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SARs Investigation of α-, β-, γ-, δ-, ε-$RuCl_2(Azpy)_2$ Complexes as Antitumor Drugs

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Abstract

Structure Activity-Relationships (SARs) of the five possible isomers of $RuCl_2(Azpy)_2$ were predicted thanks to DFT method. Azpy stands for 2-phenylazopyridine and the structure of the isomers α-$RuCl_2(Azpy)_2$, β-$RuCl_2(Azpy)_2$, γ-$RuCl_2(Azpy)_2$, δ-$RuCl_2(Azpy)_2$ and ε-$RuCl_2(Azpy)_2$ call respectively α-Cl, β-Cl, γ-Cl, δ-Cl and ε-Cl are defined according to chlorine atoms orientations. Hence, they are divided into two groups. In the first group comprising α-Cl, β-Cl and ε-Cl, both chlorine atoms are in *cis* position and Azpy ligands are intervertical. Whereas the two others isomers (γ-Cl and δ-Cl), they form the second group. Here, both chlorine are in *trans* position and Azpy are planar. The five synthesized isomers were investigated as potential antitumor agents. Then, regarding the DNA, its bases are stacked by pair. Therefore, complexes are assumed to insert and to stack on them through intercalative mode. So the electronic and geometric structures become more important to describe their SARs. Consequently, group 2 regarding γ-Cl and δ-Cl presents the best structure to allow intercalation between DNA base-pairs. Besides, the energy order of the lower unoccupied molecular orbital (LUMO) of the isomers is $E_{LUMO}(\beta\text{-Cl}) > E_{LUMO}(\alpha\text{-Cl}) > E_{LUMO}(\varepsilon\text{-Cl}) > E_{LUMO}(\gamma\text{-Cl}) > E_{LUMO}(\delta\text{-Cl})$. The energy gap between LUMO and HOMO was also sorted as $\Delta_{(L\text{-}H)}(\beta\text{-Cl}) > \Delta_{(L\text{-}H)}(\alpha\text{-Cl}) > \Delta_{(L\text{-}H)}(\varepsilon\text{-Cl}) > \Delta_{(L\text{-}H)}(\gamma\text{-Cl}) > \Delta_{(L\text{-}H)}(\delta\text{-Cl})$. In addition, the total dipole moment was classified as $\mu(\varepsilon\text{-Cl}) > \mu(\beta\text{-Cl}) > \mu(\alpha\text{-Cl}) > \mu(\gamma\text{-Cl}) > \mu(\delta\text{-Cl})$. Finally, net charge of the ligand Azpy was also classified as $Q_L(\delta\text{-Cl}) > Q_L(\gamma\text{-Cl}) > Q_L(\varepsilon\text{-Cl}) > Q_L(\alpha\text{-Cl}) > Q_L(\beta\text{-Cl})$. All those parameters show that δ-Cl isomer displays the highest activity as antitumor drug when intercalating between the DNA base-pairs Cytosine-Guanine/Cytosine-Guanine (CG/CG).

Keywords

Structure Activity-Relationship (SARs), Ru (II) Complexes, Azpy, DFT, Lanl2dz, DNA-Binding

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1. Introduction

The interest in the bidentate 2-phenylazopyridine ligand (Azpy) is due to its ability to stabilize ruthenium at a low state of oxidation [1]-[3]. The complexes performed are so far exploited as electrochemical catalysts or photochemistry sensitizers [4] [5]. Recently, the discovery of their cytotoxic activities [6] [7] has increased the interest of researchers to find out the origin of that tremendous breakthrough and to check out how those molecules eliminate the tumor cells and hence to see how to improve the process [8].

Azopyridine ligands as indicated in **Figure 1** are actually made of azo group bound to pyridine ring. This group of molecule is admitted to be the skeleton of all azopyridine ligands. They form with the metal a stable complex of a five atoms ring that limit the ruthenium at a low state of oxidation of II or III.

The 2-phenylazopyridine is assumed to be the ancestor ligand that is up today the most exploited with ruthenium atom. However, many other types of azo ligand are also being experienced [9] [10].

According to the synthesis, the complex of ruthenium performed with Azpy ligand is $RuCl_2(Azpy)_2$. However, the non symmetry of the ligand Azpy gives actually rise to five isomers complexes named α-Cl, β-Cl, γ-Cl, δ-Cl and ε-Cl [12]. The difference between them comes mainly from the position of chloride atoms (*cis* or *trans* configuration) and both Azpy ligands as indicated in **Figure 2**.

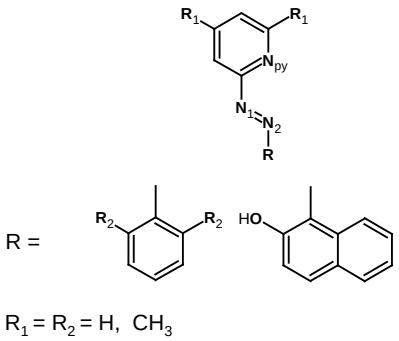

Figure 1. Skeleton of azopyridine ligand with several substituants R, R_1 and R_2. The bidentate state of the ligand consists of the ligand binding to ruthenium or central metal by N_{py} and N_2 [11]. Azpy corresponds to R_1 = R_2 = H and R equal to phenyl ring.

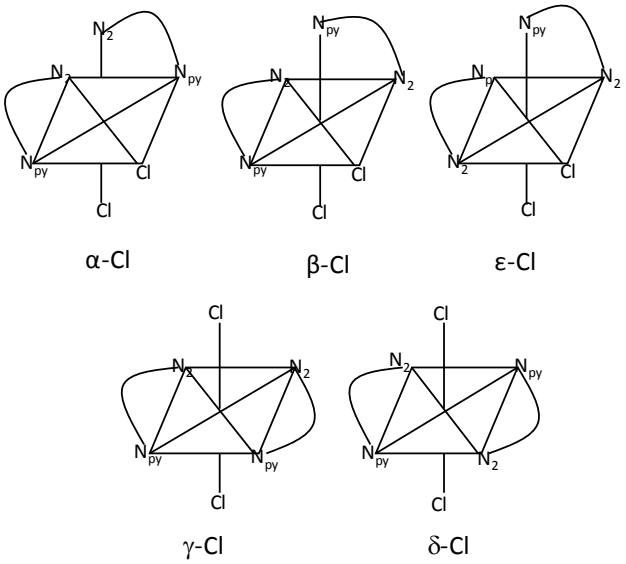

Figure 2. The five isomers of $RuCl_2L_2$ complexes. L stands for all azopyridine ligand comprising Azpy. The arcs represent azopyridine ligands highlighting their bidentate state.

In reality, they have been synthesized by different team of researchers regarding their fascinating activity. Except *β*-Cl, all of them present a C_2 symmetry. Thus, both azopyridine ligands are equivalent. Besides, regarding the symmetrical complexes, only *δ*-Cl shows different chloride atoms. Hitherto, the major synthesis consist of merging reactant $RuCl_3 \cdot 3H_2O$ with Azpy ligand [13] [14]. Here, only *γ*-Cl and *δ*-Cl are obtained with this process. Then, the three others are assumed to be obtained otherwise [15]. Nonetheless, even if the *ε*-Cl has been synthesized once [16], it has not yet been properly characterized. Therefore, the most tested isomers by experiment Among them up today remain only *α*-, *β*-, *γ*-$[RuCl_2L_2]$ where L stands for 2-phenylazopyridine (Azpy), o-tolylazopyridine and 4-methyl-2-phenylazopyridine as drug against tumors cell [17] [18]. Regarding $RuCl_2(Azpy)_2$ complexes, *α*-Cl isomer was experimentally assumed to be the most stable and the most biologically active as antitumor drug. Moreover, its activity is outstanding since it is recognized to replace the known cisplatin (*cis*-$PtCl_2(NH_3)_2$) [7]. Whereas $RuCl_2(Mazpy)_2$ (Mazpy = 4-methyl-2-phenylazopyridine), Chen *et al.* admitted by theoretical approach that the most active complex is *γ*-Cl [19] confirming the experimental work performed by Hortze *et al.* [18].

Furthermore, the cytotoxic activity consists of the complex binding to DNA that will induce the death of the cell. However, there exist two processes through which the ruthenium complexes bind to DNA base-pairs similarly to platinum drugs. The first trend indicates that the chloride atoms are actually hydrolyzed. Therefore the ruthenium is allowed to bind covalently to the nucleobase of the DNA [20] [21]. Moreover, the coordination of ruthenium can be mono or bifunctional with bases like adenine [22] [23]. The second and the most accepted trend indicates that the bonding is performed between the azopyridine ligand and the DNA base-pairs by a π-π stacking interaction [19] [24]. Here, the necessity for chloride atoms to hydrolyze is not required. Anyhow, the complex is necessarily bound to DNA base-pairs that are assumed to be the electron donor. Therefore, the aggregate must hydrolyze subsequent to the apoptosis of tumor cell owing to the coordination of the complex to the DNA base. Furthermore, it is accepted that during the coordination, the anticancer drugs insert and stack between the base-pairs of double helical DNA defining thus an intercalative binding mode [25] [26]. In reality, the stacking DNA has been theoretically studied by Kurita and Kobayashi. According to them, the stacking of DNA is made by the exclusive combination performed between Thymine pairing Adenine and Cytosine binding to Guanine by hydrogen bond according to Watson-Crick hydrogen bonds theory. Besides, three modes of combination of both bases Cytosine and Guanine through the double-helical strands were possible: Cytosine-Guanine/Cytosine-Guanine (CG/CG), Cytosine-Cytosine/Guanine-Guanine (CC/GG) and Guanine-Cytosine/Guanine-Cytosine (GC/GC). They also assumed that the most stable combination was CG/CG both with and without sugars and phosphate groups backbones [27]. Besides, it can also be assumed that the intercalation of the ruthenium complexes is the most privileged when it is only performed over this CG/CG combination with π-π interaction. Yet, CG couple is displayed in **Figure 3**.

It was reported that the possibility of the interaction between DNA and ruthenium complexes requires to know the electronic structure of both of them. It is to say that the energies, the components of the frontier molecular orbitals, the atomic charge populations and the geometrical structure of not merely ruthenium complexes but also HOMO-LUMO predictions of the DNA play an important role [17]. Thus, it becomes interesting to find out the DNA-binding affinities of complexes and further to study their structure-activity relationships (SARs).

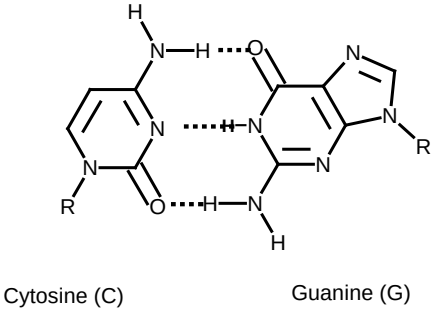

Figure 3. Combination mode of both bases of DNA according to Watson-Crick hydrogen bonds theory. That couple of bases plays the key role when DNA binds to ruthenium complexes by intercalating the ruthenium complexes through a π-π stacking interaction.

We remind that the stability of the stacked CG/CG was determined by comparing the HOMO energies of all the three isomers since it is admitted that the bonding between the stacked DNA base-pairs and the drug comes from electrons exchange. So, HOMO should belong to DNA and LUMO comes from the drug.

In this paper, we study theoretically the structure-activity relationships SARs of the five α-, β-, γ-, δ- and ε-$RuCl_2(Azpy)_2$ isomers named respectively α-Cl, β-Cl, γ-Cl, δ-Cl and ε-Cl. This will be the occasion to figure out which of all the complexes shall actually have the highest cytotoxic activity. We remember that only the first three complexes have been experimentally studied [7]. Besides, the theoretical prediction of SARs has been so far slightly studied [27]. Regarding $RuCl_2(Azpy)_2$ complexes, they have never been theoretically considered before. Therefore, this study has to compare the activity of the five isomers though their energies are very close. So, we will performed the calculation by using the widespread intercalative mode of ligands between the DNA base-pairs CG/CG.

2. Method

All the calculations were performed with DFT method using Becke's three-parameter hybrid B3LYP [28] and the double-zeta pseudo-potential LANL2DZ [29] basis set. Before each calculation, the complexes were optimized first with frequency analysis to know of the absence of eventual imaginary frequencies. 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. Regarding the HOMO energy of the staked DNA base-pairs CG/CG, it was calculated by Kurita and Kobayashi [27]. Besides, all these calculations were performed thanks to Gaussian 03 package [30].

3. Results and Discussion

3.1. Geometrical Parameters

Table 1 displays some selected computational bond lengths and angles of the five isomers. Those values have been compared to experimental data. We can see that experimental data match well with theoretical ones with almost the same slight difference for all complexes. Regarding α-Cl, it displays as well as γ-Cl, δ-Cl and ε-Cl one data per bond indicating its C_2 symmetrical structure. Whereas β-Cl isomer, it indicates two values for each actually indistinguishable pair of bonding. It confirms its non symmetrical structure. As mentioned in **Figure 2**, the five isomers are in reality divided in two groups referred to the chloride atoms positions. The first group concerns α, β and ε-$RuCl_2(Azpy)_2$ isomers where both Cl atoms are in *cis* position. Necessarily, both Azpy ligands are vertical and Cl-Ru-Cl angles are respectively 90.60˚, 90.18˚ and 94.10˚. Regarding the second group comprising γ- and δ-$RuCl_2(Azpy)_2$, they display both Cl atoms in *trans* position. Here, Cl-Ru-Cl angles are respectively 170.71˚ and 180˚. In consequence, both ligands are horizontal. Regarding γ-$RuCl_2(Azpy)_2$, the shortness value than 180˚ of Cl-Ru-Cl angle must be due to Yahn-Teller effect [31].

3.2. Electronic Structure Parameters

3.2.1. Free Enthalpy and Frontier Molecular Orbital Energies

Table 2 compares the free enthalpy and orbital frontier's energy of each isomer. As divided in two groups, the group 1 comprising α, β, ε-$RuCl_2(Azpy)_2$ displays the most stable isomers. In fact, even if all energies are closed, the most stable isomer showing up the lowest energy is α-$RuCl_2(Azpy)_2$. However, the complex that presents the highest energy is γ-$RuCl_2(Azpy)_2$. Generally speaking, we can establish the order of free enthalpy by $G^{\circ}(\alpha\text{-Cl}) < G^{\circ}(\beta\text{-Cl}) < G^{\circ}(\varepsilon\text{-Cl}) < G^{\circ}(\delta\text{-Cl}) < G^{\circ}(\gamma\text{-Cl})$.

According to the frontier orbital molecular definition, the high activity of the complex is proportional to the low value of the energy gap $\Delta E_{(L\text{-}H)}$. Otherwise, the molecule is admitted to be active if the HOMO-LUMO gap is small. Therefore, through **Table 2** we can classify the energy gap of isomers as $\Delta E(\beta\text{-Cl}) > \Delta E(\alpha\text{-Cl}) > \Delta E(\varepsilon\text{-Cl}) > \Delta E(\gamma\text{-Cl}) > \Delta E(\delta\text{-Cl})$. Thus, the most active complex is δ-Cl. Actually, it is reported regarding the intercalative mode that the biological reactivity of the complex is due to facile exchange of electron between HOMO of the DNA base-pairs and the LUMO of the complex knowing that the bonding of both molecules requires electronic interaction between them [32]. So the most active complex is assumed to display the lowest LUMO energy. In reference to that, the classification of the reactivity by LUMO energy is $E_{LUMO}(\beta\text{-Cl}) > E_{LUMO}(\alpha\text{-Cl}) > E_{LUMO}(\varepsilon\text{-Cl}) > E_{LUMO}(\gamma\text{-Cl}) > E_{LUMO}(\delta\text{-Cl})$ always confirming that the most active complex is

Table 1. Selected geometrical parameters of the five isomers calculated at B3LYP/LANL2DZ level. Distances are written in Å and angles in. They are compared to experimental data [9].

Atoms	$RuCl_2(Azpy)_2$								
	α-Cl		*β*-Cl		*γ*-Cl		*δ*-Cl		*ε*-Cl
	Theory	Experiment	Theory	Experiment	Theory	Experiment	Theory	Experiment	Theory
N_1=N_2	1.32	1.28	1.32 1.32	1.29 1.3	1.32	1.31	1.31	1.28	1.32
Ru-N_2	2.03	1.98	2.02 2.05	1.96 2.0	2.03	1.99	2.06	2.02	2.05
Ru-N_{py}	2.06	2.05	2.05 2.07	2.02 2.06	2.10	2.11	2.10	2.06	2.06
Ru-Cl_1	2.48	2.40	2.48	2.40	2.48	2.38	2.51	2.38	2.49
Ru-Cl_2	2.48	2.40	2.48	2.41	2.48	2.38	2.49	2.38	2.49
Cl_1-Ru-Cl_2	90.60	89.50	90.18	91.10	170.71	170.50	180.00	180.00	94.10
N_{py}-Ru-N_{py}	178.37	174.50	99.21	101.90	102.86	103.80	167.53	180.00	93.58
N_2-Ru-N_2	101.49	93.50	104.58	103.00	104.99	104.10	178.58	180.00	169.48

Table 2. Three enthalpy and HOMO-LUMO gaps (ΔE_{L-H}) of some frontiers orbitals in a.u.

Isomers	LANL2DZ			
	HOMO	LUMO	$\Delta E_{(L-H)}$	G°
α-$RuCl_2(Azpy)_2$	−0.205	−0.122	0.082	−1301.072
β-$RuCl_2(Azpy)_2$	−0.203	−0.118	0.0845	−1301.067
γ-$RuCl_2(Azpy)_2$	−0.198	−0.124	0.074	−1301.059
δ-$RuCl_2(Azpy)_2$	−0.192	−0.126	0.066	−1301.061
ε-$RuCl_2(Azpy)_2$	−0.198	−0.123	0.075	−1300.062

δ-Cl. Moreover, **Figure 4** displays the LUMO graphs of the isomers. We can see that the components carrying the energy come from p orbitals of N and C atoms. Therefore, we can actually assume that LUMO energy is carried by the ligand Azpy.

3.2.2. Dipole Moment

The hydrophobic value Log P that expresses the solubility of compound either in organic solvent or in water can be determined by the computed dipole moment. In fact, the dipole moment indicates the water-solubility strength of a molecule. In consequence, the high value implies the poor solubility in organic solvent and a strong solubility in water. Actually, the efficient drugs are fat-soluble since many antimetastatic drugs perform their activity in organic solvent [33]. Therefore, **Table 3** emphasizes the solubility of the isomers of $RuCl_2(Azpy)_2$ by comparing their computed dipole moment. We can observe the order as following μ(*ε*-Cl) > μ(*β*-Cl) > μ(*α*-Cl) > μ(*γ*-Cl) > μ(*δ*-Cl). It means that *δ*-Cl represents here the most active compound whose Log P is significant. Therefore, it shall display the highest cytotoxicity.

3.2.3. Atomic Net Charge

The population of atomic net charge has been determined on behalf of the natural atomic population (NPA). It shows the affinity for atom or molecule to gain electron. Regarding **Table 4**, Ru atom and Azpy ligands display the positive charge while the negative charge is carried by Cl atoms. As aforementioned that the intercalation of ruthenium complexes between DNA base-pairs allows the ligands to be the electron acceptor since they display the LUMO of the complexes and because of their electron affinity. Regarding the energy, the stacked DNA base-pairs CG/CG was predicted by Kurita and Kobayashi and its HOMO energy was −1.27 eV. However, when comparing to LUMO energies of the complexes that are comprised between −3.22 and −3.43 eV (1 a.u. = 27.21

Table 3. Dipole moment of the five isomers calculated in Debye.

	μ			
	x	y	z	Total
α-$RuCl_2(Azpy)_2$	0	0	−7.2606	7.2606
β-$RuCl_2(Azpy)_2$	−1.7373	0.9617	8.6087	8.8347
γ-$RuCl_2(Azpy)_2$	0	0	1.6738	1.6738
δ-$RuCl_2(Azpy)_2$	0	0	−1.3303	1.3303
ε-$RuCl_2(Azpy)_2$	0	0	−10.0245	10.0245

Table 4. Atomic net charge of Ru, ligand Azpy and Cl atoms in |e|.

$RuCl_2(Azpy)_2$	Total natural charge		
	Ru	Ligand Azpy	Cl
α-Cl	0.59	0.43	−1.02
β-Cl	0.58	0.42	−1.00
γ-Cl	0.55	0.48	−1.03
δ-Cl	0.55	0.52	−1.07
ε-Cl	0.58	0.48	−1.06

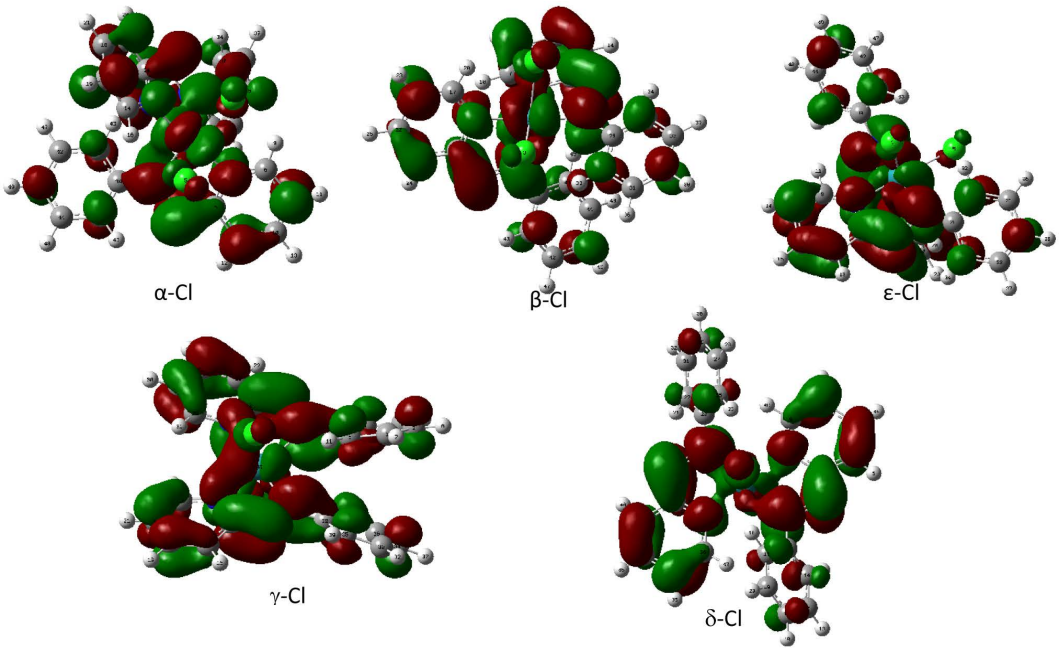

Figure 4. LUMO graphs of $RuCl_2(Azpy)_2$ complexes displayed by group. The energy of LUMO is carried by N and C atoms of both ligands Azpy.

eV), we notice that the DNA energy is higher. In consequence, ligands are reported to play a key role in affecting their binding affinity to DNA. *i.e.* the lower LUMO energy of the complex must bind easily to the DNA [19]. Moreover, the ligand displaying the highest charge is also assumed to develop a strong affinity to bind to the DNA. Therefore, the charge order computed of the ligands is $Q_L(\delta\text{-Cl}) > Q_L(\gamma\text{-Cl}) > Q_L(\varepsilon\text{-Cl}) > Q_L(\alpha\text{-Cl}) > Q_L(\beta\text{-Cl})$ stressing that the most positive charge of ligands belongs to isomer δ-Cl. So, we can assume that the most active complex to bind to DNA according to ligands positive charge is δ-$RuCl_2(Azpy)_2$.

3.3. Structure-Activity Relationships (SARs)

SARs consists of finding out a relation between electronic properties of ruthenium complexes and their cytotoxicity activities. As assumed before, Velders *et al.* [7] have already experimentally carried out the test *in vitro* of the three isomers α-Cl, β-Cl and γ-Cl of $RuCl_2(Azpy)_2$ over seven types of human cancer presented in **Table 5**.

In **Table 5**, the three isomers were compared to the Cisplatin (CPT) and the 5-Fluorouracil (5-FU) that were before admitted to be the most active molecules ever discovered. Here, α-Cl was found more antitumor active than β-Cl and γ-Cl isomers. Moreover, its activity was better than that of CPT and 5-FU regarding the breast cancer (MCF-7, EVSA-T), Melanoma (M19-MEL) and colon cancer (WIDR). Besides, the activity of the three complexes was classified as following : A(α-Cl) > A(β-Cl) > A(γ-Cl). Furthermore, three reasons seemed explain for the high activity of α-Cl isomer [34]:

1) The increase in the rate of chloride hydrolysis due the π-acceptor effect of the azopyridine ligands increasing the effective charge on the metal ion so that the hydrolysis rates are in the range of cisplatin;

2) The increased hydrophobic or intercalative interactions with DNA, which may facilitate covalent binding;

3) And the geometric effects exerted by the ligands, which may facilitate (or inhibit) protein binding to the nucleic acid.

Herewith, ruthenium complex was admitted to undergo a chloride hydrolysis before binding covalently to DNA base. Also, the bonding was performed between ruthenium and the DNA base purine and guanine derivatives [35]. However, considering that the most important DNA-binding mode remains the intercalative one since the drugs are assumed to insert and to stack between the base-pairs of DNA strands [36], it becomes necessary to consider this mode and compare then the activity of all $RuCl_2(Azpy)_2$ isomers. Therefore, the parameters affecting DNA-binding affinities of the complex must be the ligands planarity structure, the energy and population of the lowest unoccupied molecular orbital (LUMO) of the complex [37].

Regarding the geometrical structure of the complexes, their structure-activity relationship can be enhanced if both Azpy ligands are in the same plan. Hence, only γ-Cl and δ-Cl match with that structure [19]. Concerning the first group comprising α-Cl, β-Cl and ε-Cl, both ligands are perpendiculars. Therefore, the intercalative mode between the DNA base-pairs is hindered. Therefore, the most practical and competitive structures that favor the binding are γ-Cl and δ-Cl.

Considering the frontier molecular orbital, the reaction is the most efficient between two molecules when the HOMO of the first molecule (electron donor) is close to the LUMO of the second one (electron acceptor). If the HOMO is carried by the DNA base-pairs and the LUMO by ruthenium complex then the most reactive complex must have the lowest LUMO energy. In consequence, knowing the HOMO energy of DNA fixed to −1.27 eV according to Kurita and Kobayashi, the LUMO energy of isomers provided by Azpy ligands was classified as following $E_{LUMO}(\beta\text{-Cl}) > E_{LUMO}(\alpha\text{-Cl}) > E_{LUMO}(\varepsilon\text{-Cl}) > E_{LUMO}(\gamma\text{-Cl}) > E_{LUMO}(\delta\text{-Cl})$. Here again, δ-Cl is assumed to display the most available ligands to easily accept electrons from HOMO of DNA base-pairs.

Regarding the hydrophobic parameter expressed by log P, it expresses the absorption of the pharmaceutical drug. It is actually a main parameter regarding the studies of the quantitative structure-activity relationship QSAR of biological molecules [38]. Theoretically, the parameter that describes the hydrophobic factor is dipole moment. In fact, high dipole moment means high water-soluble and difficult absorption. However, the low value of dipole moment imply an efficient fat-soluble and effortless absorption. Therefore, in reference to **Table 3**, the

Table 5. IC_{50} (the concentration of material required to inhibit the growth of a pratical cell line by 50%) values (μM) of α-Cl, β-Cl, γ-Cl, Cisplatin (CPT) and 5-Fluorouracil (5-FU) against a series of tumor cell lines.

	MCF-7	EVSA-T	WIDR	IGROV	M19	A498	H266
α-$RuCl_2(Azpy)_2$	0.6	0.1	1.9	0.8	0.2	1.2	1.5
β-$RuCl_2(Azpy)_2$	4.1	1.9	11.2	7.3	2.5	8.8	10
g-$RuCl_2(Azpy)_2$	5.9	5.4	16.6	11.8	4.5	15.3	14.8
5-FU	5.8	3.7	1.7	2.3	3.4	1.1	2.6
CPT	2.3	1.4	3.2	0.6	1.9	7.5	10.9

classification of computed dipole moment of isomers as $\mu(\varepsilon\text{-Cl}) > \mu(\beta\text{-Cl}) > \mu(\alpha\text{-Cl}) > \mu(\gamma\text{-Cl}) > \mu(\delta\text{-Cl})$ shows up that δ-Cl shall display the highest cytotoxicity.

Finally, the atomic net charge of complexes must have an effect on their ability to bind by intercalation between DNA base-pairs. As DNA base-pairs possess the HOMO molecular orbitals, they carry naturally negative charge. Therefore, they bonding to ligands requires that the ligands carry high positive charge. Thus, **Table 4** presents the net charge of ligands Q_L in the isomers. The classification of ligands charges as follow $Q_L(\delta\text{-Cl}) > Q_L(\gamma\text{-Cl}) > Q_L(\varepsilon\text{-Cl}) > Q_L(\alpha\text{-Cl}) > Q_L(\beta\text{-Cl})$ highlights that the ligand Azpy in δ-Cl isomer has the highest affinity to accept electron from the DNA.

4. Conclusion

The cytotoxicity of five isomers α-$RuCl_2(Azpy)_2$, β-$RuCl_2(Azpy)_2$, γ-$RuCl_2(Azpy)_2$, δ-$RuCl_2(Azpy)_2$ and ε-$RuCl_2(Azpy)_2$ were theoretically investigated by DFT-B3LYP method using the pseudo-potential LANL2DZ basis set. Their Structure activity-relationships (SARs) were performed by analyzing their electronic and geometric structure and relating them to their cytotoxic activities. Besides, three modes regarding their bonding to DNA base-pairs are discussed nowadays. The most accepted mode that regards the insertion and the stacking of complexes between the double helical DNA base-pairs consists of intercalation of ligands. Therefore, it requires that the complex displays ligands in the same plan. Its LUMO energy must be low to allow the bonding to the electron donor DNA base-pairs. Also, the complex must possess a low dipole moment that characterizes its absorption in organic solution. Moreover, as the bonding is performed between DNA and electron acceptor ligands Azpy, the ligand must consequently display the high positive charge. Considering the aforementioned properties, we can assume that the most cytotoxic isomers by intercalative mode between DNA base-pairs is δ-$RuCl_2(Az\text{-}py)_2$.

References

[1] Goswami, S., Chakravarty, A.R. and Chakravorty, A. (1981) Chemistry of Ruthenium. 2. Synthesis, Structure, and Redox Properties of 2-(Arylazo) Pyridine Complexes. *Inorganic Chemistry*, **20**, 2246-2250. http://dx.doi.org/10.1021/ic50221a061

[2] Chakravarty, A.R. and Chakravorty, A. (1981) Synthesis, Structure, and Electrochemistry of Dihalobis (.Alpha.-Benzil Oximato) Ruthenium (III). *Inorganic Chemistry*, **20**, 275. http://dx.doi.org/10.1021/ic50215a055

[3] Krause, R.A. and Krause, K. (1982) Chemistry of Bipyridyllike Ligands. 2. Mixed Complexes of Ruthenium (II) with 2-(Phenylazo) Pyridine: A New.pi.-Bonding Probe. *Inorganic Chemistry*, **21**, 1714-1720. http://dx.doi.org/10.1021/ic00135a002

[4] Jorna, A.J., Boelrijk, A.E.M., Hoorn, H.J. and Reedijk, J. (1996) Heterogenization of a Ruthenium Catalyst on Silica and Its Application in Alcohol Oxidation and Stilbene Epoxidation. *Reactive & Functional Polymers*, **29**, 101. http://dx.doi.org/10.1016/1381-5148(96)00005-3

[5] Boelrijk, A.E.M., Jorna, A.M. and Reedjik, J. (1995) Oxidation of Octyl α-d-Glucopyranoside to Octyl α-d-Glucuronic Acid, Catalyzed by Several Ruthenium Complexes, Containing a 2-(Phenyl) Azopyridine or a 2-(Nitrophenyl) Azopyridine Ligand. *Journal of Molecular Catalysis A-Chemical*, **103**, 73. http://dx.doi.org/10.1016/1381-1169(95)00112-3

[6] Hotze, A.C.G., Velders, A.H., Ugozzoli, F., Biagini-Cingi, M., Manotti-Lanfredi, A.M., Haasnoot, J.G. and Reedijk, J. (2000) Synthesis, Characterization, and Crystal Structure of α-$[Ru(azpy)_2(NO_3)_2]$ (azpy = 2-(Phenylazo) Pyridine) and the Products of Its Reactions with Guanine Derivatives. *Inorganic Chemistry*, **39**, 3838-3844. http://dx.doi.org/10.1021/ic000066v

[7] Velders, A.H., Kooijman, H., Spek, A.L., Haasnoot, J.G., De Vos, D. and Reedijk, J. (2000) Strong Differences in the *in Vitro* Cytotoxicity of Three Isomeric Dichlorobis (2-Phenylazopyridine) Ruthenium(II) Complexes. *Inorganic Chemistry*, **39**, 2966-2967. http://dx.doi.org/10.1021/ic000167t

[8] Hotze, A.C.G., Bacac, M., Velders, A.H., Jansen, B.A.J., Kooijman, H., Spek, A.L., Haasnoot, J.G. and Reedijk, J. (2003) New Cytotoxic and Water-Soluble Bis (2-Phenylazopyridine) Ruthenium (II) Complexes. *Journal of Medicinal Chemistry*, 1743-1750. http://dx.doi.org/10.1021/jm021110e

[9] Misra, T.K., Das, D. and Sinha, C. (1997) Chemistry of Azoimidazoles: Synthesis, Spectral Characterization and Redox Properties of *bis*(N(1)-alkyl-2-(arylazo) Imidazole) Copper (I) and Silver(I) Complexes. *Polyhedron*, **16**, 4163-4170. http://dx.doi.org/10.1016/S0277-5387(97)00127-7

[10] Lu, T.-H., Misra, T.K., Lin, P.-C., Liao, F.-L. and Chung, C.-S. (2003) Synthesis and X-Ray Characterization of Two Isomeric Dichloro Bis-{1-(phenylazo) isoquinoline} Complexes of Ruthenium (II). *Polyhedron*, **22**, 535. http://dx.doi.org/10.1016/S0277-5387(02)01436-5

[11] Affi, S.T., Bamba, K. and Ziao, N. (2015) Computational Characterization of Organometallic Ligands Coordinating Metal: Case of Azopyridine Ligands. *Journal of Theoretical and Computational Chemistry*, **14**, Article ID: 1550006. http://dx.doi.org/10.1142/s0219633615500066

[12] Velders, A.H., Van der Schilden, K., Hoste, A.C.G., Reedijk, J., Kooijman, H. and Speck, A.L. (2004) Dichlorobis (2-Phenylazopyridine) Ruthenium (II) Complexes: Characterisation, Spectroscopic and Structural Properties of Four Isomers. *Dalton Transactions*, 448-455. http://dx.doi.org/10.1039/b313182c

[13] Bamba, K., Leger, J.-M., Garnier, E., Bachmann, C., Servat, K. and Kokoh, K.B. (2005) Selective Electrooxidation of D-Glucose by $RuCl_2(azpy)_2$ Complexes as Electrochemical Mediators. *Electrochimica Acta*, **50**, 3341-3346. http://dx.doi.org/10.1016/j.electacta.2004.12.007

[14] Campbell, N., Henderson, A.W. and Taylor, D.J. (1953) Geometrical Isomerism of Azo-Compounds. *Journal of the Chemical Society*, 1281. http://dx.doi.org/10.1039/jr9530001281

[15] Seal, A. and Ray, S. (1984) Structures of Two Isomers of Dichlorobis (2-Phenylazopyridine) Ruthenium (II), $[RuCl_2(C_{11}H_9N_3)_2]$. *Acta Crystallographica Section C*, **40**, 929-932. http://dx.doi.org/10.1107/s0108270184006302

[16] Popov, A.M., Egorova, M.B., Lutovinov, V.A. and Dmitrieva, R.I. (1990) Directed Synthesis of Unknown Isomer of Ruthenium Bis-Chelate Complex with 2-(phenylazo)pyridine. *Zhurnal Organicheskoi Khimii*, **60**, 2169-2170.

[17] Hotze, A.C.G., Van der Geer, E.P.L., Caspers, S.E., Kooijman, H., Spek, A.L., Haasnoot, J.G. and Reedijk, J. (2000) Coordination of 9-Ethylguanine to the Mixed-Ligand Compound α-[Ru(azpy)(bpy) Cl_2] (azpy = 2-Phenylazo-Pyridine and bpy = 2,2'-Bipyridine). An Unprecedented Ligand Positional Shift, Correlated to the Cytotoxicity of This Type of $[RuL_2Cl_2]$ (with L = azpy or bpy) Complex. *Inorganic Chemistry*, **43**, 4935-4943. http://dx.doi.org/10.1021/ic035390f

[18] Hotze, A.C.G., Caspers, S.E., De Vos, D., Kooijman, H., Spek, A.L., Flamigni, A., Bacac, M., Sava, G., Haasnoot, J.G. and Reedijk, J. (2004) Structure-Dependent *in Vitro* Cytotoxicity of the Isomeric Complexes $[Ru(L)_2Cl_2]$ (L= O-Tolylazopyridine and 4-Methyl-2-phenylazopyridine) in Comparison to $[Ru(azpy)_2Cl_2]$. *Journal of Biological Inorganic Chemistry*, **9**, 354-364. http://dx.doi.org/10.1007/s00775-004-0531-6

[19] Chen, J.C., Li, J., Qian, L. and Zheng, K.C. (2005) Electronic Structures and SARs of the Isomeric Complexes α-, β-, γ-$[Ru(mazpy)_2Cl_2]$ with Different Antitumor Activities. *Journal of Molecular Structure*: *THEOCHEM*, **728**, 93-101. http://dx.doi.org/10.1016/j.theochem.2005.05.005

[20] Hartmann, M., Finhauser, T.J. and Keppler, B.K. (1996) Two Antitumour Ruthenium(III) Complexes Showing Selectivity in Their Binding towards Poly(dG)·poly(dC) and Poly(dA)·poly(dT). *Chemical Communications*, 1741-1742. http://dx.doi.org/10.1039/cc9960001741

[21] Velders, A.H., Ugozzoli, F., Biagini-Cingi, M., Manotti-Lanfredi, A.M., Haasnoot, J.G. and Reedijk, J. (1999) A Unique Fourfold Intramolecular Hydrogen Bonding Stabilises the Structure of *trans*-Bis(2-amino-5,7-dimethyl [1,2,4]triazolo [1,5-*a*]pyrimidine-N^3) Aquatrichlororuthenium(III) Monohydrate. *European Journal of Inorganic Chemistry*, **1999**, 213-215. http://dx.doi.org/10.1002/(SICI)1099-0682(19990202)1999:2<213::AID-EJIC213>3.0.CO;2-2

[22] Hotze, A.C.G., Broekhuisen, M.E.T., Velders, A.H., Van der Schilden, K., Haasnoot, J.G. and Reedijk, J. (2002) Unusual Coordination of the Rare Neutral Imine Tautomer of 9-Methyladenine Chelating in the *N*6, *N*7-Mode to Ruthenium(II) Complexes. *European Journal of Inorganic Chemistry*, **2002**, 369-376. http://dx.doi.org/10.1002/1099-0682(20022)2002:2<369::AID-EJIC369>3.0.CO;2-H

[23] Velders, A.H. (2000) "*Ruthenium Complexes with Heterocyclic Nitrogen Ligands*" Design, Synthesis, Structural and Conformational Characterization, Biological Activity and the Binding to DNA Model Bases. PhD Thesis, Leiden University, Leiden.

[24] Hotze, A.C.G., Kooijman, H., Spek, A.L., Haasnoot, J.G. and Reedijk, J. (2004) Synthesis and Characterization of Ruthenium(II) Complexes with the New Ligand 2-Phenylazopyridine-5-Sulfonic Acid (Hsazpy): In Search for New Anticancer Agents. *New Journal of Chemistry*, **28**, 565-569. http://dx.doi.org/10.1039/b313746e

[25] Ji, L.N. and Zhang, Q.L. (2001) DNA Structure, Binding Mechanism and Biology Functions of Polypyridyl Complexes in Biomedicine. *Science China* (*Series B*), **44**, 246-259. http://dx.doi.org/10.1007/BF02879615

[26] Lawrence, D., Vaidyanathan, V.G. and Nair, B.U. (2006) Synthesis, Characterization and DNA Binding Studies of Two Mixed Ligand Complexes of Ruthenium(II). *Journal of Inorganic Biochemistry*, **100**, 1244-1251. http://dx.doi.org/10.1016/j.jinorgbio.2006.02.003

[27] Kurita, N. and Kobayashi, K. (2000) Density Functional MO Calculation for Stacked DNA Base-Pairs with Backbones. *Computers & Chemistry*, **24**, 351-357. http://dx.doi.org/10.1016/S0097-8485(99)00071-6

[28] Becke, A.D. (1993) A New Mixing of Hartree-Fock and Local Density-Functional Theories. *The Journal of Chemical Physics*, **98**, 1372-1377. http://dx.doi.org/10.1063/1.464304

[29] Foresman, J.B. and Frisch, Æ. (1996) Exploring Chemistry with Electronic Structure Methods. Second Edition, Gaussian Inc., Pittsburgh.

[30] Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Montgomery Jr., J.A., Vre-

ven, T., Kudin, K.N., Burant, J.C., Millam, J.M., Iyengar, S.S., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, G.A., Nakatsuji, H., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Klene, M., Li, X., Knox, J.E., Hratchian, H.P., Cross, J.B., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Ayala, P.Y., Morokuma, K., Voth, G.A., Salvador, P., Dannenberg, J.J., Zakrzewski, V.G., Dapprich, S., Daniels, A.D., Strain, M.C., Farkas, O., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Ortiz, J.V., Cui, Q., Baboul, A.G., Clifford, S., Cioslowski, J., Stefanov, B.B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Martin, R.L., Fox, D.J., Keith, T., Al-Laham, M.A., Peng, C.Y., Nanayakkara, A., Challacombe, M., Gill, P.M.W., Johnson, B., Chen, W., Wong, M.W., Gonzalez, C. and Pople, J.A. (2003) Gaussian, Inc., Pittsburgh.

[31] Shriver, D.F. and Atkins, P.W. (1999) Inorganic Chemistry. 3rd Edition, Oxford University Press, New York, 235-236.

[32] Fleming, I. (1976) Frontier Orbital and Organic Chemical Reaction. Wiley, New York.

[33] Zhao, R.I., Al-Said, N.H., Sternbach, D.L. and William Lown, J. (1997) Camptothecin and Minor-Groove Binder Hybrid Molecules: Synthesis, Inhibition of Topoisomerase I, and Anticancer Cytotoxicity *in Vitro*. *Journal of Medicinal Chemistry*, **40**, 216-225. http://dx.doi.org/10.1021/jm9605804

[34] Clarke, M.J. (2003) Ruthenium Metallopharmaceuticals. *Coordination Chemistry Reviews*, **236**, 209-233. http://dx.doi.org/10.1016/S0010-8545(02)00312-0

[35] Hotze, A.C.G., Broekhuisen, M.E.T., Velders, A.H., Kooijman, H., Spek, A.L., Haasnoot, J.G. and Reedijk, J. (2002) Crystallographic and NMR Evidence of the Unusual *N*6, *N*7-Didentate Chelation of 3-Methyladenine Coordinated to the Cytotoxic α-Dichlorobis(2-Phenylazopyridine) Ruthenium(II) Complex. *Journal of the Chemical Society, Dalton Transactions*, 2809-2810. http://dx.doi.org/10.1039/b204665m

[36] Jain, S.C., Tsai, C.C. and Sobell, H.M. (1977) Visualization of Drug-Nucleic Acid Interactions at Atomic Resolution. II. Structure of an Ethidium/Dinucleoside Monophosphate Crystalline Complex, Ethidium: 5-Iodocytidylyl (3'-5') Guanosine. *Journal of Molecular Biology*, **114**, 317-331. http://dx.doi.org/10.1016/0022-2836(77)90253-4

[37] Zheng, K.C., Deng, H., Liu, X.W., Li, H., Chao, H. and Ji, L.N. (2004) Electronic Structures, DNA-Binding and Related Properties of Complexes $[Ru(bpy)_2L]^{2+}$ (L = ip, pip, hpip). *Journal of Molecular Structure*: *THEOCHEM*, **682**, 225-233. http://dx.doi.org/10.1016/j.theochem.2004.05.026

[38] Wu, W.J., Lai, R., Zheng, K.C. and Yun, F. (2005) Quantitative Structure-Activity Relationship of Indolo[1,2-b]quinazoline Derivatives with Antitumor Activity. *Acta Physico-Chimica Sinica*, **21**, 28.