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# Theoretical investigation of the Structure Activity Relationships (SARs) of a series of five isomeric α, β, γ, δ, ε ruthenium complexes $RuCl_2L_2$ with azopyridine ligands [L= azpy, tazpy, 4mazpy, 5mazpy]

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**ABSTRACT**
Theoretical study of a series of isomeric α-, β-, γ-, δ-, ε-$RuCl_2L_2$ (L= azpy, tazpy, 4mazpy, 5mazpy) complexes is carried out using the density functional theory (DFT) method at B3LYP/LanL2DZ level. The effects of the ligand on the electronic structures and related properties, e.g. the components and the energies of some frontier molecular orbital, the net charge populations of some main atoms of the complexes, the effect of substituent methyl as well as the Structure Activity-Relationships (SARs) of the complexes were investigated. The results show that the sterical differences between isomeric structures of these complexes have serious influence on their electronic structures and related properties. First and foremost, the geometric configuration of δ-Cl and γ-Cl isomers must be advantageous to the conjugative ligand to intercalate between DNA-base-pairs in comparison with α-Cl, β-Cl and ε-Cl complexes. Secondly, the energy order of the lowest unoccupied molecular orbital (LUMO) of the isomers is $E_{LUMO}$(δ-Cl) < $E_{LUMO}$(γ-Cl) < $E_{LUMO}$(ε-Cl) < $E_{LUMO}$(α-Cl) < $E_{LUMO}$(β-Cl). And their HOMO-LUMO gap energy is classified as ΔE(δ-Cl) < ΔE(γ-Cl) < ΔE(ε-Cl)< ΔE(α-Cl)< ΔE(β-Cl). Thirdly, the dipole moments (μ) of the isomers, expressing the hydrophobic parameters of the molecules, was also classified as μ(ε-Cl) > μ(β-Cl) > μ(α-Cl) > μ(γ-Cl) > μ(δ-Cl). Finally, the net charge of the ligands azopyridine that defines the aptitude for the ligand to accept the electron from DNA, are classified as $Q_L$(*δ*-Cl) > $Q_L$(*γ*-Cl) > $Q_L$(*ε*-Cl) > $Q_L$(*α*-Cl) >$Q_L$(*β*-Cl). These electronic and geometric structural characteristics can be used to explain the trend in the anticancer-activities (A) of isomeric α-, β-, γ- $RuCl_2L_2$ (L= azpy, tazpy, 4mazpy) or to predict the order of activity of the five δ-Cl, *γ*-Cl, *α*-Cl, *β*-Cl and ε-Cl isomers of the three complexes $RuCl_2(azpy)_2$, $RuCl_2(tazpy)_2$ and $RuCl_2(4mazpy)_2$. They are also suitable to predict the activity of five non synthesized isomers of $RuCl_2(5mazpy)_2$ since the three azopyridine ligands tazpy, 4mazpy and 5mazpy display the same number of electrons.

***Keywords:*** Ru(II) complexes ; Anticancer activity ; Structure Activity-Relationships (SARs) ; azopyridine (azpy) ; DFT.

## I. INTRODUCTION

Azopyiridine constitutes a very famous class of ligands in the synthesis of a large variety of ruthenium complexes due to their strong π-acidity [1-2], their stability and various applications in DNA probing [3], as chemotherapeutic drugs [4], and in anticancer activity [5-7] or as well as electrochemical catalysts [8-9]. This fact is due to its ability to stabilize ruthenium at a low state of oxidation [10] and to obtain complexes with remarkable electronic transfer charge compared to bipyridine complexes [1]. So, researchers have been focusing more attention on ruthenium complexes with azopyridine and their derivatives in an effort to improve their properties [11].

Actually, azopyridine ligands as indicated in Figure 1 are all bidentates owing to their mode of coordination to a metal ion. They bind to metal through the lone pairs on the nitrogen atoms of both the pyridine and the azo groups, thereby forming a stable chelating 5-membered ring [12]. The 2-phenylazopyridine (azpy), which represents the first azopyridine compound ever studied, is the most exploited with ruthenium atom. Also the methylated ligands such as o-tolyazopyridine (tazpy), 4-methyl-2-phenylazopyridine (4mazpy) and 5-methyl-2-phenylazopyridine (5mazpy) (Figure 1) and many other types of azopyridine ligands have been described [13-17]. Besides, the azopyridine ligand is an asymmetric molecule. Therefore, the azopyridine ruthenium complexes $RuCl_2L_2$ (L= azopyridine ligand) are theoretically expected to exist in five different isomeric forms named α-Cl, β-Cl, γ-Cl, δ-Cl and ε-Cl as shown in Figure 2 [13] [18,19]. The difference between them comes mainly from the

position of both chloride atoms. While α, β and ε adopt the *Cis*-geometry with respect to the two chloride atoms, γ and δ isomers are in the *Trans* configuration where both chloride atoms form 180° including the center ruthenium ion. In addition, except β-Cl all isomers are $C_2$ symmetrical [20].

Regarding their fascinating activity, they were synthesized in different teams of researchers [21] [6]. However, only three isomers, namely α-, β- and γ-$RuCl_2L_2$ (L= azpy, 4mazpy and tazpy) are so far tested for their cytotoxicity in the panel of cell lines MCF-7 (breast cancer), EVSA-T (breast cancer), WIDR (colon cancer), IGROV (ovarian cancer), M19 (melanoma), A498 (renal cancer) and H226 (non-small cell lung cancer) [5-6]. In contrast to the reported inactivity of *Cis* or γ-[$RuCl_2(bpy)_2$] [22], Hotze et al. discovered generally that α-Cl and γ-Cl present a very high cytotoxicity[5-6]. Whereas the inactivity of β-Cl, it may be due either to the steric constraints provided by the position of the azopyridine ligands or to the lack of symmetry in the complex.

Possible reasons of substantial activity against several cell lines of azopyridine complexes can be explained by: (1) The decrease in the rate of chloride aquation due the π-acceptor effect of the imine ligands increasing the effective charge on the metal ion so that hydrolysis rates are in the range of the cisplatin molecule. (2) The increased hydrophobic or intercalative interactions with DNA, which may facilitate the covalent binding. (3) And a geometric effects exerted by the ligands, which may facilitate (or inhibit) protein to bind to the nucleic [23].

In addition to experimental studies, azopyridine complexes have attracted a great number of theoretical researchers that have tried to correlate the experiment results with theoretical predictions. In this way, Chen et al. confirmed by theoretical approach the experimental work for $RuCl_2(4mazpy)_2$ and $RuCl_2(azpy)_2$ [24] [25]. They admitted that the most active complex is γ-Cl. However, in a previous work where we investigate the five isomers of $RuCl_2(azpy)_2$ and predicted thanks to DFT method Structure Activity Relationships (SARs), we found out that δ-Cl isomer is assumed to display the highest activity as antitumor drug [20].

Even though, SARs of $RuCl_2(azpy)_2$ has recently been reported [20], we report herewith some theoretical results on effects caused by the added methyl group using the DFT method at the B3LYP/LanL2DZ level. The effects of methylated azpy ligand on the electronic structures, the related properties, e.g. the compositions and the energy of some frontier molecular orbitals, the spectral properties, the net charge on some main atoms, the relative stabilities of isomers and the main bond lengths of the complexes, as well as the Structures Activity Relationships (SARs) in order to understand theoretically the differences between these isomers regarding the cytotoxic activity are investigated. So, we perform the calculation by using the widespread intercalative mode of ligands between the DNA base-pairs CytosineGuanine/ CytosineGuanine (CG/CG).

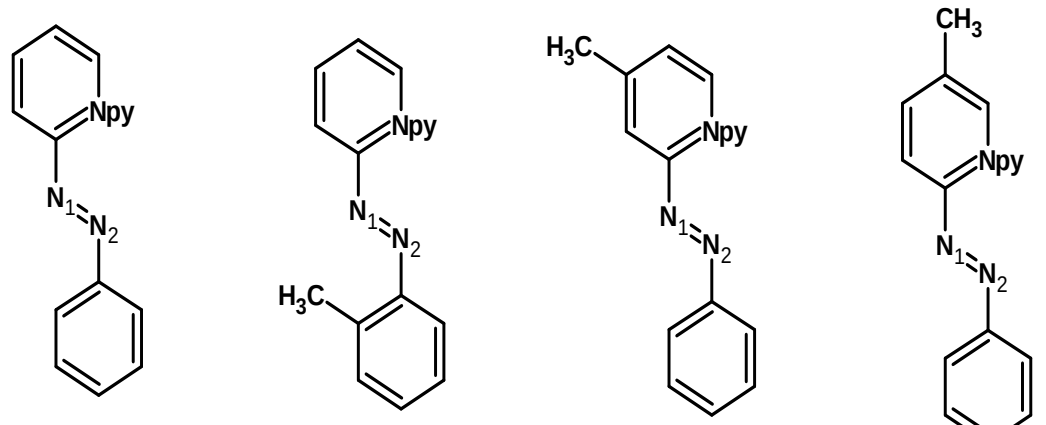


**Figure 1:** Structures of azopyridine ligands, from II to IV, hydrogen atom was substituted by methyl group. Hence, the three complexes are isoelectronical. The three first ligands have already been experimentally studied. But, the fourth ligand IV has never been studied before. Therefore, its results will be predicted thanks to complexes I to III.

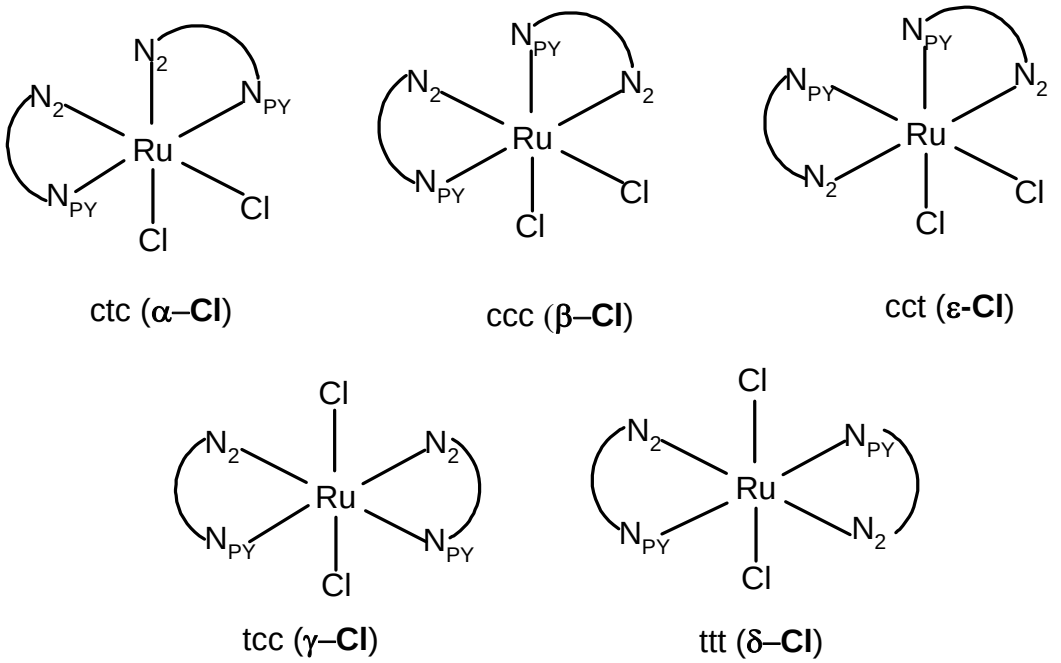


**Figure 2:** The five expected isomeric forms of azopyridine complexes $RuCl_2L_2$. L represents azopyridine ligand. The three-letter code indicates the mutual *cis*(c) or *trans*(t) orientation of the chlorides (Cl), the pyridine ($N_{py}$) and the azo nitrogens ($N_2$). The arcs represent the azopyridine ligands.

## II. METHOD

All the calculations were performed with DFT method using Becke's three-parameter hybrid B3LYP [26] and the double-zeta pseudo-potential LANL2DZ [27] basis set. Before each calculation, the complexes were optimized first with frequency analysis to know of the absence of eventual imaginary vibrational data. This method allows to perform calculations over the most stable molecules in their ground states. The energy of the frontier molecular orbital (HOMO and LUMO) was analyzed. The natural orbital population analysis NPA was also carried out at the same level. Regarding the HOMO energy of the staked DNA base-pairs CG/CG, it was calculated by Kurita and Kobayashi [28]. Besides, all these calculations were performed thanks to Gaussian 03 package [29].

## III. RESULTS AND DISCUSSIONS

### 3.1 Geometrical Parameters

Both computed results and experimental data [5-6] on the main bond lengths and bond angles of isomeric complexes are shown in Table 1. Comparing the computed results with experimental data, we can find that the computational Ru-X (X=Cl, $N_2$, $N_{py}$) bond lengths agree with that of the corresponding experimental ones in all isomeric complexes. It shows the DFT method to be reliable. Moreover, we can see that the substitution of hydrogen atom by the methyl group hasn't any real effect on the main bond lengths nor on bond angles of the complexes. Some slight computation errors from experiments may nevertheless come mainly from two factors: One is that theoretical calculations do not consider the effects of chemical environment (calculations are performed on isolated molecules), and the other one is that the method of calculation and basis set used are still approximate in certain extent [30].

In the optimized structures of $RuCl_2L_2$ complexes, the environment of ruthenium adopts as usual, a distorted octahedral coordination geometry. However, the geometry of ligand remains essentially unchanged within the isomeric complexes. Anyway, the lost of octahedral geometry of these complexes that doesn't hamper the $C_2$ symmetry of α-, γ-, δ-, ε-$RuCl_2L_2$ must be due to Yahn Teller effect [31].

**Table 1:** Comparison between the computational and experimental data for selected geometrical parameters of five isomers $RuCl_2L_2$ calculated at B3LYP/LANL2DZ level. Distances are written in Å and bond angles in deg.

| a) | | | $N_1=N_2$ | Ru-$N_2$ | Ru-$N_{py}$ | Ru-$Cl_1$ | Ru-$Cl_2$ | $Cl_1$-Ru-$Cl_2$ | $N_{py}$-Ru-$N_{py}$ | $N_2$-Ru-$N_2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | α-Cl | Calc | 1.32 | 2.03 | 2.06 | 2.48 | 2.48 | 90.60 | 178.37 | 101.49 |
| | | exp | 1.28 | 1.98 | 2.05 | 2.40 | 2.40 | 89.50 | 174.50 | 93.50 |
| | β-Cl | Calc | 1.32 | 2.02 | 2.05 | 2.48 | 2.48 | 90.18 | 99.21 | 104.58 |
| | | | 1.32 | 2.05 | 2.07 | | | | | |
| | | exp | 1.29 | 1.96 | 2.02 | 2.40 | 2.41 | 91.10 | 101.90 | 103.00 |
| | | | 1.3 | 2.00 | 2.06 | | | | | |
| **I** | γ-Cl | Calc | 1.32 | 2.03 | 2.10 | 2.48 | 2.48 | 170.71 | 102.86 | 104.99 |
| | | exp | 1.31 | 1.99 | 2.11 | 2.38 | 2.38 | 170.50 | 103.80 | 104.10 |
| | δ-Cl | Calc | 1.31 | 2.06 | 2.10 | 2.51 | 2.49 | 180.00 | 167.53 | 178.58 |
| | | exp | 1.28 | 2.02 | 2.06 | 2.38 | 2.38 | 180.00 | 180.00 | 180.00 |
| | ε-Cl | Calc | 1.32 | 2.05 | 2.06 | 2.49 | 2.49 | 94.10 | 93.58 | 169.48 |
| | α-Cl | Calc | 1.32 | 2.04 | 2.07 | 2.48 | 2.48 | 91.67 | 178.14 | 93.64 |
| | β-Cl | Calc | 1.32 | 2.01 | 2.06 | 2.48 | 2.49 | 91.01 | 99.72 | 104.86 |
| | | | | 2.04 | 2.07 | | | | | |
| **II** | γ-Cl | Calc | 1.32 | 2.03 | 2.10 | 2.48 | 2.48 | 170.85 | 102.60 | 105.16 |
| | | exp | 1.30 | 1.961 | 2.085 | 2.38 | 2.37 | 167.83 | 105.65 | 102.23 |
| | | | | 1.975 | 2.103 | | | | | |
| | δ-Cl | Calc | 1.30 | 2.05 | 2.10 | 2.50 | 2.50 | 180.00 | 173.13 | 179.11 |
| | ε-Cl | Calc | 1.32 | 2.06 | 2.06 | 2.49 | 2.49 | 94.09 | 95.78 | 170.31 |
| | α-Cl | Calc | 1.32 | 2.03 | 2.07 | 2.48 | 2.48 | 92.21 | 178.46 | 90.43 |
| | | exp | 1.30 | 1.98 | 2.04 | 2.39 | 2.39 | 94.40 | 174.10 | 88.40 |
| **III** | β-Cl | Calc | 1.32 | 2.05 | 2.05 | 2.48 | 2.48 | 90.30 | 99.16 | 104.55 |
| | | | | 2.02 | 2.02 | | | | | |
| | γ-Cl | Calc | 1.32 | 2.03 | 2.10 | 2.48 | 2.48 | 170.87 | 105.10 | 102.90 |
| | δ-Cl | Calc | 1.31 | 2.06 | 2.10 | 2.51 | 2.49 | 180.00 | 167.88 | 179.08 |
| | ε-Cl | Calc | 1.32 | 2.05 | 2.06 | 2.49 | 2.49 | 94.03 | 93.65 | 169.29 |
| | α-Cl | Calc | 1.32 | 2.03 | 2.07 | 2.48 | 2.48 | 92.19 | 178.73 | 90.39 |
| | β-Cl | Calc | 1.32 | 2.05 | 2.05 | 2.48 | 2.48 | 90.27 | 99.27 | 99.27 |
| | | | | 2.02 | 2.07 | | | | | |
| **IV** | γ-Cl | Calc | 1.32 | 2.04 | 2.10 | 2.48 | 2.48 | 171.08 | 102.87 | 105.07 |
| | δ-Cl | Calc | 1.31 | 2.06 | 2.10 | 2.51 | 2.49 | 180.00 | 167.77 | 178.35 |
| | ε-Cl | Calc | 1.31 | 2.05 | 2.06 | 2.49 | 2.49 | 94.01 | 93.75 | 169.57 |

I to IV stands respectively for $RuCl_2(Azpy)_2$, $RuCl_2(tazpy)_2$, $RuCl_2(4mazpy)_2$ and $RuCl_2(5mazpy)_2$ complexes.

### 3.2 Electronic Structure Parameters

#### 3.2.1. Free Enthalpy and Frontier Molecular Orbital Analysis

Table 2 compares the free enthalpy and orbital frontier's energy of each isomer. They are all negative. Which indicates that the synthesis of all complexes is possible at 273.15 K and 1 atm. Moreover, α-$RuCl_2L_2$ is the most stable isomers which shows the lowest energy, regardless the nature of ligand. However, apart from γ-$RuCl_2(tazpy)_2$, γ-$RuCl_2L_2$ is the less stable isomer since it presents the highest energy. Furthermore, regarding the type of isomers, we can remark that the free enthalpy increases when azpy is substituted by methylated ligand thereby destabilizing the complex of ruthenium. The ligands 4mazpy and 5mazpy, which present the methyl substituent on the pyridine ring keep the same order of stability of isomers as well as azpy: $\Delta G°(\alpha\text{-Cl}) < \Delta G°(\beta\text{-Cl}) < \Delta G°(\varepsilon\text{-Cl}) < \Delta G°(\delta\text{-Cl}) < \Delta G°(\gamma\text{-Cl})$. It means that a methyl group on pyridine ring, regardless its position has nothing to do with the rank of stability of the complexes. And the most stable isomer are those where both chloride atoms are in *cis* position. However, when the methyl group is bound to the phenyl ring through $RuCl_2(tazpy)_2$, the order is modified as follow: $\Delta G°(\alpha\text{-Cl}) < \Delta G°(\delta\text{-Cl}) < \Delta G°(\beta\text{-Cl}) < \Delta G°(\gamma\text{-Cl}) < \Delta G°(\varepsilon\text{-Cl})$. Here, the stability of δ-Cl and γ-Cl is increased when ε-Cl destabilizes. Anyhow, α-Cl remains the most stable isomer no matter how methyl group is linked to azpy ligand.

The frontier molecular orbitals (FMOs), in particular, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) [32] determine the way a given molecule interacts with other species such as DNA and play a major role in governing many chemical reactions of molecules [33]. The HOMO energy determines the ability of a compound to donate an electron while the LUMO energy determines its

ability to accept an electron [34] [35]. The composition of the FMOs of α-, β-, γ-, δ- and ε-$RuCl_2L_2$ have been calculated and are presented in Table 2.

We can see therein that the HOMOs of the complexes $RuCl_2L_2$ come mainly from d orbitals of the Ru ion while the components of LUMOs come from p orbitals of C and N atoms in azopyridine ligands, i.e. they are characterized by p orbitals of the ligands. Besides, Figures 3-6 emphasize this allocation through the iso-surfaces of the FMOs of the complexes. Therefore, the electronic transitions between them are assigned to singlet metal-to-ligand charge-transfer transitions ($^1$MLCT) [36]. It results from this analysis that the components characteristic of FMOs of all studied $RuCl_2L_2$ complexes are similar to those of $RuCl_2(azpy)_2$.

According to the frontier molecular orbital theory [37] [38] and the perturbation molecular orbital theory [39] [40], the reactions are controlled by orbital's interactions between reactant molecules. The high activity of the complex is proportional to the low value of the gap energy $\Delta E_{L\text{-}H}$. it characterizes also photochemical sensitiveness of the molecule. Therefore, through Table 2, the increase of the order of the gap energy of isomers $RuCl_2L_2$ is as follow: ΔE(δ-Cl)< ΔE(γ-Cl)< ΔE(ε-Cl)< ΔE(α-Cl)< ΔE(β-Cl). In addition, the methylated ligands reduce drastically the gap energy of α-Cl isomers comparatively to α-$RuCl_2(azpy)_2$. Whereas in both γ-Cl and ε-Cl isomers, the gap energy increases less. However in β-Cl, it remains slightly the same. Anyway, it comes of this analysis that the most active as photo sensitive complex is assumed to be δ-Cl isomers regardless the nature of azopyridine ligand. In addition, the highest energy for HOMO of DNA and the lowest one for LUMO of the azopyridine complexes $RuCl_2L_2$ are more advantageous regarding the reaction or electronic interaction between them [38]. According to Chen et al. [24], ligands play a key role in affecting their binding affinity to DNA, i.e. the lowest LUMO's energy of the complex must bind easily to the DNA. So the most active complex binding to DNA is assumed to display the lowest LUMO energy. Therefore, the energy of LUMO for the isomers $RuCl_2(azpy)_2$ and $RuCl_2(5mazpy)_2$ are in the order of $E_{LUMO}$(δ-Cl)<$E_{LUMO}$(γ-Cl)<$E_{LUMO}$(ε-Cl)< $E_{LUMO}$(α-Cl)< $E_{LUMO}$(β-Cl). However, regarding $RuCl_2(tazpy)_2$ and $RuCl_2(4mazpy)_2$, the orders of the energy of LUMO are respectively $E_{LUMO}$(δ-Cl) <$E_{LUMO}$(γ-Cl) < $E_{LUMO}$(α-Cl) < $E_{LUMO}$(ε-Cl) < $E_{LUMO}$(β-Cl) and $E_{LUMO}$(δ-Cl)< $E_{LUMO}$(ε-Cl)< $E_{LUMO}$(γ-Cl)< $E_{LUMO}$(α-Cl)< $E_{LUMO}$(β-Cl). It confirms that the most active complex remains δ-$RuCl_2L_2$. Also, we can assume that the complex is more active towards DNA molecule when both chloride atoms are in *trans* position forming then a perpendicular angle with the azopyridine ligands.

**Table 2 :** Free enthalpy (in kcal) and HOMO-LUMO gaps of some frontiers orbitals (in ev).

| | Isomers | $E_{HOMO}$ | $\mathcal{E}_{LUMO}$ | $\Delta E_{L\text{-}H}$ | ΔG° |
|---|---|---|---|---|---|
| *$RuCl_2(azpy)_2$* | α-Cl | -5.554 | -3.333 | 2.221 | -16.989 |
| | β-Cl | -5.525 | -3.224 | 2.301 | -13.796 |
| | γ–Cl | -5.386 | -3.366 | 2.020 | -9.010 |
| | δ-Cl | -5.229 | -3.429 | 1.800 | -10.110 |
| | ε-Cl | -5.402 | -3.363 | 2.039 | -10.889 |
| *$RuCl_2(tazpy)_2$* | α-Cl | -5.461 | -3.296 | 2.165 | -16.077 |
| | β-Cl | -5.494 | -3.190 | 2.304 | -14.336 |
| | γ–Cl | -5.398 | -3.317 | 2.081 | -11.267 |
| | δ-Cl | -5.251 | -3.455 | 1.796 | -15.476 |
| | ε-Cl | -5.397 | -3.274 | 2.123 | -9.033 |
| *$RuCl_2(4mazpy)_2$* | α-Cl | -5.312 | -3.216 | 2.096 | -13.142 |
| | β-Cl | -5.407 | -3.103 | 2.304 | -10.369 |
| | γ–Cl | -5.266 | -3.233 | 2.033 | -6.086 |
| | δ-Cl | -5.101 | -3.305 | 1.796 | -7.437 |
| | ε-Cl | -5.276 | -3.236 | 2.040 | -7.568 |
| *$RuCl_2(5mazpy)_2$* | α-Cl | -5.302 | -3.189 | 2.113 | -16.808 |
| | β-Cl | -5.401 | -3.088 | 2.313 | -13.721 |
| | γ–Cl | -5.250 | -3.235 | 2.015 | -8.594 |
| | δ-Cl | -5.110 | -3.293 | 1.817 | -10.478 |
| | ε-Cl | -5.292 | -3.224 | 2.068 | -10.926 |

**Table 3** Frontier molecular orbital and compositions of the ground states of α-, β-, γ-, δ- and ε-$RuCl_2L_2$, at the B3LYP/Lanl2DZ.

| | Isomers | Orbital | | | composition % | | | |
|---|---|---|---|---|---|---|---|---|
| | | index | orbital | Energy (eV) | Ru | Cl | Ligand | Main bond type |
| | α-Cl | 112 | L | -3.333 | 11 | 2 | 87 | d(Ru) + p(Cl) + π(azpy) |
| | | 111 | H | -5.554 | 46 | 34 | 20 | d(Ru) + p(Cl) + π(azpy) |
| | β-Cl | 112 | L | -3.224 | 14 | 2 | 84 | d(Ru) + p(Cl) + π(azpy) |
| | | 111 | H | -5.525 | 45 | 35 | 20 | d(Ru) + p(Cl) + π(azpy) |
| I | γ -Cl | 112 | L | -3.366 | 8 | 3 | 89 | d(Ru) + p(Cl) + π(azpy) |
| | | 111 | H | -5.386 | 53 | 26 | 21 | d(Ru) + p(Cl) + π(azpy) |
| | δ -Cl | 112 | L | -3.429 | 4 | 3 | 93 | d(Ru) + p(Cl) + π(azpy) |
| | | 111 | H | -5.229 | 60 | 30 | 10 | d(Ru) + p(Cl) + π(azpy) |
| | ε-Cl | 112 | L | -3.363 | 6 | 1 | 93 | d(Ru) + p(Cl) + π(azpy) |
| | | 111 | H | -5.402 | 55 | 35 | 10 | d(Ru) + p(Cl) + π(azpy) |
| | α-Cl | 120 | L | -3.296 | 1 | 1 | 98 | d(Ru) + p(Cl) + π(tazpy) |
| | | 119 | H | -5.461 | 51 | 23 | 26 | d(Ru) + p(Cl) + π(tazpy) |
| | β-Cl | 120 | L | -3.19 | 13 | 2 | 85 | d(Ru) + p(Cl) + π(tazpy) |
| | | 119 | H | -5.494 | 46 | 33 | 21 | d(Ru) + p(Cl) + π(tazpy) |
| II | γ -Cl | 120 | L | -3.317 | 8 | 3 | 89 | d(Ru) + p(Cl) + π(tazpy) |
| | | 119 | H | -5.398 | 54 | 26 | 20 | d(Ru) + p(Cl) + π(tazpy) |
| | δ -Cl | 120 | L | -3.455 | 2 | 3 | 95 | d(Ru) + p(Cl) + π(tazpy) |
| | | 119 | H | -5.251 | 58 | 26 | 16 | d(Ru) + p(Cl) + π(tazpy) |
| | ε-Cl | 120 | L | -3.274 | 6 | 2 | 92 | d(Ru) + p(Cl) + π(tazpy) |
| | | 119 | H | -5.397 | 56 | 32 | 12 | d(Ru) + p(Cl) + π(tazpy) |
| | α-Cl | 120 | L | -3.216 | 1 | 2 | 97 | d(Ru) + p(Cl) + π(4mazpy) |
| | | 119 | H | -5.312 | 42 | 34 | 24 | d(Ru) + p(Cl) + π(4mazpy) |
| | β-Cl | 120 | L | -3.103 | 14 | 2 | 84 | d(Ru) + p(Cl) + π(4mazpy) |
| | | 119 | H | -5.407 | 46 | 34 | 20 | d(Ru) + p(Cl) + π(4mazpy) |
| III | γ -Cl | 120 | L | -3.233 | 8 | 3 | 89 | d(Ru) + p(Cl) + π(4mazpy) |
| | | 119 | H | -5.266 | 53 | 25 | 22 | d(Ru) + p(Cl) + π(4mazpy) |
| | δ -Cl | 120 | L | -3.305 | 2 | 3 | 95 | d(Ru) + p(Cl) + π(4mazpy) |
| | | 119 | H | -5.101 | 58 | 26 | 16 | d(Ru) + p(Cl) + π(4mazpy) |
| | ε-Cl | 120 | L | -3.236 | 6 | 2 | 92 | d(Ru) + p(Cl) + π(4mazpy) |
| | | 119 | H | -5.276 | 56 | 32 | 12 | d(Ru) + p(Cl) + π(4mazpy) |
| | α-Cl | 120 | L | -3.189 | 1 | 2 | 97 | d(Ru) + p(Cl) + π(5mazpy) |
| | | 119 | H | -5.302 | 43 | 33 | 24 | d(Ru) + p(Cl) + π(5mazpy) |
| | β-Cl | 120 | L | -3.088 | 13 | 3 | 84 | d(Ru) + p(Cl) + π(5mazpy) |
| | | 119 | H | -5.401 | 46 | 34 | 20 | d(Ru) + p(Cl) + π(5mazpy) |
| IV | g-Cl | 120 | L | -3.235 | 7 | 3 | 90 | d(Ru) + p(Cl) + π(5mazpy) |
| | | 119 | H | -5.25 | 54 | 25 | 21 | d(Ru) + p(Cl) + π(5mazpy) |
| | δ -Cl | 120 | L | -3.293 | 3 | 3 | 94 | d(Ru) + p(Cl) + π(5mazpy) |
| | | 119 | H | -5.11 | 60 | 30 | 10 | d(Ru) + p(Cl) + π(5mazpy) |
| | ε-Cl | 120 | L | -3.224 | 6 | 1 | 93 | d(Ru) + p(Cl) + π(5mazpy) |
| | | 119 | H | -5.292 | 55 | 34 | 11 | d(Ru) + p(Cl) + π(5mazpy) |

### 3.2.2. Dipole Moment

The highest cytotoxicity depends on Log P which can be qualitatively analyzed by using the dipole moment. The high value of dipole moment implies the poor solubility in organic solvent and a strong solubility in water [20]. Table 4 gives the computed dipole moment of the five isomers $RuCl_2L_2$ for the four azopyridine complexes. Within this Table, the order of the total dipole moment (μ) of the isomers $RuCl_2L_2$ is μ(ε-Cl) > μ(β-Cl) > μ(α-Cl) > μ(γ-Cl) > μ(δ-Cl). It means that for each complex, δ-Cl isomer represents here the most soluble molecule compound in organic solution and shall display the highest cytotoxicity [25] [20]. Besides, the methyl group in azpy increases the dipole moment in γ-Cl and δ-Cl isomers comparatively to the reference $RuCl_2(Azpy)_2$. Anyway, the dipole moment enhances in both γ-Cl and δ-Cl stressing that chloride atoms are in *trans* position .

**Table 4.** Dipole moment of the five isomers calculated in Debye.

| | | μ | | | |
|---|---|---|---|---|---|
| | **isomers** | **x** | **y** | **z** | **Total** |
| **$RuCl_2(azpy)_2$** | α-Cl | 0 | 0 | -7.2606 | 7.2606 |
| | β-Cl | -1.7373 | 0.9617 | 8.6087 | 8.8347 |
| | γ–Cl | 0 | 0 | 1.6738 | 1.6738 |
| | δ-Cl | 0 | 0 | -1.3303 | 1.3303 |
| | ε-Cl | 0 | 0 | -10.0245 | 10.0245 |
| **$RuCl_2(tazpy)_2$** | α-Cl | 0 | 0 | -7.8575 | 7.8575 |
| | β-Cl | -3.2116 | 0.6980 | 7.9602 | 8.6120 |
| | γ–Cl | 0 | 0 | 2.5473 | 2.5473 |
| | δ-Cl | 0 | 0 | -1.5043 | 1.5043 |
| | ε-Cl | 0 | 0 | -10.3571 | 10.3571 |
| **$RuCl_2(4mazpy)_2$** | α-Cl | 0 | 0 | -7.5132 | 7.5132 |
| | β-Cl | 2.2009 | 3.2763 | 8.5112 | 9.3823 |
| | γ–Cl | 0 | 0 | 2.8452 | 2.8452 |
| | δ-Cl | 0 | 0 | -1.7997 | 1.7997 |
| | ε-Cl | 0 | 0 | -11.1797 | 11.1797 |
| **$RuCl_2(5mazpy)_2$** | α-Cl | 0 | 0 | -6.7355 | 6.7355 |
| | β-Cl | 3.1066 | 2.3961 | 8.4286 | 9.2970 |
| | γ–Cl | 0 | 0 | 3.3813 | 3.3813 |
| | δ-Cl | 0 | 0 | -1.6551 | 1.6551 |
| | ε-Cl | 0 | 0 | -10.9747 | 10.9747 |

### 3.2.3. Atomic Net Charge

Atomic charge population in a molecule through computations permits to emphasize its electrophilic or nucleophilic reactions and the charge interactions between two molecules. Table 5 displays the net charge populations regarding the Ru ion, the azopyridine ligands (azpy, tazpy, 4mazpy and 5mazpy), and the Cl atoms in the five isomers of $RuCl_2L_2$.

From Table 5, the atomic charge populations in the five isomers, irrespective to the shape of the ligand are almost the same. Ru ion and azopyridine ligands display the positive charge while the negative charge is carried by Cl atoms. Chen et al. showed that the ligand displaying the highest charge is also assumed to develop a strong affinity to bind to the DNA [24] [25]. So, we can find that the next charge order of the ligands for $RuCl_2(azpy)_2$ and $RuCl_2(4mazpy)_2$ is $Q_L(\delta\text{-Cl})>Q_L(\gamma\text{-Cl})>Q_L(\varepsilon\text{-Cl})>Q_L(\alpha\text{-Cl})>Q_L(\beta\text{-Cl})$.
However, this order is slightly modified with $RuCl_2(tazpy)_2$ and $RuCl_2(5mazpy)_2$ as $Q_L(\delta\text{-Cl})>Q_L(\gamma\text{-Cl})> Q_L(\alpha\text{-Cl})>Q_L(\varepsilon\text{-Cl})> Q_L(\beta\text{-Cl})$ and reveals that the most active isomer anyhow to bind to DNA base pairs according to ligands positive charge remains $\delta$-$RuCl_2L_2$. Besides, it comes of this analysis that the *trans* position of both chloride atoms is by far favorable to increase the ligand's charge regardless its nature.

**Table 5.** Atomic net charge of Ru, ligand azopyridine and Cl atoms in .le

| | Total natural charge | | | |
|---|---|---|---|---|
| | isomers | Ru | Ligand | Cl |
| $RuCl_2(azpy)_2$ | α-Cl | 0.590 | 0.434 | -1.024 |
| | β-Cl | 0.580 | 0.420 | -1.000 |
| | γ–Cl | 0.550 | 0.480 | -1.030 |

| | | | | |
|---|---|---|---|---|
| | δ-Cl | 0.550 | 0.517 | -1.067 |
| | ε-Cl | 0.580 | 0.476 | -1.056 |
| $RuCl_2(tazpy)_2$ | α-Cl | 0.585 | 0.471 | -1.056 |
| | β-Cl | 0.597 | 0.428 | -1.025 |
| | γ–Cl | 0.560 | 0.486 | -1.046 |
| | δ-Cl | 0.549 | 0.531 | -1.080 |
| | ε-Cl | 0.581 | 0.469 | -1.050 |
| $RuCl_2(4mazpy)_2$ | α-Cl | 0.588 | 0.466 | -1.054 |
| | β-Cl | 0.588 | 0.432 | -1.020 |
| | γ–Cl | 0.550 | 0.486 | -1.036 |
| | δ-Cl | 0.551 | 0.525 | -1.076 |
| | ε-Cl | 0.589 | 0.473 | -1.062 |
| $RuCl_2(5mazpy)_2$ | α-Cl | 0.585 | 0.479 | -1.064 |
| | β-Cl | 0.587 | 0.429 | -1.016 |
| | γ–Cl | 0.547 | 0.493 | -1.040 |
| | δ-Cl | 0.544 | 0.531 | -1.075 |
| | ε-Cl | 0.587 | 0.475 | -1.062 |

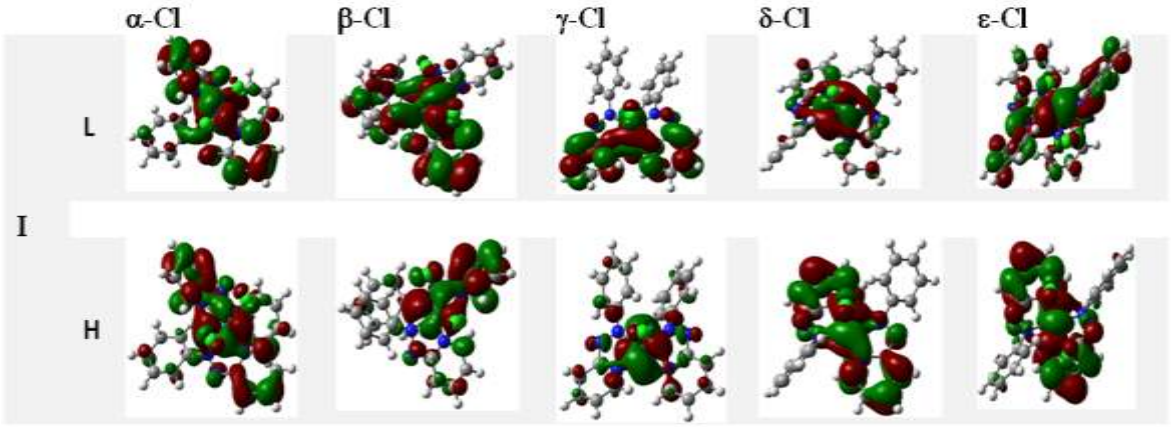


**Figure 3** Frontier molecular orbitals and associated electronic transition for $RuCl_2(azpy)_2$ calculated at B3LYP/LanL2DZ in Gas.

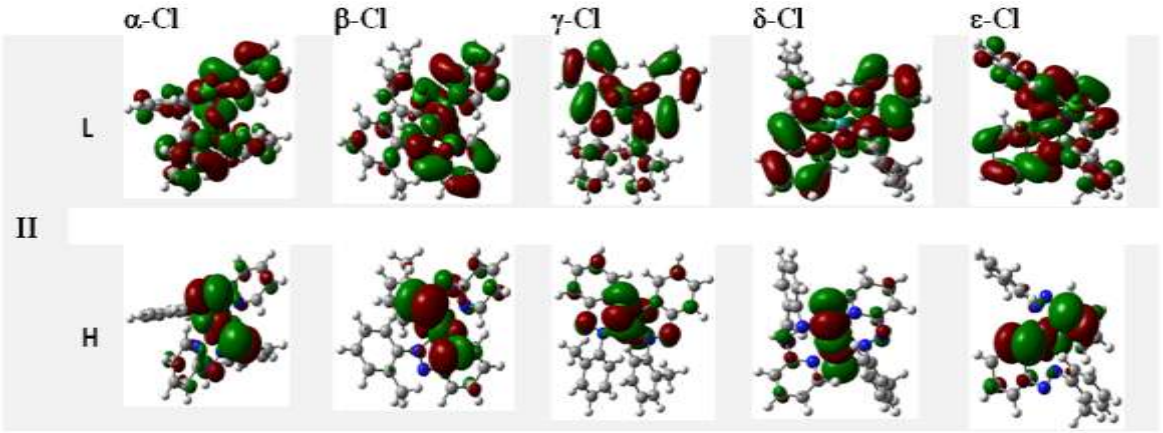


**Figure 4** Frontier molecular orbitals and associated electronic transition for $RuCl_2(tazpy)_2$ calculated at B3LYP/LanL2DZ in Gas.

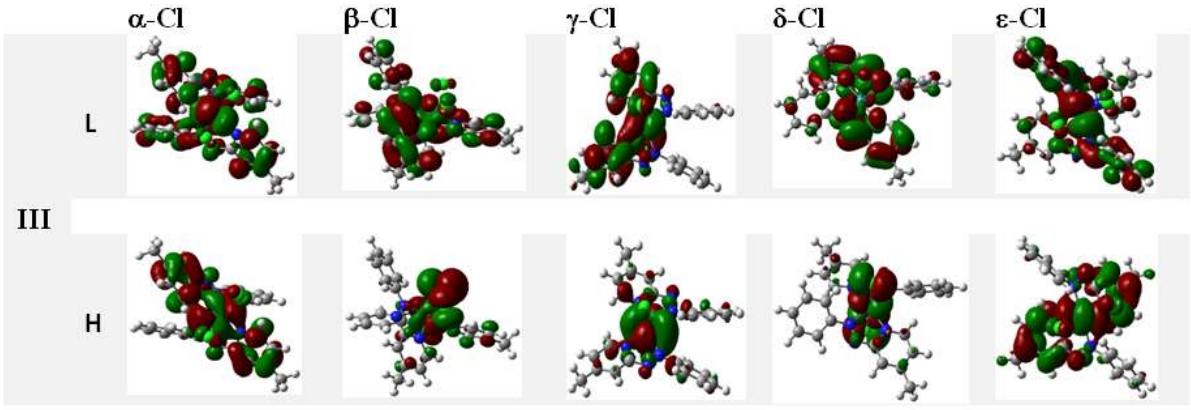


**Figure 5** Frontier molecular orbitals and associated electronic transition for $RuCl_2(4mazpy)_2$ calculated at B3LYP/LanL2DZ in Gas.

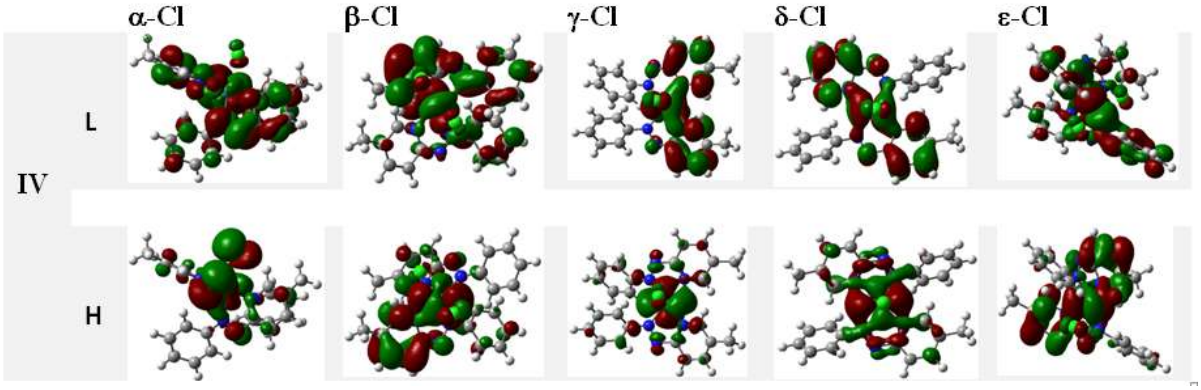


**Figure 6** Frontier molecular orbitals and associated electronic transition for $RuCl_2(5mazpy)_2$ calculated at B3LYP/LanL2DZ in Gas.

**3.3 Structure-Activity Relationships of $RuCl_2L_2$ (L= azpy, tazpy, 4mazpy)**

The $IC_{50}$ values of three isomeric α-, β- and γ-$RuCl_2L_2$ (L= azpy, tazpy, 4mazpy) of azopyridine ruthenium(II) complexes against a series of tumor-cell lines were determined by Hotze et al. [6]. These values compared to the cisplatin and 5-fluorouracil are reported in Table 6. Those known values are then compared to those of $RuCl_2(5mazpy)_2$ so that its reactivity can be predicted.

The trend in DNA-binding affinities of the complexes can be theoretically explained as follow. Regarding the intercalation mode, when there is interaction between the complex and the DNA, then the base pairs on DNA are electron-donors and the complex is electron-acceptor [28, 41]. Therefore, based on the frontier molecular orbital theory, the DNA-binding affinity of the complex should mainly rely on two factors:

The first factor that is the LUMO energy of the complex must be lower to receive the electrons provided by the HOMO of the DNA base pairs.

The second one is the planarity area of the intercalative ligand that is required to be the larger possible to favorise the interaction between DNA and the complex [42]. This latter factor can be determined by considering the structure of the five isomers. In fact, the five isomers are divided in two groups in respect of both chloride atoms as displayed in Fig. 2. The first group comprise the two isomers δ-Cl and γ-Cl where both Cl atoms are in *trans* configuration. In this case, both azopyridine ligands are parallel and planar. However, the second group including α-Cl, β-Cl and ε-Cl presents both chloride atoms in *cis* configuration where both ligands are assumed to be perpendicular. Based on this analysis and according to the abovementioned statement, we can admit that only δ-Cl and γ-Cl isomers must be advantageous to bind to DNA base pairs.[20]

From Table 6, the activity of the three isomeric of $RuCl_2(azpy)_2$ and $RuCl_2(4mazpy)_2$ complexes was classified as following: A(γ-Cl) > A(α-Cl) >A(β-Cl). Whereas, the order of activity of the three isomers of $RuCl_2(tazpy)_2$ was A(α-Cl) > A(γ-Cl)> A(β-Cl). The modification of the rank of activity can be explained by the steric hindrance created by the substituent methyl group on the phenyl ring.

In addition, the frontier molecular orbital play an important role in the reaction. A higher HOMO energy of a reactant molecule and a lower LUMO energy of another are more profitable to the

reaction between the two molecules. By the DFT method, Kurita et al. reported a series of energies of molecular orbitals for stacked DNA-base-pairs with backbone [28]. The three occupied MOs lying near the HOMO of DNA (-1.27, -1.33, -1.69, -1.79 eV) were higher and provide with the DNA molecule a good electron-donor. From Table 2, the LUMO energies of isomers are much lower than the above energies of the HOMOs of the DNA-base-pairs. These results show that the LUMO of the complex must easily accept the electrons offered by the HOMO of DNA-base-pairs due to the orbital interaction. So, in respect to the HOMO energy of the DNA, the LUMO energy of isomers provided by azopyridine ligand was classified as following $E_{LUMO}$(δ-Cl)<$E_{LUMO}$(γ-Cl)<$E_{LUMO}$(ε-Cl)< $E_{LUMO}$(α-Cl)< $E_{LUMO}$(β-Cl), and thus their binding affinities (*B*) to DNA should be *B*(δ-Cl)>*B*(γ-Cl)>*B*(ε-Cl)>*B*(α-Cl)>*B*(β-Cl). Such order is in agreement with that of the anticancer activity (*A*) of three isomers, i.e., *A*(γ-Cl) > *A*(α-Cl) >*A*(β-Cl). In addition, the reactivity of the molecule can be predicted by the HOMO-LUMO gap. The smaller HOMO-LUMO gaps $\Delta E_{L\text{-}H}$ is at the origin of a great reactivity of the molecule. The order of the HOMO-LUMO gap which is ΔE(δ-Cl)< ΔE(γ-Cl)< ΔE(ε-Cl)< ΔE(α-Cl)< ΔE(β-Cl), is also in agreement with that in their anticancer-activity.

Considering the hydrophobic parameter expressed by log P, it is used to express the absorption of the pharmaceutical drug. The fat-solubility of complexes indicates their ability to penetrate cells and to bind to the target. Theoretically, the parameter that describes the hydrophobic factor is dipole moment. In this way, the low value of dipole moment implies an efficient fat soluble and effortless absorption. From Table 4, the order of the total dipole moments (μ) of the isomers being μ(ε-Cl) > μ(β-Cl) > μ(α-Cl) > μ(γ-Cl) > μ(δ-Cl), it results from this analysis that δ-Cl shall display the highest cytotoxicity regardless the ligand structure.

At last, the populations of the atomic net charges may have some effects on the anticancer-activity of the isomers. Since DNA-base-pairs carry an abundance of negative charge, the intercalative ligand with more positive charge must be advantageous to accepting the electrons from DNA-base-pairs. Therefore, the order of the net charge populations on the ligands azpy, 4mazpy and tazpy, is commonly $Q_L$(*δ*-Cl) > $Q_L$(*γ*-Cl) > $Q_L$(*ε*-Cl) > $Q_L$(*α*-Cl) >$Q_L$(*β*-Cl) in agreement with that in their anticancer-activities. In consequence for all azopyridine ligand, δ-Cl has the highest affinity to accept electron from the DNA.

### 3.4 Prediction for activities of isomeric complexes □-, □-, □-, □-□-[Ru(5mazpy)$_2$Cl$_2$]

The isomeric complexes α-, β-, γ-, δ-, ε-Ru(5mazpy)$_2$Cl$_2$ are calculated by using the same method and basis set. The geometric and electronic parameters are listed in Tables 1-5 respectively. Regarding the geometrical structure of the complexes, their structure-activity relationship can be enhanced if both 5mazpy ligands are in the same plan. Hence, only γ-Cl and δ-Cl match with that structure [24] [25]. Concerning the first group comprising α-Cl, β-Cl and ε-Cl, both ligands are perpendicular. Therefore, the intercalative mode between the DNA base-pairs is hindered. Therefore, the most practical and competitive structures that are allowed to bind to DNA are γ-Cl and δ-Cl. The energies ($E_{LUMO}$) of the LUMO are in sequence of $E_{LUMO}$(δ-Cl)<$E_{LUMO}$(γ-Cl)<$E_{LUMO}$(ε-Cl)< $E_{LUMO}$(α-Cl)< $E_{LUMO}$(β-Cl), and the gap energy (ΔE) between the LUMOs and HOMOs, are in sequence of ΔE(δ-Cl)< ΔE(γ-Cl)< ΔE(ε-Cl)< ΔE(α-Cl)< ΔE(β-Cl). The total dipole moments (μ) of the isomers are in sequence of μ(ε-Cl) > μ(β-Cl) > μ(α-Cl) > μ(γ-Cl) > μ(δ-Cl). Moreover, the order of positive charges ($Q_L$) in the 5mazpy ligand is $Q_L$(*δ*-Cl) > $Q_L$(*γ*-Cl) > $Q_L$(*ε*-Cl) > $Q_L$(*α*-Cl) >$Q_L$(*β*-Cl). Based on the above discussions regarding the structure-activity relationship of the ruthenium azopyridine complexes in previous paragraph, we can predict that δ-[Ru(5mazpy)$_2$Cl$_2$] has also the highest anticancer activity according to the above-mentioned main factors, i.e., geometric configuration, $E_{LUMO}$, ΔE, μ and $Q_L$.

**Table 6.** IC50 values (lμM) of a series of ruthenium(II) complexes, cisplatin and 5-fluorouracil against a series of tumor-cell lines (MCF-7, EVSA-T, WIDR, IGROV, M19, A498 and H266)

| Tested compound | A498 | EVSA-T | H226 | IGROV | M19 | MCF-7 | WIDR |
|---|---|---|---|---|---|---|---|
| **α-RuCl$_2$(azpy)$_2$** | 0.27 | 0.063 | 0.48 | 0.27 | 0.064 | 0.27 | 0.27 |
| **β-RuCl$_2$(azpy)$_2$** | 8.8 | 0.96 | 13 | 3.4 | 0.75 | 6.2 | 11 |
| **γ-RuCl$_2$(azpy)$_2$** | 0.2 | 0.019 | 0.17 | 0.041 | 0.017 | 0.052 | 0.065 |
| **α-RuCl$_2$(tazpy)$_2$** | 0.36 | < 0.0056 | 0.03 | 0.0088 | < 0.006 | 0.021 | 0.045 |
| **β-RuCl$_2$(tazpy)$_2$** | 74 | 17 | 29 | 30 | 15 | 32 | 52 |
| **γ-RuCl$_2$(tazpy)$_2$** | 1.2 | 0.011 | 0.083 | 0.077 | 0.019 | 0.093 | 0.23 |
| **α-RuCl$_2$(4mazpy)$_2$** | 1.1 | 0.079 | 0.46 | 0.22 | 0.065 | 0.42 | 0.8 |
| **β-RuCl$_2$(4mazpy)$_2$** | 43 | 2.5 | 18 | 14 | 4.8 | 15 | 21 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **γ-$RuCl_2(4mazpy)_2$** | 0.5 | 0.013 | 0.17 | 0.14 | < 0.006 | 0.079 | 0.2 |
| **Cisplatin** | 7.5 | 1.4 | 11 | 0.6 | 1.9 | 2.3 | 3.2 |
| **5-Fluorouracil** | 11 | 3.7 | 2.6 | 2.3 | 3.4 | 5.8 | 1.7 |

## IV. CONCLUSION

Theoretical studies of the five isomers α-Cl, β-Cl, γ-Cl, δ-Cl and ε-Cl of $RuCl_2L_2$ (with L= azpy, 4mazpy, 5mazpy and tazpy) show that the different ligands have some interesting structure activity-relationships. Their structures are closely correlated to their anticancer activities by analyzing their electronic and geometric structure and relating them to their cytotoxic activities. First, it requires that the complex displays ligand in the same plan for the best intercalation of ligands between the double helical DNA base pairs. In this condition, δ-Cl and γ-Cl are admitted to be the most advantageous. Second, the order of LUMO energies of the all complexes greatly increases in the order of $E_{LUMO}$(δ-Cl)<$E_{LUMO}$(γ-Cl)<$E_{LUMO}$(ε-Cl)<$E_{LUMO}$(α-Cl)<$E_{LUMO}$(β-Cl) and the energy differences ΔE between the LUMOs and HOMOs, are in the sequence of ΔE(δ-Cl)< ΔE(γ-Cl)< ΔE(ε-Cl)< ΔE(α-Cl)< ΔE(β-Cl). So, δ-Cl is still the best indicated to accept the electrons of DNA base-pairs in π-π stacking interactions. Third, the order of total dipole moments of the isomers, being closely relative to the hydrophobic parameters of the molecules are in order μ(ε-Cl) > μ(β-Cl) > μ(α-Cl) > μ(γ-Cl) > μ(δ-Cl). Herewith, the lower dipole moment of δ-Cl indicates that the isomer is the best absorbed in organic solution. Fourth, the ability to accept the electron from DNA was performed thanks to the positive charges in the ligand. According to the charge classification, $Q_L(\delta\text{-Cl}) > Q_L(\gamma\text{-Cl}) > Q_L(\varepsilon\text{-Cl}) > Q_L(\alpha\text{-Cl}) > Q_L(\beta\text{-Cl})$, δ-Cl isomers display the high positive charge. All these properties are advantageous to the DNA-binding affinity of δ-$RuCl_2L_2$. Furthermore, when comparing the activity of all the four (4) δ-Cl complexes regardless the structure of the ligand, we can remark that δ-$RuCl_2(azpy)_2$ remains the most active.

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