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Benzothiazole metallic complexes with Tc and Pt as a potential alternative for cancer diagnosis

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Dissertation presented to the Federal University of Lavras, as part of the requirements of the Postgraduate Program in Agrochemistry, area of concentration in Computational Chemistry, to obtain the title of Master

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Advisor

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Benzothiazole metallic complexes with Tc and Pt as a potential alternative for cancer diagnosis

Complexos metálicos de benzotiazol com Tc e Pt como potencial alternativa para o diagnóstico de câncer.

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agroquímica, área de concentração em Química Computacional aplicada na agricultura, para a obtenção do título de Mestre.

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Orientador

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2019

A Deus, por me guiar
aos meus Pais, Milton e Maria Lúcia,
aos meus irmão, Eduardo e Bárbara,
ao Delano, por todo amor e carinho,
à minha segunda família, Tadeu, Zezé, Izabela, Marcelo e Túlio.
Dedico.

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MEU MUITO OBRIGADA!

*“The whole purpose of education is to turn mirrors into windows” (Sydney J. Harris***)**

RESUMO

Atualmente, o câncer é um dos principais problemas de saúde no mundo, sendo responsável por muitas mortes. Entre os tipos mais comuns de câncer, pode-se destacar o câncer de mama, uma vez que é uma das manifestações mais frequentes da doença, levando muitas vezes o paciente a óbito. Os principais tratamentos utilizados contra o câncer de mama são quimioterapia, radioterapia e mastectomia. No entanto, essas técnicas são invasivas para o corpo e a mente do paciente. Portanto, o diagnóstico precoce é um passo importante, uma vez que permite encontrar tumores no estágio inicial da doença e assim facilitar o tratamento. Compostos derivados de benzotiazol pertencem a uma classe de moléculas que vem sendo estudada para aumentar a seletividade dos agentes antitumorais. Assim, o objetivo desta pesquisa foi estudar a aplicação de complexos de platina e tecnécio ligados ao derivado 2-(4'aminofenil)benzotiazol no diagnóstico do câncer, a fim de propor esses complexos como potenciais compostos para o diagnóstico do câncer de mama em estágio inicial. Este estudo foi dividido em duas partes. Na primeira, o complexo de Tc foi estudado como potencial agente de contraste na ressonância magnética (RMI). Essa parte do estudo foi realizada avaliando a influência do núcleo tecnécio nos hidrogênios da água, considerando os parâmetros de EPR por meio de métodos DFT. Por outro lado, o complexo de platina foi estudado como sonda em sistemas biológicos, no qual o deslocamento químico da platina foi avaliado através de métodos DFT em diferentes ambientes (vácuo, solvente implícito e no sítio ativo da enzima P13K). Como resultado, para o tecnécio, os parâmetros analisados se aproximaram dos valores de gadolínio relatados na literatura. Para os complexos de platina, o deslocamento químico variou nos diferentes ambientes, assim como esperado. Assim, ambos os complexos apresentam potencial aplicação no diagnóstico precoce do câncer de mama. Além disso, ambos os metais são amplamente utilizados na medicina, o que facilita sua aplicação em técnicas de diagnóstico.

Palavras-chave: Câncer de mama. Diagnóstico. Benzotiazol. Platina. Tecnécio. Agente de contraste. RMN

ABSTRACT

Currently, cancer is one of the major health problems in the world, which is responsible for many deaths. Among the most common types of cancer, breast cancer can be highlighted, once it is one of the most frequent manifestations of the disease, in which often leads to death. The main treatments used against breast cancer are chemotherapy, radiation therapy and mastectomy. However, these techniques are invasive to the body and mind of the patient. Therefore, early diagnosis is an important step, since it allows to find tumors in the early stage of the disease, and thus facilitate the treatment. Compounds derived from benzothiazole belong to a class of molecules that have been studied to increase the selectivity of antitumor agents. Thus, the objective of this research was to study the application of platinum and technetium complexes of 2- (4'-aminophenyl) benzothiazole derivative in the cancer diagnosis, in order to propose these complexes as potential compounds for diagnosis of initial stage. This study was divided into two parts. In the first, the Tc complex was studied as a potential contrast agent in magnetic resonance imaging (MRI). This part of the study was carried out by evaluating the influence of the technetium nucleus on water hydrogens considering the EPR parameters, which were performed using DFT methods. On the other hand, the platinum complex was studied as a probe in biological systems, in which the platinum chemical shift was evaluated through DFT methods in different environments (vacuum, implicit solvent and inside P13K enzyme active site). As a result, for technetium, the analyzed parameters approximated to the gadolinium values reported in the literature. For the platinum complexes, the chemical shift varied in different environments, as expected. Thus, both complexes present potential application in the early diagnosis of breast cancer. In addition, both metals are widely used in medicine, which facilitates their application in diagnostic techniques.

Key-words: Breast cancer. Diagnosis. Benzothiazole. Platinum. Technetium. Contrast agent. NMR

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SUMMARY

FIRST PART

1. INTRODUCTION

Currently, cancer is one of the most discussed and studied diseases. This disease is characterized by cells uncontrolled growth, which can spread affecting organs and tissues in the body. There are several cancer types, such as breast cancer, brain cancer, kidney cancer and prostate cancer. These types of cancer have solid tumors, while leukemia (cancer of blood) has not. Regardless of the cancer type, this disease is one of the most important barriers for increasing life expectancy in the world in the 21st century.

Breast cancer has been studied thoroughly in the last years, once itis the leading cause of deaths related with cancer. This cancer type presents high incidence, every four cancer diagnoses one of it is breast cancer, moreover affecting with one in eight women worldwide. Among the most commonly used techniques for breast cancer diagnosis are mammography, positron-emission tomography (PET), computed tomography (CT) and magnetic resonance imaging (MRI). In this sense, it is important the awareness that early diagnosis is extremely important to achieve good treatment results.

The main treatment against breast cancer are chemotherapy, radiotherapy and mastectomy. However, the mastectomy has been decreased due to mammographic screening techniques and targeted therapies. Benzothiazole derivatives compounds have been studied as selective class of antitumor agents. The 2-(4-aminophenyl)benzothiazole analogues belongs to this class, which presents activity against several cancers, including breast cancer. Benzothiazole compounds are capable of growth inhibition, once they are tyrosine kinase inhibitors.

In this regard, some studies have shown that 2- (4'-aminophenyl)benzothiazole as an organic ligand in metallic complexes could be used in cancer treatment, once its activity against cancer is known. In this respect, Tzanopoulou and collaborators (2006) synthesized the ^{99m}Tc complex bonded to with 2-(4’aminophenyl)benzothiazole derivative to study its activity against cancer cells. In this line, Mavroidi and collaborators (2016) performed a synthesis, structural characterization and in vitro biological evaluation of Pt complex with 2-(4’aminophenyl)benzothiazole organic ligand to study its activity against cancer.

It is known that platinum (Pt) complexes are used in cancer treatment. The first platinum complex used in this sense was cisplatin, widely known for its cytotoxic activity. Afterwards, different drugs using Platinum were developed to improve treatment of different types of cancer. On the other hand, technetium (Tc) complexes are used in nuclear medicine

for cancer diagnosis, once it has nuclear properties which are used for medical imaging and therapy.

Accordingly, the aim of this work was to study both Pt and Tc complexes with 2-(4'aminophenyl)benzothiazole with application in cancer diagnosis to propose these complexes as potential compounds for early breast cancer diagnosis, once that both complexes have already been studied in the cancer treatment. The Tc complex was studied as potential contrast agent in magnetic resonance imaging (MRI) through evaluation of its nucleus influence in water hydrogens of the human body considering EPR parameters. Differently, the platinum complex was studied as probe in biological system. In other words, we evaluated the ^{195}Pt chemical shifts in different chemical environments as vacuum, implicit solvent and inside P13K enzyme active site.

2. THEORETICAL REFERENCE

2.1 Breast cancer

Cancer is a group of related diseases in which body's cells suffer certain changes that cause uncontrolled cell growth. There are types of cancer that result in visible growths called tumors, as breast and brain cancer, while others, such leukemia, do not present solid tumors. Cancerous tumors are malignant, it means that their cells can spread into or invade nearby tissues. When these tumors grow, some of their cells can break away and travel to distant places through the blood or lymph system, which allows the growth of new tumors in cells far from the initial tumor (BRAY et al., 2018; SIEGEL; MILLER; JEMAL, 2018).

A Brazilian research estimates the occurrence of 600 thousand new cases of cancer for each year for the 2018-2019 biennium. The most common types registered in Brazil are prostate, lung, breast, colon and rectal cancer. Furthermore, cervix, stomach and esophagus cancer also have high rates of incidence, according to National Cancer Institute (INCA).

Breast cancer is the most common cancer in the female population around the world. Every year, 1.5 million women are diagnosed with breast cancer and almost 15% of them die due to the advanced stage and complications of this disease. Therefore, the early detection of breast cancer is a vital step, once it increases the treatment possibilities(WAKS; WINER, 2019; ZONOUZY et al., 2019)

2.2 Breast cancer diagnosis

One of the many challenges for achieving effective results in cancer treatments is its diagnosis in early stages, once, as mentioned before, this increases the treatment possibilities. Thus, diagnostic techniques have been studied with the aim of improving and/or developing new methods to increase the diagnostic rates in early stages. Among several procedures for diagnosis, imaging techniques have been used as the main tool in breast cancer diagnosis (JAFARI et al., 2018). This feature can also assist in surgeries, once it obtains high resolution images of the environment which may be useful to identify possible tumors in regions where the intervention will be performed (JAFARI et al., 2018; WAKS; WINER, 2019).

In this sense, imaging techniques are essential tools in early stage cancer diagnosis. Nonetheless, for the specific case of breast neoplasm, the breast self-examination and regular

breast exam are also important procedures for success in early diagnosis. Some imaging techniques are used as main approaches in breast cancer diagnosis and other cancer types, as glioma, oral cancer and melanoma, among them are mammography, ultrasound, magnetic resonance imaging (MRI), positron-emission tomography (PET), and computed tomography (CT)(COSTELLOE et al., 2009; JAFARI et al., 2018; KESHAVARZI et al., 2017; ROSTAMZADEH et al., 2017).

Mammography is a routine exam in women's life. This technique is known as the gold technique in breast cancer diagnosis, once it is an exam frequently performed in which many tumors are found in early stages. Mammography presents some benefits as high sensitivity and specificity and it is an accessible exam to most women, due to its low cost. The use of mammography can reduce the mortality rate caused by breast cancer, but the negligence in performing this exam annually hampers the early diagnosis. Some limitations associated with this technique are also reported as patient's pain and anxiety, false alarms and radiation risks(DHEEBA; SINGH; SELVI, 2014; FLOWERS et al., 2015).

One of the many advantages of magnetic resonance imaging (MRI) is its high ability in differentiating tissues. Moreover, the MRI is not an invasive technique, once it utilizes magnetic properties of the ^{1}H nuclei, which is abundantly present in the human body. However, due some technical limitations, in this methodology it is required the use of external resources to improve image resolution. The compounds used for improving the resolution are called contrast agents and they allow the MRI technique to be more effective in diagnosing and monitoring several types of cancer (ALCANTARA et al., 2014; JAFARI et al., 2018; KUHL, 2008).

2.3 Nuclear magnetic resonance

The nuclear magnetic resonance spectroscopy, commonly called NMR is a powerful structural elucidation tool. This technique has its principle based on the intrinsic magnetic moment of the nucleus, which occurs when a nucleus has spin different than zero (I=0). When a nucleus is in the presence of an external magnetic field, the spin determines its nuclear magnetic moment(μ), as shown in the equation below (HOFFMANN; FORSÉN; GESTBLOM, 1971):

$$\mu = \gamma I\hbar \qquad (1)$$

where, *I* is the spin, γ is the nucleus gyromagnetic ratio and ħ is the Planck constant.

The application of a magnetic field (B_0) divides the energies of the spin states; for example, a nucleus with spin will have $2I + 1$ possible orientations. Then, a nucleus with spin 1/2 will have 2 possible orientations: with low energy level when the spins are aligned with B_0 and with high energy level when the spins are aligned against B_0. Thus, each spin state has an associated energy (Equation 2), which will exhibit resonance with the applied radiofrequency, thus generating the signals of the NMR spectrum (HOFFMANN; FORSÉN; GESTBLOM, 1971).

$$E = -\mu B_0 \tag{2}$$

The NMR technique is based on radiofrequency waves absorption by nucleus due to the action of a magnetic field; however, the electrons also feel the action of this field. Then, due to this fact, the electrons produce an induced magnetic field, which is opposite to B_0. Thus, the magnetic field that the nucleus feels is smaller than B_0. To this new field is given the name shielding (σ)(HOFFMANN; FORSÉN; GESTBLOM, 1971).

The shielding is very essential in NMR, once it is from the shielding that arises the chemical shift (δ), which is an important NMR parameter. The nuclei chemical shift (δ) is related to the chemical environment, and it is represented by the position of the signal in the NMR spectrum. The chemical shifts are usually related to some standard reference. Then, a reference compound is used, and the chemical shift is the measure of the distance between the sample signal and the reference compound signal. This is obtained from the shielding of these compounds, as shown in Equation 3: (HOFFMANN; FORSÉN; GESTBLOM, 1971).

$$\delta = \sigma_{reference} - \sigma_{nucleus} \tag{3}$$

Another important NMR parameter is the coupling constant (J). While the chemical shift shows in which spectrum region the nucleus absorbs, the coupling constant provides information about the nucleus neighborhood. Therefore, as well as the chemical shift, the coupling constant (J) allows structural elucidation(HOFFMANN; FORSÉN; GESTBLOM, 1971).

In this sense, these NMR parameters present medical applications, which can help in the diseases treatment and diagnosis. For instance, the magnetic resonance imaging (MRI) is an example of medical application of nuclear magnetic resonance (NMR). The spins of a nucleus, when in the presence of a magnetic field, assume different states of energy and when the relaxation process occurs, there is a conversion of this non-equilibrium population to a normal population. The spin relaxation rates can be measured and used in imaging applications (PYKETT et al., 1982).

2.4 Magnetic resonance imaging

The magnetic resonance imaging (MRI) technique is based on fundaments of nuclear magnetic resonance (NMR). Therefore, for understanding MRI is important to learn the main NMR principles. This technique is based on magnetic properties of the ^{1}H nucleus, which is a NMR-active nucleus. The hydrogen atom is the most common element employed because it has some important spectroscopy properties, such as having spin = 1/2; another important feature of hydrogen atom is its abundance in the human body. The ^{1}H nuclei amount in diseased tissues is different than in healthy ones and thus, it is possible to identify different magnetic properties in healthy and diseased tissues(GROVER et al., 2015; MAZZOLA, 2009; PYKETT et al., 1982).

The signals of relaxation time are generated from spin relaxation processes, which are related to the time required or the spins to return to the ground state after the release of the absorbed energy by NMR spectroscopy (GROVER et al., 2015). The emitted signals are measured, and the Fourier transformation is used to convert this frequency information from signal to image, which is displayed as gray shades in a matrix arrangement of pixels. The relaxation processes are characterized in two different types, the spin-lattice, also called longitudinal relaxation times (T_1) and the spin-spin, also called transverse relaxation (T_2)(GROVER et al., 2015; PYKETT et al., 1982; TANG et al., 2018).

The longitudinal relaxation time is the time constant which determines the relaxation rate (R_1) when excited protons return to equilibrium. In other words, T_1 corresponds to the time required for the protons to realign with the external magnetic field. The transverse relaxation time is a constant which determines the relaxation rate (R_2) when excited protons reach the equilibrium or go out of phase with each other. Then, T_2 is the time required for the

protons to lose phase coherence.(GROVER et al., 2015; PYKETT et al., 1982; TANG et al., 2018).

When paramagnetic species interact with water molecules, it can dramatically reduce the water molecules relaxation time, as described in Equations (4) and (5), which show the longitudinal and transverse relaxation time induced by paramagnetic ions in aqueous solution, respectively(GONÇALVES et al., 2014; LEPAGE; GORE, 2004).

$$R_1 = \frac{1}{T_1} \cong \frac{1}{15}\frac{S(S+1)g_e^2\beta^{2g^2{}_N}\beta^2{}_N}{\hbar^{2r^6}} + \left(\frac{A}{\hbar}\right)^2\frac{S(S+1)}{3}\left[\frac{2\tau_e}{1+(\omega_I\tau_e)^2}\right] \quad (4)$$

$$R_2 = \frac{1}{T_2} \cong \frac{1}{15}\frac{S(S+1)g^2\beta^2g^2{}_N\ \beta^2{}_N}{\hbar^2r^6} + \left(\frac{A}{\hbar}\right)^2\frac{S(S+1)}{3}\left(\tau_c + \frac{\tau_c}{1+\omega_S{}^2\tau^2{}_e}\right) \quad (5)$$

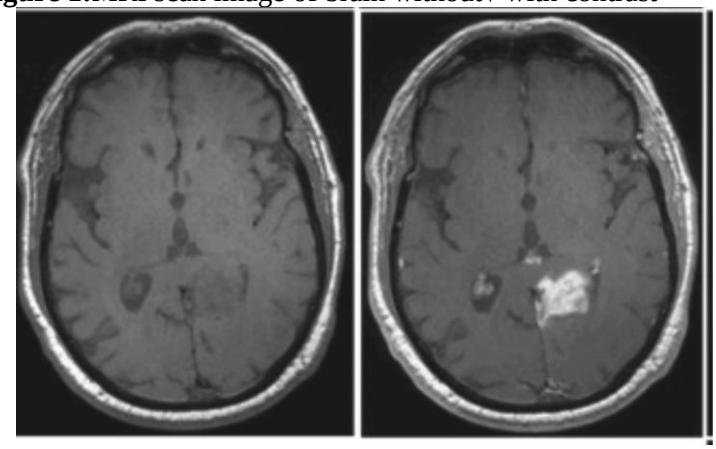
The longitudinal and transverse relaxation time constants depend on the electron spin (S), the electronic and proton g factors (ge and gN, respectively), the Bohr magneton (β), the nuclear magneton (βN), the hyperfine coupling constant (A), the ion-nucleus distance (r), and the Larmor frequencies for the proton and electron spins (ωI and ωS, respectively). The rate of change of the interactions between the paramagnetic species and neighboring protons are related with the correlation times τc and τe(GONÇALVES et al., 2014; LEPAGE; GORE, 2004).

In this sense, it is possible to obtain MRI image through the natural contrast formed by the water molecules present in the tissues. However, sometimes, the image resolution is not satisfactory. Thus, paramagnetic compounds, called contrast agents (ACs), are used to improve the MRI image resolution and are described in details in the next section(WAHSNER et al., 2019).

2.5 Contrast agents

The magnetic resonance imaging (MRI) technique is one of the most important tools in cancer diagnosis. However, not always the MRI can differentiate normal tissues from abnormal tissues. Thereby, contrast agents (ACs) are widely used to increase the image resolution difference between normal and abnormal tissues as shown in Figure 1 (WAHSNER et al., 2019):

Figure 1:MRI scan image of brain without / with contrast



Source: WAHSNER et al.(2019)

Currently, the most famous contrast agents in MRI are either paramagnetic gadolinium ion complexes or super paramagnetic magnetite particles (WAHSNER et al., 2019; XIAO et al., 2016). The Gadolinium(III) Gd^{3+} metal atom is the most commonly used, once it has a high magnetic moment and it is the most stable ion with unpaired electrons. These gadolinium contrast agents shorten the longitudinal and transverse relaxation time of the water protons around the metal (GONÇALVES et al., 2014; WAHSNER et al., 2019; XIAO et al., 2016).

The use of contrast agents in magnetic resonance imaging is important, once it improves the contrast of the images and allow more accurate diagnoses. These compounds are also used in combination with target molecules for visualization of specific tissues. However, the high toxicity of these contrast agents is a major obstacle to research in this field(WAHSNER et al., 2019; XIAO et al., 2016).

Currently, many studies have shown that the use of biomarkers can aid imaging techniques and make them more selective. The use of these biomarkers can contribute to the early diagnosis and follow up of the disease treatment(DUFFY et al., 2017). Protein tyrosine kinases are enzymes normally found in cancer cells, including in breast cancer. Thus, this class of proteins has been extensively studied for the biomarker therapy advancement and it is better discussed in the next section.

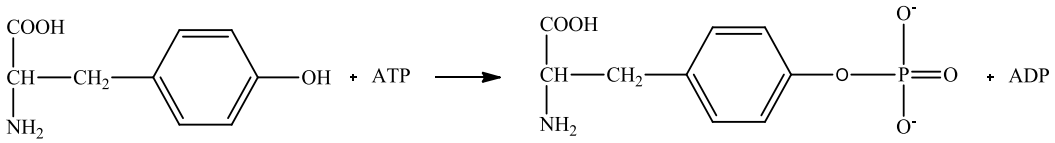
2.6 Molecular targets: tyrosine kinase proteins

Protein kinases are part of a large protein family responsible for intracellular control, regulation and cell growth. Therefore, the detailed understanding of these protein is of great

importance, and because of that, their action mechanism, and inhibition procedures have been widely studied(GONCALVES; HOPKINS; CANTLEY, 2018; KANNAIYAN; MAHADEVAN, 2018; LEITE; CALLADO; RIBEIRO, 2012). In this sense, many studies have helped to better understand the cancer biology, the molecular structures and signaling pathways of receptor tyrosine kinases. This fact has become important for the target therapy advancements(GONCALVES; HOPKINS; CANTLEY, 2018; LEITE; CALLADO; RIBEIRO, 2012).

More specifically, tyrosine kinases (TKs) are enzymes responsible for catalyzing the transfer of the γ–phosphate group of the adenosine triphosphate (ATP) to hydroxyl groups of tyrosine residues, as shown in Figure 2:

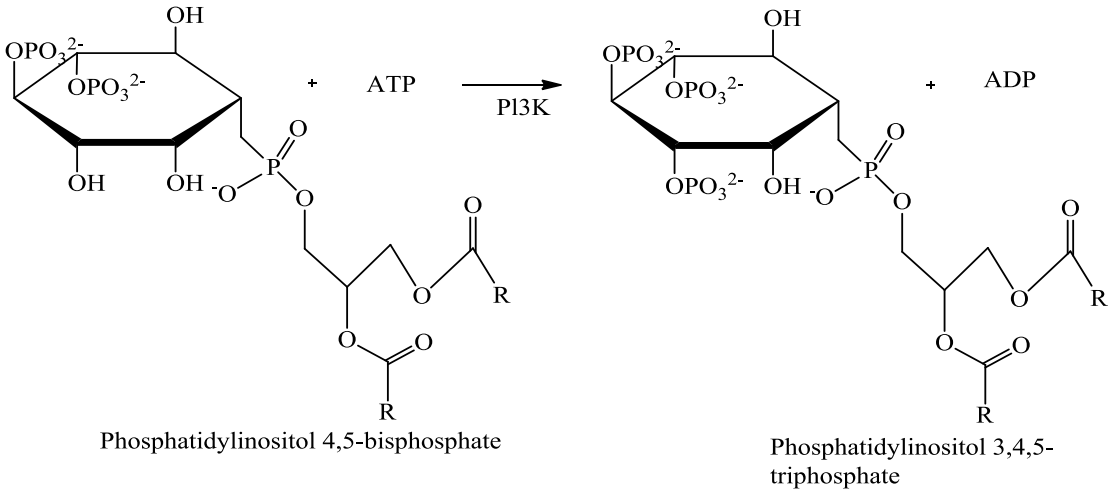
Figure 2: mechanism of phosphorylation of protein kinases



Tyrosine Phosphotyrosine

Currently, 58 human tyrosine kinase receptors are known, which are divided into different classes based on structural domain characteristics (NEBEN et al., 2019). The main growth-related signaling pathways involve the phosphatidylinositol-3-kinase (P13K), which is responsible for the phosphorylation of the 3'-hydroxyl group of phosphatidylinositol 4,5-bisphosphate (Figure 3). This enzyme has an important role in cell growth and metabolism. Moreover, the P13K signaling pathway has an important role in apoptosis, once this pathway reduces apoptosis and promotes tumor cell growth(KIM et al., 2011; SHRIVASTAVA et al., 2015).

Figure 3: phosphorylation of the 3'-hydroxyl group of phosphatidylinositol 4,5-bisphosphate

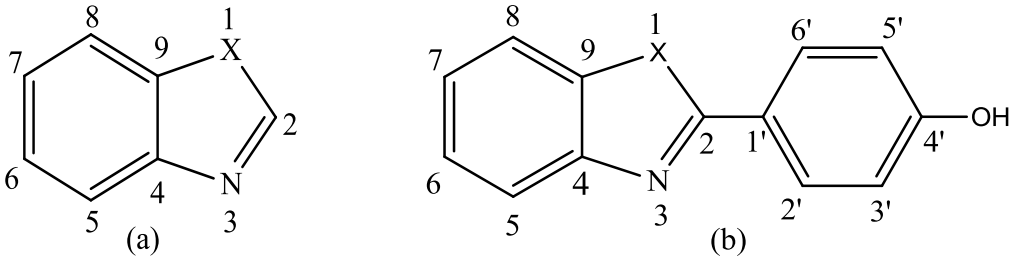


The protein kinases present sites where ATP binds and triggers the phosphorylation (KATZ et al., 2011).Some studies show that the inhibition of these enzymes by benzothiazole compounds could be used as a possible target therapy, once these compounds can compete with ATP for binding sites in the enzyme causing its inhibition (LEITE; CALLADO; RIBEIRO, 2012).

2.7 Benzazolic heterocyclic compounds

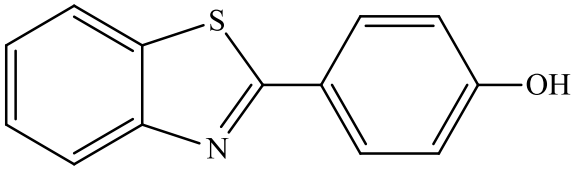
Benzazolic heterocycles are azole derivatives condensed to a benzene ring. The azole ring is structured by a five membered ring, which has heteroatoms at positions 1 and 3, and the heteroatom at position 3 is always nitrogen (Figure 4a). When an azolic derivative binds to a phenyl group at position 2, it forms the 2- (4'-hydroxyphenyl) benzazole heterocycles, also known as phenylbenzazoles. (Figure 4b).

Figure 4: (a) azole ring; (b) phenylbenzazole compound.



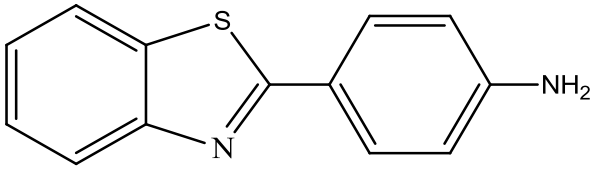
The introduction of different heteroatoms at position 1 of the azole ring generates different phenylbenzazole derivatives, such as phenylbenzoxazole (X= O), phenylbenzimidazole (X = NH) and phenylbenzothiazole (X= S) (Figure 5) (KERI et al., 2015; TZANOPOULOU et al., 2010).

Figure 5: phenylbenzothiazole (X = S)



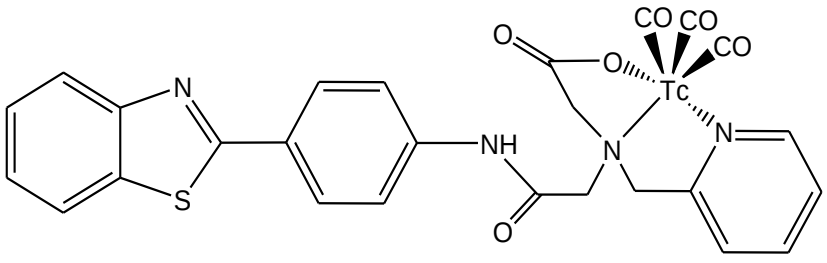
It is known that 2- (4'-aminophenyl)benzothiazole (ABT) members (Figure 6) have highly selective antitumor properties against cancer cells. Its chemical activity is related to the interaction between this compound and the protein tyrosine kinase, which is an enzyme responsible for the cell proliferation control(BASNIWAL et al., 2006; MANCINI et al., 2014; TZANOPOULOU et al., 2010; YURTTAŞ; TAY; DEMIRAYAK, 2015).

Figura 6: 2- (4'-aminophenyl) benzothiazole (ABT) compound



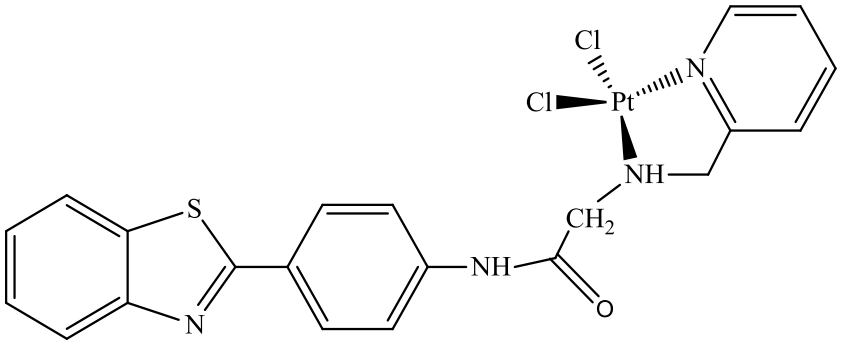
In this sense, studies have been performed for evaluating benzothiazole compounds bonded to metals for biological systems application. Therefore, Tzanopoulou and collaborators synthesized the ^{99m}Tc complex bonded to 2-(4’aminophenyl)benzothiazole derivative (Figure 7)(TZANOPOULOU et al., 2006, 2010).

Figure 7: ^{99m}Tc $(CO)_3$-(NNO) bonded to 2-(4’aminophenyl)benzothiazole derivative



Another study was carried out by Mavroidi and collaborators, in which it was performed a synthesis, structural characterization and in vitro biological evaluation of the cis-dichloro(2'pyridinyl)methylamineplatinum(II)bonded to 2-(4'aminophenyl)benzothiazole derivative (Figure 8). In this research, it was possible to conclude that the synthesized complexes were stable and interacted strongly with DNA (MAVROIDI et al., 2016).

Figure 8: cis-dichloro(2'pyridinyl)methylamineplatinum(II) bonded to 2-(4'aminophenyl)benzothiazolederivative.



2.8 Technetium

Technetium is a chemical element that has applications in different areas, among them it can be highlighted its use in nuclear medicine. However, for better understanding this specific application, it is important first to look at its chemical properties. Technetium is a transition metal represented by the symbol Tc in the periodic table and has atomic number equals to 43. The most common technetium oxidation states are +7, +5, and +4. Furthermore, technetium has several isotopes, but most of them have too short half-lives. The most common technetium isotope found is the ^{99}Tc, but the nuclear isomer ^{99m}Tc is the radioactive isotope used in medical tests(PASQUALI et al., 2018; SRIVASTAVA, 1996).

Technetium-99m has interesting properties that make it efficient in diagnosis. This Tc isotope emits readily detectable gamma rays with a photon energy of 141 keV; it has 89% abundance and its half-life is 6 h, which means that 93.7% of it decays to ^{99}Tc in a short period of time. Because of this, the ^{99m}Tc isotope is used as radioactive tracer, once it allows scanning processes that collect data rapidly, which means that the patient will be exposed to radiation only for a short period of time (MANCINI et al., 2014; PAPAGIANNOPOULOU, 2017; TZANOPOULOU et al., 2006, 2010).

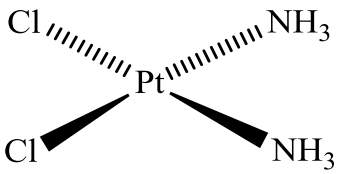
Some technetium complexes are already used in breast cancer diagnosis as adjuncts to mammography with the aim of improving sensitivity and specificity in breast cancer detection. In this perspective, Tzanopoulou and collaborators synthesized the ^{99m}Tc complex bonded to 2-(4'aminophenyl)benzothiazole derivative (Figure 7), once 2-(4'aminophenyl)benzothiazole presents activity *in vitro* against breast cancer cells.(MUKIZA et al., 2018; PAPAGIANNOPOULOU, 2017; TZANOPOULOU et al., 2006, 2010).

2.9 Platinum

Platinum is a noble transition metal with many applications in industrial and medical chemistry, mainly in cancer treatment. This metal has an atomic number equals to 78 and Pt^{+2} and Pt^{+4} are the most common oxidation states. However, it is possible to find this element with other oxidation states, but it is less common(SILVA; GUERRA, 2010).

Platinum forms several coordination compounds, which have relevant properties in medicinal chemistry, such as the cis-diamminedichloroplatinum(II) complex, cis-$[Pt(NH_3)_2Cl_2]$ (Figure 9), commonly called "cisplatin", which presents a square planar arrangement. This compound was first synthesized in 1844 by Michele Peyrone, but its antitumor properties were demonstrated by Barnett Rosenberg et al. Thus, began the use of several platinum complexes as cytotoxic agents, which are compounds capable of killing or damaging cancer cells (FREZZA et al., 2010; ROSERNBERG; VANCAMP; KRIGAS, 1965; SILVA; GUERRA, 2010).

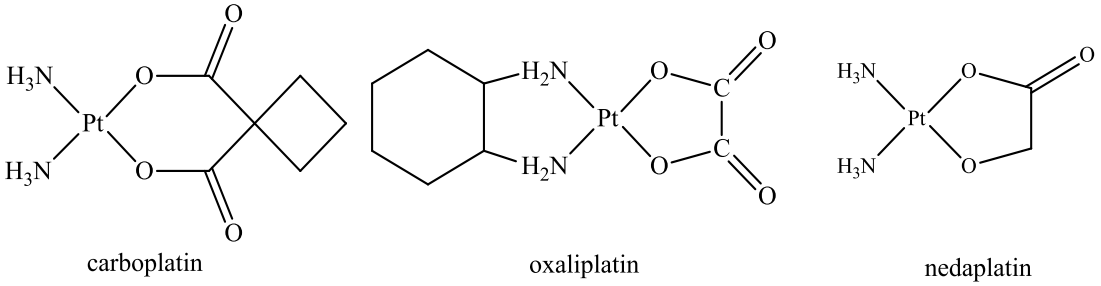
Figure 9 : Cisplatin



However, several side effects have appeared with cisplatin use in cancer treatment; consequently, many researchers have sought to understand the action mechanism of platinum complexes in the biological environment, in order to minimize these effects in new classes of drugs. In this sense, cisplatin-analogues have been developed. The most common analogues of platinum are carboplatin, oxaliplatin and nedaplatin(BRABEC; HRABINA;

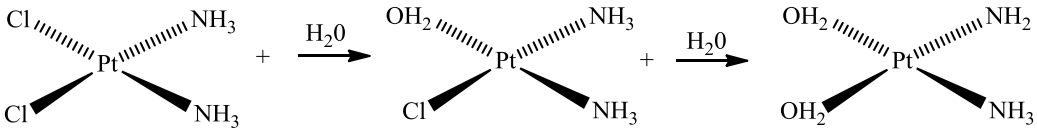
KASPARKOVA, 2017; DASARI; TCHOUNWOU, 2014; DEO et al., 2018), which are represented below:

Figure 10: analogues of cisplatin complex



Currently, it is well known that the platinum complexes activity is related to their DNA coordination. However, many studies have been carried out to understand the structural changes that occur so that the complex can bind to the DNA. A known step in the mechanism of action of these complexes is the aquation, in which the chlorine atoms are replaced by water molecules in a two steps mechanism (Figure 11)(AHMAD, 2017; CORINTI et al., 2017; DASARI; TCHOUNWOU, 2014).

Figure 11: cisplatin aquation



Several studies have shown that the monoaqua cisplatin complex {cis-$[Pt(NH_3)_2(H_2O)Cl]^{+1}$ } is the active hydrolyzed specie at 310 K that binds to DNA, once the diaquo complex { cis-$[Pt(NH_3)_2(H_2O)_2]^{+2}$ } is the less likely specie in physiological pH(AHMAD, 2017).

The platinum complex is administered clinically intravenously with a sodium chloride solution, and then the compound penetrates the cell by diffusion. Subsequently, the substitution of chlorine ligands by water is favored, once the chloride concentration in the blood plasma is higher than in the cytoplasm. The mechanism responsible for this exchange of ligands is called associative and it involves a trigonal bipyramidal transition state distortion, in which the coordination with the input group (H_2O) is totally formed before the leaving groups (Cl) exit (AHMAD, 2017).

Different platinum complexes have been studied in several areas of chemistry, but mainly in nuclear magnetic resonance (NMR) spectroscopy. Platinum has 32 isotopes, three of them are more abundant than the others, ^{194}Pt (32.9%), ^{195}Pt (33.8%) and ^{196}Pt (25.3%)(PASCHOAL et al., 2016; SILVA; GUERRA, 2010; STILL et al., 2007; URSINI, 1997).Platinum-195 is the only active isotope in nuclear magnetic resonance, this nucleus has nuclear spin (*I)* = ½, property that makes its activity possible. Many studies involving ^{195}Pt NMR have been performed since the 1960s when the sensitivity of the ^{195}Pt chemical shift was studied for the first time(STILL et al., 2007).

The chemical shift of ^{195}Pt is very sensitive to the bond nature and the coordination sphere around the Platinum. For example, a σ bond (L → M) reduces the electron density on the ligand and thereby it increases on Platinum; however, if there is a π bond(M → L) the electron density decreases on Platinum and increases on the ligand. These are some factors that involve the Metal - ligand bonds and can influence in the chemical displacement of Platinum(STILL et al., 2007; URSINI, 1997).

2.10 Computational chemistry

Computational chemistry has become a very important tool for studies into chemical, physical and biological systems, once it complements experimental studies in order to better understand some problems. There are three general classes of computational methods used in simulations of these systems, that are divided in two groups: those that use classical physics principles and those which use quantum physics principles(ELKIN; NEWHOUSE, 2018; JENSEN, 2007; MORGON; COUTINHO, 2007).

The classical methods are based on calculations that do not employ wave functions; in this sense, the atom electronics' natures not considered, and the chemical bonds are studied as elastic potential. This broad area is called molecular mechanics, which is very important for theoretical studies concerning biological systems, such as the investigation of new drugs, studies of interactions between ligands and enzymes, investigations of diseases mechanisms, among others(JENSEN, 2007; MORGON; COUTINHO, 2007).

The quantum methods solve – by performing approximations- the Schrödinger wave equation, directly or indirectly. Among them are the semi-empirical methods, which are based on parameterized experimental measurements, and the ab initio methods, which use the physics fundamental constants exclusively, such as gravity, speed of light, among others. Calculations using semi-empirical methods begin with the resolution of the Schrödinger

equation and, then, some experimental considerations are used. The first developed ab initio method was the so-called Hartree-Fock (HF), and although effective, it presented some limitations that lead to the development of many other methods called post HF methods. Each of these methods present advantages and drawbacks, because of that new solutions have been investigated, such as the density functional theory (DFT). DFT is a different approach for solving the Schrodinger's equation, in which the interactions between electrons are treated indirectly by electronic density; this consideration has proven to be effective for treating the electronic aspects of many different atomic systems and it is also much cheaper than the *ab initio* methods. Therefore, this methodology is discussed in more details in the next section(JENSEN, 2007; MORGON; COUTINHO, 2007).

2.11 Density functional theory

The density functional theory (DFT) emerged as an alternative to ab initio and semi-empirical methods. This method is based on the concept that the electron density (ρ) contains all information that can be obtained from the wave function for systems with many electrons. This is an advantageous method, once the wave function requires 3 variables to describe each electron state, that is, 3Ne wave functions for each electron, while the electronic density is a function of just 3 variables (BURKE, 2012; JENSEN, 2007; MORGON; CUSTODIO, 1995; KOHN e SHAM, 1965).

The resolution of the time independent Schrödinger equation from the operator known as Hamiltonian allows to determine the energy of systems with many electrons. This operator is applied to a function called wave function and thus, the system energy is obtained(MORGON; COUTINHO, 2007; KOHN e colab., 1996). (Equation 6):

$$\hat{H}\Psi = E\Psi \qquad 6)$$

In which H is described by the Equation 7

$$\hat{H} = \hat{T}n + \hat{T}e + \hat{V}ne + \hat{V}ee + \hat{V}nn \qquad (7)$$

in which the $\hat{T}n$ and $\hat{T}e$ terms represent the nucleus and electron kinetic energy respectively, and the $\hat{V}ne + \hat{V}ee + \hat{V}nn$ terms represent the attraction energy between the

nucleus-electron, and the repulsion energy between nucleus-nucleus and electron-electron, respectively. However, the exact analytical solution of the Schrödinger equation is highly complex, so some approximations are necessary(BURKE, 2012; JENSEN, 2007).

The Born Oppenheimer approaches one of the most important approximations employed in quantum mechanics. This approximation considers that the nucleus mass is much larger than the electrons mass, then, the core cannot keep up with the rapid movements of the electrons; therefore, the core can be considered fixed. Thus, the core kinetic energy term is considered zero ($T_n = 0$), and the nucleus-nucleus repulsion term is considered constant (V_{nn} = constant); in this way the Hamiltonian can be described by Equation 8(BURKE, 2012; MORGON; CUSTODIO, 1995; KOHN ; SHAM, 1965).

$$\hat{H} = \hat{T}e + \hat{V}ne + \hat{V}ee \tag{8}$$

However, even with this approximation, it is still difficult to obtain the Schrödinger equation resolution. In this sense, the theory of density functional seems interesting, once it reduces computational complexity (the electronic density is a function of 3 variables)(BURKE, 2012; JENSEN, 2007).

The DFT is based on Hohemberg-Kohn Theorems. The first one establishes that the electronic density determines an external potential, which is produced by the atomic nucleus. From this potential and electron density, it is possible to find the Hamiltonian of the system, and then the ground state energy (E_0) is determined by the electron density(BURKE, 2012; JENSEN, 2007; MORGON; CUSTODIO, 1995; KOHN e colab., 1996; KOHN; SHAM, 1965)(Equation 9).

$$E_0 = E_0v[\rho] \tag{9}$$

In which v shows the dependence of external potential. The second theorem considers the variational principle of energy. In this one the calculated energy will always be greater than or equal to the exact energy of the system. The ground state energy is obtained from Equation 10:

$$E_0 = \int \rho_0(r)v(r)\,dr + HK[\rho_0] \tag{10}$$

The functional of Hohemberg and Kohn (HK) includes the kinetic energy functional and interelectronic interaction, as shown in Equation 11:

$$HK[\rho 0] = T[\rho 0] + Vee[\rho 0] \quad (11)$$

Konh and Sham later included an exchange and a correlation term in the functional of Hohemberg and kohn, in order to improve the energy calculation, as shown in Equation 12:

$$HK[\rho_0] = T[\rho_0] + Vee[\rho_0] + EXC[\rho_0] \quad (12)$$

The difference among the DFT methods is in the choice of the exchange and correlation functional, once several approximations are performed in the functional $EXC[\rho_0]$ in order to obtain a better energy for the system. The first exchange and correlation functional developed was the Local Density Approximation (LDA), in which it is possible to approximate a system of non-homogeneous electronic density in a homogeneous system sum. Many approaches have been developed and other exchange and correlation functional have been used and showing satisfactory results(BURKE, 2012; JENSEN, 2007; MORGON; CUSTODIO, 1995).

2.12 Molecular docking

Molecular docking is an important tool in drug development, once this method is used to study the behavior of a ligand inside the enzyme active site, which allows a better understanding of the drug action mechanism. It is worth mentioning that in docking studies it is necessary that the protein three dimensional structure had already been elucidated(MENG et al., 2012; MORGON; COUTINHO, 2007). The docking is used to find the most likely ligand-enzyme complex structure, in which all interactions are analyzed taking into account the ligand conformational flexibility (DU et al., 2016; STARK; POWERS, 2015).

When the ligand is approaching the enzyme active site its conformation is altered to obtain a better fit in the enzyme active site (induced-fit model). Thus, conformational changes also occur in the enzyme, due the interactions between ligand and amino acid residues present in the enzyme active site. These changes in the enzyme conformation are responsible for the

its inhibition or activation(DU et al., 2016; MORGON; COUTINHO, 2007; TAYLOR; JEWSBURY; ESSEX, 2002).

Molecular docking determines the conformation of the most likely ligand-enzyme complex taking into account the interactions performed and the energy of the complex. Technically, an algorithm is used to generate the various poses that the ligand can assume, as well as their respective poses at the binding site of the analyzed enzyme(DU et al., 2016; MORGON; COUTINHO, 2007; STARK; POWERS, 2015).

In this sense, some criteria for the use of this methodology must be adopted in a way that a good balance between effectiveness and computational cost be established. Because of this, the biggest challenge in a docking analysis is how to efficiently deal with protein flexibility, once it is not possible taking into account all degrees of freedom, but, at the same time, excluding them generates poor poses prediction results. Thus, in order to improve the technique, the choice of the region must be done carefully(DU et al., 2016; MENG et al., 2012).

A function called score function is used to estimate the free energies of binding for the poses obtained (DU et al., 2016; MENG et al., 2012; MORGON; COUTINHO, 2007),and is defined by Equation 13:

$$E_{score} = E_{inter} + E_{intra} \tag{13}$$

In which E_{inter} is the intermolecular interaction energy (protein and ligand) and E_{intra} is related to ligand internal energy, as shown in Equations 14 and 15

$$E_{inter} = \sum_{i=ligand} \sum_{j=protein} \left[E_{PLP}(r_{ij}) + 332{,}0 \, \frac{q_i q_j}{4{r_{ij}}^2} \right] \tag{14}$$

In which the E_{PLP} term is the potential energy representing factors related to steric effects and hydrogen bonds. The other term is the Coulomb potential, which describes the electrostatic interactions between molecules with charges(DU et al., 2016; MORGON; COUTINHO, 2007).

$$E_{inter} = \sum_{i=ligand} \sum_{j=ligand} E_{PLP}\,(r_{ij}) \; + \sum_{flexiblebonds} A\,[1 - \cos(m\theta - \theta_0)] + \; E_{\text{clash}} \tag{15}$$

The sums are related to the ligand pair of atoms, excluding those connected by two bonds, the next term refers to torsional energy, in which θ is the bond angle. Finally, the E_{clash} term, is a penalty for nearby atoms (distance below 2.0Å), in which the numerical value assumed is 1000(DU et al., 2016; MORGON; COUTINHO, 2007).

3 GENERAL CONCLUSIONS

In this research, technetium and platinum complexes of 2-(4'aminophenyl)benzothiazole derivatives were studied to purpose both complexes as alternative in cancer diagnosis, once that both compounds have been already studied in biological systems. This work was divided in two parts. In the first one, technetium complex was evaluated as potential contrast agent in magnetic resonance image using EPR parameters through DFT calculation. In the last one, platinum complexes were studied as probe in biological systems using ^{195}Pt chemical shifts performing NMR calculation through DFT methods. These calculations were performed in three different chemical environments: vacuum, implicit solvent and inside active site of enzyme P13K.

For technetium complexes the parameter analyzed was similar to gadolinium, that is the most used compound as contrast agent. In the same line, platinum complexes presented different chemical shifts in the different proposed chemical environment, as expected, once the ^{195}Pt chemical shifts are very sensitive to chemical environment, coordination sphere, ligand and other factors.

In this context, these complexes are potential compounds for cancer diagnosis. It is worth mentioning that technetium complexes already used in medicine and it is less toxic than gadolinium. In the same way, platinum complexes have been used in cancer treatment since long time ago. Thus, to study these complexes is important for finding more effective and less toxic compounds for cancer diagnosis since they are already used in medicine, but not for diagnosis. Therefore, this research finding shed light the importance to study new compounds with in order to improve the diagnosis tools. Thus, experimental studies using Tc and Pt complexes will be an important step for the sequence of this work.

REFERENCES

AHMAD, S. Kinetic aspects of platinum anticancer agents. **Polyhedron**, v. 138, p. 109–124, 2017.

ALCANTARA, D. et al. Molecular imaging of breast cancer: present and future directions. **Frontiers in Chemistry**, v. 2, p. 1–11, 2014.

BASNIWAL, P. et al. 2-(4-Aminophenyl) Benzothiazole: A Potent and Selective Pharmacophore with Novel Mechanistic Action Towards Various Tumour Cell Lines. **Mini-Reviews in Medicinal Chemistry**, v. 6, p. 633–637, 2006.

BRABEC, V.; HRABINA, O.; KASPARKOVA, J. Cytotoxic platinum coordination compounds . DNA binding agents. **Coordination Chemistry Reviews journal**, v. 351, p. 2–31, 2017.

BRAY, F. et al. Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. **A Cancer Journal for Clinicians**, v. 68, p. 394–424, 2018.

BURKE, K. Perspective on density functional theory Perspective on density functional theory. **The Journal of Chemical Physics**, v. 136, p. 1–9, 2012.

CORINTI, D. et al. Hydrolysis of cis- and transplatin: structure and reactivity of the aqua complexes in a solvent free environment. **RSC Advances**, v. 7, p. 15877–15884, 2017.

COSTELLOE, C. M. et al. Imaging bone metastases in breast cancer: techniques and recommendations for diagnosis. **The Lancet Oncology**, v. 10, p. 606–614, 2009.

DASARI, S.; TCHOUNWOU, P. B. Cisplatin in cancer therapy : Molecular mechanisms of action. **European Journal of Pharmacology**, v. 740, p. 364–378, 2014.

DEO, K. M. et al. Platinum coordination compounds with potent anticancer activity. **Coordination Chemistry Reviews**, v. 375, p. 148–163, 2018.

DHEEBA, J.; SINGH, N. A.; SELVI, S. T. Computer-aided detection of breast cancer on mammograms: A swarm intelligence optimized wavelet neural network approach. **Journal of Biomedical Informatics**, v. 49, p. 45–52, 2014.

DU, X. et al. Insights into protein–ligand interactions: Mechanisms, models, and methods. **International Journal of Molecular Sciences**, v. 17, p. 1–34, 2016.

DUFFY, M. J. et al. Clinical use of biomarkers in breast cancer : Updated guidelines from the European Group on Tumor Markers (EGTM). **European Journal of Cancer**, v. 75, p. 284–298, 2017.

ELKIN, M.; NEWHOUSE, T. R. Computational chemistry strategies in natural product synthesis. **Chemical Society Reviews**, v. 47, p. 7830–7844, 2018.

FLOWERS, C. R. et al. Breast Cancer Screening for Women at Average Risk. **Obstetrical &**

Gynecological Survey, v. 314, n. 15, p. 1599–1614, 2015.

FREZZA, M. et al. Novel metals and metal complexes as platforms for cancer therapy. **Current pharmaceutical design**, v. 16, p. 1813–1825, 2010.

GONÇALVES, M. A. et al. Dynamics, NMR parameters and hyperfine coupling constants of the Fe 3O4(1 0 0)-water interface: Implications for MRI probes. **Chemical Physics Letters**, v. 609, p. 88–92, 2014.

GONCALVES, M. D.; HOPKINS, B. D.; CANTLEY, L. C. Phosphatidylinositol 3-Kinase, Growth Disorders, and Cancer. **New England Journal of Medicine**, v. 379, n. 21, p. 2052–2062, 2018.

GROVER, V. P. B. et al. Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians. **Journal of Clinical and Experimental Hepatology**, v. 5, p. 246–255, 2015.

HOFFMANN, R. A.; FORSÉN, S.; GESTBLOM, B. **NMR Basic Principles and Progress**. NY.

JAFARI, S. et al. Breast cancer diagnosis : Imaging techniques and biochemical markers. **Journal of Cellular Physiology**, v. 233, p. 5200–5213, 2018.

JENSEN, F. **Introduction to Computational Chemistry**. Second ed. Chichester: John Wiley & Sons, 2007.

KANNAIYAN, R.; MAHADEVAN, D. A comprehensive review of protein kinase inhibitors for cancer therapy. **Expert Review of Anticancer Therapy**, v. 18, n. 12, p. 1249–1270, 2018.

KATZ, S. et al. Activation of the PI3K/Akt signaling pathway through P2Y2 receptors by extracellular ATP is involved in osteoblastic cell proliferation. **Archives of Biochemistry and Biophysics**, v. 513, n. 2, p. 144–152, 2011.

KERI, R. S. et al. A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry. **European Journal of Medicinal Chemistry**, v. 89, p. 207–251, 2015.

KESHAVARZI, M. et al. Molecular Imaging and Oral Cancer Diagnosis and Therapy. **Journal of Cellular Biochemistry**, v. 118, n. 10, p. 3055–3060, 2017.

KIM, O. et al. Design and synthesis of imidazopyridine analogues as inhibitors of phosphoinositide 3-kinase signaling and angiogenesis. **Journal of Medicinal Chemistry**, v. 54, n. 7, p. 2455–2466, 2011.

KOHN, W. BECKE, A. D. e PARR, R. G. Density functional theory of electronic structure. **Journal of Physical Chemistry,** v. 100, n. 31, p. 12974–12980, 1996.

KOHN, W. SHAM, L. J. Self-Consistent Equations Including Exchange and Correlation Effects. **Physical Review**, v. 140, n. 4A, p. A11333–A1138, 1965.

KUHL, C. The Current Status of Breast MR Imaging Part I. Choice of Technique, Image Interpretation, Diagnostic Accuracy, and Transfer to Clinical Practice. **Radiology**, v. 244, n. 2, p. 356–378, 2008.

LEITE, C. A. V. G.; CALLADO, R. B.; RIBEIRO, R. A. Receptores tirosina-quinase : implicações terapêuticas no câncer. **Revista Brasileira de Oncologia Clínica**, v. 8, n. 29, p. 130–142, 2012.

LEPAGE, M.; GORE, J. C. Contrast mechanisms in magnetic resonance imaging. **Journal of Physics: Conference Series**, v. 3, p. 78–86, 2004.

MANCINI, D. T. et al. 99 Tc NMR as a promising technique for structural investigation of biomolecules : theoretical studies on the solvent and thermal effects of phenylbenzothiazole complex. **Magnetic Resonance in Chemistry**, v. 53, p. 129–137, 2014.

MAVROIDI, B. et al. Palladium (II) and platinum (II) complexes of derivatives of 2- (4 0 - aminophenyl) benzothiazole as potential anticancer agents. **Inorganica Chimica Acta**, v. 444, p. 63–75, 2016.

MAZZOLA, A. A. Ressonância magnética : princípios de formação da imagem e aplicações em imagem funcional Magnetic resonance : principles of image formation and applications in funcional imaging. **Revista Brasileira de Física Médica**, v. 3, n. 1, p. 117–129, 2009.

MENG, X.-Y. et al. Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. **Current Computer Aided-Drug Design**, v. 7, n. 2, p. 146–157, 2012.

MORGON, N. H.; COUTINHO, K. **Métodos de química teórica e modelagem molecular**. Primeira ed. São Paulo: Livraria da Física, 2007.

MORGON, N. H.; CUSTODIO, R. Teoria do funcional de densidade. **Química nova**, v. 18, p. 44–55, 1995.

MUKIZA, J. et al. A Review on Technetium and Rhenium Based Radiopharmaceuticals for Diagnostic Imaging and Therapeutic Nuclear Medicine. **The Rwanda Medical Journal**, v. 75, p. 14–22, 2018.

NEBEN, C. L. et al. Feedback regulation of RTK signaling in development. **Developmental Biology**, v. 447, n. 1, p. 71–89, 2019.

PAPAGIANNOPOULOU, D. Technetium-99m radiochemistry for pharmaceutical applications. **Journal of Labelled Compounds and Radiopharmaceuticals**, v. 60, n. 11, p. 502–520, 2017.

PASCHOAL, D. et al. Predicting Pt-195 NMR Chemical Shift Using New Relativistic All-Electron Basis Set. **Journal of Computational Chemistry**, v. 37, p. 2360–2373, 2016.

PASQUALI, M. et al. Technetium Complexes and Radiopharmaceuticals with Scorpionate Ligands. **Molecules**, v. 23, n. 8, p. 2039, 2018.

PYKETT, I. L. et al. Principles Resonance of Nuclear Imaging Magnetic. **Nuclear Magnetic resonance**, v. 143, p. 157–168, 1982.

ROSERNBERG, B.; VANCAMP, L.; KRIGAS, T. Inhibition of Cell Division in Escherichia coli by Electrolysis Products from a Platinum Electrode. **Nature**, v. 205, p. 698–699, 1965.

ROSTAMZADEH, A. et al. MicroRNAs-Based Imaging Techniques in Cancer Diagnosis and Therapy. **Journal of Cellular Biochemistry**, v. 118, n. 12, p. 4121–4128, 2017.

SHRIVASTAVA, S. et al. Anticancer effect of celastrol on human triple negative breast cancer: Possible involvement of oxidative stress, mitochondrial dysfunction, apoptosis and PI3K/Akt pathways. **Experimental and Molecular Pathology**, v. 98, n. 3, p. 313–327, 2015.

SIEGEL, R. L.; MILLER, K. D.; JEMAL, A. Cancer Statistics , 2018. **A Cancer Journal for Clinicians**, v. 68, n. 1, p. 7–30, 2018.

SILVA, P. P.; GUERRA, W. Platina. **Química Nova na Escola**, v. 32, n. 2, p. 1–2, 2010.
SRIVASTAVA, S. C. Is there life after technetium what is the potential for developing new broad-based radionuclides? **Seminars in Nuclear Medicine**, v. 26, n. 2, p. 119–131, 1996.

STARK, J. L.; POWERS, R. Application of NMR and Molecular Docking in Structure-Based Drug Discovery. v. 200, n. 1, p. 165–187, 2015.

STILL, B. M. et al. 195Pt NMR - theory and application. **Chemical Society Reviews**, v. 36, n. 4, p. 665–686, 2007.

TANG, X. et al. Magnetic resonance imaging relaxation time in Alzheimer's disease. **Brain Research Bulletin**, v. 140, n. May, p. 176–189, 2018.

TAYLOR, R. D.; JEWSBURY, P. J.; ESSEX, J. W. A review of protein-small molecule docking methods - Taylor-JCAMD2002.pdf. p. 151–166, 2002.

TZANOPOULOU, S. et al. Synthesis, characterization, and biological evaluation of M(I)(CO) 3(NNO) complexes (M = Re, 99mTc) conjugated to 2-(4-aminophenyl)benzothiazole as potential breast cancer radiopharmaceuticals. **Journal of Medicinal Chemistry**, v. 49, n. 18, p. 5408–5410, 2006.

TZANOPOULOU, S. et al. Evaluation of Re and 99mTc complexes of 2-(4???-aminophenyl) benzothiazole as potential breast cancer radiopharmaceuticals. **Journal of Medicinal Chemistry**, v. 53, n. 12, p. 4633–4641, 2010.

URSINI, C. V. Ressonância magnética nuclear de Platina-195 em compostos organometálicos. **Química Nova na Escola**, v. 20, n. 1, p. 72–82, 1997.

WAHSNER, J. et al. Chemistry of MRI contrast agents: Current challenges and new frontiers. **Chemical Reviews**, v. 119, n. 2, p. 957–1057, 2019.

WAKS, A. G.; WINER, E. P. Breast Cancer Treatment: A Review. **JAMA - Journal of the American Medical Association**, v. 321, n. 3, p. 288–300, 2019.

XIAO, Y. D. et al. MRI contrast agents: Classification and application (Review). **International Journal of Molecular Medicine**, v. 38, n. 5, p. 1319–1326, 2016.

YURTTAŞ, L.; TAY, F.; DEMIRAYAK, Ş. Synthesis and antitumor activity evaluation of new 2-(4-aminophenyl)benzothiazole derivatives bearing different heterocyclic rings. **Journal of Enzyme Inhibition and Medicinal Chemistry**, v. 30, n. 3, p. 458–465, 2015.

ZONOUZY, V. T. et al. An educational intervention based on the extended parallel process model to improve attitude , behavioral intention , and early breast cancer diagnosis : a randomized trial. **International Journal of Women's Health**, v. 11, p. 1–10, 2019.

SECOND PART

ARTCLE 1

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Exploring EPR parameters of ^{99}Tc complexes for designing new MRI probes: coordination environment, solvent and thermal effects on the spectroscopic properties

Abstract

In this work, we have evaluated the solvent and thermal effects on spectroscopic parameters of ^{99}Tc complexes coordinated to explicit water molecules. Molecular dynamics simulations were performed followed by hyperfine coupling constant calculations (A_{iso}). Our results show a significant increase of $A_{iso,}$ which demonstrates that the studied compounds can be promising contrast agents in MRI.

Keywords:^{99}Tc; MRI; Contrast Agents; Hyperfine constant.

1. Introduction

Currently, cancer is one of the most serious health problems faced by humanity. Studies show a large increase in incidence of this disease, including a growing number of deaths [1].Among the several types of cancer, breast cancer is the most frequent in the world, incidence being more common in women [2].

Generally, breast cancer is diagnosed in advanced stages and consequently presents the highest mortality rates in the entire world [2, 3].However, more recently, some modern techniques, such as tomography and Magnetic Resonance Imaging (MRI) [4], which allow the diagnosis in early stages, have been utilized for breast cancer diagnosis. The MRI has been considered a very effective technique due its high sensitivity in finding small tumors and nodules in the breast [5]. In fact, MRI has been shown as a strong tool for early stage breast cancer diagnosis [5].

The MRI is considered a non-invasive technique for diagnosis and is based on the magnetic properties of the ^{1}H and ^{17}O nuclei, which are the most abundant elements in the human body [6]. However, with only the natural relaxation of the water molecules in the body, it is often not possible to obtain clear MRI images. Thus, aiming to improve the image resolution contrast agents (CAs) are used [7].

The CAs are paramagnetic compounds able to decrease longitudinal and transverse relaxation times of water molecules in the proximity of their structure, thus facilitating breast cancer diagnosis [8]. Based on this context, it is necessary to understand the relaxation mechanisms of water molecules and the influence of paramagnetic effects on the ^{1}H and ^{17}O hyperfine coupling constant (A_{iso}) values [7].

More recently, radioisotopes of Technetium (Tc) have been considered promising nuclei for the NMR (Nuclear Magnetic Resonance) and MRI techniques [9-11]. Based on this context, in 2006 Tzanopoulou *et al.* synthesized the $(^{99m}Tc)(CO)_3(NNO)$ complex conjugated to the antitumor agent 2-(4'-aminophenyl)benzothiazole [ABT; see Complex **1**, Figure 1] [12].The ABT compound presents nanomolar activity *in vitro* against some breast cancer cells in humans. In addition, this compound can be utilized for transporting the radioisotope of choice to the diseased tissue, facilitating the diagnostic and therapeutic applications against breast cancer [13-15].

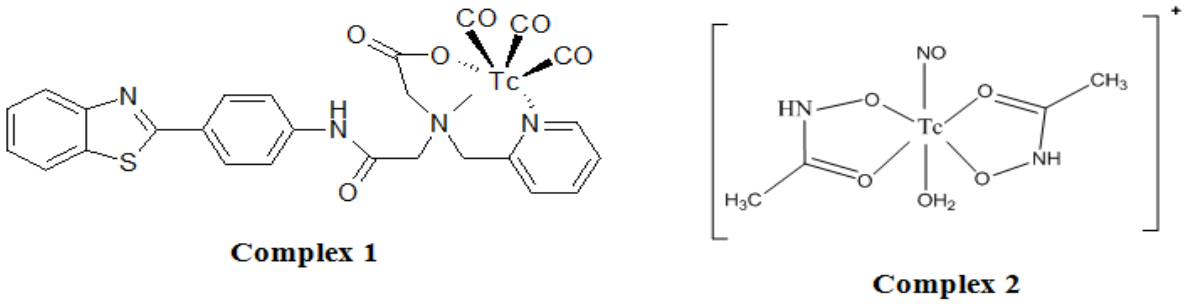


Figure 1*:* (^{99m}Tc)(CO)3(NNO) conjugated with 2-(4'-aminophenyl)benzothiazole(1); oxotechnetium(V) complex with the ligand N(2(1-Hpyrolmethyl) N'-(4-pentene-3-one-2)ethane-1,2-diamine (2)

The ^{99m}Tc is a meta stable nucleus and emitter of gamma energy (Eγ = 140 keV), with a relatively short half-life (t1/2=6.02h). The^{99m}Tc disintegrates by emission of gamma radiation to yield ^{99}Tc,which is less toxic, more stable and presents along half-life ($t_{1/2}$ = 2.2 x 10^5 years) [16]. The ^{99}Tc nuclei presents a quadrupole moment equal to 0.19 x 10^{-28} m^2 and a large spin, I = 9/2. It should be kept in mind that these characteristics can make of the ^{99}Tc a promising nucleus for the EPR (Electron Paramagnetic Resonance) spectroscopy and MRI studies [17, 18]. According to literature, the ^{99m}Tc complexes are used in 85% of cancer diagnosis cases in hospitals [19].

As another paramagnetic complex, $[Tc(NO)(aha)_2(H_2O)]^+$ (Complex **2**, Figure 1), was used to evaluate the Tc coordination environment effect on the ^{1}H and ^{17}O hyperfine coupling constant (A_{iso}) values. The EPR parameters of a set Tc(II) nitrosyl complexes was reported in the previous studies in the literature [20].

In order to validate our calculation strategy for ^{1}H and ^{17}O A_{iso} values, the complex $[Mn(H_2O)_6]^{2+}$ was used. Within this context, the goal of this work is to explore the spectroscopic properties of the (^{99m}Tc)$(CO)_3$(NNO) complex conjugated with the ABT compound in solution, evaluating the thermal and solvent effects on the ^{1}H and^{17}O hyperfine coupling constant values and thus propose this compound as a new MRI contrast agent. In addition, different coordinating modes, as well as solvent and thermal effects on A_{iso} values for Tc complexes were investigated.

2. Computational methods

2.1 Optimization and molecular dynamics procedure

Geometries were fully optimized using the gradient-corrected density functional BP86 [21] and LanL2dz basis set [22]. Water molecules were introduced in the system using the ADF(Amsterdam Density Functional) software [24], the solvent sphere was radius 15 Å and the solute factor was approximately 1.0 Å. Since the time-scale accessible to the atom-centered density matrix propagation (ADMP) [24] method is very restricted, no extensive equilibration is possible and care must be taken to start from reasonably well pre-equilibrated configuration. To this end, we first prepared a classical system through an MD simulation using the modified force field (GROMOS96) [25, 26] with the GROMACS 5.1 package [27]. As usual, periodic boundary conditions (PBC) and a cutoff distance of 9.0 Å were applied. Using the last configuration from classical MD as the starting point, we subsequently started quantum MD simulations using the ADMP method at the DFT level [24] (BP86/LanL2dz). These procedures have been employed with success in previous studies [11].

ADMP employs an extended Lagrangian similar to the well-known Car-Parrinello molecular dynamics. It can treat all electrons quantum-mechanically and can control the deviations from the Born-Oppenheimer surface precisely [24, 28]. In this work, a temperature of 310K (physiologic temperature) was used throughout the simulation. In fact, this temperature is suitable to simulate the behavior of compounds in biological systems. After an equilibrium time of 1 ps, in which a temperature of 310K was maintained via velocity rescaling, statistical averages and snapshots for hyperfine coupling constant (HFCC) calculations were collected from subsequent unconstrained micro canonical runs of 1 ps, obtained a total of 1000 conformations. Snapshots were taken every 25 fs generating a total of 40 conformations [11] for use in HFCC calculations. A new method of selecting structures it has been successfully used, OWSCA, this method uses the wavelet transform to decompose the DM signal, the signal decomposed by the transform is able to represent well and with a minimum error the entire DM signal [29]. All optimization and quantum MD calculations were carried out using Gaussian 09 software [30].

2.2 Hyperfine coupling constant (HFCC) calculations

After MD simulations, the structures of Complexes 1 and 2 (Figure 1) with water molecules were used for the hyperfine coupling constant (HFCC) calculations. For both complexes, the A_{iso} calculations were performed using the functional PBE1PBE [31] with the basis set aug-cc-pVTZ-J and EPR-III [32] for hydrogen and oxygen atoms; 6-31G [33] for the carbon, nitrogen and sulfur atoms and LanL2dz for the technetium atom in the Gaussian 09 program [30]. For the discussions of A_{iso} calculations, we used the following notation: level of A_{iso} computation//level of geometry optimization or MD simulation. For example: ((PBE1PBE (H_2O) // BP86 (H_2O)) means A_{iso} computation with explicit solvent//geometry optimization with explicit solvent; ((PBE1PBE (H_2O) / PCM // BP86 (H_2O)) means A_{iso} computation with explicit and implicit solvent (PCM)//geometry optimization with explicit solvent molecules.

The (PBE1PBE (H_2O) // BP86 (H_2O)) structures were used as the starting point for the MD simulations. The same notation is utilized when including the dynamic effect (MD simulation).

2.3 QTAIM Calculations and Spin Density Distributions.

The Quantum Theory of Atoms in Molecules *(*QTAIM*)* is firstly an extension of quantum mechanics to sub domains, properly defining an atom as an open system. The QTAIM is important to describe the properties of atoms (such as the nature of the chemical bond and the strength of hydrogen bonding) [34, 35]. AIM analysis was performed using calculations in the AIMALL [36] and QTAIMQB program [35], the analysis of the results were made by the AIMSTUDIO program [36], both are part of the AIMALL program package [36].

The QTAIM calculations were performed with the optimized geometries at the level BP86/Lanl2dz. Atomic spin density was evaluated using the natural population analysis (NPA) performed in the Gaussian 09 program [30] the contour surface was fixed at 0.0004 a.u value.

3. Results and Discussion

3.1 Validation of the Hyperfine Coupling Constant (HFCCs) calculations.

This stage of the work was performed to validate the theoretical methodology used to calculate the EPR parameters, because, to our knowledge, there is no ^{1}H and ^{17}O hyperfine coupling constant data for the proposal complexes in Fig. 1 are ported in the literature so far.

It is known that the relaxation theory of NMR has been the subject of many books and scientific articles [37]. Thus, the NMR relaxation parameters have been considered one of the most useful and versatile method for the investigation of MRI probes. The relaxation time (T_1 e T_2) is given by paramagnetic ions that are able to interact with the water molecules, drastically reducing the relaxation times. Equations 1 and 2 show the relaxation times T_1 and T_2 induced by paramagnetic ions in aqueous solution, respectively [38, 39].

Equation 1:

$$R_1 = \frac{1}{T_1} \cong \frac{1}{15}\frac{S(S+1){g_e}^2\beta^2{g^2}_N\ {\beta^2}_N}{\hbar^2 r^6} + (\frac{A}{\hbar})^2\ \frac{S(S+1)}{3}[\frac{2\tau_e}{1+(\omega_I\tau_e)^2}]$$

Equation 2:

$$R_2 = \frac{1}{T_2} \cong\ \frac{1}{15}\frac{S(S+1)g_e^2\beta^2g_N^2\beta_N^2}{h^2\ r^6} + (\frac{A}{\hbar})^2\ \frac{S(S+1)}{3}[\tau_C + \frac{\tau_C}{1+(\omega_S\tau_e)^2}]$$

The relaxation time constant (T_1 and T_2) depends on the electron spin (S), the electronic and proton g factors (g_e and g_N, respectively), the Bohr magnet on (β), the nuclear magnet on (β_N), the hyperfine coupling constant (A), the ion-nucleus distance (r), and the Larmor frequencies for the proton and electron spins ($\omega_I$and ω_S, respectively). The correlation times τ_c and τ_e are characteristic of the rate of change of the interactions between the metallic species and neighboring protons. In fact, the order parameter, the overall molecular rotational correlation time (τ_c) and the internal rotational correlation time (τ_e) are essential motional parameters to obtain pictures of molecular motion [38, 39]. In the Equations 1 and 2, each of the relaxation rates (R_1 and R_2) is a sum of two terms. The first term comes from the dipolar coupling and the second term from the scalar coupling [38, 39].

In order to validate our calculation strategy for ^{1}H and ^{17}O hyperfine coupling constant values, the complex $[Mn(H_2O)_6]^{2+}$ was used [40, 41]. In this line, theoretical and experimental values are reported in Table 1. The theoretical calculations were performed using two

different basis sets (EPR-III and aug-cc-pVTZ-J) with the functional PBE1PBE. Among other basis sets, the choice of the basis sets EPR-III and aug-cc-pVTZ-Jwith the functional PBE1PBE improved the results, so that they are completely satisfactory. Both basis sets have been shown to be satisfactory in similar structures [30, 40]. The smaller basis sets are unsatisfactory and the larger are too expensive.

For the HFCCs calculations of the static equilibrium structure in the presence of explicit solvent A_{iso}^{eq}((PBE1PBE (H_2O) // BP86 (H_2O)), we obtained 0.62 and 0.76 MHz for ^1H and values of 1.98 and 3.14 MHz for ^{17}O at the EPR-III and aug-cc-pVTZ-J levels, respectively. For the static equilibrium structure with explicit and implicit solvent A_{iso}^{eq}(PBE1PBE(H_2O)/PCM// BP86 (H_2O)), the values 0.23 and 0.66MHz for ^1H and 2.08 and 1.41 MHz were obtained for ^{17}O at the EPR-III and aug-cc-pVTZJ levels, respectively. Taking in to consideration now the structures selected during the MD simulation,A_{iso}^{310K} (MD(H_2O)//MD(H_2O)), values of 0.81 and 1.00 MHz for ^1H, and 2.23 and 4.96 MHz for ^{17}O, with the basis set EPR-III and aug-cc-pVTZ-J were obtained, respectively. The experimental values for $[Mn(H_2O)_6]^{2+}$ are 0.81 and 5.38 MHz for the ^1H and ^{17}O, respectively. Thus, it is possible to observe that the aug-cc-pVTZ-J basis set is in much better agreement with the experimental values, on the other hand, the ^{17}O values at the EPR-III level is far from the experimental value.

Table 1: Calculated hyperfine coupling constants (A_{iso}) [MHz] for static equilibrium structure (A_{iso}^{eq}) and for the structures selected during the MD simulation (A_{iso}^{310K})

$[Mn(H_2O)_6]^{2+}$		**A_{iso}**	
Type	Method	^1H[MHz][b]	^{17}O [MHz][b]
A_{iso}^{eq}((PBE1PBE (H_2O) // BP86 (H_2O))	EPR-III	0.62	1.98
	AUG-cc-pVTZ-J	0.76	3.14
A_{iso}^{eq}(PBE1PBE(H_2O)/PCM// BP86 (H_2O))	EPR-III	0.23	2.08
	AUG-cc-pVTZ-J	0.66	1.41
A_{iso}^{310K}(MD(H_2O)//MD(H_2O))[c]	EPR-III	0.81 ± 0.10[a]	2.23 ± 0.83[a]
	AUG-cc-pVTZ-J	1.00 ± 0.10[a]	4.96 ± 0.90[a]
Experimental[40]		**0.81**	**5.38**

[a]Standard deviations values.
[b]A_{iso}values for ^{17}O and ^1H correspond to average values for all water molecules present in the system.
[c]A_{iso} average values for the 40 conformations selected of MD.

3.2 Thermal and solvent effects on the Hyperfine Coupling Constant (HFCCs) for Complex 1

According to Tzanopoulou *et al.*, (Figure 1) is a potential candidate for imaging techniques (^{99m}Tc), facilitating breast cancer diagnosis [12, 13].In view of that, we have performed EPR calculations evaluating thermal and solvent effects on the Hyperfine Coupling Constant (HFCCs) in Complex **1**.

In Table 2, it is possible to observe that theA_{iso}^{eq}values for the static equilibrium structure with the explicit solvent ((PBE1PBE (H_2O) // BP86 (H_2O)),were 0.89 and 3.39 MHz for ^{1}H and ^{17}O, respectively.For the static equilibrium structure with explicit and implicit solvent A_{iso}^{eq}(PBE1PBE(H_2O)/PCM//BP86(H_2O)), we obtained ^{1}H and ^{17}O values of 0.84 for 3.79 MHz, respectively. A slight difference was observed between the explicit and explicit / implicit solvent of 0,05MHz for the ^{1}H and 0,4 MHz for ^{17}O (Table 2). This small difference between the explicit and explicit / implicit solvent is to be expected,which shows that the number of water molecules added during the A_{iso} calculation describes the systemwell. Considering now the structures selected during the MD simulation,A_{iso}^{310K} (MD(H_2O)//MD(H_2O)), values of 1.07 and 1.50 MHz were obtained for ^{1}H and ^{17}O respectively. Thus, it can be seen that the thermal effects greatly influence the system, particularly the water molecule ^{17}O atoms, as can be seen in Table 2.

It is important to observe in Table 2 that, the $A_{iso}^{eq}$and A_{iso}^{310K}values for Complex **1** are higher than the Gadolinium complex in solution, which is the most used contrast agent currently. According to the literature, the A_{iso} experimental values for $[GdL(H_2O)]^{n+/-}.xH_2O$ complexes can vary between 0.5 – 0.6 MHz [42, 43].

It should be kept in mind, however, that, the use of gadolinium complexes as MRI contrast agents has revealed serious problems due to their high toxicity [44].Based on this context, an alternative is the use of technetium complexes as MRI contrast agents, which present lower toxicity and show good (A_{iso}) results in solution (see Table 2).

Table 2: Calculated hyperfine coupling constants (A_{iso}) [MHz] for static equilibrium structure (A_{iso}^{eq}) and for the structures selected during the MD simulation (A_{iso}^{310K}) with aug-cc-pVTZ-J level.

Complex 1	**A_{iso}**	
Type	^{1}H [MHz][b]	^{17}O [MHz][b]
A_{iso}^{eq}(PBE1PBE(H_2O)// BP86 (H_2O))	0.89	3.39
A_{iso}^{eq}(PBE1PBE(H_2O)/PCM// BP86 (H_2O))	0.84	3.79
A_{iso}^{310K}(MD(H_2O)//MD(H_2O))[c]	1.07± 0.16[a]	1.50± 0.65[a]

[a]Standard deviations values.
[b]A_{iso}values for ^{17}O and ^{1}H correspond to average values for all water molecules present in the system.
[c]A_{iso} average values for the 40 conformations selected of MD.

3.3 Thermal and solvent effects on the Hyperfine Coupling Constant (HFCCs) for the Complex 2.

In this work, we have also performed EPR calculations, evaluating thermal and solvent effects on the Hyperfine Coupling Constants (HFCCs) on Complex **2**(Figure 1), in order to investigate also the influence of the Tc coordination environments on the A_{iso} values.

In Table 3, it is possible to observe that, for the static equilibrium structure with the explicit solvent A_{iso}^{eq}(PBE1PBE(H_2O)//BP86 (H_2O)) the values 1.28 MHz for ^{1}H and 2.30 MHz for ^{17}O were obtained. For the static equilibrium structure with explicit and implicit solvent A_{iso}^{eq} (PBE1PBE(H_2O)/PCM//BP86 (H_2O)) the values 1.02 MHz for ^{1}H and 2.40 MHz for ^{17}O were obtained. Thus, there is a small difference between both solvation models (explicit and explicit / implicit solvents) of 0.26 MHz and 0.10 MHz for ^{1}H and ^{17}O, respectively.It is possible to notice that explicit water molecules is sufficient to realistically represent our system.

By analyzing the structures selected during the MD simulation, A_{iso}^{310K} (MD(H_2O)//MD(H_2O)), the ^{1}H and ^{17}O values of 1.41 and 2.78 MHz were obtained, respectively. Thus, it can be seen that the thermal effects are very important for spectroscopic calculations.

It is important to observed that the Tc coordination environments are different for both Complexes, $Tc(CO)_3(NNO)$ and $[Tc(NO)(aha)_2(H_2O)]^+$, Complex **1** and 2, respectively. For equilibrium geometry, comparing the A_{iso} values for Complex **1** (Table 2) with Complex **2** (Table 3), the changes in the A_{iso}^{eq} values for ^{17}O were up to 1.09 MHz. On the other hand, the changes in the A_{iso}^{eq} values for ^{1}H were up to 0.39 MHz. Also it is important to notice that, when the thermal effect was included in system, it was observed a difference in the A_{iso}^{310K} values of 1.28 and 0.34 MHz for the ^{17}O and ^{1}H atoms, respectively (see Tables 2 and 3). Based on this context, our findings point out that, changes in the coordination environment of Tc complexes can significantly influence the A_{iso} results.

Table 3: Calculated hyperfine coupling constants (A_{iso}) [MHz] for static equilibrium structure (A_{iso}^{eq}) and for the structures selected during the MD simulation (A_{iso}^{310K}) with aug-cc-pVTZ-J level

Complex 2	**A_{iso}**	
Type	^{1}H [MHz][b]	^{17}O [MHz][b]
A_{iso}^{eq}(PBE1PBE(H_2O)// BP86 (H_2O))	1.28	2.30
A_{iso}^{eq}(PBE1PBE(H_2O)/PCM// BP86 (H_2O))	1.02	2.40
A_{iso}^{310K}(MD(H_2O)//MD(H_2O))[c]	1.41 ± 0.14[a]	2.78 ± 0.31[a]

[a]Standard deviations values.
[b]A_{iso}values for ^{17}O and ^{1}H correspond to average values for all water molecules present in the system.
[c]A_{iso} average values for the 40 conformations selected of MD.

3.4 Analysis of Quantum Theory of Atoms in Molecules (QTAIM) and Spin Density Distributions.

The QTAIM methodology is a quantum model considered innovative in studies of chemical bonds, but also is effective in characterizing intramolecular and / or intermolecular interactions [45].Thus, QTAIM calculations are very important to check the influence of hydrogen bonds in the A_{iso} values [39-50] this model was developed by Richard Bader [46]. Table 4 shows the values of the analyzed parameters, **1a**, **1b,1c**(complex 1) and **2a, 2b, 2c** (complex 2)are the interactions analyzed for. In according to the Koch and Popelier parameter [47], the atoms in **1a**possess $\nabla^2\rho(r) > 0$ and $H(r) < 0$, suggesting partial covalent interactions.

Now, the other analyzed interactions (**1a**,1b, 1c, 2a, 2b and 2c) possess $\nabla^2\rho(r) > 0$ and H(r) > 0, suggesting non-covalent interactions.

Table 4: QTAIM parameters obtained at the hydrogen bond BCPs for the structures of **1**-**2**(au) (Structures 1: Complex **1** with waterand2: Complex **2**with water molecules).

Structure	ρ(r)	$\nabla^2\rho(r)$	ε	V(r)	G(r)	H(r)
1a$_{(Oa\cdots Ha)}$	+0.033326	+0.120236	+0.149635	-0.031505	+0.030782	-0.000723
1b$_{(Ob\cdots Hb)}$	+0.027099	+0.099372	+0.057624	-0.023879	+0.024361	+0.000482
1c$_{(Na\cdots Hc)}$	+0.014533	+0.049406	+0.477539	-0.009003	+0.010677	+0.001674
2a$_{(Oa\cdots Ha)}$	+0.027257	+0.115545	+0.083698	-0.025766	+0.027326	+0.00156
2b$_{(Ob\cdots Hb)}$	+0.019167	+0.076374	+0.094193	-0.015995	+0.017544	+0,001549
2c$_{(Oc\cdots Hc)}$	+0.010155	+0.040305	+0.230208	-0.006249	+0.008162	+0.001913

From the rigorous concepts of QTAIM, the BCPs (bond critical points) are located on hydrogen bonds formed by proton donors and electrons π, Figures 2 and 3 [33, 42], thus the low ρ(r) values along with the positive results of the Laplacian ($\nabla^2\rho(r)$) indicate the formation of hydrogen bonds in each intermolecular BCP. Thus, we can see that when putting the water molecules in the system, interactions of free water molecules with the oxygen of complex (HO···H) can take place.

Interestingly, some hydrogen bonds (HO···H) among free water molecules in solution were broken and new hydrogen bonds taken place with the complex, thereby conferring extra stability to the system.

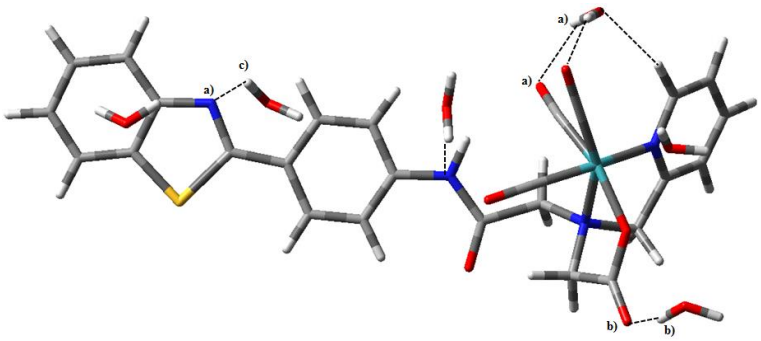


Figure 2*:* Geometry for Complex 1 with water molecules.

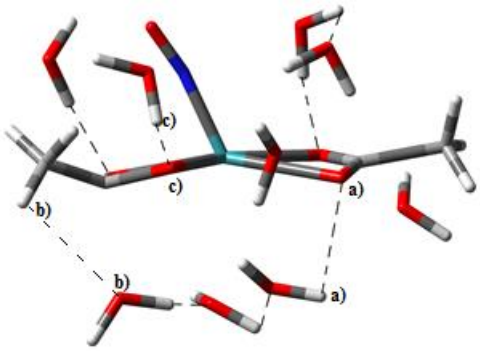
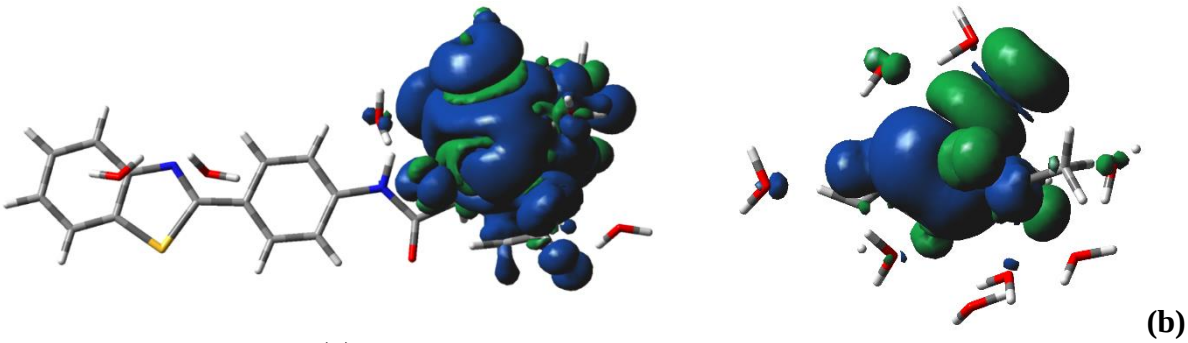


Figure 3: Geometry for Complex 2 with water molecules.

Figure 4 shows the spin density around the atoms complex, the distribution of the spin density in a given paramagnetic molecule indicates the contributions due to electrons with the majority spin (α) and the minority spin (β) [48, 49]. In Figure 4 these regions are represented by the colors blue (spin α) and green (spin β). Thus, Figure 4 shows a high spin density around the complex, especially the part around the metal and it is possible to note that their electron transfer from the metal (spin α) to the ligands of the complex (spin β). A characteristic indication of a spin-polarization effect is the presence of alternate spin density signs along the pathway of the bonded atoms radiating out from the paramagnetic atom. For our complex, the density is negative around the ^{17}O nucleus of coordinated water molecules and positive at their ^{1}H nuclei. Thus, the significant increase of A_{iso} values is due to the strong hydrogen bonding of water molecules with the complex and also due to the transfer of electrons around the complex [50].



(a) **(b)**

Figure 4: Spin-density map of the compounds (the iso surface contour value is 0.0004). (a) Complex 1 (b) Complex 2

4. Conclusions

In this work, the performance of two bases sets, EPR-III and aug-cc-pVTZ-J,^{17}O and ^{1}H HFCCs calculations in $[Mn(H_2O)_6]^{2+}$ were evaluated. Our results show that the aug-cc-pVTZ-J bases set presents a realistic description of the system ($[Mn(H_2O)_6]^{2+}$) in different environments, because good agreement was observed between theoretical and the experimental results for A_{iso} values.

Furthermore, it was possible to theoretically determine the A_{iso} values for ^{17}O and ^{1}H in Complexes **1** and **2**. Thermal and solvent effects were also studied computationally by quantum-chemical methods. It is worth noting that these effects are important for^{17}O and ^{1}H HFCC calculations.

It is well-known that the use of gadolinium complexes as MRI contrast agents has generated several problems due to their high toxicity [9]. However, our theoretical findings point out that an alternative to this traditional approach is to use technetium complexes as MRI contrast agents. They present lower toxicity and show good A_{iso} results in solution. Motivated by this idea, we report a theoretical proof-of-principle study on the use of Tc complexes for designing new MRI probes. To our knowledge, this is the first application of this approach in the condensed phase.

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References

1.B. Dunn, “Cancer: Solving an age-old problem,” *Nature Reviews Cancer, vol.*483, pp. S2-S6, 2012.

2.E. R.Galvão, L. M. Martins,J.O Ibiapina, H. MAndrade, S. J. Monte, “Breast câncer proteomics: a review for clinicians,”*Journal of Cancer Research and Clinical Oncology,*vol. 137, pp. 915-925, 2011.

3.A. Jemal, R. Siegel, J. Xu, E. Ward, “Cancer Statistics,”*CA: A Cancer Journal for Clinician,*vol. 60, pp. 277-300, 2010.

4.C. Angelis, R. F. Brizzi, R. Pellicano, "Endoscopic ultrasonography for pancreatic cancer: current and future perspectives,"*The Journal of Gastrointestinal Oncology, vol.* 4, pp. 220-230, 2013.

5. L. F. Chala, N. Barros, "Avaliação das mamas com métodos de imagem,"*Radiologia Brasileira,* vol.40, pp.4-6, 2007.

6. A. A. Mazzola, "Ressonância magnética: princípios de formação da imagem e aplicações em imagem funcional,"*Revista Brasileira de Física Médica,* vol.3, pp.117-129, 2009.

7. G. H. Im, S. M. Kim, D. G. Lee, W. J Lee, J. H. Lee, I. S. Lee, "Fe(3)O(4)/MnO hybrid nanocrystals as a dual contrast agent for both T(1)- and T(2)-weighted liver MRI,"*Biomaterials,* vol.34, pp. 2069-2076, 2013.

8. P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, "Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics, and Applications,"*Chemical reviews,* vol.99, pp. 2293-2352, 1999.

9.M. Sagnou, S. Tzanopoulou, C. P. Raptopoulou, V. Psycharis, H. Braband, R. Alberto, I. C. Pirmettis, M. Papadopoulos, M. Pelecanou, "A Phenylbenzothiazole Conjugate with the Tricarbonyl *fac*-$[M(I)(CO)_3]^+$ (M = Re, ^{99}Tc, ^{99m}Tc) Core for Imaging of β-Amyloid Plaques,"*Journal of Inorganic Chemistry,* vol.27, pp. 4279-4286, 2012.

10.Tarasov VP, Muravlev BY, German KE, Popova NN, "99Tc NMR of Supported Technetium Nanoparticles,"*Doklady Physical Chemistry,* vol.377, pp. 71-76, 2001.

11.D. T. Mancini, E. F. Souza, M. S. Caetano, T. C. Ramalho, "99Tc NMR as a promising technique for structural investigation of biomolecules: theoretical studies on the solvent and thermal effects of phenylbenzothiazole complex,"*Magnetic Resonance in Chemistry* vol. 52, pp.129-137, 2014.

12.S. Tzanopoulou, I. C. Pimettis, G. Patsis, M. Paravatou-Petsotas, E. Livaniou, M. Papadopoulos, M. Pelecanou, "Synthesis, characterization, and biological evaluation of M(I)(CO)3(NNO) complexes (M = Re, 99mTc) conjugated to 2-(4-aminophenyl) benzothiazole as potential breast cancer radiopharmaceuticals,"*Journal of Medicinal Chemistry* vol. 49, pp. 5408-5410, 2006.

13.S. Tzanopoulou, M. Sagnou, M. Paravatou-Petsotas, E. Gourni, G. Loudos, S. Xanthopoulos, D. Lafkas, H. Kiaris, A. Varvarigou, I. C. Pirmettis, M. Papadopoulos, M. Pelecanou, "Evaluation of Re and (99m)Tc complexes of 2-(4'-aminophenyl) benzothiazole as potential breast cancer radiopharmaceuticals,"*Journal of Medicinal Chemistry*vol. 53, pp. 4633-4641, 2010.

14. D. F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fichtner, M. F. G. Stevens, "Antitumor benzothiazoles. 3. Synthesis of 2-(4-aminophenyl)benzothiazoles and evaluation of their activities against breast cancer cell lines in vitro and in vivo,"*Journal of Medicinal Chemistry*vol. 39, pp.3375-3384, 1996.

15.D. T. Mancini, K. Sen, M. Barbatti,W. Thiel, T. C. Ramalho, "Excited-State Proton Transfer Can Tune the Color of Protein Fluorescent Markers,"*Physical Chemistry Chemical Physics* vol.16, pp.3444-3449, 2015.

16.U. Abram, R. Alberto, "Technetium and Rhenium - Coordination Chemistry and Nuclear Medical Applications,"*Journal of the Brazilian Chemical Society* vol.17, pp.1486-1500, 2006.

17.N. I. Gorshkov, A. A. Lumpov, A. E. Miroslavov, V. A. Mikhalev, D. N Suglobov, "^{99}Tc NMR study of complexation of $[Tc(CO)_3(H_2O)_3]^+$ with halide and thiocyanate ions in aqueous solutions,"*Czechoslovak Journal of Physics* vol.53, pp.A745-A749, 2003.

18.K. J. Franklin, C. J. L Lock, B. G. Sayer, G. J. Schrobilgen, "Chemical applications of 99Tc NMR spectroscopy: Preparation of novel Tc(VII) species and their characterization by multinuclear NMR spectroscopy,"*Journal of the American Chemical Society*,vol.104, pp.5303-5306, 1982.

19.S. S. Jurisson,L. D.Lydon, "Potential technetium small molecule radiopharmaceuticals,"Chemical Reviews,vol. 99, pp.2205-2218, 1999.

20.S. M. Balasekaran,J. Spandl,A. Hagenbach,K. Köhler,M. Drees, U. Abram, "Fluoridonitrosyl Complexes of Technetium(I) and Technetium(II). Synthesis, Characterization, Reactions, and DFT Calculations,"*Inorganic Chemistry*, vol.53, pp.5117-5128, 2014.

21. a) A. D.Becke, "Density-functional exchange-energy approximation with correct asymptotic behavior,"*Phys RevA*, vol.38, pp.3098-3100, 1988. b) J. P. Perdew, "Density-functional approximation for the correlation energy of the inhomogeneous electron gas, "*Physical Review B*, vol.33, pp.8822-8824, 1986. c) J. P. Perdew, "Erratum: Density-functional approximation for the correlation energy of the inhomogeneous electron gas,"*Physical Review B*, vol.34, pp.7406, 1986.

22. T. H. Dunning Jr, P. J. Hay, "in Modern Theoretical Chemistry; Methods of Electronic Structure Theory," edited by Schaefer H.F. III Ed., Plenum Press, New York.vol. 3, pp.1-28, 1977.

23.G. Tevelde, F. M. Bickelhaupt, E. J. Baerends, C. F. Guerra, S. J. A. Van Gisbergen, J. G. Snijders, T. Ziegler, "Chemistry with ADF,"*Journal of Computational Chemistry*, vol.22, pp.931-967, 2001.

24.S. S. Iyengar, H. B. Schlegel, G. A. Voth, "Atom-Centered Density Matrix Propagation (ADMP): Generalizations Using Bohmian Mechanics." *Journal of Physical Chemistry A*,vol.107, pp.7269-7277, 2003.

25. D. Van der Spoel, A. R. van Buuren, E. Apol, P. J. Meulenhoff, D. P. Tieleman, A. L. T. M. Sijbers, B. Hess, K. A. Feentra, E. Lindahl, R. van Drunen, H. J. C. Berendsen, GROMACS user manual, version 3.0, 2001.

26. A. P. Guimarães, A. A. Oliveira, E. F. F. da Cunha, T. C. Ramalho, T. C. C. França, "Analysis of Bacillus anthracis nucleoside hydrolase via in silico docking with inhibitors

and molecular dynamics simulation," *Journal of Molecular Modeling* vol.17, pp.2939–2951, 2011.

27.T. C. Ramalho, M. Bühl, "Probing NMR parameters, structure and dynamics of 5-nitroimidazole derivatives. Density functional study of prototypical radiosensitizers," *Magnetic Resonance in Chemistry*, vol.43, pp.139-146, 2005.

28.T. C. Ramalho, D. H. Pereira, W. Thiel, "Thermal and solvent effects on NMR indirect spin-spin coupling constants of a prototypical Chagas disease drug,"*Journal of Physical Chemistry A*, vol.115, pp. 13504-13512, 2011.

29. M. A. Gonçalves, L. S. Santos, D. M. Prata, F. C. Peixoto, E. F. F. da Cunha, T. C. Ramalho, "Optimal wavelet signal compression as an efficient alternative to investigate molecular dynamics simulations: application to thermal and solvent effects of MRI probes,'' *Theoretical Chemistry Accounts*, vol. 136, pp. 2-13, 2017.

30.Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery Jr JA, reven T, Kudin KN, Burant JC et al, Gaussian 09, Revision C.02. Gaussian, Inc, 2004.

31.J. P. Perdew, K. Burke, M. Ernzerhof, "Generalized Gradient Approximation Made Simple,"*Physical Review Letters*, vol.77, pp.3865-3868, 1996.

32.E. D. Hedegård, J. Kongsted, S. P. Sauer, "Improving the calculation of electron paramagnetic resonance hyperfine coupling tensors for d-block metals,"*Physical Chemistry Chemical Physics*, vol.14, pp.10669-10676, 2012.

33.R. Ditchfield, W. J. Hehre, J. A. Pople, "Self-Consistent Molecular-Orbital Methods. IX. An Extended Gaussian-Type Basis for Molecular-Orbital Studies of Organic Molecules,"*Journal of Chemical Physics*, vol.54, pp.724-728, 1971.

34. F. Cortes-Guzman, R. F. W. Bader, "Complementarity of QTAIM and MO theory in the study of bonding in donor–acceptor complexes,"*Coordination Chemistry Reviews*, vol.249, pp.633-662, 2005.

35.Bader RFW, Principle of stationary action and the definition of a proper open system,*Physical Review B*, vol.49, pp.13348-13355, 1994.

36.T. A. Keith, AIM ALL (version 10.05.04), TK Gristmill Software, Overland Park KS, 2010.

37. M. Kaupp, M. Bühl, V. V. G. Malkin, (Eds.).Weinheim432, 2004.

38.M. Lepage, J. C. Gore, "Contrast mechanisms in magnetic resonance imaging."*Journal of Physics*, vol. 3, pp.78-86, 2004.

39.O. V. Yazyev, L. Helm, V. G. Malkin, O. L. Malkina, "Quantum chemical investigation of hyperfine coupling constants on first coordination sphere water molecule of gadolinium(III) aqua complexes,"*Journal of Physical Chemistry A*, vol.109, pp.10997-11005, 2005.

40. (a) M. A. Gonçalves, E. F. F. da Cunha, F. C. Peixoto, T. C. Ramalho, "Probing thermal and solvent effects on hyperfine interactions and spin relaxation rate of δ-FeOOH(100) and [MnH3buea(OH)]$^{2-}$: Toward new MRI probes,"*Computational and Theoretical Chemistry*,vol.1069, pp.96-104, 2015. (b) M. A. Gonçalves, F. C. Peixoto,E. F. F. da Cunha, T. C. Ramalho, "Dynamics, NMR parameters and hyperfine coupling constants of the Fe3O4(100)–water interface: Implications for MRI probes,"*Chemical Physics Letters*, vol.609, pp.88-92, 2014.

41.G. A. Rolla, C. P. Iglesias, M. Botta, L. Tei, L. Helm, "1H and 17O NMR relaxometric and computational study on macrocyclic Mn(II) complexes,"*Inorganic Chemistry,* vol.52, pp.3268-3279, 2013.

42.D. H. Powell, O. M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer, A. E. Merbach, "Structural and Dynamic Parameters Obtained from 17O NMR, EPR, and NMRD Studies of Monomeric and Dimeric Gd^{3+} Complexes of Interest in Magnetic Resonance Imaging: An Integrated and Theoretically Self-Consistent Approach,"*Journal of the American Chemical Society,* vol.118, pp.9333-9346, 1996.

43.S. Laurent, L. Vander Elst, S. Houz, N. Gurit, R. N. N. Muller,"Synthesis and characterisation of various benzyl diethylenetriaminepentaacetic acids (dtpa) and their paramagnetic complexes, Potential contrast agents for magnetic resonance imaging,"*Helvetica Chimica Acta,* vol.83, pp.394-406, 2000.

44.K. Kattel, J. Y. Park, W. Xu, B. A. Bony, W. C. Heo, T. Tegafaw, C. R. Kim, M. W. Ahmad, S. Jin, J. S. Baeckl, *Biomaterials,* vol.33, pp.3254-3261, 2012.

45.B. G. Oliveira,R. C. M. U. Araujo, M.N. Ramos, "A topologia molecular QTAIM e a descrição mecânico-quântica de ligação de hidrogênio e ligações de di-hidrogênio,"*Química Nova,* vol.33, pp.1155-1162, 2010.

46.R. F. W. Bader, "Atoms in Molecules-A Quantum Theory;" Oxford University Press: Oxford, U.K, 1990.

47.M. Pecul, J. Lewandiwski, J. Sadlej, "Benchmark calculations of the shielding constants in the water dimer," *Chemical Physics Letters,* vol.333, vol.139-145, 2001.

48.D. E. Gómez, A. Blas, T. R. Blas, L. Helm, C. P. Iglesias, "Hyperfine coupling constants on inner-sphere water molecules of Gd(III)-based MRI contrast agents,"*ChemPhysChem*,vol.16, pp.3640-3650, 2012.

49.E. Ruiz, J. Cirera,S. Alvarez, "Spin density distribution in transition metal complexes,"*Coordination Chemistry Reviews,* vol.249, pp.2649-2660, 2005.

50.E. Alarcon, M. Gonzalez-Bejar, S. Gorelsky, R. Ebensperger, C. Lopez-Alarcon, J. C. Netto-Ferreira, J. C. Scaiano, "Photophysical characterization of atorvastatin (Lipitor®) ortho-hydroxy metabolite: role of hydroxyl group on the drug photochemistry,"*Photochemical & Photobiological Sciences*, vol.9, pp.1378-1384, 2010.

ARTICLE 2

Submitted for publication

^{195}Pt NMR of phenylbenzothiazole complexes as spectroscopic technique for the cancer diagnosis

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Abstract: Platinum complexes have been studied for cancer treatment since long time ago. However, an important platinum characteristic is related to its chemical shifts, in which some studies show the ^{195}Pt chemical shifts are very sensitive to the environment, coordination sphere and oxidation state. Based on this feature, it is possible to propose Pt complexes as potential probes for NMR Spectroscopy, once in the different tissues (healthy and damaged) the signals of chemical shifts may be different. In this paper, the main goal was to investigate the behavior of Pt chemical shifts in the different environments, in which calculations were carried out in vacuum, implicit solvent and inside the active site of P13K enzyme using DFT method. Moreover, to investigate platinum complexes with a selective moiety can improve the early diagnosis. In line with that, the Pt complexes used in this paper present a selective moiety for some types of cancer, the 2-(4'aminophenyl)benzothiazole derivative. In this paper two Pt complexes were used, once some studies have been shown that Platinum complexes coordinated to chlorine atoms may suffer hydrolyses inside the cell due to the low chloride ion concentration. Thus, the same calculations for the first platinum complex were performed for the second complex, in which a chlorine atom was replaced by a water molecule. This paper shows that Platinum complexes can be a potential probe in biological systems and they should be studied not only cancer treatment but also in diagnosis.

Keywords: Platinum complexes; ^{195}Pt NMR; biological systems; cancer diagnosis

1. Introduction

Currently, cancer is one of the most discussed diseases, once it presents incredible high mortality rates [1,2]. More specifically, cancer is a term employed for a group of diseases characterized by the acute growth of abnormal cells. Breast cancer is the class with the highest incidence in women and it is associated with the high mortality rates in women with cancer [3–5].

Diagnosis in early stages is very important for cancer treatment, once it can reduce mortality rates and increase the treatment possibilities [6–8]. Some imaging techniques are used as main approaches in cancer diagnosis, among them are mammography, magnetic resonance imaging (MRI), positron-emission tomography (PET), Computed tomography (CT). Furthermore, the use of these techniques with the help of biochemical biomarkers, such as proteins, could improve diagnosis [3,9,10]

The use of imaging techniques associated to biomarkers as proteins, RNAs, microRNAs and enzymes could increase the chances of early diagnosis [3,11,12]. Furthermore, the use of compounds that have already been employed in medicine could be a useful possibility to probe diseases [3,13,14]. In this context, Mavroid and colleagues synthesized the *cis*-dichloro(2'pyridinyl)methylamineplatinum(II) bonded to with 2-(4'aminophenyl)benzothiazole derivative in order to study its properties as an antitumor agent [13].

This complex has two important moieties in cancer treatment, the platinum and the benzothiazole group. The Platinum had its carcinogenic properties discovery in 1965 with the emergence of Cisplatin (important drug in chemotherapy) [15–17].Since then, different drugs using Platinum has been developed to improve treatment of different types of cancer [16,18,19]. Regarding the benzothiazole moiety, some studies have revealed that these types of molecules showed potent and selective antitumor activity in vitro and in vivo against breast cancer cells, among other type of cancers [14,20,21].

Accordingly, the purpose of this work was to use the *cis*-dichloro(2'pyridinyl)methylamineplatinum(II) bonded to with 2-(4'aminophenyl)benzothiazole derivative(Figure 1) as a potential probe in the breast cancer diagnosis. It is also important to mention that this coordinated complex has already been investigated as a potential anticancer agents [13]. Furthermore, the strategy chosen for this work was to perform chemical shift analysis of Platinum-195 in different chemical environments (vacuum, implicit solvent and inside active enzyme P13K), which was done through NMR calculations. These calculations were performed for monoaquated complex (Figure 2), once it is well known that platinum complexes are hydrolyzed inside the cell[18,19,22].

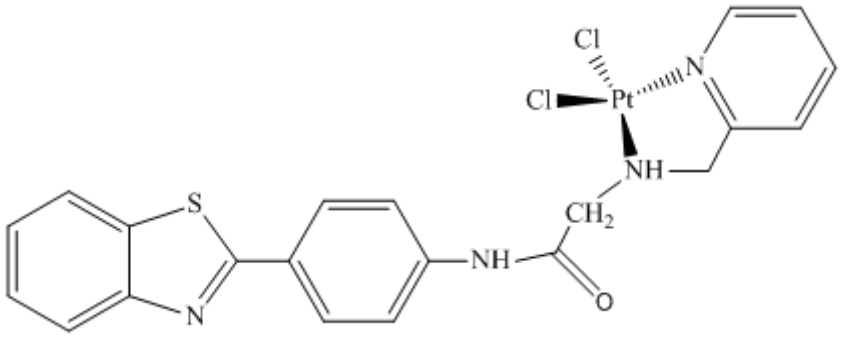

Figure 1.cis-dichloro(2'pyridinyl)methylamineplatinum(II) bonded to with 2-(4'aminophenyl)benzothiazole derivative.

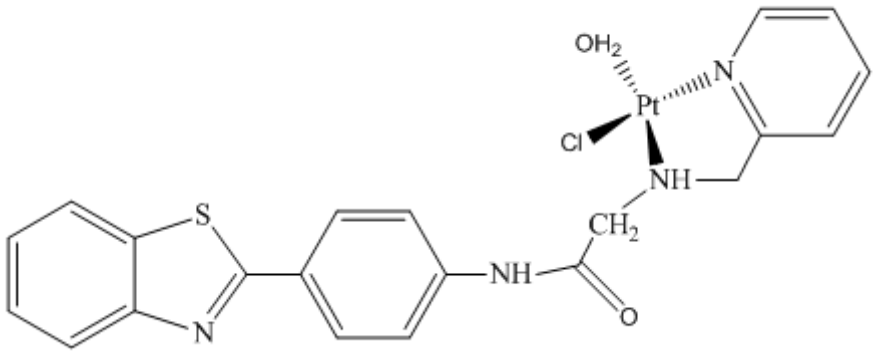

Figure 2. Monoaquated complex: monoaquamonochloro(2'pyridinyl)methylamineplatinum(II) ion bonded to 2-(4'aminophenyl)benzothiazole derivative.

2. Methodology

2.1. Optimization and molecular dynamics (MD) procedure

Geometries were fully optimized at the B3LYP theory level [23], with the LanL2dz [24] basis set with effective core potential (ECP) for the Pt atom and Def2-TZVPP [32] for the ligand molecule in the Gaussian 09 program [25]. These calculations were performed either in vacuum or in the presence of polarizable continuum model, using the dielectric constants of water [denoted (PCM)(H_2O)] [14,26-30].

The MD simulations were performed using the atom centered density matrix propagation (ADMP) [31], which is a molecular dynamics model. Furthermore, the same level of theory, i.e. B3LYP/Lanl2dz, was chosen for MD. It is worth mentioning that the temperature of 310K was included in all MD simulations to reproduce the regular biological temperature.

From the obtained MD conformations, the uncorrelated structures were selected employing the statistical inefficiency method, available in the SciLab 2.7 software [32]. This procedure was performed in order to select conformations for further NMR analysis.

2.2. Nuclear Magnetic Resonance (NMR) calculations

The magnetic shielding parameters (σ) were obtained for optimized geometries as well as for selected conformations from molecular dynamics. These calculations were performed with the gauge-including atomic orbitals (GIAO) - DFT method involving the PBEPBE functional [26,33–37]. In these calculations was used the NMRDKH basis set [26], such presents doubly polarized triple-zeta characteristic.

The ^{195}Pt NMR chemical shifts δ were calculated relative to cisplatin, it presented experimental chemical shift [δ (^{195}Pt) = -2097 ppm][26]. For this compound it was employed the same theory level described above.

2.3. Molecular Docking studies

The complexes were docked inside of the PI3K enzyme, which presents crystallographic structure complexed with N-{6-[2-(methylsulfanyl)pyrimidin-4-yl]-1,3-benzothiazol-2-yl}acetamide (active ligand) (Protein Data Bank (PDB) code 3QJZ[38]; resolution= 2,90 Å) using the Molegro Virtual Docker (MVD®) software according to the same procedure adopted previously [14,39].

The binding site was limited into a spherical cavity with variation in the radius of 10–12 Å, and the residues was considered flexible inside a radius of 11 Å around the ligand. For these analyses around 50 poses were obtained for each compound. Then, it was made the analysis of the ligand–protein interactions were performed and the overlaps with the active ligand inside PI3K. The best conformation of the compound was selected according to its degree of structural similarity between the active ligand and the complexes, the intermolecular interaction energy was also used for choosing the best conformation. The accommodation in the cavity is other important factor that helps evaluating the best energy of interaction with the enzyme.[39].

The appropriate conformation of ligand at the active site of the enzyme, in which it was considered the residues around the ligand was selected through NMR analysis. Furthermore, the same level of theory, i.e. PBEPBE/NMRDKH, was chosen for these calculations.

3. Results and Discussion

This discussion is divided into three main parts following the same order established in the methodology section. Initially, it is worth mentioning that the validation step of the theoretical methodology was based on another work about NMR platinum complexes developed by PASCHOAL and collaborators (2017) [26]. In this research, the authors developed a basis set with relativistic correction for Pt(II) chemical shifts calculations [26]. In the second part, the ^{195}Pt NMR chemical shift for complex 1 (Figure 1) was calculated in the gas phase, in solution using implicit solvent (PCM)

model and in the P13K enzyme active site. In order to evaluate the thermal effects, the dynamic simulations were also carried out. In the last part, the same calculations were performed for the monoaquated complex (Figure **2**).

For NMR chemical shifts calculations, the notation was the following: level of shielding computation (PBEPBE)//level of geometry optimization (B3LYP) or MD simulation (ADMP). For example: (PBEPBE//B3LYP) means shielding computation in vacuum//geometry optimization in vacuum; [PBEPBE/PCM(H_2O)//B3LYP/PCM(H_2O)] means shielding computation with implicit solvent (PCM)//geometry optimization with implicit solvent (PCM)PCM. The same notation was employed when including the dynamic effect (ADMP). For example: (PBEPBE//ADMP) means shielding computation in vacuum//dynamic simulations in vacuum; [PBEPBEPCM(H_2O)//ADMP/PCM(H_2O)]means shielding computation with implicit solvent (PCM)//dynamic simulations with implicit solvent (PCM).

3.1. Validation of ^{195}Pt NMR chemical shifts theoretical methodology

In the study developed by PASCHOAL and collaborators, a basis set (labeled as NMR-DKH) was developed for ^{195}Pt NMR, which allows the recovering of relativistic effects. The authors performed several chemical shift calculations for many Pt(II) complexes using different theoretical methodologies. Then, a comparison was made among the three different methodologies [26].

Initially, in that case, the studied complexes were optimized using two different basis set. After this step, NMR calculations were performed using NMR-DKH basis set, as following: (PBEPBE/NMR-DKH/IEFPCM(UFF)//B3LYP/LANL2DZ/Def2-TZVPP/IEFPCM(UFF)) (model 2) and (PBEPBE/NMR-DKH/IEFPCM(UFF)//B3LYP/LANL2DZ/Def2-SVP/IEFPCM(UFF)) (model 3) Then, a comparison was made using a Hamiltonian relativistic operator (ZORA), as following: COSMO-PBE-SO-ZORA/TZ2P (model 1)[27]. A statistical study, in which the absolute deviation (MAD) and the relative deviation (MRD) were used for methodologies comparison. The statistical results showed that the MAD and the MRD were 200 ppm and 6% (Model 1),182 ppm and 6% (Model 2) and 168 ppm and 5% (Model 3) respectively [26].

As a result, the methodology chosen by PASCHOAL and collaborators showed an excellent agreement between the different models used. In the same way, the theoretical values obtained by relativistic calculations using NMR-DKH basis set agreed with the experimental data [26].

3.2. The ^{195}Pt nuclear magnetic resonance chemical shift

It is well known the importance of investigating how temperature, coordination and chemical environment effects influence the chemical shifts, even more when the goal is to use this NMR parameter to propose a compound as probe in biological systems. Keeping that in mind, the importance of geometry, chemical environment, solvent and thermal effects for ^{195}Pt chemical shift in the complex **1** (Figure 1) were analyzed. The corresponding $\delta(^{195}Pt)$ values were collected and are reported in Table 1.

Table 1.^{195}Pt NMR chemical shifts for Complex (Figure 1) computed at the GIAO–PBEPBE/ NMR-DKH.

Level of approximation	**^{195}Pt (ppm)**
δ_e(PBEPBE//B3LYP)	-1960.49
δ_e[PBEPBE//B3LYP/PCM(H_2O)]	-2296.13
δ_e[PBEPBE/PCM(H_2O)//B3LYP/PCM(H_2O)]	-1783.80
δ^{310K}(PBEPBE//ADMP)	-2256.00
δ^{310K} [PBEPBE/PCM(H_2O)//ADMP/PCM(H_2O)]	-4069.56

Analyzing the geometry effect on the ^{195}Pt chemical shifts (Table 1), it can observe that the δ values reveal a variation of 335.64 ppm when comparing δ_e(PBEPBE//B3LYP) and δ_e[PBEPBE//B3LYP/PCM(H_2O)]. Regarding the chemical shift effects for ^{195}Pt, when comparing the NMR calculations with and without implicit solvent {δ_e[PBEPBE//B3LYP/PCM(H_2O)] and δ_e[PBEPBE/PCM(H_2O) //B3LYP/PCM(H_2O)]},it was observed a variation of 512.33 ppm (Table 1).It is worth mentioning that this large difference in the chemical shifts values for this different methodologies (vacuum and implicit solvent) was already expected. According to the literature, the Platinum chemical shift is very sensitive to geometry, electronic parameters and chemical environments [40].

In this line, thermal and dynamic effects are other important contributions to be analyzed when studying chemical shifts. Regarding the dynamic effect, when it was included in the gas phase {comparing δ_e(PBEPBE//B3LYP) and δ^{310K}(PBEPBE//ADMP)}, the chemical shift value decreased 295.51 ppm (Table 1). Moreover, when implicit solvent effect was considered along with dynamic effect {comparing δ_e[PBEPBE/PCM(H_2O)//B3LYP/PCM(H_2O)] and δ^{310K}[PBEPBE/PCM(H_2O)//ADMP/PCM(H_2O)]}, the chemical shift value decreased 2285. 76 ppm.

Indeed, the methodology including thermal and dynamic effects with implicit solvent was the closest approximation to real systems used in this work. In this sense, this methodology was compared to Pt chemical shift inside the enzyme in the next topic. This is an important step, once the environment inside the enzyme and considering solvent and thermal effects are different.

3.2.1. The ^{195}Pt chemical shift in PI3K enzyme active site

Docking calculations are a fundamental tool for studies involving ligand and receptor systems [39]. In this work, a docking study was carried out to analyze the ^{195}Pt chemical shift inside the enzyme PI3K, which is related to breast cancer. Then, the overlap between the complex conformations, generated during docking analysis, and the active ligand (PDB ligand) (benzothiazole derived) was performed inside the active site of the enzyme. The best superposition between the two structures is shown in Figure 3.

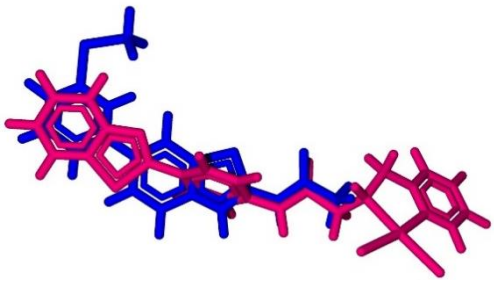

Figure 3. Complex 1 (pink) docked in the active site of PI3K, the active ligand is shown in blue.

Furthermore, the complex behavior inside of the receptor active site was also evaluated to check the stability of the complex in this chemical environment. The complex established hydrogen bonds with Val882 and Asp 884 amino acids..

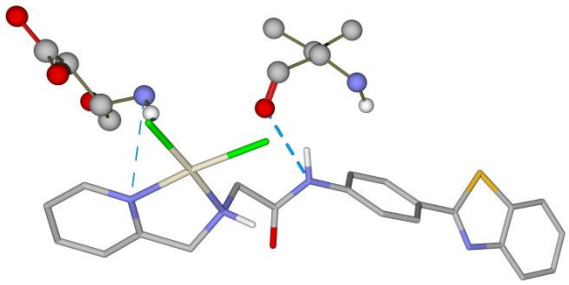

Figure 4.a) Intermolecular interaction between Platinum complex,Val 882 and Asp 884 .

Furthermore, other interactions happen to give stability to the system receptor-ligand, as electrostatic and hydrophobic interactions, for example. In this sense, the electrostatic interactions were also shown in the Figure 5.

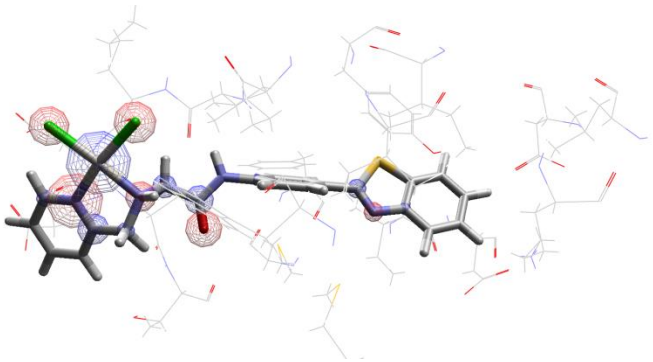

Figure 5: Electrostatic interactions for complex inside active site of PI3K.

Accordingly, it can be suggested that the Platinum complex is stable inside the active site of the enzyme, once it formed these interactions inside the active site of enzyme besides it presented intermolecular interaction energy around to – 150 kcal mol^{-1}.To validate this methodology, a redocked was performed. However, the docking study is a theoretical research and the preliminary results do not take into account pharmacokinetic proprieties. Therefore, it is necessary an additional *in vitro* test for evaluating the interactions between this Platinum complex and P13K enzyme.

At last, for analyzing the Platinum complex (Figure 1) as a potential probe in biological systems, it was also necessary to perform NMR calculations for the ^{195}Pt chemical shifts of the most stable complex conformation in the active site of P13K enzyme, which resulted in a $\delta(^{195}Pt)$ of -1919.70 ppm. The result is according to the expected value, once it is well known that the chemical environment inside the active site is hydrophobic, then the chemical shift should be close to that chemical shift obtained through the calculations without solvent δe (PBEPBE//B3LYP) for the complex (Table 1). Furthermore, the comparison between chemical shift for complex after docking and using thermal, dynamic and solvent effects $\{\delta^{310K}$ [PBEPBE/PCM(H2O)//ADMP/PCM(H2O)]} is important to understand the behavior of the complex in different environments. In this sense, the difference found when comparing these both methodologies was 2149.86 ppm.

With that in mind, this results are very interesting for this proposal, once the environment inside the protein and considering thermal and solvent effects are different, which show the sensitivity of Pt chemical shift. In addition, platinum complexes are already used in medicine for cancer treatment, which may facilitate its use as a diagnosis compounds.

3.3. The ^{195}Pt chemical shift for monoaqua Platinum complex

Platinum (II) complexes are important drugs in cancer treatment since 1965, when carcinogenic properties of Cisplatin were discovered [15–17]. The Platinum drugs present activity against cancer due their ability of binding to the N7 atom of guanine from the DNA. An important step in the interaction between Platinum and DNA is the aquation of Platinum complexes, which is crucial for the following binding step [18,22]. Several studies have shown that the monoaqua Cisplatin complex {cis-$[Pt(NH_3)_2(H_2O)Cl]^{+1}$} is the active hydrolyzed specie at 310 K that binds to DNA, once the diaquo complex {cis-$[Pt(NH_3)_2(H_2O)_2]^{+2}$} is the less likely specie in physiological pH [18].

In this context, it is also important to analyze the behavior of the monoaqua complex, once it may be found in this way inside the cell. Thus, for proposing platinum complexes as a potential probe, this aspect needs to be considered., . In this sense, for monoaqua complex (Figure 2) calculations were performed including thermal and dynamic effects with implicit solvent {δ^{310K} [PBEPBEPCM(H_2O)//ADMP/PCM(H_2O)]}. Therefore, for the study of the monoaqua compound this methodology was employed for the evaluation of the thermal, dynamic and solvent effects, and ^{195}Pt chemical shift calculations were also performed inside the active site of the enzyme. Besides, these both results were compared to analyze the behavior of chemical shift in these different environments.

The corresponding $\delta(^{195}Pt)$ value for monoaqua Platinum complex was -2813.79 ppm. Comparing this result to result for the complex before hydrolyses (Figure 1) (Table 1), it was observed a variation in the ^{195}Pt chemical shift values obtained. Therefore, when a water molecule was added in place of a Chlorine atom the respective value increased 1255.79 ppm. This was expected, since it is known that platinum chemical shifts are sensitive to the coordination sphere

In this sense, the docking study was performed for better understanding the interaction of monoaqua complex inside the active site of the enzyme. The most important moiety of the complexes for this step is the benzothiazole, once the ligand inside the enzyme is a benzothiazole derived, as already mentioned in the docking topic. From the docking studies, it was observed that the monoaquated complex stablished the hydrogen bonds similar to what was observed for the complex 1; the associated intermolecular interaction energy was around -140 kcal mol^{-1}.

At last, the ^{195}Pt chemical shift of the most stable complex conformation in the active site of PI3K enzyme was performed for the complex 2, which resulted in a δ (195Pt) of -1783.36 ppm. The result is according to the expected value and presents a big difference when comparing with results for {δ^{310K} [PBEPBEPCM(H_2O)//ADMP/PCM(H_2O)]} methodology, that was -2813.79 ppm.Accordingly, the results for the monoaqua Platinum complex (a possible specie for the Platinum complex in biological system) indicate a similar behavior between this specie and the first complex

4. Conclusion

In this research, two Platinum complexes were investigated in different chemical environments (vacuum, implicit solvent and inside active site of P13K enzyme) to propose complex 1 (Figure 1) as probe in biological systems. This proposal is possible because the ^{195}Pt chemical shifts are very sensitive to the chemical environment. Keeping this in view, the ^{195}Pt chemical shift value is a good spectroscopic parameter to propose platinum complexes as a potential probe in biological systems. In this context, we performed chemical shift analysis of Platinum-195 complexes in these chemical environments using NMR-DKH basis set. The difference found in ^{195}Pt chemical shifts, when comparing the value in each chemical environment, was expected and indicates that Platinum complexes may be a potential probe in biological systems. This is possible due to the difference found when comparing the values inside and outside the enzyme. This paper proposes the use of Platinum complexes not only cancer treatments but also in diagnosis, once these complexes have already been studied for cancer treatment.

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References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer Statistics , 2018. *A Cancer J. Clin.* [https://doi.org/10.3322/caac.21442]**2018**, *68*, 7–30.

2. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *A Cancer J. Clin.***2018**, *68*, 394–424. [https://doi.org/10.3322/caac.21492]

3. Jafari, S.; Saadatpour, Z.; Salmaninejad, A.; Momeni, F.; Mokhtari, M.; Nahand, J.S.; Rahmati, M.; Mirzaei, H.; Kianmehr, M. Breast cancer diagnosis : Imaging techniques and biochemical markers. *J. Cell. Physiol.***2018**, *233*, 5200–5213.[https://doi.org/10.1002/jcp.26379]

4. Park, J.V.; Park, S.J.; Yoo, J.S. Finding characteristics of exceptional breast cancer subpopulations using subgroup mining and statistical test. *Expert Syst. Appl.***2019**, *118*, 553–562.[https://doi.org/10.1016/j.eswa.2018.10.016]

5. Wang, Y.; Zhang, G.; Hao, X.; Ma, Y.; Ma, M.; Yan, X.; Jiang, X.; Bie, F.; Yuan, N. Potential biomarker for breast cancer screening : A systematic review and meta-analysis. *Futur. Gener. Comput. Syst.***2019**, *91*, 518–526.[https://doi.org/10.1016/j.future.2018.09.030]

6. Zonouzy, V.T.; Niknami, S.; Ghofranipour, F.; Montazeri, A. An educational intervention based on the extended parallel process model to improve attitude , behavioral intention , and early breast cancer diagnosis : a randomized trial. *Int. J. Women's Heal.***2019**, *11*, 1–10.[https://doi.org/10.2147/IJWHS182146]

7. Heneghan, H.M.; Miller, N.; Kelly, R.; Newell, J.; Kerin, M.J. Systemic miRNA-195 Differentiates Breast Cancer from Other Malignancies and Is a Potential Biomarker for Detecting Noninvasive and Early Stage Disease. *Oncologist***2010**, *15*, 673–682.[https://doi.org/10.1634/theoncologist.2010-0103.]

8. Cui, X.; Li, Z.; Zhao, Y.; Song, A.; Shi, Y. Breast cancer identification via modeling of peripherally circulating miRNAs. *PeerJ***2018**, *6*, 1–12.[https://doi.org/10.7717/peerj.4551]

9. Khalkhali, I.; Mena, I.; Diggles, L. Review article of breast cancer : a new role of prone scintimammography using technetium-99m sestamibi. *Eur. J. Nucl. Med.***1994**, *21*, 357–362.[https://www.ncbi.nlm.nih.gov/pubmed/8005161]

10. Pereira, B.T.L.; Silva, E.F.; Gonçalves, M.A.; Mancini, D.T.; Ramalho, T.C. Exploring EPR Parameters of99Tc Complexes for Designing New MRI Probes: Coordination Environment, Solvent, and Thermal Effects on the Spectroscopic Properties. *J. Chem.***2017**, 1–8.[https://doi.org/10.1155/2017/8102812]

11. Duffy, M.J.; Harbeck, N.; Nap, M.; Molina, R.; Nicolini, A.; Senkus, E.; Cardoso, F. Clinical use of biomarkers in breast cancer : Updated guidelines from the European Group on Tumor Markers (EGTM). *Eur. J. Cancer***2017**, *75*, 284–298.[https://doi.org/10.1016/j.ejca.2017.01.017]

12. Kurozumi, S.; Yamaguchi, Y.; Kurosumi, M.; Ohira, M.; Matsumoto, H.; Horiguchi, J. Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes. *J. Hum. Genet.***2017**, *62*, 15–24.[https://doi.org/10.1038/jhg.2016.89]

13. Mavroidi, B.; Sagnou, M.; Stamatakis, K.; Paravatou-petsotas, M.; Pelecanou, M.; Methenitis, C. Palladium (II) and platinum (II) complexes of derivatives of 2- (4 0 -aminophenyl) benzothiazole as potential anticancer agents. *Inorganica Chim. Acta***2016**, *444*, 63–75.[https://doi.org/10.1016/j.ica.2016.01.012]

14. Mancini, D.T.; Souza, E.F.; Caetano, M.S.; Ramalho, T.C. 99 Tc NMR as a promising technique for structural investigation of biomolecules : theoretical studies on the solvent and thermal effects of phenylbenzothiazole

complex. *Magn. Reson. Chem.***2014**, *53*, 129–137.[https://doi.org/10.1002/mrc.4043]

15. Rosernberg, B.; Vancamp, L.; Krigas, T. Inhibition of Cell Division in Escherichia coli by Electrolysis Products from a Platinum Electrode. *Nature***1965**, *205*, 698–699.[https://www.ncbi.nlm.nih.gov/pubmed/14287410]

16. Weiss, R.B.; Christian, M.C. New Cisplatin Analogues in Development. A review. *Drugs***1993**, *46*, 360–377.[https://doi.org/10.2165/00003495-199346030-00003]

17. Dasari, S.; Tchounwou, P.B. Cisplatin in cancer therapy : Molecular mechanisms of action. *Eur. J. Pharmacol.***2014**, *740*, 364–378.[https://doi.org/10.1016/j.ejphar.2014.07.025]

18. Deo, K.M.; Ang, D.L.; Mcghie, B.; Rajamanickam, A.; Dhiman, A.; Khoury, A.; Holland, J.; Bjelosevic, A.; Pages, B.; Gordon, C.; et al. Platinum coordination compounds with potent anticancer activity. *Coord. Chem. Rev.***2018**, *375*, 148–163.[https://doi.org/10.1016/j.ccr.2017.11.014]

19. Brabec, V.; Hrabina, O.; Kasparkova, J. Cytotoxic platinum coordination compounds . DNA binding agents. *Coord. Chem. Rev. J.***2017**, *351*, 2–31.[https://doi.org/10.1016/j.ccr.2017.04.013]

20. Solomon, V.R.; Hu, C.; Lee, H. Hybrid pharmacophore design and synthesis of isatin – benzothiazole analogs for their anti-breast cancer activity. *Bioorg. Med. Chem.***2009**, *17*, 7585–7592.[https://doi.org/10.1016/j.bmc.2009.08.068]

21. Abdelgawad, M.A.; Belal, A.; Omar, H.A.; Hegazy, L.; Rateb, M.E. Synthesis, Anti-Breast Cancer Activity, and Molecular Modeling of Some Benzothiazole and Benzoxazole Derivatives. *Arch. der Pharm. - Chem. Life Sci.***2013**, *346*, 534–541.[https://doi.org/10.1002/ardp.201300044]

22. Ahmad, S. Kinetic aspects of platinum anticancer agents. *Polyhedron***2017**, *138*, 109–124.[https://doi.org/10.1016/j.poly.2017.09.016]

23. Lee, C.; Yang, W.; Parr, R.G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B1998, 37, 785–789.[https://www.ncbi.nlm.nih.gov/pubmed/9944570]

24. Hay, P.J.; Wadt, W.R. Ab initio effective core potentials for molecular calculations . Potentials for the transition metal atoms Sc to Hg. J. Chem. Phys.1985, 82, 270–283.[https://doi.org/10.1063/1.448799]

25. Weigend, F.; Ahlrichs, R. Balanced Basis Sets of Split Valence, Triple Zeta Valence and Quadruple Zeta Valence. Phys. Chem. Chem. Phys. 2005, 7, 3297–3305.[http://dx.doi.org/10.1039/b508541a]

26. Paschoal, D.; Guerra, C.F.; de Oliveira, M.A.; Ramalho, T.C.; Dos Santos, H.F. Predicting Pt-195 NMR Chemical Shift Using New Relativistic All-Electron Basis Set. J. Comput. Chem.2016, 37, 2360–2373.[https://doi.org/10.1002/jcc.24461]

27. Frisch, M. J.; Trucks, G.W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.;Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.. S.D. Gaussian 09 2009.

28. Barone, V.; Cossi, M.; Tomasi, J. Geometry optimization of molecular structures in solution by the polarizable continuum model. J. Comput. Chem.1998, 19, 404–417.[https://doi.org/10.1002/(SICI)1096-987X(199803)19:4<404::AID-JCC3>3.0.CO;2-W]

29. Cancès, E.; Mennucci, B.; Tomasi, J. A new integral equation formalism for the polarizable continuum model : Theoretical background and applications to isotropic and anisotropic dielectrics. J. Chem. Phys.1997, 107, 3032–3041.[https://doi.org/10.1063/1.474659]

30. Gonçalves, M.A.; Santos, L.S.; Prata, D.M.; Peixoto, F.C.; da Cunha, E.F.F.; Ramalho, T.C. Optimal wavelet signal compression as an efficient alternative to investigate molecular dynamics simulations: application to thermal and solvent effects of MRI probes. Theor. Chem. Acc.2017, 136, 1–13.[https://doi.org/10.1007/s00214-016-2037-z]

31. Iyengar, S.S.; Frisch, M.J. Effect of time-dependent basis functions and their superposition error on atom-centered density matrix propagation (ADMP): Connections to wavelet theory of multiresolution analysis. J. Chem. Phys.2004, 121, 5061–5070. [https://doi.org/10.1063/1.1780157]

32. SciLab v 2.7. 1989–2003 INRIA/ENPC.

33. Wolinski, K.; Hinton, J.F.; Pulay, P. Efficient Implementation of the Gauge-Independent Atomic Orbital Method for NMR Chemical Shift Calculations. J. Am. Chem. Soc.1990, 112, 8251–8260. [https://doi.org/10.1021/ja00179a005]

34. Cheeseman, J.R.; Trucks, G.W.; Keith, T.A.; Frisch, M.J. A comparison of models for calculating nuclear magnetic resonance shielding tensors. J. Chem. Phys.1996, 104, 5497–5509.[https://doi.org/10.1063/1.471789]

35. Perdew, J.P.; Burke, K.; Ernzerhof, M. Generalized gradient approximation made simple. Phys. Rev. Lett.1996, 77, 3865–3868.[https://doi.org/10.1103/PhysRevLett.77.3865]

36. Perdew, J.P.; Burke, K.; Ernzerhof, M. Generalized Gradient Approximation Made Simple. Phys. Rev. Lett.1997, 78, 1396.[https://doi.org/10.1103/PhysRevLett.78.1396]

37. Ramalho, T.C.; Pereira, C.M.P.; Martins, T.L.C.; Figueroa-Villar, J.D.; Flores, A.F.C. Theoretical and experimental13C and15N NMR investigation of guanylhydrazones in solution. Magn. Reson. Chem. 2003, 41, 983–988.[DOI: 10.1002/mrc.1299]

38. D'Angelo, N.D.; Kim, T.S.; Andrews, K.; Booker, S.K.; Caenepeel, S.; Chen, K.; Amico, D.D.; Freeman, D.; Jiang, J.; Liu, L.; et al. Discovery and Optimization of a Series of Benzothiazole Phosphoinositide 3-Kinase (PI3K)/ Mammalian Target of Rapamycin (mTOR) Dual Inhibitors. J. Med. Chem.2011, 54, 1789–1811.[https://doi.org/10.1021/jm1014605]

39. Thomsen, R.; Christensen, M.H. MolDock : A New Technique for High-Accuracy Molecular Docking. J. Med. Chem.2006, 49, 3315–3321.[https://doi.org/10.1021/jm051197e]

40. Still, B.M.; Kumar, P.G.A.; Aldrich-Wright, J.R.; Price, W.S. 195Pt NMR - theory and application. Chem. Soc. Rev.2007, 36, 665–686. [https://doi.org/10.1039/B606190G]