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Semi-Empirical Study of the Drug Riociguat, an Important Drug for Oral Treatment against Chronic Thromboembolic Pulmonary Hypertension

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***Abstract*—** *The drug Riociguat is an orally administered medication, unique in the treatment of Chronic Thromboembolic Pulmonary Hypertension and still effective in common Pulmonary Hypertension. The aim of the present study was to use the semi-empirical method Parametrical Model 7 (QM-PM7) to perform an in silico characterization of the drug Riociguat using semi-empirical calculations based on quantum mechanics. The molecular structure of the anti-CTPH compound, Riociguat, was geometrically optimized, until its conformation was as stable as possible and the stationary minimum-energy minimum point (Self Consistent Field) was reached, reaching -51.14967 KJ mol^{-1} and its Heat of formation (-12.22507 KCAL mol^{-1}). It can be observed that the best area for nucleophilic reactions is located in the nucleophilic sites, nitrogen and oxygen atoms, which is demonstrated by the GAP values that indicated several regions with high reactivity, As well as a high Gap value. These data generated during the production of this article, aim at a better understanding of the structure and the drug, which can serve as the basis for a later work of elaboration of more biologically efficient analogues, or for the realization of molecular docking studies with Riociguat.*

***Keywords*—** *HOMO, LUMO, MESP, Parametrical Model 7, Riociguat.*

I. INTRODUCTION

The design of drug design, from a totally new structure or modifying a pre-existing structure (Drug Design), aims to identify a drug candidate (suitable for clinical trial) with a greater biological activity, associated to a decrease in the effects Collaterals. Intermolecular forces (lipophilic, polar, electrostatic and stereo interactions) determine the interactions of a drug with its biological receptor [1]. In this way, substances that have biological action on their biological receptor, have a three-dimensional structure, modulated by the medium that is coupled so that the dispositions of their functional groups ester a greater complementarity to the binding site, providing a decrease in the potential energy of the molecule [1].

There are several methods that use mathematical parameters and adjustments to understand the structural and electronic properties of the molecule such as classical methods (molecular mechanics, molecular dynamics) and quantum methods based on the resolution of the Schroedinger equation (Ab initio methods, semi-empirical methods , Density functional theory (DFT) [2]. Dewar and collaborates [3] aiming to increase the accessibility of modelling software have developed a series of programs for semi-empirical molecular orbitals calculations that also provide chemically accurate structural information such as the AM1 (Austin Model 1) method and the PM3 (Parametric Method 3) (STEWART, 1989) and recently the PM7 (Parametric Method 7) was developed [4]. All of these methods have several similarities but differ in their parameterization.

Using previously established data and neglecting some integrals involved in solving the Schrödinger equation, semi-empirical quantum methods reduce computational requirements, thus enabling the ability to study more complex molecules. Chronic Thromboembolic Pulmonary Hypertension (CTPH) is an uncommon and poorly understood disease characterized by obstruction of the pulmonary vascularization system caused by thrombi spread through the pulmonary arteries, which leads to a significant increase in mean pulmonary artery pressure of ≥ 25mmHg and Pulmonary artery occlusion at ≤15mmHg, resulting in a pulmonary hypertension (HP), which may lead to a failure of the right ventricle [5], [6]. The only treatment considered effective when it comes to CTPH is pulmonary endarterectomy (PAD), but only about 63% of the cases are considered operable, and 36.6% are not, and there is still a chance of developing HP residual in 16.7% of the cases after PAD [6], [7]. Thus, for patients who are non-operable and those who develop residual PH, treatment through drug administration becomes the only available treatment [5]. To understand the action of the drug in the treatment of CTPH, it is necessary to understand the function of nitric oxide (NO) in the organism through the process NO-sGC-cGMP; NO is produced in the healthy lung by alveolar epithelial cells, vascular endothelium and airways as a whole and diffuses through the smooth vascular musculature, binding to the prosthetic groups sGC (soluble guanylate cyclase). After the active sGC is activated, it synthesizes the cGMP, a secondary messenger responsible for decreasing the concentration of calcium in the smooth muscles, thus inhibiting the contraction of its cells, which is one of the main causes of vasoconstriction and which is closely related to the appearance Of HP [8]. Due to the availability of new pharmacological agents and advances in the pathophysiological knowledge of pulmonary arterial

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hypertension, the treatment by drug administration for the same has been evolving significantly in the last decade. However, in relation to chronic pulmonary thromboembolic pulmonary hypertension, this treatment has been fundamentally Without much focus on pharmacological production [9]. The drug Riociguat, a tetracyclic drug developed by Bayer Healthcare® registered under the name Adempas®, has been approved for use in the treatment of CTPH in Canada and subsequently by the US FDA in 2013 [10], is innovative in its proposal Because unlike the other medicated HP treatments that acted as inhibitors of the PDE-5is enzyme avoiding the degradation of the cyclic guanosine monophosphate [cGMP] or increasing NO production, Riociguat has a double action involving the sGC group, Both increases the response of the sGC group in the NO bonds, and stimulates the sGC group independently of the availability of NO, also increasing the level of cGMP, which in turn causes vasodilation, being an alternative oral treatment to pulmonary endoarterectomy and not Is only effective in combating CTPH but also in the common HP [8], [9], [11]. The use of three-dimensional images of nanoscopic systems has been fostered by the development of computers with high processing performance, allowing the creation of increasingly precise software, capable of performing calculations with great speed, thus allowing a better visualization of the models adopted by the Scientific community to represent the atomic arrangements and processes involved in chemical phenomena. In this context, the present work aimed to characterize the drug molecule Riociguat, using the semi-empirical method PM7 (Parametric Method 7), adding to this characterization descriptors available in repositories of biological molecules.

II. METHODOLOGY

All the computations were performed on Dell Inspiron personal computer with intel® Core ™ i7-4510U processor, 16 GB RAM, 2GB AMD Radeon® video card and Microsoft Windows 10® as operating system. At the first moment, a search was made at the Drugbank repository (http://www.drugbank.ca/), using the descriptor Riociguat, where structural, taxonomic and pharmacological information were collected. Following the methodology proposed by Dewar and collaborates [3] to optimize the structure and to obtain energy parameters (total energy, nuclear energy, electronic energy, molecular orbital energy of εHOMO (HOMO-Highest Occupied Molecular Orbital and εLUMO (LUMO- (Unoccupied Molecular Orbital), the Molecular Orbital Package (MOPAC2016), Version 16.111W 2016, was configured to perform the semi-empirical method Parametric Method 7 (PM7) using the Hartree-Fock approximation) (Self-consistent field method), for wave function, having the vacuum as the mean, considering the molecule in the ground state. Using the output file generated by the optimization of the structure, the frontier orbitals, HOMO and LUMO, and the Surface Map of the electrostatic potential (MESP) [12] were generated.

To correlate structure and reactivity of the molecules, the values of the border orbitals were used to calculate the GAP (Equation 1) [1]. Using the output file generated by the optimization of the structure, the frontier orbitals, HOMO and LUMO, and the Surface Map of the electrostatic potential (MESP) [12] were generated.

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$$GAP = (\varepsilon_{HOMO} - \varepsilon_{LUMO}) \quad (1)$$

III. RESULTS AND DISCUSSION

The drug Riociguat (Fig. I) has iupac name methyl N-[4,6-diamino-2- [1 - [(2-fluorophenyl) methyl] pyrazolo [3,4-b] pyridin-3-yl] pyrimidine -5-yl] -N-methylcarbamate, has a molecular formula $C_{20}H_{19}FN_8O_2$ Available on drugbank under code: DB08931, has CAS number "625115-55-1".

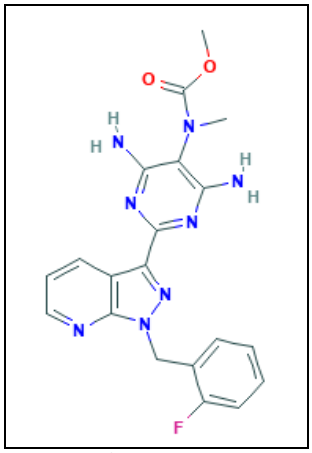

Fig. I. Two-dimensional structure of Riociguat drug available at the Drugbank Repository (https://www.drugbank.ca/drugs/DB08931).

It can be classified as a heterocyclic compound, with presence of amino groups and a characteristic ester group. It has Hydrogen Bond Acceptor Count 9 hydrogen atoms per molecule, and Hydrogen Bond Donor Count 2 atoms, has low solubility in Water (0.0682 mg mL-1), and observable in the partition coefficient LogS with absolute value of -3.8. The partition coefficient LogP representing the water / octanol-1 ratio can be considered an indication of hydrophobicity, which the drug had a value of 2.69, along with other properties reported by DrugBank (Table I).

TABLE I. Predicted properties for the drug molecule Riociguat.

Property	Value
Water Solubility	0.0682 mg/mL
logP	2.69
logS	-3.8
pKa (Strongest Acidic)	18.9
pKa (Strongest Basic)	3.5
Physiological Charge	0
Hydrogen Acceptor Count	8
Hydrogen Donor Count	2
Polar Surface Area	138.07 Å^2
Rotatable Bond Count	5
Refractivity	135.25 m^3·mol^{-1}
Polarizability	41.9 Å^3

The Drugbank repository provides us with a number of data on drug-drug interactions (Antihypertensive Agents,

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Antihypertensive for Pulmonary Arterial Hypertension, Azoles, BCRP / ABCG2 Substrates, Cardiovascular System, Cytochrome P-450 CYP2C8 Substrates, Cytochrome P-450 CYP3A4 Substrates, Guanylate Cyclase, Heterocyclic Compounds Heterocyclic Compounds, 1-Ring, Hypotensive Agents), infusion (CTEPH), (WHO Group 4) after surgical treatment, or inoperable CTEPH, to improve exercise capacity and WHO functional class, Riociguat is indicated for the treatment of adults with pulmonary arterial hypertension (PAH), (WHO Group 1), to improve exercise capacity, WHO functional class and to delay clinical worsening. (25%) PAH (61%) or PAH associated with connective tissue diseases (25%) as well as several other characteristics (Table II)

TABLE II. Pharmacology drug Riociguat

<table>
<tr><th colspan="2">Pharmacology</th></tr>
<tr><td>Indication</td><td>Riociguat is indicated for the treatment of adults with persistent/recurrent chronic thromboembolic pulmonary hypertension (CTEPH), (WHO Group 4) after surgical treatment, or inoperable CTEPH, to improve exercise capacity and WHO functional class. Riociguat is indicated for the treatment of adults with pulmonary arterial hypertension (PAH), (WHO Group 1), to improve exercise capacity, WHO functional class and to delay clinical worsening. Efficacy was shown in patients on Riociguat monotherapy or in combination with endothelin receptor antagonists or prostanoids. Studies establishing effectiveness included predominately patients with WHO functional class II–III and etiologies of idiopathic or heritable PAH (61%) or PAH associated with connective tissue diseases (25%).</td></tr>
<tr><td>Structured Indications</td><td>Chronic Thromboembolic Pulmonary Hypertension
Pulmonary Arterial Hypertension (PAH)</td></tr>
<tr><td>Mechanism of action</td><td>Riociguat is a stimulator of soluble guanylate cyclase (sGC), an enzyme in the cardiopulmonary system and the receptor for nitric oxide (NO). When NO binds to sGC, the enzyme catalyzes synthesis of the signaling molecule cyclic guanosine monophosphate (cGMP). Intracellular cGMP plays an important role in regulating processes that influence vascular tone, proliferation, fibrosis and inflammation. Pulmonary hypertension is associated with endothelial dysfunction, impaired synthesis of nitric oxide and insufficient stimulation of the NO-sGC-cGMP pathway. Riociguat has a dual mode of action. It sensitizes sGC to endogenous NO by stabilizing the NO-sGC binding. Riociguat also directly stimulates sGC via a different binding site, independently of NO. Riociguat stimulates the NO-sGC-cGMP pathway and leads to increased generation of cGMP with subsequent vasodilation.
<table>
<tr><th>Target</th><th>Kind</th><th>Actions</th><th>Organism</th><th>UniProt ID</th></tr>
<tr><td>Guanylate cyclase soluble subunit alpha-2</td><td>Protein</td><td>agonist stimulator</td><td>Human</td><td>P33402</td></tr>
</table>
</td></tr>
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The semi-empirical methods have become a solution for the modeling of medium-sized molecules, since they use experimentally determined parameters, reducing the number of integrals to be solved for the solution of the Schrödinger equation (2) [13].

$$-\frac{\hbar^2}{2m}\nabla^2\psi + E_p\psi = i\hbar\frac{\partial\psi}{\partial t} \qquad (2)$$

Where Ep represents potential energy in the region considered, m is the mass of the particle associated with this wave function and $\partial\psi / \partial t$ represents the derivative parc

Ial of the wave function in order to time; 2Ψ is the so-called Laplacian of Ψ.

The total energy was used to estimate the stability of the molecule, and corresponds to the sum of the energy of nuclear repulsion with the electronic energy. The electronic energy was determined by the Born-Oppenheimer approximation, which dissociates the electronic and nuclear movements assuming a fixed position of the nuclei, and the Schrödinger (2) was solved in order to find the electronic energy of the molecule.

All the geometries of the drug were optimized in internal coordinates with complete optimization of all the geometric parameters, obtaining the structure theoretically more stable because it has the lowest total energy 51.14967 KJ mol^{-1}, where we can observe the dihedral angles C15C14N13 (113.5º) And C2 N5 C6 (119.6) Fig II and b respectively. It also has a heat of formation (12.22507 KCAL mol-1), total energy (-5257.26279 EV) electronic energy (-42899.73903 EV), core-core repulsioN (37642.47624 EV), gradient norm (0.96866), dipole (5.45459 DEBYE) And ionization potential (8.786887 EV).

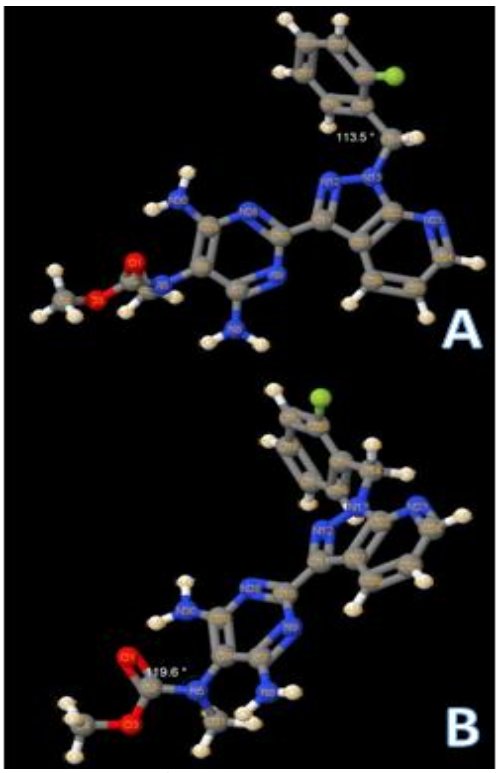

Fig. II. Optimized structure of the drug Riociguat, with the dihedral angles C15C14N13 (A) C2N5C6 (B).

With respect to the surface area 418.32 $Å^2$, there was a significant discrepancy from that reported by the DrugBank repository, which was 138 $Å^2$ (Table I). Regarding the volume, the calculated value was 471.76 Å3, observable in the Van der Walls structure (Fig. III).

Molecular modeling, with the aid of modern computation, has become an important tool in the process of rational drug development. The knowledge of the charge distribution,

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identifying its partial densities, helps to understand the form of interaction between one molecule and another [14] [15].

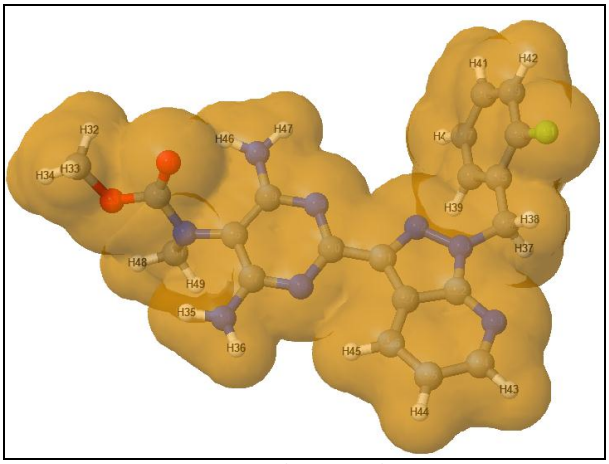

Fig. III. Van der Waals Surface Map of the drug Riociguat.

The Surface Map of Electrostatic Potential (MESP) provides a visual method that helps identify the relative polarity of the compounds [16], identifying the nucleophilic and electrophilic sites and which together with the dipole moment of the molecule can be [17] [18], but it is also an important tool in the study of the interaction between biological molecules and their receptors Of new drugs [19]. In Figure 4, we can identify that the area where there is the highest concentration of electrons, nucleophilic region (in red), is located at the extremities formed by fluorine (Fig IV), by the nitrogen (N9, N12, N23, N28) and by the oxygens (O1, O3), the region of the cycle formed by the carbons (C15, C16, C17, C18, C19, C20) is in a neutral area, while the rest of the molecule remains in the electron-poor area called the electrophilic region.

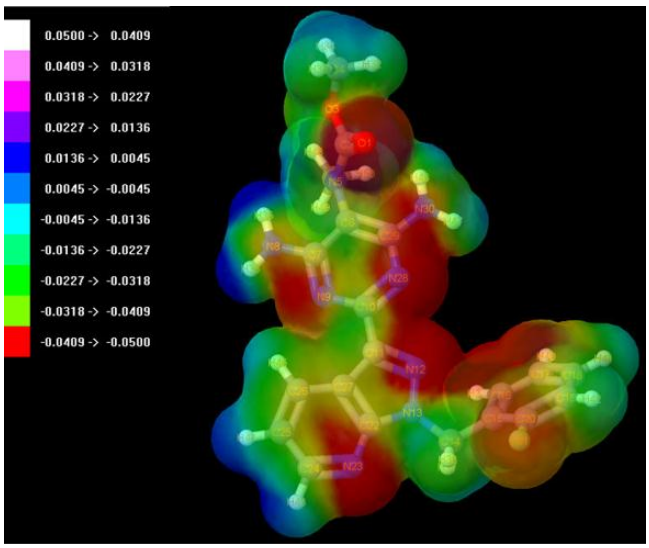

Fig. IV. Surface map of the electrostatic potential (MESP) of the drug Riociguat.

The theory of boundary orbitals is based on the principle that when the molecular orbitals of two reactants begin to interact and overlap, it leads to the formation of two new molecular orbitals, a ligand of lower energy and, another, anti-ligand, Of higher energy [2]. We can exemplify them in a reaction that uses the second order nucleophilic substitution mechanism, where the reaction begins with the interaction of the highest Occupied Molecular Orbital (HOMO), which contains the pair of electrons to be donated (LUMO), so we can relate the energy of the HOMO (εHOMO) and LUMO (εLUMO) orbitals to the attracting force of the pair of Electrons that can be donated (high energy of HOMO), as well as we can relate this variation of energy with the capacity to receive electrons (low energy of LUMO). These energies can be better visualized by the calculation of the GAP (Eq. 2) [1], because the smaller the Gap the more facilitated the reaction. In Figure V, we can observe a small variation in the GAP values, indicating, therefore, a high reactivity for this molecule. It is possible to observe the homo orbital (Fig. V) that it is formed by the contribution of the atoms C6, N30, N8, N9, N28, C11, C27, N12, N13, N23, C26 and C25 and small scale C10 and C15, The C 2, C 2, C 2, C 2, C 2, C 2, C 2, C 2, C 2, C 2, C 2, C 2, C 2, C 2, N 2 and C 2 atoms. Of LUMO.

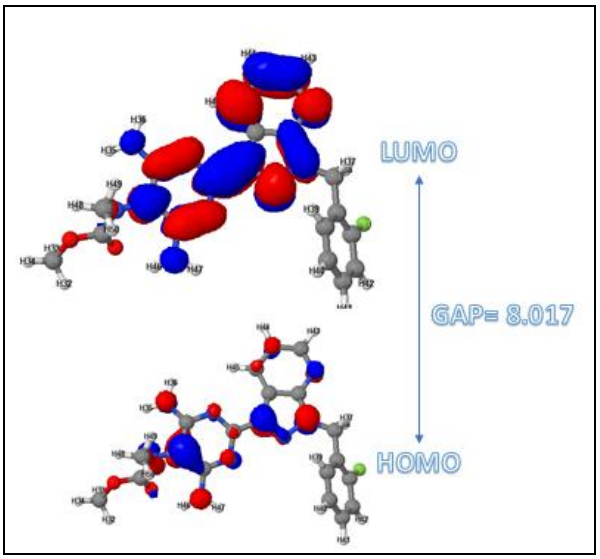

Fig. V. Border Orbitals (HOMO and LUMO) and GAP values of the drug Riociguat.

IV. Conclusions

Molecular modeling, with the aid of modern computation, has become an important tool in the rational drug development process, allowing the structural characterization, as observed in the modeling of the drug Riociguat. Using semi-empirical calculations based on quantum mechanics, the molecular structure of the anti-CTPH compound, Riociguat was geometrically optimized, until its conformation was as stable as possible and the stationary point of least potential minimum energy (Self Consistent Field) was reached, reaching The value -51.14967 KJ mol^{-1} and its heat of formation (-12.22507 KCAL mol^{-1}). It is possible to observe that the best area for nucleophilic reactions is located in the nucleophilic bridge that has nucleophilic sites on the nitrogen and oxygen atoms. This

is demonstrated by the GAP values that indicated several regions with High reactivity as well as a high Gap value. These data generated during the production of this article aim at a better understanding of the structure and the drug, which can serve as a basis for further work on the elaboration of biologically efficient analogues or for the realization of molecular docking studies with Riociguat.

This work constitutes an initial step for the improvement of the drug, since from the complete understanding of the characteristics that influence the reactivity of the compound, we can begin the planning of new compounds through structural modifications (Drug designer).

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