

Research Article

Exploring EPR Parameters of ^{187}Re Complexes for Designing New MRI Probes: From the Gas Phase to Solution and a Model Protein Environment

Gustavo A. Andolpho ¹, Elaine F. F. da Cunha ¹, and Teodorico C. Ramalho ^{1,2}

¹Chemistry Department, Natural Sciences Institute, Federal University of Lavras, Lavras, Brazil

²Center for Basic and Applied Research, Faculty of Informatics and Management, University Hradec Kralove, Hradec Kralove, Czech Republic

Correspondence should be addressed to Elaine F. F. da Cunha; elaine_cunha@ufla.br and Teodorico C. Ramalho; teo@dqi.ufla.br

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Breast cancer is one of the major types of cancer around the world, and early diagnosis is essential for successful treatment. New contrast agents (CAs), with reduced toxicology, are needed to improve diagnosis. One of the most promising Magnetic Resonance Imaging (MRI) CA is based on rhenium conjugated with a benzothiazole derivate (ReABT). In this sense, DFT has been used to evaluate the best methodology for calculating the hyperfine coupling constant (A_{iso}) of ReABT. Then, a thermodynamic analysis was performed to confirm the stability of the complex. Furthermore, a docking study of ReABT at the enzyme PI3K active site and A_{iso} calculations of ReABT in the enzyme environment were carried out. The best methodology for the A_{iso} calculation of ReABT was using the M06L functional, SARC-ZORA-TZVP (for Re) and TZVP (for all other atoms) basis set, relativistic Hamiltonian, and the CPCM solvation model with water as the solvent which confirm that the relativistic effects are important for calculating the A_{iso} values. In addition, thermodynamic analysis indicates that ReABT presents a higher stability and a lower toxicity than Gd-based CAs. The docking studies point out that ReABT interacts with amino acids residues of alanine, aspartate, and lysine from the PI3K active site. Considering the enzyme environment, A_{iso} values decrease significantly. These findings indicate that the CA candidate ReABT could be a good candidate for a new contrast agent.

1. Introduction

Female breast cancer is expected to be the major type of cancer, besides non-melanoma skin tumors, in Brazil between 2020 and 2022, affecting over 66 thousand cases per year according to the National Institute of Cancer José Alencar (INCA) [1]. Across the world, data from the American Cancer Society estimate that in 2020 more than 275,000 cases should be diagnosed in the United States and, according to the European Cancer Information System, ECIS, in the European Union more than 355,000 diagnoses are expected by 2020 [2, 3].

Early diagnosis is fundamental to the successful treatment of breast cancer. In this context, numerous techniques can help with diagnoses, such as mammography, PET, CT,

SPECT, and MRI [4]. The last one has aroused great interest because of its high sensitivity for the identification and characterization of breast cancer and its success in early diagnosis [5–7].

This technique is based on the principles of NMR spectroscopy, where the signals come from the relaxation time of the hydrogen and oxygen nuclei after the application of a radio frequency [8]. Using more efficient contrast agents (CAs) leads to a better resolution of the images and can cause a change in the longitudinal and transversal relaxation times, T_1 and T_2 , respectively, [5, 8, 9]. Hyperfine interactions, which are related to interactions between nuclear and electron spins, of paramagnetic species change relaxation times and can be obtained by EPR spectroscopy [9, 10].

The hyperfine coupling (A) depends on the contributions of isotropic (A_{iso}) and dipolar (A_{dip}) hyperfine coupling constants (equation (1)) [9, 10]. The first one comes from an unpaired spin in an s orbital, and the second one results from a nonlocal and local dipolar constant. Nevertheless, the dipolar hyperfine coupling constant is close to zero when fluid solutions are considered [9, 10]. These parameters, since they affect relaxation times and therefore present activity in the EPR, are crucial for studying and designing new contrast agents for MRI [9, 10]:

$$A = A_{\text{iso}} + A_{\text{dip}}. \quad (1)$$

Traditionally, CAs are metal-based structures like Gd, Pt, and Tc, for example. The study of new CA is important due to the need for increasingly efficient agents, providing better images from the relaxation time of water molecules [11, 12]. Among these metals, we can highlight Tc, highly applied and studied as a contrast agent, and Re, a metal that has great potential as CA because of its low toxicity [5, 13–17].

Re compounds, especially those that have the *fac*-[Re(CO)₃]⁺ fragment, have been extensively studied as a diagnostic agent for breast cancer. Tzanopoulou et al. presented this fragment conjugated to the phenylbenzothiazolic derivative, 2-(4-Aminophenyl) benzothiazole (ABT) (Figure 1), as an agent with potential application for MRI [15]. The ABT compound shows great biological activity as antibacterial, antifungal, antiallergic, anti-inflammatory, and, in our interest, anticancer and inhibiting some enzymes, such as the enzyme kinase [14, 18], especially the phosphoinositide 3-kinase (PI3K) enzyme, which is related with the growth of tumors, like breast cancer [19].

Rhenium compounds, especially those with ¹⁸⁶Re and ¹⁸⁸Re, present properties, such as β emission and half-life time equal to 3.8 and 0.7 d, respectively, that give them a potential for medicinal applications [11]. The *fac*-[Re(CO)₃] core presents a low spin. This characteristic, allied with the characteristics previously presented, provides high interest in this family of compounds for use as radiopharmaceuticals [14]. This leads to a broad study of the application of these compounds in EPR spectroscopy and MRI [14] and the need to evaluate the solvent and environment effects on the EPR parameters, which module the action of this compound as a potential MRI contrast agent. Therefore, the aim of this work was to study solvent and relativistic effects on spectroscopic properties (A_{iso} values for ¹H and ¹⁷O) of the complex Re(CO)₃(NNO) conjugated with 2-(4'-aminophenyl) benzothiazole.

2. Materials and Methods

2.1. Structural Evaluation. The first step was to perform a geometry optimization calculation for the Re(CO)₃(NNO) conjugated to 2-(4'-aminophenyl) benzothiazole, ReABT. This calculation was performed using the DFT method, using the ω B97X-D3 hybrid-GGA functional [20], using TZVP basis set for all atoms except Re and SARC-ZORA-

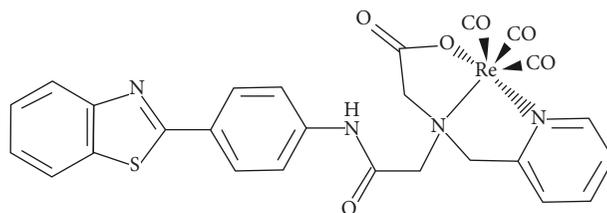


FIGURE 1: Re(CO)₃(NNO) conjugated with 2-(4'-aminophenyl) benzothiazole.

TZVP [21] for rhenium, and considering the zero order regular approximation (ZORA) [22] on software ORCA 4.2.1 [23].

2.2. Spectroscopic Calculations. For the hyperfine coupling constant calculations (A_{iso}), the density functional theory was also used, taking the geometry of minimum energy got and applying different approaches to define which methodology works better in our compound. To do that, three different functionals were used, the GGA functional of Perdew-Burke-Ernzerhof, PBE [24], the hybrid-GGA functional PBE0, which mixes the PBE exchange energy with the Hartree-Fock exchange energy [25], and the meta-hybrid GGA functional Minnesota 06-L, M06-L [26].

The effect of the solvent was also analyzed by theoretical calculations carried out under vacuum and in the presence of water as a solvent [27]. The solvation method used was the Conductor-like Polarizable Continuum Model, CPCM, since it considers the solvent as a polarizable conductor, so it can describe molecules that interact with polar solvents, like water [28].

So, in the first step, the solvent effect was analyzed. In the second step, relativistic effects were evaluated; to this end, calculations considering these effects in the Hamiltonian with ZORA, using the SARC-ZORA-TZVP basis set for rhenium atom and TZVP for all other atoms, and nonrelativistic calculations were performed with def2-TZVP and TZVP basis sets for rhenium and other atoms, respectively. These three steps will provide us with a methodology that fits better with the compound under study.

2.3. Thermodynamics Investigation and Molecular Docking Calculation. To understand whether the complex is thermodynamically stable, a frequency calculation was performed, using the M06L functional, SARC-ZORA-TZVP basis set for rhenium and TZVP for all other atoms and relativistic effects in the Hamiltonian with ZORA, in the ReABT complex, in the Re(CO)₃⁺ core (Figure 2(c)), and in the tridentate ligand, (NNO) ABT (Figure 2(b)), both in the gas phase, to obtain the ΔG_g , and in solution, to obtain ΔG_{solv} (ReABT), ΔG_{solv} (Re(CO)₃), and ΔG_{solv} ((NNO) ABT) values. This analysis will provide the free energy of complexation of the complex (Figure 2(a) and equation (2)), allowing us to understand if the rhenium core is strongly bonded in the complex [29]:

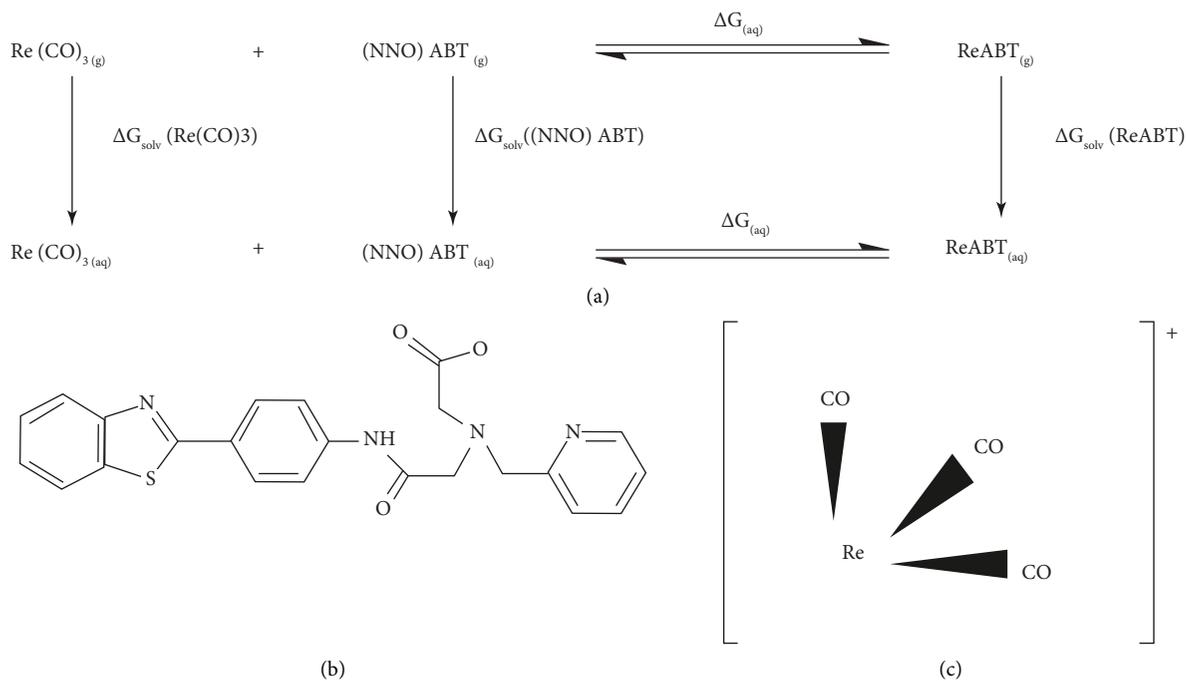


FIGURE 2: (a) Thermodynamic cycle. (b) (NNO) ABT. (c) $\text{Re}(\text{CO})_3^+$.

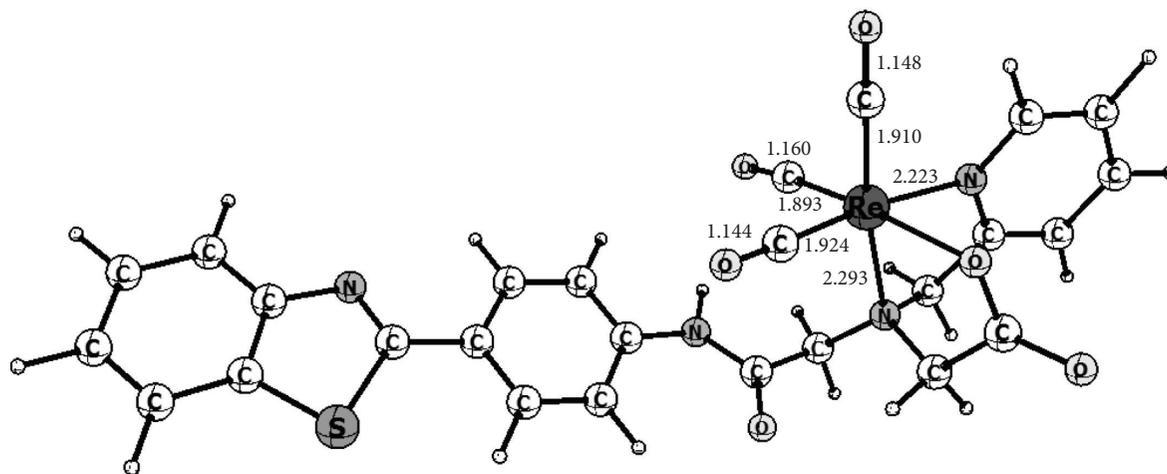


FIGURE 3: $\text{Re}(\text{CO})_3(\text{NNO})$ conjugated to 2-(4'-aminophenyl) benzothiazole optimized structure with some bond lengths highlighted in Angstrom.

$$\Delta G_{Aq} = \Delta G_{\text{SOLV}}(\text{ReABT}) - [\Delta G_{\text{SOLV}}(\text{Re}(\text{CO})_3) + \Delta G_{\text{SOLV}}((\text{NNO})\text{ABT})] + \Delta G_g \quad (2)$$

The molecular docking of the complex was performed at the active site of the PI3K enzyme to obtain A_{iso} values for the compound [7, 17]. For this, the complex was docked inside the PI3K enzyme (Protein Data Bank (PDB) code 3QJZ [30]) using the Molegro Virtual Docking (MVD) software [31]. The conformation that most closely matches the active ligand of the PI3K enzyme, with the amino acid residues that perform hydrogen bonds (H-Bonds) in the PI3K active site, was selected to calculate the hyperfine coupling constant values using the methodology defined previously.

3. Results and Discussion

The results provided in this paper were divided into three major parts. The first one is the validation of the methodology, where the structural results were analyzed and the best theoretical methodology to calculate A_{iso} was defined. The second part will discuss the thermodynamics of the complex to evaluate the free energy of complexation. In the last part of the results, the methodology defined previously was applied to analyze how the A_{iso} values of the ReABT complex

TABLE 1: Lengths of some bonds of the ReABT (theoretical) and ReAPBT (experimental) complexes.

Bond lengths	Length (Å)		Difference in bond lengths
	Theoretical	Experimental	
Re-C _{ax}	1.910	1.897	0.013
Re-C _{eq1}	1.924	1.901	0.023
Re-C _{eq2}	1.893	1.908	0.015
Re-N _{ax}	2.293	2.179	0.114
Re-N _{eq}	2.223	2.244	0.021
C _{ax} -O	1.148	1.157	0.009
C _{eq1} -O	1.160	1.155	0.005
C _{eq2} -O	1.144	1.151	0.007

changed when the complex was docked at the active site of PI3K.

3.1. Validation of the Methodology. The geometry optimization of complex ReABT was performed using DFT calculation with the functional ω B97X-D3, considering relativistic effects by using ZORA, with TZVP basis sets for H, C, O, N, and S atoms and SARC-ZORA-TZVP basis sets for Re. The optimized geometry is presented in Figure 3, with the main bond lengths highlighted. These bond lengths are presented in Table 1. As, to our knowledge, there are no crystallographic data for ReABT, we use the experimental results obtained by Machura et al. for the complex Re(CO)₃Cl conjugated to 2-(2-aminophenyl) benzothiazole (ReAPBT) [13]. This complex was chosen since both compounds present the *fac*-[Re(CO)₃]⁺ core and coordinated in the rhenium, with one equatorial and one axial Re-N bond.

In order to evaluate if whether the theoretical values for the bonds of our complex (Table 1) are in agreement with experimental data and structural parameters from our theoretical findings were compared to Machura’s work [13]. It should be kept in mind, however, that the equatorial bond between Re-O in our complex was replaced by a Re-Cl bond in the compound studied by Machura and co-workers [13], so this change can affect the other bonds. The main difference is in the Re-N_{ax} bond, with 0.114 Å longer in the theoretical result. The C-O bonds are shorter, ranging from 0.005 to 0.009 Å. Differences in bond lengths have a medium value of 0.026 Å.

To improve structural analysis, we compared the bond angles among theoretical and experimental values obtained

TABLE 2: Dihedral angles of some connections in ReABT (theoretical) and ReAPBT (experimental).

Bond angles	Angle (°)		Difference in bond angles
	Theoretical	Experimental	
Re-C-O _{ax}	178.84	175.8	3.040
Re-C-O _{eq1}	174.45	176.5	2.050
Re-C-O _{eq2}	177.21	179.9	2.690
N _{eq} -Re-N _{ax}	76.70	77.15	0.450
C _{eq} -Re-C _{eq}	89.03	88.78	0.250
C _{eq2} -Re-C _{ax}	86.51	87.9	1.390
C _{eq1} -Re-C _{ax}	89.57	84.5	5.070

TABLE 3: Hyperfine coupling constants, A_{iso} , in MHz, for three different functionals, in the gas phase and solution, using the relativistic effect (SARC-ZORA-TZVP for Re, TZVP for all other atoms, and ZORA in the Hamiltonian).

Functionals		¹ H	¹⁷ O	¹⁸⁷ Re
M06L	Gas phase	2.937	2.528	96.658
	Solution	2.656	1.445	36.311
PBE	Gas phase	1.694	1.171	51.851
	Solution	1.588	0.629	24.903
PBE0	Gas phase	1.956	0.669	9.560
	Solution	1.947	0.586	9.195
<i>Exp</i> [35]	—	—	—	38.000

The italic value is to call attention for the experimental value.

from the work of Machura et al. (Table 2). The differences in bond angles, presented in Table 2, range from 0.25°, for the C_{eq}-Re-C_{eq} bond and 5.07° for the C_{eq1}-Re-C_{ax} bond when compared with the experimental data of Machura and co-workers’ paper [13]. These results of bond lengths and bond angles show that our result of geometry optimization is in agreement with the experimental results.

Since the geometry of the complex was optimized, hyperfine coupling constant calculations, A_{iso} , were carried out. Equations (3) and (4) define the relaxation times (T_1 and T_2), i.e., the parameter that came from the interaction of the paramagnetic substances with water. This interaction causes a large drop in the relaxation times. This relationship was one of the most important parameters of the NMR relaxation for the study of MRI probes [5, 9, 32, 33]:

$$\frac{1}{T_1} \approx \frac{1}{15} \frac{S(S+1)g_e^2\beta^2g_N^2\beta_N^2}{\hbar^2r^6} + \left(\frac{A}{\hbar}\right)^2 \frac{S(S+1)}{3} \left[\frac{2\tau_e}{1+(\omega_s\tau_e)^2} \right], \quad (3)$$

$$\frac{1}{T_2} \approx \frac{1}{15} \frac{S(S+1)g_e^2\beta^2g_N^2\beta_N^2}{\hbar^2r^6} + \left(\frac{A}{\hbar}\right)^2 \frac{S(S+1)}{3} \left[\tau_C + \frac{\tau_c}{1+(\omega_s\tau_e)^2} \right]. \quad (4)$$

TABLE 4: Hyperfine coupling constants, A_{iso} , in MHz, for three different functionals, in solution, and without relativistic effect.

Functionals	^1H	^{17}O	^{187}Re
M06L	3.433	15.789	0.006
PBE	2.068	2.558	0.017
PBE0	3.127	6.051	0.016
<i>Exp</i> [35]	—	—	<i>38.000</i>

The italic value is to call attention for the experimental value.

These two equations show the dependence of the relaxation time constant (T_1 and T_2) of the electron spin (S), the electronic and proton g factors (g_e and g_N), the Bohr magneton (β), the distance between the nucleus and the ion (r), the Larmor frequencies for proton and electron spins (ω_1 and ω_2), the correlation times τ_C and τ_S , corresponding to the molecular rotational correlation time and the internal rotational correlation time, respectively, and the hyperfine coupling constant (A), which is described by equation (1) [5, 9, 32, 33].

First, the solvent effect has been evaluated on the A_{iso} values. For that, theoretical calculations considering ReABT in the gas phase and solution with relativistic effects were performed [34]. Our theoretical findings are presented in Table 3.

Table 3 shows that the differences among A_{iso} results in solution are small. The larger differences are between the gas phase and solution, for oxygen, about 1.084 MHz, using the M06L functional, 0.543 MHz, for the PBE functional, and 0.083 MHz, when the functional used is PBE0. For hydrogen atoms, these differences are 0.281, 0.106, and 0.009 MHz, for M06L, PBE, and PBE0 functionals, respectively. This result shows that the complex in water, as occurring in the biological environment and human body, can decrease the hyperfine coupling constant values when compared to the result in the gas phase.

A large difference between the A_{iso} results of Re can be observed, except for the PBE0 functional, where the difference is about 0.365 MHz, which is smaller in the solvent. For the PBE and M06L functionals, the decrease was approximately 26.948 and 60.347 MHz, respectively, when the solvation model was applied.

Despite its great importance, to our knowledge, this is the first investigation of the hyperfine coupling constant values $^{185|187}\text{Re}$ for the ReABT complex. To compare our result, we use the result obtained by Lunsford et al. which points to the A_{iso} value for $^{185|187}\text{Re}$ in a $\text{Re}(\text{CO})_3$ core as 38 MHz [35]. Thus, our findings point out that the calculation using the Minnesota 06-L functional in the solution agrees with the result obtained in the literature.

Keeping in mind that although applying the relativistic effects in the Hamiltonian and the basis set leads to an improvement in the accuracy of the calculation, it also leads to an increase in computational demand. With this, we perform a nonrelativistic calculation to investigate the impact of the relativistic effects on the A_{iso} values of the spectroscopic probe ReABT (Figure 1). Since the previous results show that the solvent effect was important, all calculations were performed, including the solvent effect. These

results are presented in Table 4 and, as expected, show poor results for nonrelativistic calculations of $^{185|187}\text{Re}$ hyperfine coupling constant values. So, our findings put in evidence that the relativistic effects are needed for calculating more accurate A_{iso} values of ReABT.

The results presented in Table 4 point out that, as expected, the A_{iso} value decreases drastically for rhenium, being 9.179, 24.886, and 36.305 MHz smaller for PBE0, PBE, and M06L functionals, respectively, when the relativistic effect was not included in the calculations. For the oxygen and hydrogen atoms, the A_{iso} value increased when the relativistic effect was not present. For hydrogen, the increase was 1.180, 0.480, and 0.777 MHz for PBE0, PBE, and M06L functionals, respectively, and for oxygen, the increase was 5.465, 1.929, and 14.344 MHz for PBE0, PBE, and M06L functionals, respectively.

From these A_{iso} theoretical findings, it is possible to define the best theoretical strategy, combination of functional, solvent model, and relativistic effect. For the spectroscopic probe ReABT, the best combination is using the M06L functional considering relativistic effects on zeroth order regular approximation, with the SARC-ZORA-TZVP basis set for Re and solvent effects evaluated at the CPCM level.

3.2. Thermodynamics Evaluation for the Complex Stability: Toxicologic Properties. Currently, most contrast agents (CAs) used for MRI are Gd-based compounds. They perform well for the improved images provided by MR. However, in the early 2000s, a life-threatening disease, Nephrogenic Systemic Fibrosis, was associated with these CAs, since they release free Gd ions. Since then, the search for new CAs with low toxicity and high performance has intensified [36, 37].

Rhenium-based CAs, such as ReABT, were studied due to their potential as spectroscopy probes and low toxicity [15]. It is important to understand the behavior of the complex in solution, i.e., if the ReABT complex will release free $\text{Re}(\text{CO})_3^+$ ions in solution. For that, the thermodynamic cycle (Figure 2) has been used to obtain the Gibbs free energy of complexation. Although a study by Breitz et al. shows that compounds with rhenium atoms, despite their accumulation in the kidneys, do not influence renal activity [38], it is important to understand the stability of ReABT complex in solution.

The result obtained for the Gibbs free energy of complexation, -744.37 kJ/mol, at the M06-L level, with the ZORA and SARC-ZORA-TZVP basis sets for rhenium and TZVP for all other atoms, is strongly negative and indicates that the complex is stable in solution. Experimental results in the literature indicate that Gd-DOTA, one of the most common commercial MRI probes, has a Gibbs free energy of complexation around -60 kJ/mol [39]. We are quite aware of the limitations of direct comparison between theoretical and experimental result for the Gibbs free energy of complexation, but as shown in other publications, at least for small sized complexes, the theoretical strategy does not introduce significant changes in the mechanism and energetics of

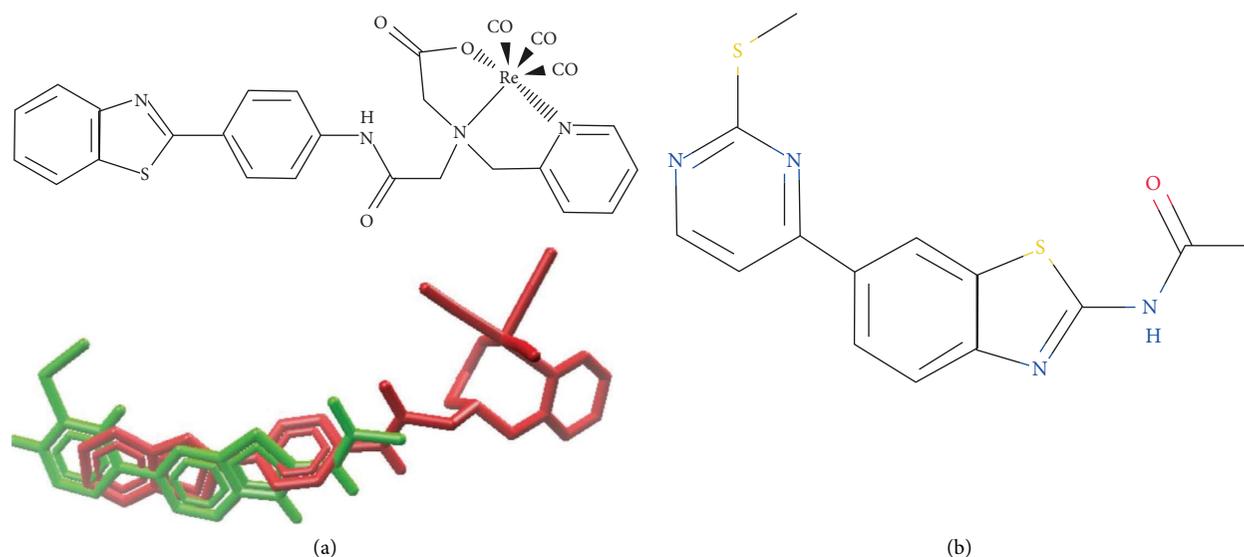


FIGURE 4: ReABT in red(a) and active ligand in green (b) docked at the PI3K (PDB code 3QJZ) active site.

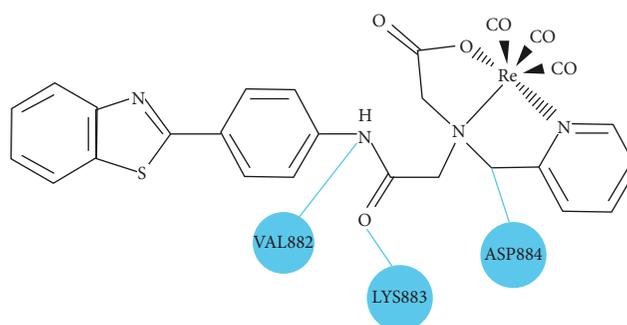


FIGURE 5: H-bonds performed in molecular docking simulation with the ReABT-PI3K system.

complexation reactions [40, 41]. Therefore, the mechanism of the reaction, as well as the relative energies, can be well established with this model.

Therefore, it can be understood that the ReABT complex is significantly more stable when compared to Gd-DOTA. In this way, the complex ReABT shows low toxicity due to its high stability, which will theoretically not show the dissociation of Re ions. Now, our findings have pointed out that the complex shows high stability in solution; it is possible to move on to the next step of this paper, where the hyperfine coupling constant values for ReABT when docked at the active site of the enzyme will be evaluated.

3.3. Molecular Interaction between ReABT and the Target Protein: Docking Studies. Early diagnosis is crucial for the successful treatment of any disease, especially breast cancer [4]. For that, the use of spectroscopic probes that can interact with specific molecular targets is important. In breast cancer, this molecular target is the enzyme phosphoinositide 3-kinase (PI3K), which is closely related to the growth of cancer cells in this tissue [19]. The compounds of the benzothiazole

family are known inhibitors of the enzyme kinase family, especially the PI3K enzyme [7, 17, 42, 43]. In this context, the study of the interaction between the complex ReABT and the enzyme PI3K is important and was investigated in our work using docking calculations.

First, it is possible to check the position of the compound in the active site by taking the overlap and orientation according to the active ligand, N-{6-[(2-(methylsulfonyl)pyrimidin-4-yl)-1,3-benzothiazol-2-yl]} acetamide, a benzothiazole derived (Figure 4).

To continue the analysis of the docking calculation, we can investigate the hydrogen bonds that the ReABT performs inside the active site. In Figure 5, it is possible to observe that ReABT interacts by means of H-bonds, with amino acid residues Val882, Lys883, and Asp884. It is important to note that the active ligand of the PI3K enzyme interacts with the amino acid residue Val882. The intermolecular interaction energy of the active ligand was equal to $-121.17 \text{ kcal}\cdot\text{mol}^{-1}$ and $-163.62 \text{ kcal}\cdot\text{mol}^{-1}$ for the ReABT. These results pointed out that the complex ReABT could have better stability in the active site of the PI3K enzyme. Meanwhile, it should be noted that these results do not consider pharmacokinetic properties.

Now, the theoretical methodology defined in the hyperfine coupling constant study has been applied to analyze the change in the A_{iso} value when the ReABT complex is in the active site of the PI3K enzyme. The calculation was performed using the DTF, with M06L functional, ZORA in the Hamiltonian, SARC-ZORA-TZVP basis sets for Re, and TZVP for all other atoms. This calculation shows that the A_{iso} value for ^{187}Re decreased from 36.311 MHz to 0.0571 MHz, while for ^1H the decrease was from 2.656 MHz to 0.0236 MHz and from 1.445 MHz to 0.0018 MHz for ^{17}O . This dramatic change indicates that ReABT shows activity on EPR at the active site of PI3K demonstrating its potential for the development of a new spectroscopic probe.

4. Conclusions

In this work, we study a potential spectroscopic probe, the complex $(\text{CORE})_3(\text{NNO})$ conjugated to 2-(4'-aminophenyl) benzothiazole, to obtain the EPR activity of this complex in an enzymatic environment. From our data, the best calculation level for obtaining the hyperfine coupling constant (A_{iso}) for the complex ReABT is with the M06L functional, SARC-ZORA-TZVP basis set for Re and TZVP for all other atoms, considering the relativistic effects in the Hamiltonian with ZORA and using implicit solvation method CPCM. Thermodynamic analysis shows that the ReABT complex is stable and should not release free rhenium into biological systems. In this sense, the toxicological risks associated with the complex are considerably reduced.

The best theoretical strategy has been selected and applied to ReABT. This complex was docked in the PI3K active site, presenting electrostatic interactions with the amino acids residues of alanine, aspartate, and lysine. Our findings put in evidence, in this environment, a huge decrease in the A_{iso} values for ^1H , ^{17}O , and ^{187}Re , showing that the ReABT complex has activity on EPR spectroscopy. This finding reveals that the complex $\text{Re}(\text{CO})_3(\text{NNO})$ conjugates to 2-(4'-aminophenyl) benzothiazole has the potential to lead to the development of a new spectroscopic probe for diagnosis cases of breast cancer.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

- [1] R. de Janeiro, "INCA, Instituto Nacional de Câncer José Alencar Gomes da Silva," *Estimated 2018: Cancer Incidence in Brazil*, INCA RJ, Portuguese, 2019.
- [2] American Cancer Society, *Breast Cancer Facts & Figures 2019-2020*, American Cancer Society, Inc, Atlanta, 2019.
- [3] European Cancer Information System, *Breast Cancer burden in EU-27*, European Union, Brussels, 2020.
- [4] S. H. Jafari, Z. Saadatpour, A. Salmaninejad et al., "Breast cancer diagnosis: Imaging techniques and biochemical markers," *Journal of Cellular Physiology*, vol. 233, no. 7, pp. 5200–5213, 2018.
- [5] B. T. L. Pereira, É.F. Silva, M. A. Gonçalves, D. T. Mancini, and T. C. Ramalho, "Exploring EPR parameters of ^{99}Tc complexes for designing new MRI probes: coordination environment, solvent, and thermal effects on the spectroscopic properties ^{99}Tc complexes for designing new MRI probes: coordination environment, solvent, and thermal effects on the spectroscopic properties," *Journal of Chemistry*, vol. 2017, Article ID 8102812, 8 pages, 2017.
- [6] Q. Hu, H. M. Whitney, and M. L. Giger, "A deep learning methodology for improved breast cancer diagnosis using multiparametric MRI," *Scientific Reports*, vol. 10, no. 1, pp. 10536–10611, 2020.
- [7] S. Corrêa, I. A. Rosa, G. A. Andolpho et al., "Hybrid materials based on magnetic iron oxides with benzothiazole derivatives: a plausible potential spectroscopy probe," *International Journal of Molecular Sciences*, vol. 22, no. 8, p. 3980, 2021.
- [8] N. Long and W. Wong, *The Chemistry of Molecular Imaging*, John Wiley & Sons, NY, USA, 2014.
- [9] M. A. Gonçalves, E. F. F. da Cunha, F. C. Peixoto, and T. C. Ramalho, "Probing thermal and solvent effects on hyperfine interactions and spin relaxation rate of $\delta\text{-FeOOH}$ (100) and $[\text{MnH}_3\text{buea}(\text{OH})_2]^{2-}$: toward new MRI probes," *Computational and Theoretical Chemistry*, vol. 1069, pp. 96–104, 2015.
- [10] D. C. Look and D. R. Locker, "Time saving in measurement of NMR and EPR relaxation times," *Review of Scientific Instruments*, vol. 41, no. 2, pp. 250–251, 1970.
- [11] J. Wahsner, E. M. Gale, A. Rodríguez-Rodríguez, and P. Caravan, "Chemistry of MRI contrast agents: current challenges and new frontiers," *Chemistry Review*, vol. 119, no. 2, pp. 957–1057, 2019.
- [12] M. A. Gonçalves, L. S. Santos, F. C. Peixoto, E. F. F. da Cunha, T. C. Silva, and T. C. Ramalho, "Comparing structure and dynamics of solvation of different iron oxide phases for enhanced magnetic resonance imaging," *ChemistrySelect*, vol. 2, no. 31, pp. 10136–10142, 2017.
- [13] B. Machura, M. Wolff, I. Gryca, A. Palion, and K. Michalik, "Novel Re (I) tricarbonyl complexes of chelating ligands with aromatic N-heterocycle ring and aliphatic amine donor – synthesis, spectroscopic characterization, X-ray structure and DFT calculations," *Polyhedron*, vol. 30, no. 13, pp. 2275–2285, 2011.
- [14] B. Machura, M. Wolff, E. Benoist, and Y. Coulais, "Tricarbonyl rhenium (I) complex of benzothiazole – synthesis, spectroscopic characterization, X-ray crystal structure and DFT calculations," *Journal of Organometallic Chemistry*, vol. 724, pp. 82–87, 2013.

- [15] S. Tzanopoulou, I. C. Pirmettis, G. Patsis 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*, vol. 49, no. 18, pp. 5408–5410, 2006.
- [16] S. Tzanopoulou, M. Sagnou, M. Paravatou-Petsotas et al., "Evaluation of Re and 99mTc complexes of 2-(4'-aminophenyl) benzothiazole as potential breast cancer radiopharmaceuticals," *Journal of Medicinal Chemistry*, vol. 53, no. 12, pp. 4633–4641, 2010.
- [17] D. T. Mancini, E. F. Souza, M. S. Caetano, and 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, no. 4, pp. 129–137, 2014.
- [18] B. Mavroidi, M. Sagnou, K. Stamatakis, M. Paravatou-Petsotas, M. Pelecanou, and C. Methenitis, "Palladium (II) and platinum (II) complexes of derivatives of 2-(4'-aminophenyl) benzothiazole as potential anticancer agents," *Inorganica Chimica Acta*, vol. 444, pp. 63–75, 2016.
- [19] F. Janku, "Phosphoinositide 3-kinase (PI3K) pathway inhibitors in solid tumors: from laboratory to patients," *Cancer Treatment Reviews*, vol. 59, pp. 93–101, 2017.
- [20] Y. S. Lin, G. D. Li, S. P. Mao, and J. D. Chai, "Long-range corrected hybrid density functionals with improved dispersion corrections," *Journal of Chemical Theory and Computation*, vol. 9, no. 1, pp. 263–272, 2013.
- [21] D. A. Pantazis, X. Y. Chen, C. R. Landis, and F. Neese, "All-electron scalar relativistic basis sets for third-row transition metal atoms," *Journal of Chemical Theory and Computation*, vol. 4, no. 6, pp. 908–919, 2008.
- [22] C. van Wüllen, "Molecular density functional calculations in the regular relativistic approximation: method, application to coinage metal diatomics, hydrides, fluorides and chlorides, and comparison with first-order relativistic calculations," *The Journal of Chemical Physics*, vol. 109, no. 2, pp. 392–399, 1998.
- [23] F. Neese, "Software update: the ORCA program system, version 4.0," *WIREs Computational Molecular Science*, vol. 8, no. 1, p. e1327, 2018.
- [24] J. P. Perdew, K. Burke, and M. Ernzerhof, "Generalized gradient approximation made simple," *Physical Review Letters*, vol. 77, no. 18, pp. 3865–3868, 1996.
- [25] C. Adamo and V. Barone, "Toward reliable density functional methods without adjustable parameters: the PBE0 model," *The Journal of Chemical Physics*, vol. 110, no. 13, pp. 6158–6170, 1999.
- [26] Y. Zhao and D. G. Truhlar, "A new local density functional for main-group thermochemistry, transition metal bonding, thermochemical kinetics, and noncovalent interactions," *The Journal of Chemical Physics*, vol. 125, no. 19, p. 194101, Article ID 194101, 2006.
- [27] E. J. L. Toledo and T. C. Ramalho, "Controversies about hydrogen bonds in water molecules on the influence of high magnetic fields: implications on structural and electronic parameters," *Molecular Simulation*, vol. 47, no. 14, pp. 1159–1167, 2021.
- [28] M. Cossi, N. Rega, G. Scalmani, and V. Barone, "Energies, structures, and electronic properties of molecules in solution with the C-PCM solvation model," *Journal of Computational Chemistry*, vol. 24, no. 6, pp. 669–681, 2003.
- [29] L. S. Braga, ÉF. Silva, D. T. Mancini, E. P. da Rocha, E. F. F. da Cunha, and T. C. Ramalho, "Detection of chemical weapon agents using spectroscopic probes: a computational study," *Journal of Chemistry*, vol. 2020, Article ID 1312403, 11 pages, 2020.
- [30] N. D. D'Angelo, T. S. Kim, K. Andrews et al., "Discovery and optimization of a series of benzothiazole phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) dual inhibitors," *Journal of Medicinal Chemistry*, vol. 54, no. 6, pp. 1789–1811, 2011.
- [31] R. Thomsen and M. H. Christensen, "MolDock: a new technique for high-accuracy molecular docking," *Journal of Medicinal Chemistry*, vol. 49, no. 11, pp. 3315–3321, 2006.
- [32] O. V. Yazyev, L. Helm, V. G. Malkin, and O. L. Malkina, "Quantum chemical investigation of hyperfine coupling constants on first coordination sphere water molecule of gadolinium (III) aqua complexes," *The Journal of Physical Chemistry A*, vol. 109, no. 48, pp. 10997–11005, 2005.
- [33] M. Lepage and J. C. Gore, *Contrast Mechanisms in Magnetic Resonance Imaging*, Vol. 3, IOP Publishing, , Bristol, UK, 2004.
- [34] P. Pyykkö, "Relativistic effects in chemistry: more common than you thought," *Annual Review of Physical Chemistry*, vol. 63, no. 1, pp. 45–64, 2012.
- [35] A. M. Lunsford, K. F. Goldstein, M. A. Cohan et al., "Comparisons of MN 2 S 2 vs. bipyridine as redox-active ligands to manganese and rhenium in (L-L) M'(CO) 3 Cl complexes," *Dalton Transactions NO VOL NO. to 2009; 2010 is vol. 39*, vol. 46, no. 16, pp. 5175–5182, 2017.
- [36] K. M. Hasebroock and N. J. Serkova, "Toxicity of MRI and CT contrast agents," *Expert Opinion on Drug Metabolism and Toxicology*, vol. 5, no. 4, pp. 403–416, 2009.
- [37] L. Chehabeddine, T. Al Saleh, M. Baalbaki, E. Saleh, S. J. Khoury, and S. Hannoun, "Cumulative administrations of gadolinium-based contrast agents: risks of accumulation and toxicity of linear vs. macrocyclic agents," *Critical Reviews in Toxicology*, vol. 49, no. 3, pp. 262–279, 2019.
- [38] H. B. Breitz, J. S. Durham, D. R. Fisher et al., "Pharmacokinetics and normal organ dosimetry following intraperitoneal rhenium-186-labeled monoclonal antibody," *Journal of Nuclear Medicine*, vol. 36, no. 5, pp. 754–761, 1995.
- [39] A. D. Sherry, P. Caravan, and R. E. Lenkinski, "Primer on gadolinium chemistry," *Journal of Magnetic Resonance Imaging*, vol. 30, no. 6, pp. 1240–1248, 2009.
- [40] T. L. C. Martins, T. C. Ramalho, J. D. Figueroa-Villar, A. F. Flores, and C. M. P. Pereira, "Theoretical and experimental ¹³C and ¹⁵N NMR investigation of guanilylhydrazones in solution," *Magnetic Resonance in Chemistry*, vol. 41, no. 12, pp. 983–988, 2003.
- [41] T. C. Ramalho, E. F. F. da Cunha, and R. Bicca de Alencastro, "A density functional study on the complexation of ethambutol with divalent cations," *Journal of Molecular Structure: THEOCHEM*, vol. 676, no. 1-3, pp. 149–153, 2004.
- [42] A. F. Pereira, I. G. Prandi, and T. C. Ramalho, "Parameterization and validation of a new force field for Pt (II) complexes of 2-(4'-amino-2'-hydroxyphenyl) benzothiazole," *International Journal of Quantum Chemistry*, vol. 121, no. 6, Article ID e26525, 2021.
- [43] E. F. F. da Cunha, W. Sippl, T. de Castro Ramalho, O. A. Ceva Antunes, R. B. de Alencastro, and M. G. Albuquerque, "3D-QSAR CoMFA/CoMSIA models based on theoretical active conformers of HOE/BAY-793 analogs derived from HIV-1 protease inhibitor complexes," *European Journal of Medicinal Chemistry*, vol. 44, no. 11, pp. 4344–4352, 2009.