**Structure-Activity Relationships of Substituted Ruthenium Azopyridine Complexes: A DFT and QSAR Study for Anticancer Applications**

**Abstract:**

The anarchic proliferation of cells in all types of organs in the human body leads to tumours called cancer. Chemotherapy remains an alternative treatment. Transition metal-based agents such as cisplatin have been shown to be effective. However, their use is hampered by unwanted side effects. One alternative to platinum is ruthenium. Ruthenium azopyridine complexes are promising anticancer agents for the treatment of certain cancers by chemotherapy. The aim of this work is to design ruthenium azopyridine complexes with enhanced cytotoxic activity. These compounds are all derived from δ-Cl isomer of the ruthenium azopyridine complex RuCl2(Tazpy)2 by substituting toluene group of the Tazpy ligand with different benzene derivatives. The methyl substituent in toluene group is replaced by various substituents (-COCH3, -COOH, -CH2-COOH, -CH2-OH, -COH, -CONH2, -CNH2, CH3-O-, -CH2-CH3, OH-, -CH2-CH2-CH3 and -CH=CH2). A series of thirteen ruthenium complexes is studied using density functional theory (DFT) and time-dependent DFT (TD-DFT) at the B3LYP/Lanl2DZ level. Examination of the geometrical parameters of the compounds after optimization and frequency calculation indicates that all complexes have the same geometry whatever the nature of the substituent. This assumes an identical mode of interaction with the DNA base. The formation of compounds C4 and C7 is possible at temperatures above 298K. Also, the substituents -OH and -CH2-CH2-CH3 increase the chemical reactivity of the complex, while the binding and interaction affinity with DNA is amplified by the alkyl substituents (-CH2-CH3, -CH2-CH2-CH3) and -CH=CH2. The cytotoxic activity of the compounds against A498, H226, IGROV, MCF-7 and WIDR cancers is predicted using QSAR models. The substituents -CH2-COOH, -CH2-CH3, -CH2-CH2-CH3 and -CH=CH2 enhance the cytotoxicity of the ruthenium azopyridine complex. These compounds absorb in the visible range at wavelengths above 600nm. These metal ligand transitions are an advantage when it comes to using them in the photodynamic treatment of cancer or as a dye in dye-sensitized solar cells.

**Keywords:** Cancer, Ruthenium complexes, DFT, TD-DFT, Cytotoxic activity, QSAR

1. **Introduction**

Cancer is a metastatic disease that attacks every cell in the human body. There are various methods of treating cancer, including surgery, radiotherapy, phototherapy and chemotherapy. Chemotherapy, which appears to be the most effective and viable means of treating cancer, is based on powerful chemotherapeutic agents. Different modes of action for these drugs are listed. They act by targeting and destroying cancer cells through DNA alkylation (cyclophosphamide and cisplatin), by interrupting cancer cell growth by mimicking the nutrients required for DNA and RNA synthesis (Methotrexate and 5-fluorouracil), and by inhibiting cell replication (Vincristine and Paclitaxel). In a nutshell, Chemotherapeutic drugs are essential in the treatment of cancer, but their use can lead to side effects due to their impact on healthy cells (Hamroun, et al., 2018) (Ben Kridis, et al., 2021). In the case of cisplatin, which is used to treat various types of cancer, including testicular, ovarian and bladder cancers, adverse effects can include nausea, vomiting, kidney toxicities, and myelo-suppression (reduced blood cell production). Ongoing research consists on improving the efficacy and reducing the toxicity of these treatments. Ruthenium complexes are an emerging class of anticancer drugs, including compounds such as ruthenium(II) and ruthenium(III) (Ana, et al., 2019) (Affi, et al., 2015) (Anna, et al., 2005). Like cisplatin, ruthenium complexes can interact with DNA, but they also exhibit other mechanisms of action, such as:

1. The generation of free radicals, which can induce oxidative stress in cancer cells.
2. Inhibition of tumor cell-specific signaling pathways.

Thus, ruthenium complexes could offer a promising alternative for cisplatin-resistant patients. Thanks to their selectivity, ruthenium complexes could target cancer cells more specifically, reducing the effects on healthy cells. In addition, ruthenium complexes show reduced toxicity compared to cisplatin, offering the hope of fewer side effects while maintaining anti-tumor efficacy. The biological activity of ruthenium complexes is greatly influenced by the geometry and nature of the ligand (Kouakou, et al., 2017). Polypyridine ligands, in particular azopyridine ligands, have been shown to enhance the cytotoxic properties of ruthenium complexes (N'Guessan, et al., 2018) (Kafoumba, et al., 2016) (Affi, et al., 2015). These organic ligands consist of a pyridine ring and an azo group (-N=N-) linked to another aromatic ring. They bidentate bond to ruthenium from the pyridine nitrogen atom and through the azo nitrogen atom. Depending on the arrangement of the ligands in the RuCl2(azpy)2 complex, five stable isomers were formed (N'Guessan, et al., 2017). In the previous work, the study of the activity-structure relationship of these compounds has shown that molecular flatness, ligand charge (QL), dipole moment (µd), LUMO energy and the energy gap between HOMO and LUMO can be used to correlate the evolution of cytotoxic activity (N’guessan, et al., 2017). The δ-RuCl2(Azpy)2 isomer has also proved to be the most active and effective isomer for cancer treatment. In addition, quantitative structure activity relationship (QSAR) modeling was performed to explain and predict the cytotoxic activities of a series of ruthenium azopyridine complexes on cancer stem cells (kidney cancer (A498), lung cancer (H226), ovarian cancer (IGROV), breast cancer (MCF-7) and colon cancer (WIDR)) with theoretical descriptors (Table 1). The models present the following statistical indicators: regression correlation coefficient R2 =0.986 - 0.905, standard deviation S=0.516 - 0.153, Fischer test F = 106.718 - 14.220, cross-validation correlation coefficient Q2cv= 0.985 - 0.895 and R2 -Q2cv = 0.010 - 0.001. These descriptors are accredited as excellent static indicators. A strong correlation was observed between experimental and predicted values of cytotoxic activity, indicating the validity and quality of the QSAR models obtained. Furthermore, examination of the five (05) models obtained shows that the quantum chemical descriptors (QL, ΔfG° and μd) are useful for predicting the cytotoxic activity (-log(IC50\*10-6 )) of ruthenium azopyridine complexes. The present work is part of the investigation for new more effective chemotherapeutic agents. Its aim is to design and predict the cytotoxicity of ruthenium complexes derived from the δ-Cl isomer of the ruthenium azopyridine complex RuCl2(Tazpy)2 with enhanced activity. Quantum Chemical methods, in particular Density Functional Theory (DFT) and time-dependent DFT, are employed as study methods.

**Table 1.** QSAR models of cytotoxic activity on A498, H226, GROV, MCF-7 and WIDR cancer cells.

|  |  |  |
| --- | --- | --- |
| Cancer cells | Code | QSAR Models |
| Renal cancer | A498 | pIC50 = -22.805+0.222\*µ+58.264\*QL |
| Lung **cancer** | H226 | pIC50 = -18.394-0.122\*ΔrG°+50.088\*QL |
| Ovarian cancer | IGROV | pIC50 = -20.477-0.162\*ΔrG°+53.937\*QL |
| Breast cancer | MCF-7 | pIC50 = -18.740-0.102\*ΔrG°+51.530\*QL |
| Colon cancer | WIDR | pIC50 = -17.264-0.101\*ΔrG°+47.707\*QL |

**2. Method and methodology**

**2.1. Molecule presentation**

The structures of the thirteen (13) azopyridine ligands used to complex ruthenium from the RuCl3,3H2O base are listed in Table 2. These ligands are all derived from O-tolyazopyridine by substitution of the methyl group in the ortho position on the phenyl (Affi, et al., 2015).

Table 2. Structures of azopyridine ligands studied

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | | | | |
| Code | R | ligand | **Code** | R | ligand |
| C1 |  | O-éthanone-  phénylazopyridine | **C8** |  | O-méthoxy-  phénylazopyridine |
| C2 |  | O-acide benzoïqueazopyridine | **C9** |  | O-éthyl-  phénylazopyridine |
| C3 |  | O-acide acetique-phénylazopyridine | **C10** |  | O-phénolazopyridine |
| C4 |  | O-méthanol-phénylazopyridine | **C11** |  | O-propyl-phénylazopyridine |
| C5 |  | O-benzaldéhyde  azopyridine | **C12** |  | O-tolyazopyridine |
| C6 |  | O-benzamide  azopyridine | **C13** |  | O-vinyl-phénylazopyridine |
| C7 |  | O-anilineazopyridine |  |  |  |

**2.2 Calculation method**

All molecules were optimized using GAUSSIAN 09 software (Frisch, et al., 2009). DFT and TD DFT were employed at the B3LYP/Lanl2DZ and TD-B3LYP/Lanl2DZ levels of theory respectively (N'guessan, et al., 2015) (Chiodo, et al., 2006). Due to the high number of core electrons in ruthenium, the Lanl2DZ pseudopotential is used to consider the relativist state. From the ground state, the variation of thermodynamic reaction quantities is given by the relation:

∆rΦ0(298K) = (1)

Here Φ represents the thermodynamic quantity.

Natural bond orbital (NBO) analysis provides information on the interactions between the orbitals of a molecule (MOHD & ANJANI, 2014). An interaction between electron donor and acceptor, which leads to stabilization of the system, is quantified by second-order perturbation theory. The electronic stabilization energy E(2) associated with electron delocalization between donor and acceptor is estimated by second-order perturbation theory (Theivarasu & Murugesan, 2016):

(2)

Fi,j is an element of the Fock matrix, qi represents the occupancy of the donor orbital, εi and εj are the energies of the donor and acceptor NBO orbitals respectively.

The overall reactivity of the complexes formed is elucidated from descriptors such as gap ∆E, electronegativity (χ), chemical hardness (η), overall softness (σ).

These fundamental quantities in the theory of acids and bases as developed by Pearson (Pearson, 1963) can be expressed as a function of the ionization potential PI and the electronic affinity AE:

(3)

Chemical potential (µ) and electronegativity (χ) as a function of ionization potential PI and electron affinity AE :

The electron affinity AE and ionization potential PI can be calculated using the Koopmans approximation (Koopmans, 1934), according to which :

1. **AE = - ELUMO**: characterizes the molecule's suitability for nucleophilic attack.
2. **PI = - EHOMO**: characterizes the molecule's suitability for electrophilic attack.

QSAR models are used to assess the cytotoxic activity of compounds. However, these models come up against the question of the reliability of their predictions (Roy, et al., 2015). These reliable predictions generally only concern chemicals that are structurally similar to the compounds used to build the model (Sheridan, et al., 2004) . The principle of the applicability domain obliges users to specify the scope of the proposed models, thus defining the limits of the model with regard to its structural domain. The leverage method is used to determine the domain of applicability of a QSAR model. It is based on the variation of the standardized residuals of the dependent variable with the distance between the values of the descriptors and their means, called levers. If a compound has a residual and a lever that exceed the threshold **h\*=3p/n** (where p is the number of descriptors plus 1 and n the number of observations), this compound is considered outside the applicability domain of the model developed. Minitab software is used to determine the leverage. The range of applicability will be discussed using the Williams diagram, which plots standardized prediction residuals against hi lever values (Software, 2000). For each compound i in the original space of independent variables (Xi), the value of hi is calculated by the following relationship (Sahigara, et al., 2012):

where i = (1 ... n)

With Xi is the row vector of descriptors for compound i, X (n\*k-1) is the model matrix deduced from the values of descriptors in the training set; Index T denotes the transposed matrix. The critical value of the lever (h\*) is set (Netzeva, et al., 2005) at:

**h\* = 3(k+1) / n** (6)

Where n is the number of test compounds used; k is the number of model descriptors.

If hi < h\*, the probability of agreement between the measured and predicted values of compound "i" is as high as that of the compounds in the database. Compounds with hi > h\* strengthen the model when they belong to the training set, but will otherwise have dubious predicted values without necessarily being outliers, as residuals can be low (Dearden, 2016).

**3. Results and Discussions**

**3.1 Geometric study of ruthenium azopyridine complexes**

The ruthenium complex δ-RuCl2L2 (where L is an azopyridine ligand) has been shown to have C2 symmetry. These complexes, which should have an octahedral geometry, adopt this C2 symmetry due to the Yann Teller effect. So, based on the geometries (with C2 geometry constraint), we carried out geometry optimization and frequency calculations. The optimized geometries of the complexes studied are shown in Figure 1.

|  |  |  |
| --- | --- | --- |
| **C1** | **C2** | **C3** |
| **C4** | **C5** | **C6** |
| **C7** | **C8** | **C9** |
| **C10** | **C11** | |
| **C12** | **C13** |  |

**Figure 1.** Geometric structures of the ruthenium complexes studied

The effect of substitution on the structural properties of δ-RuCl2(Tazpy)2 is elucidated using Ru-X bond lengths (where X = Npy, N2 and Cl). Comparison of the bond lengths of δ-RuCl2(Tazpy)2 derivatives with those of δ-RuCl2(Tazpy)2 indicates that methyl substitution does not alter the values of bond lengths of the reference complex (**Table 3**). These values are very close to those of δ-RuCl2(azpy)2. This result shows that the nature of the substituent does not affect the geometry of the complex. Thus, the action of these compounds on DNA takes place by intercalation between two DNA strands, due to the flatness of the ligands.

**Table 3**. Comparison of bond lengths (in Å) of complexes obtained in vacuum at the B3LYP/LanL2DZ level

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Complexes | **Ru-N2** | **Ru-Npy** | **Ru-Cl1** | **Ru-Cl2** |
| C1 | 2.06 | 2.09 | 2.47 | 2.53 |
| C2 | 2.05 | 2.09 | 2.52 | 2.47 |
| C3 | 2.06 | 2.10 | 2.50 | 2.50 |
| C4 | 2.08 | 2.05 | 2.34 | 2.33 |
| C5 | 2.05 | 2.10 | 2.49 | 2.50 |
| C6 | 2.05 | 2.10 | 2.48 | 2.50 |
| C7 | 2.09 | 2.10 | 2.51 | 2.50 |
| C8 | 2.06 | 2.09 | 2.48 | 2.52 |
| C9 | 2.05 | 2.10 | 2.50 | 2.51 |
| C10 | 2.06 | 2.09 | 2.52 | 2.48 |
| C11 | 2.05 | 2.10 | 2.51 | 2.50 |
| C12 | **2.05** | **2.10** | **2.50** | **2.50** |
| C13 | 2.05 | 2.10 | 2.50 | 2.50 |

**3.2 Enthalpy and free enthalpy variation of reaction of formation of modeled complexes**

Thermodynamic quantities variations of formation reactions are calculated and reported in Table 4. The enthalpy variation reflects the thermicity of a chemical reaction, while the free enthalpy variation provides information on the spontaneity with which a chemical reaction occurs. The free enthalpy of compounds C4 and C7 are positive. This result indicates the formation of these compounds are not favourable at 298K. However, for the another compound, the free enthalpy of reaction variations recorded are negative, reflecting spontaneous formation reactions of these compounds at 298K.

**Table 4.** Thermodynamic reaction quantities and dipole moment

|  |  |  |  |
| --- | --- | --- | --- |
| Compounds | ΔH(kcal/mol) | ΔG(kcal/mol) | µD (Debye) |
| C1 | 7.788 | -1.535 | 3.751 |
| C2 | 1.106 | -7.596 | 0.318 |
| C3 | -6.906 | -13.311 | 1.635 |
| C4 | 43.397 | 37.902 | 3.091 |
| C5 | -2.163 | -11.394 | 5.178 |
| C6 | 2.384 | -6.706 | 3.774 |
| C7 | 11.112 | 2.449 | 3.236 |
| C8 | -1.984 | -12.628 | 3.536 |
| C9 | -3.229 | -14.591 | 1.676 |
| C10 | -1.512 | -10.003 | 2.218 |
| C11 | -3.640 | -15.543 | 1.664 |
| **C12** | **-3.006** | **-14.606** | **1.499** |
| C13 | -8.421 | -17.853 | 0.817 |

Synthesis of C4 and C7 complexes is not possible at this temperature. The values of the free enthalpy variation allow us to establish the following order:

***C13 ˂ C11 ˂ C12 ˂ C9 ˂ C3 ˂ C8 ˂ C5 ˂ C10 ˂ C2 ˂ C6 ˂ C1 ˂ C7 ˂ C4***

This ranking shows that the formation of compound C13 is the most spontaneous. The ethyl and propyl substituents also increase the spontaneity of the reaction.

The values for the change in enthalpy of reaction for compounds C3, C5, C8, C9, C10, C11, C12 and C13 are negative, indicating exothermic formation reactions. However, the formation reactions of compounds C1, C2, C4, C6 and C7 are endothermic due to the positive enthalpy of formation reaction values.

In addition to the thermodynamic quantities of reaction formation, the solubility of these molecules is gauged by using the dipole moment. The solubility of compounds in aqueous solution can be assessed by determining the partition coefficient log P. However, this quantity can be approximated by calculating the dipole moment μ. The dipole moment indicates the stability of a molecule in water. A high dipole moment indicates low solubility in organic solvents and high solubility in water. The theoretical values obtained for the δ-Cl isomers of ruthenium azopyridine complexes suggest the following descending order of dipole moment:

***μd( C2) ˂ μd(C13) ˂ μd (C12) ˂ μd (C3) ˂ μd (C11) ˂ μd (C9) ˂ μd (C10) ˂ μd (C4) ˂ μd (C7) ˂ μd (C8) ˂ μd (C1) ˂ μd (C6) ˂ μd (C5)***

Compound C2, which has the lowest dipole moment value, is the most soluble in organic solvents. Compound C5, on the other hand, is the most soluble in aqueous solution. Moreover, substituents containing the -COOH acid function and alkyls (ethyl and propyl) boost solubility in the organic phase. Substituents with ketone, aldehyde and amide functions are more soluble in aqueous phase (Carley & Sundberg, 2000).

**3.3 Global reactivity of ruthenium azopyridine complexes**

The HOMO and LUMO frontier orbitals govern their reactivity and chemical stability. The isodensity surfaces of these molecular orbitals are shown in Figure 2. From this figure, it can be seen that, whatever the compound, the electron density for the HOMO level in the ground state is localized on ruthenium. This orbital, the highest occupied, is made up mainly of the atomic orbitals (t2g) of ruthenium (table), materializing its metallic character. In the HOMO expression, ruthenium has the highest coefficient. It is therefore the atom subject to electrophilic attack and the electron donor site. It is therefore at the origin of the photodynamic process in photodynamic therapy. As for the LUMO, its electron density is distributed over the azopyridine ligand and chlorine atoms (figure 2). The expression of this first empty orbital shows that it is composed of the px and py orbitals of the pyridine nitrogen atoms Npy and the azo group N2, thus conferring a π-acceptor character on the azopyridine ligand. Thus, a light-induced electron shift from the HOMO of these compounds to its LUMO corresponds to a t2g→π\* MLCT transition.

In addition, based on DFT-optimized structures at the B3LYP/LanL2DZ level of theory, global reactivity parameters have been determined. These parameters are energy gap ΔE, ionization potential PI, electron affinity, chemical potential µ and overall hardness. The values of these parameters are recorded in Table 5. Figure 2 compares the energy levels of the HOMO and LUMO frontier orbitals of the thirteen compounds studied. Energy gaps are also compared.

|  |  |  |
| --- | --- | --- |
| Compounds | HOMO | LUMO |
| C1 |  |  |
| C2 |  |  |
| C3 |  |  |
| C5 |  |  |

**Figure 2.** Comparison of the isodensity surfaces of the frontier orbitals of some of the complexes studied

**Table 5.** Composition and expression of frontier orbitals

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compounds | OM | Energy (eV) | composition ( en %) | | | atomic contribution |
| Ru | Cl | Ligand |
| C1 | L | -3,494 | 12 | 8 | 80 | 0,22(N2) + 0,12 (N2) + 0,12 (Ru) |
| H | -5,348 | 42 | 17 | 41 | 0,42(Ru) + 0,12 (N2) + 0,11 (Cl) |
| C2 | L | -3,386 | 2 | 3 | 95 | 0,14(N2) + 0,14(N2) + 0,12(Npy) + 0,12(Npy) |
| H | -5,233 | 60 | 28 | 12 | 0,60(Ru) + 0,18(Cl) + 0,10(Cl) |
| C3 | L | -3,478 | 1 | 3 | 96 | 0,15(N2) + 0,14(N2) + 0,12(Npy) + 0,12(Npy) |
| H | -5,286 | 65 | 25 | 10 | 0,65(Ru) + 0,15(Cl) + 0,10(Cl) |
| C4 | L | -3,546 | 11 | 9 | 80 | 0,18(N2) + 0,16(N1) + 0,11(Ru) |
| H | -5,547 | 48 | 17 | 35 | 0,48(Ru) + 0,10(N1) 0,09(Cl) + 0,08(Cl) |
| C5 | L | -3,889 | 3 | 3 | 94 | 0,15(N2) + 0,14(N2) + 0,12(Npy) + 0,12(Npy) |
| H | -5,699 | 62 | 27 | 11 | 0,62(Ru) + 0,15(Cl) + 0,12 (Cl) |
| C6 | L | -3,882 | 4 | 3 | 93 | 0,15(N2) + 0,15 (N2) + 0,12(Npy) + 0,12(Npy) |
| H | -5,65 | 58 | 29 | 13 | 0,58(Ru) + 0,15(Cl) + 0,14(Cl) |
| C7 | L | -3,215 | 3 | 2 | 95 | 0,13(N2) + 0,13(N2) + 0,09(Npy) + 0,09(Npy) |
| H | -5,027 | 62 | 24 | 14 | 0,62(Ru) + 0,14(Cl) + 0,10 (Cl) |
| C8 | L | -3,204 | 1 | 3 | 96 | 0,14(N2) + 0,14(N2) + 0,11(Npy) + 0,11(Npy) |
| H | -4,949 | 61 | 27 | 12 | 0,61(Ru) + 0,17(Cl) + 0,11(Cl) |
| C9 | L | -3,465 | 1 | 3 | 96 | 0,15 (N2) + 0,14 (N2) + 0,12 (Npy) + 0,12 (Npy) |
| H | -5,246 | 64 | 26 | 10 | 0,64 (Ru) + 0,14 (Cl) + 0,12 (Cl) |
| C10 | L | -2,739 | 4 | 3 | 93 | 0,12(N2) + 0,12 (N2) + 0,09 (Npy) + 0,09(Npy) |
| H | -4,357 | 48 | 19 | 33 | 0,48 (Ru) + 0,11 (Cl) + 0,09 (Cl) |
| C11 | L | -2,907 | 4 | 3 | 93 | 0,12 (N2) + 0,12(N2) + 0,09(Npy) + 0,09 (Npy) |
| H | -4,529 | 47 | 19 | 34 | 0,47(Ru) + 0,10(Cl) + 0,10 (Cl) |
| C12 | L | -3,454 | 1 | 3 | 96 | 0,14 (N2) + 0,14 (N2) + 0,11(Npy) + 0,11(Npy) |
| H | -5,251 | 63 | 26 | 11 | 0,63 (Ru) + 0,14(Cl) + 0,12 (Cl) |
| C13 | L | -3,495 | 63 | 26 | 11 | 0,16(N2) + 0,14(N2) + 0,12(Npy) + 0,12(Npy) |
| H | -5,308 | 2 | 3 | 95 | 0,63(Ru) + 0,14(Cl) + 0,12(Cl) |

Interactions between two molecular entities take place between the boundary orbitals of these two entities. In the case of cancer therapy, interactions take place between the HOMO of the DNA and the LUMO of the complex. According to Kurita et al (Kurita & Kobayashi, 2000), the DNA responsible for cytotoxicity is the Cytosine-Guanine/Cytosine-Guanine (CG/CG) pair, whose HOMO energy is -1.27 eV. DNA will interact with the complex whose LUMO energy value is closest to that of the HOMO of the CG/CG pair. The order of LUMO energy (EL) values of the compounds studied is as follows (Table 6):

*EL(C5) ˂ EL(C6) ˂ EL(C4) ˂ EL(C13) ˂ EL(C1) ˂ EL(C3) ˂ EL(C9) ˂ EL(C12) ˂ EL(C2) ˂ EL(C7) ˂ EL(C8) ˂ EL(C11) ˂ EL(C10)*

Compounds C5, C6, C4, C13, C1, C3 and C9 will interact faster with DNA than the reference compound δ-RuCl2(Tazpy)2 (compound C12).

In addition to LUMO energy, we were interested in the energy gap between HOMO and LUMO of the compounds. The recorded energy gap values allow us to establish the following order of reactivity:

***C10 ˂ C11 ˂ C8 ˂ C6 ˂ C9 ˂ C12 ˂ C3 ˂ C5 ˂ C7 ˂ C13 ˂ C2 ˂ C1 ˂ C4***

**Table 6.** Global reactivity parameters of ruthenium azopyridine complexes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compounds | *Ehomo* | E*lumo* | ΔE | µ( -χ) | η |
| C1 | -5.348 | -3.494 | 1.854 | -4.421 | 1.854 |
| C2 | -5.233 | -3.386 | 1.847 | -4.310 | 1.847 |
| C3 | -5.286 | -3.478 | 1.808 | -4.382 | 1.808 |
| C4 | -5.547 | -3.546 | 2.001 | -4.547 | 2.001 |
| C5 | -5.699 | -3.889 | 1.810 | -4.794 | 1.810 |
| C6 | -5.650 | -3.882 | 1.768 | -4.766 | 1.768 |
| C7 | -5.027 | -3.215 | 1.812 | -4.121 | 1.812 |
| C8 | -4.949 | -3.204 | 1.745 | -4.077 | 1.745 |
| C9 | -5.246 | -3.465 | 1.781 | -4.356 | 1.781 |
| C10 | -4.357 | -2.739 | 1.618 | -3.548 | 1.618 |
| C11 | -4.529 | -2.907 | 1.622 | -3.718 | 1.622 |
| C12 | -5.251 | -3.454 | 1.797 | -4.353 | 1.797 |
| C13 | -5.308 | -3.495 | 1.813 | -4.402 | 1.813 |

Compounds C10 and C11 are the most reactive due to their smaller gap values. Therefore, we can assume that Substitution of methyl (C12) by OH-, CH3-CH2-CH2-, CH3-O-, -CONH2 and CH3-CH2- groups increases the chemical reactivity of the azopyridine complex.

In addition to the energy gap, global reactivity descriptors namely chemical potential (µ) and chemical hardness (η) were calculated (Table 6).

The chemical potential (μ) reflects the propensity of a molecular system to attract electrons to itself. In Table 6, the values of the chemical potential (μ) of the compounds studied make it possible to establish the following ascending order:

***C5 ˂ C6 ˂ C4 ˂ C1 ˂ C13 ˂ C3 ˂ C9 ˂ C12 ˂ C2 ˂ C7 ˂ C8 ˂ C11 ˂ C10***

From this order, it emerges that compounds C2, C7, C8, C11 and C10 are more electrophilic than compound C12. Compared to C12, these compounds are the most electron accepting of DNA.

With regard to chemical hardness, which reflects the system's resistance to electron transfer, the values recorded enable the following ranking:

***C10 ˂ C11 ˂ C8 ˂ C6 ˂ C9 ˂ C12 ˂ C3 ˂ C5 ˂ C7 ˂ C13 ˂ C2 ˂ C1 ˂ C4***

This ranking shows that compounds C3, C5, C7, C13, C2, C1 and C4 are the most resistant to electron loss or gain compared to δ-RuCl2(Tazpy)2 (C12). These compounds are therefore more stable than C12. In contrast, compounds C10, C11, C8, C6 and C9 have a greater tendency to exchange electrons with DNA than C12.

**3.4. Electronic structure analysis of ruthenium azopyridine complexes**

The formation of azopyridine complexes results from the pooling of valence electrons from the ruthenium of the RuCl3,2H2O base and the Npy and N2 nitrogen atoms of the azopyridine ligand. These electrons are then delocalized in the Ru-X bond (Npy and N2). NBO analysis helps elucidate the distribution of these electrons and the resulting stabilizing interactions between bonds. In Table 7, the NBO charges of the main entities (Ru, ligand and Cl) making up the azopyridine complex are presented. Ruthenium and azopyridine ligands have positive NBO charge values, while chlorine atoms display negative values, whatever the compound studied. Ligand charge is an important parameter for predicting the cytotoxic activity of ruthenium azopyridine complexes. The net atomic charge of the complexes contributes to their ability to bind by intercalation to DNA bases. DNA binding to ruthenium azopyridine complexes is conditioned by the higher positive charge of the ligands within the complex. A high positive charge of the ligand promotes a high affinity of the ligand to bind DNA. The ligand charge values **QL**calculated for the different complexes studied enable us to establish the following ascending order:

***C4 ˂ C1 ˂ C6 ˂ C5 ˂ C7 ˂ C2 ˂ C8 ˂ C10 ˂ C9 = C11 = C12 = C13 ˂ C3***

This order of evolution of QL charge suggests the greater affinity of compound C3 for DNA interaction. Compounds C9, C11 and C13 have the same DNA affinity as C12.

**Table 7**. Comparison of NBO charge

|  |  |  |  |
| --- | --- | --- | --- |
| Complexes | Ru | Ligand | Cl |
| C1 | 0.55 | 0.48 | -0.55 |
| C2 | 0.55 | 0.52 | -0.51 |
| C3 | 0.55 | 0.54 | -0.55 |
| C4 | 0.56 | 0.27 | -0.42 |
| C5 | 0.55 | 0.51 | -0.52 |
| C6 | 0.56 | 0.49 | -0.54 |
| C7 | 0.56 | 0.51 | -0.53 |
| C8 | 0.55 | 0.52 | -0.52 |
| C9 | 0.55 | 0.53 | -0.54 |
| C10 | 0.55 | 0.52 | -0.55 |
| C11 | 0.55 | 0.53 | -0.54 |
| C12 | 0.55 | 0.53 | -0.54 |
| C13 | 0.55 | 0.53 | -0.54 |

**3.5. Predicting the cytotoxicity of model complexes**

The cytotoxicity of a compound is its ability to induce apoptosis in cancer cells, or to inhibit the action of enzymes responsible for tumor proliferation. The prediction of this anticancer activity involves the theoretical establishment of mathematical functions called QSAR models. The QSAR models used to predict the activity of our ruthenium azopyridine complexes were established by N'guessan et al. (Kouakou, et al., 2017).

**3.5.1. Model applicability**

The range of applicability of the QSAR models used was determined from the standardized residuals of the descriptors used in the various models. Threshold levers were determined using Minitab software. Williams’s diagrams for each cancer type are shown in figure 3.

**Figure 3**. Graph of standardized residues of cytotoxic activity as a function of levers for the different models used

These graphs show standardized residuals as a function of hii levers. Analysis of the graphs shows that the threshold lever, independently on the model, is 1.5. Thus, an azopyridine compound belongs to the same field of applicability if its hi lever is less than 1.5. In this case, the model can be used to predict the compound's activity. In the opposite case, i.e. if hI˃1.5, the compound does not belong to the field of applicability and the predicted activity is erroneous.

**3.5.2. Determination of the theoretical cytotoxic potential of the azopyridine complexes studied**

The QSAR models developed by N'guessan et al. were used to determine the inhibition potentials of the complexes modeled. In addition to cytotoxic potentials, the levers of these compounds are determined according to the descriptors involved in the model considered. The values of cytotoxic potentials and levers are given in Table 8. The cytotoxic activities of c4 and C7 were not determined because their formation reaction is not favourable at at 298K due to positive ΔG values.

**Table 8.** Theoretical values of pIC50 inhibition potentials for model complexes

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compounds | theoretical activities pIC50 | | | | | Levier | |
| A498 | H226 | IGROV | MCF-7 | WIDR | hii (ΔG;Ql) | hii (ΔG;D) |
| C1 | 5.996 | 5.835 | 5.662 | 6.151 | 5.791 | 0.072 | 0.149 |
| C2 | 7.505 | 8.525 | 8.749 | 8.778 | 8.266 | 0.084 | 0.001 |
| C3 | 8.846 | 10.121 | 10.647 | 10.287 | 9.704 | 0.090 | 0.028 |
| C5 | 8.061 | 8.536 | 8.880 | 8.701 | 8.222 | 0.081 | 0.284 |
| C6 | 6.351 | 6.764 | 6.825 | 6.987 | 6.602 | 0.074 | 0.151 |
| C8 | 8.453 | 9.337 | 9.781 | 9.496 | 8.967 | 0.085 | 0.133 |
| C9 | 8.389 | 9.876 | 10.424 | 10.006 | 9.452 | 0.087 | 0.030 |
| C10 | 7.927 | 8.817 | 9.140 | 9.023 | 8.510 | 0.084 | 0.052 |
| C11 | 8.445 | 10.042 | 10.632 | 10.154 | 9.597 | 0.088 | 0.029 |
| C12 | 8.175 | 9.728 | 10.264 | 9.853 | 9.311 | 0.086 | 0.024 |
| C13 | 8.257 | 10.323 | 11.007 | 10.389 | 9.831 | 0.087 | 0.007 |

Calculated leverage values for modeled compounds are below 1.5. Consequently, the models developed can be used to predict the activity of these compounds, as they fall within the scope of each model.

The inhibitory potential is defined as the opposite of the logarithm to the base ten of the inhibitory concentration:

**pIC50= -log(IC50**)

Where IC50 is the concentration required to inhibit 50% of cancer cells. A high pIC50 value induces a low IC50 value and therefore greater cytotoxicity. The pIC50 values of compounds recorded for cancer A498 indicate that compounds C13, C9, C11, C8 and C3 have a higher cytotoxicity than C12. Here, compound C3 has the highest activity against cancer A498. For H226, IGROV, MCF-7 and WIDR cancers, compounds C9, C11, C3 and C13 have higher inhibition potentials than C12. These compounds are therefore more effective cytotoxic agents than the reference compound C12 (RuCl2(Tazpy)2. Thus, we can assume that the substituents -CH2-COOH, -CH2-CH3, -CH2-CH2-CH3 and -CH=CH2 enhance the cytotoxicity of the ruthenium azopyridine complex. Here, we can see that increasing the carbon chain length of the substituent increases cytotoxic activity.

**3.6. TD-DFT study of the absorption spectrum of model complexes**

The absorption spectra of the complexes studied are determined using time-dependent DFT at the B3LYP/Lanl2DZ level. These spectra (Figure 4) are plotted from the values of excitation energy, wavelength and oscillator strength of the various possible electronic transitions (Table 9). The compounds studied absorb in the visible and infrared wavelengths. The spectra recorded generally show two types of transition. Intra-ligand transitions of the π→π\* type are observed at wavelengths below 400nm. The second type of transition corresponds to a charge transfer from metal to ligand (MLCT) of the t2g→π type. The absorption wavelengths for these transitions observed for the compounds studied range from 630.581 nm to 913.438 nm. The absorption spectrum of the reference compound RuCl2(Tazpy)2 noted C2 shows this transition at 803.063 nm. A hypsochromic effect (decrease in wavelength or shift to shorter wavelengths) is observed for a substitution of the methyl group (-CH3) of Tazpy by the substituents -CH2-COOH, -CH2-CH2-CH3, -CH=CH2 and -CH2-OH. In addition, these compounds C4, C3, C13 and C11 absorb at wavelengths below 803.063 nm. As for the other substituents, they have a bathochromic effect (increasing the absorption wavelength or shifting to higher wavelengths). These MLCT transitions observed beyond the therapeutic window (Sibata, et al., 2000) can be exploited in photodynamic therapy.

**Table 9.** Excitation energies ΔE, wavelengths λ, oscillator strength f and electronic transitions of the absorption maxima of each isomer.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compounds | Excitation energy (eV) | Wavelength λ(nm) | Oscillator force f | Electronic transition |
| C1 | 1.353 | 913.438 | 0.057 | H-1→ L |
| C2 | 1.518 | 816.549 | 0.052 | H-1→ L |
| C3 | 1.692 | 732.598 | 0.046 | H→ L |
| C4 | 1.966 | 630.581 | 0.074 | H→ L+1 |
| C5 | 1.537 | 806.511 | 0.059 | H-1→ L |
| C6 | 1.455 | 852.364 | 0.047 | H-2→ L |
| C7 | 1.442 | 860.11 | 0.083 | H-1→ L |
| C8 | 1.462 | 848.282 | 0.058 | H-1→ L |
| C9 | 1.542 | 803.896 | 0.063 | H-1→ L |
| C10 | 1.453 | 853.126 | 0.058 | H-1→ L |
| C11 | 1.547 | 801.557 | 0.063 | H-1→ L |
| C12 | 1.544 | 803.063 | 0.061 | H-2→ L |
| C13 | 1.564 | 792.894 | 0.063 | H-1→ L |



**Figure 4.** Comparison of theoretical absorption spectra of modeled complexes

**Conclusion**

In this work, the physicochemical, spectroscopic and cytotoxic properties of a series of thirteen azopyridine ruthenium complexes have been investigated using density functional theory at the B3LYP/LanL2DZ level of theory. These compounds are derived from the δ-Cl isomer of the RuCl2(Tazpy)2 complex by substitution of the methyl group of the Tazpy ligand by other substituents. These complex isomers are being investigated in the purpose of designing more effective anti-cancer agents. Structural, electronic and spectroscopic properties determined from ground-state structures are used as descriptors to predict the cytotoxicity against various cancers. Study of the complex geometry revealed that these compounds adopt C2 symmetry. Moreover, substitution of the methyl group by other substituents hardly affects the geometry. The δ-Cl isomer formation reactions of the complexes studied, with the exception of compounds C4 and C7, are spontaneous at 298K. In addition, the -COOH acid function and alkyl groups promote the solubility of azopyridine complexes in the human body. With regard to electronic properties, examination of the frontier orbitals, specifically the LUMO energy, showed that compounds C5, C6, C4, C13, C1, C3 and C9 have a higher binding or interaction affinity with DNA bases than δ-RuCl2(Tazpy)2. As for the analysis of the ligand's natural charges in the complexes, this study confirmed that C9, C11 and C13 have an interaction affinity with DNA identical to that of δ-RuCl2(Tazpy)2. However, compounds C10 and C11 are found the most chemically active. All the compounds studied fall within the range of applicability of the QSAR models developed by N'guessan et Coll [6]. This activity prediction shows that the substituents -CH2-COOH, -CH2-CH3, -CH2-CH2-CH3 and -CH=CH2 increase the cytotoxicity of the ruthenium azopyridine complex. Furthermore, exploitation of the boundary orbital composition indicates a metallic character of the HOMO and a predominance of the px and py orbitals of the ligand's N2 and Npy nitrogen atoms. This composition of boundary orbitals favors MLCT transitions. This result was confirmed by the study of UV-visible absorption spectra. The MLCT transitions observed in the therapeutic window make these compounds potentially efficient photosensitizers for photodynamic therapy applications. A theoretical study will be carried out to evaluate the performance of these ruthenium azopyridine complexes in photodynamic therapy.

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