups. Similarly, the Soret band of the δ-Nazpy isomer, which is also the band of major therapeutic interest of the MLCT type appears at 810 nm. This transition is from HOMO-1 to LUMO with a percentage of 91% and an oscillation strength of 0.081.

Table 7. *Electronic transitions, transition coefficients, transition percentage, nature of transitions, wavelength, excitation energy and oscillation strengths of isomers of $RuCl_2L_2$ complexes with L= Azpy or Nazpy.*

complexes	S	TE	CET	Pt	NT	OM_i		OM_j		λ_{cal} (nm)	E (eV)	f> 0,030
						%M	%L	%M	%L			
δ-$RuCl_2(Azpy)_2$	3	H-1→ L	0, 642	83	MLCT	39	61	2	98	821	1,51	0,051
	15	H-9→ L	-0,194	57	LLCT/ILCT	18	82	2	98	418	2,96	0,058
	18	H-7→ L	-0,139	70	LLCT/ILCT	23	77	2	98	393	3,15	0,161
δ-$RuCl_2(Nazpy)_2$	S3	H-1→L	0,221	91	MLCT	18	82	0	100	810	1,53	0,081
	S4	H-3→L	-0,135	92	MLCT	45	55	0	100	752	1,65	0,041
	S6	H-4→L	0,146	90	MLCT	61	39	0	100	624	1,99	0,061
	S10	H-2→L+1	-0,373	62	MLCT	1	99	18	82	535	2,32	0,049

3.2.3. Absorption and Emission Characteristics of $RuCl_2L_2$ Complexes with L= Azpy or Nazpy of Ruthenium in Vacuum

To determine the ability of these ruthenium azopyridine complexes to be engaged in a fluorescence process, we evaluated several fluorescence parameters such as the absorption wavelength λ_{Abs}, the stokes shift (Ss), the transition lifetime still called transition lifetime (τ), the attachment energy of an exciton Eb, the extinction coefficient, the light harvesting efficiency (LHE). The fluorescence properties of these two series of isomers of $RuCl_2L_2$ complexes with L=Azpy or Nazpy were determined using the TDDFT method. The results obtained are reported in Tables 8 and 9 at the B3LYP/ Lanl2DZ level.

Table 8. *Absorption and emission wavelengths (nm), stokes displacements and anti-stokes displacements (nm) and molar extinction coefficient ($M^{-1}cm^{-1}$) of azopyridine complexes in vacuum at B3LYP/ Lanl2DZ level.*

Complexes	λ_{Abs}	f_{Abs}	ε_{Abs}	λ_{Em}	f_{Em}	Δstokes /anti stokes
β-Ru-Azpy	388	0,097	11300	888	0,018	500
δ-Ru-Azpy	393	0,161	9000	518	0,087	125
β-Ru-Nazpy	559	0,041	5400	691	0,085	132
δ-Ru-Nazpy	810	0,081	6200	584	0,064	-226

Table 8 shows that the absorption bands of Azpy complexes are between 300 nm and 500 nm while their Nazpy analogues have absorption bands between 500 nm and 900 nm. Thus the isomers of Nazpy complexes have much more favorable absorption bands for therapeutic use than their Azpy analogues. This is because all Nazpy isomers absorb in the therapeutic window except the β-isomer. The isomer with the highest fluorescence intensity is the β-Ru-Azpy isomer. The molar extinction coefficient of this complex is 11300 $M^{-1}cm^{-1}$. The absorption and emission light distinguishing parameters indicate two trends: isomers with stokes shifts and isomers with anti-stokes shifts. All isomers in both series of complexes have stokes shifts except for the δ-Ru-Nazpy isomer which has an antistokes shift. The fundamental difference between these two types of fluorescence is in the position of the absorption and emission bands. Stokes luminescence shift converts low excitation wavelengths into high emission wavelengths. In this case, detection is better when the stokes shift value is high. Some commercial fluorophores such as rhodamine, fluorescein, cyanines, and boron dipyrromethenes admit low stoke shift values (smaller than 25 nm); therefore, some undesirable effects will be detected when using these fluorophores [40]. When looking at the stoke shift values in Table 8, it can be deduced that the β-Ru-Azpy isomer admits the best fluorescence signal detection with a stoke shift value of 500 nm. Moreover, all these stokes displacements are higher than 25 nm. The lowest value is 132 nm [40]. Therefore, these fluorophores will produce a better signal detection in the absence of competitive fluorescence effects. In the case of an anti-stokes shift, we see a conversion from high excitation wavelengths to small emission wavelengths [41]. This is a special optical process. This spectacular optical process is crucial for biological applications and therapeutic methods associated with light. Indeed, the high wavelengths of the excitation sources of these molecules, generally in the near IR, have a deep penetration of cellular tissues offering a much wider field of application of these compounds in the medical field. Moreover, the luminescent signal generated by an antistokes shift is distinct from the auto-fluorescence of biological cells, thus reducing the interference phenomena of stray radiation from biological cells [41]. Furthermore, the excitation (τ_{exc}) and emission (τ_{emi}) lifetime, the photon attachment energy (Eb) and the light harvesting efficiency (LHE) [8] of the complexes were evaluated. These parameters make it possible to evaluate the behavior of these complexes in the presence of light. The values obtained during this evaluation have been recorded in Table 9.

Table 9. *Excitation (τ_{exc}) and emission lifetime (τ_{emi}), photon attachment energy (E_b) and light harvesting efficiency (LHE) in vacuum at the B3LYP/ Lanl2DZ level.*

Complexes	Eb	τexc	τémi	LHE
β-Ru-Azpy	0,914	145,61	653,73	0,200
δ-Ru-Azpy	-0,594	11,08	46,07	0,31
β-Ru-Nazpy	0,465	889,72	84,33	0,090
δ-Ru-Nazpy	-0,173	7,95	79,88	0,170

Table 9 shows that the δ isomers of the Azpy and Nazpy complexes have the lowest values of exciton attachment energies relative to the β isomers. The values of these energies are -0.594 and -0.173 for Azpy and Nazpy respectively. This shows that these isomers will be able to emit much more easily compared to the other isomers. This energy decreases with substitution in the β isomer and increases with substitution in the δ isomer. Thus, the extent of conjugation influences the attachment energy of the excitations. The lowest value of excitation and emission lifetime is from the δ isomers for the Azpy and Nazpy complexes. The lowest values most favor fluorescence activity and relatively minimize other forms of energy conversion [42]. The values of light collection efficiency calculated at the two sets of complexes vary between 0.200 and 0.310 for Azpy complexes and between 0.090 and 0.170 for Nazpy complexes. Among these isomers, the δ-Ru-Azpy and Nazpy isomers collect better the light. From the above, we can say that the substitution of the phenyl group with the naphthol group decreases the light collection efficiency of these azopyridine complexes. This decrease is related to the extent of conjugation within these complexes.

3.2.4. Comparative Study of the Fluorescence Characteristics of the Two Series of Azopyridine Complexes with Commonly Used Fluorophores

Table 10. *Comparative study of the characteristics of Nazpy and Azpy complexes with some commonly used fluorophores.*

Complexes	λ_{Abs}	λ_{Em}	Δ_{stokes}	ε_{Abs}
β-Ru-Azpy	388	888	500	11300
Y66H	382	459	77	25000
δ-Ru-Azpy	393	518	125	9000
GFPuv	395	509	114	27000
β-Ru-Nazpy	559	691	132,2	5000
BODIPY 558/568[b]	558	559	11	97000
δ-Ru-Nazpy	810	584	-226	6200
ICG	830	845	15	24200

Analysis of the fluorescence parameters of the two series of azopyridine complexes (Azpy and Nazpy) in Table 10 in comparison with those of some commonly used

fluorophores including Y66H, GFPuv, BODIPY 558/568, indocyanine green (ICG) shows several trends [15, 43, 44]. Firstly, at the level of emission bands, all complexes show stoke shifts or anti-stoke shifts higher than those of the commonly used fluorophores cited in this work. The values of these stoke shifts or anti-stoke shifts are in absolute value between 125 nm and 500nm while those of the reference compounds are between 10 nm and 114 nm. This finding indicates that these complexes distinguish better between emitted and absorbed light compared to the reference fluorophores. In addition, the δ-Ru-Nazpy isomer exhibits an anti-stoke shift that results in a form of fluorescence that is distinctly different from the auto fluorescence generated by biological cells. Secondly, the fluorescence intensity of these azopyridine complexes is lower than those of the reference compounds. This is evidenced by the values of the extinction coefficients. They are all lower than those of the reference compounds. Of the two series of complexes, the Nazpy isomers are better suited for treatments requiring deep penetration. From the above, we can say that the substitution of the phenyl group with the naphthol group improves the fluorescence.

4. Conclusion

The impact of the substitution of the phenyl group by the naphthol group on the cytotoxicity and fluorescence properties of the azopyridine isomers and complexes (Azpy and Nazpy) has been studied by means of DFT and TDDFT methods at the B3LYP/ LANL2DZ level. The structure-activity relationship (SAR) of these complexes was explored by analyzing their electronic and geometrical structures. The parameters of these structures were related to their cytotoxic activities. It was found that the most cytotoxic isomers by mode of intercalation between DNA base pairs are the δ-$RuCl_2$ $(Azpy)_2$ and δ-$RuCl_2$ $(Nazpy)_2$ isomers. Analysis of the fluorescence parameters indicates that the δ-$RuCl_2$ $(Nazpy)_2$ isomer specifically is best suited for medical imaging. This complex absorbs in the therapeutic window and has a significant antistokes shift. Furthermore, the substitution of the phenyl group with the naphthol group improves the cytotoxicity and fluorescence properties of these azopyridine complexes. In view of the above mentioned properties, it can be said that the δ-$RuCl_2$ $(Nazpy)_2$ isomer can be a good theranostic agent for the treatment of breast, colon, ovarian, melanoma and tongue cancers. In order to increase the efficacy of this complex, a photodynamic study could be an asset.

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