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In Silico Characterization of Hypoglycemic Agent Phenformin Using Classical Force Field MMFF94

Miquéias Reges [1], Márcia Machado Marinho[2], Emmanuel Silva Marinho[3]
[1]*Department of Chemistry, University State of Ceará, Brazil*
[2]*Departamento of Pharmacy, Federal University of Ceará,Brazil*
[3]*Department of Chemistry, University State of Ceará, Brazil*
Email: [1]miqueias.reges @aluno.uece.br, [2]marinho.marcia@gmail.com.br, [3]emmanuel.marinho@uece.br

Abstract **- Diabetes mellitus type 2 affects 11% of the Brazilian population over 40 years old (about 5 million people are also currently one of the main causes of morbidity in adults in Brazil, reaching between 30 and 40% of them.) The compound Phenformin is then a biguanide oral hypoglycemic agent, widely used.The present work aimed to perform the structural and electronic characterization of the drug Phenformin, using the force field MMFF94.According to the cited literature and results obtained using the Marvin Sketch ©, Arguslab® and Avogrado® computational freeware software, the calculations performed allowed to obtain basic properties of the treprostinil molecule, and the electronic and structural characterization of this drug, which identified a stable energy conformation, potential energy of -1081.3789 kcal.mol-1 and formation heat -57.388 Kcal.mol-1 through the semi-empirical method (PM3). The calculations also allowed to observe the contributions in The molecular structure of the Phenformin drug was geometrically optimized by means of classical force field calculations using the Avogadro® freeware set up at MMFF94 steepest descent until reaching the point of least potential energy reaching the theoretically more stable conformation of its native form, obtaining at the end of this process the energy of [-794.4979 kJ · mol-1]. It's mass [205.132751 Da] and null dipole moment were also characterized. With the optimized structure, it was possible to calculate the connection lengths, the angles between connections and the dihedral angles, with special emphasis on the connection (C6 - C7) because it is the only one to have rotatability. In addition to the calculations, the Van der Waals surface of the structure was rendered. The obtained data consist of an initial stage for future studies of molecular semi-empirical modeling and molecular docking, seeking to optimize this compound and its possible analogues in biological potential.**

Keywords **– Diabetes, MMFF94, molecular modeling, theoretical chemistry.**

I. INTRODUCTION

Type 2 Diabetes Mellitus affects 11% of the Brazilian population over 40 years old (about 5 million people) [1]. It is currently one of the main causes of adult morbidity in Brazil, reaching 30 to 40% of them [2]. This alarming percentage comes from increased urbanization mixed with high-fat diets and minimization of physical activity and quantity [3]. In addition to prevention, the treatment of diabetes consists mainly of glycemic control, a control that is obtained through the joint effort between specific diet, physical activity and medication, which may be insulin or hypoglycemic agents [4].

Among the hypoglycemic agents, or oral hypoglycemic agents, there are two main classes: sulfonylureas and biguanides. The compound Phenformin is then a biguanide, introduced into the clinical setting in 1957[5], so far referred to as phenethyl-biguanide and of acceptable clinical toxicity [6] Similar to metformin, it works by lowering glucose uptake by the gut, while decreasing glucose production in the liver and promoting the body's ability to use it more effectively [7][8]; it then acts to raise the patient's sensitivity to insulin. The mechanisms of action of this oral drug have as a principle the binding to protein kinase through the activation of AMPK that, when activated, can deceive insulin-sensitive cells, promoting a greater use of glucose, by causing the body to think that levels of insulin levels are low [8]. The drug, however, was banned commercially in the

United States in 1977 because of the occurrence of lactic acidosis in cases treated with Phenformin; and due to its action causing increased anaerobic glucose utilization, patients receiving biguanides are permanently lactic and pyruvic acid and increased ketone bodies [6].

Rational drug planning allows, through molecular modeling, determining parameters that relate structure and activity [9] to study the biological activity of specific compounds and to promote the creation of analogs that maintain the biological potential with less toxicity and better clinical acceptanceThe design of new drugs and the improvement of existing drugs, uses molecular modeling, which can be defined as a system of software and tools that allow the construction, editing, visualization and analysis of molecular structures, providing the Barreiro and collaborates[10]. In the present work, we present the results of the present study. Thus, computational chemistry, through in silico methods and theoretical calculations, allows the complete characterization of compounds [11], generating structures with high fidelity rate to the native structures and with stable conformational geometry, as well as some indexes relevant for the planning of drugs such as: formation heat, minimum potential energy, HOMO (Lowest Occupied Molecular Orbital) and LUMO energies, dipole moment and the specific arrangement of each atom in the molecule[12]. In this perspective, through molecular modeling methodology the present work aimed to perform the structural and electronic characterization of the drug Phenformin, using the force field MMFF94.

II. METHODOLOGY

For the accomplishment of this work, we used Computer simulation softwares with free license for academic and research purposes. All simulations were performed on a personal computer, based on the Microsoft Windows ® Operating System. This work was carried out in five steps: (1) the two-dimensional molecular structure of the compound Phenformin and its nomenclatures were originally obtained from the Drugbank® virtual repository [www.drugbank.ca] [13-15]; (2) through the virtual repositories ChemSpider® (http://www.chemspider.com) [16] and Drugbank® [13-15] obtained the physicochemical properties of the structure as well as the pharmacodynamics and mechanisms of action of the same; (3) in the third step were performed using the softwareMarvinSketch and MarvinView[17-18], to perform the theoretical elemental analysis. (4) by means of classical force field calculations, Merck Molecular Force Field (MMFF94)[19], performed through the software Avogadro®[20], configured for cycles of 500 interactions of the steepest descent algorithm, the geometric optimization of the molecule was performed, thus obtaining its conformation of lower potential energy and (5) characterizing the links, torsion angles and dihedral angles, as well as (6) visualizing the dipole moment and rendering the Van der Waals surface maps.

III. RESULTS AND DISCUSSIONS

In the areas of biochemistry and theoretical chemistry, the virtual simulation tools provide a considerable minimization of the resources used in the geometric optimization processes of a molecular structure, as well as the calculations of specific properties of the same structure or the docking of this with a specific ligand. Obtaining a molecular structure can be linked to some molecular modeling program, where it will be built step-by-step, or can be freely downloaded from virtual databases containing thousands of structures in its catalogs. As for the structure in question, Phenformin, the Drugbank® virtual repository (https://www.drugbank.ca) was used to obtain the initial structure (Fig.1) and some properties and nomenclatures; some of these important data found were the CAS identification number (114-86-3), its correct name according to IUPAC (1-carbamimidamido-N- (2-phenylethyl) methanimidamide) and still some other physical-chemical properties (Table I), which were used to study the structure in molecular modeling. The partition coefficients LogP (-0.72) and LogS (-3) and the water solubility (0.232 mg / ml) apolar) used in docking or molecular dynamics tests.

Table I
Physicochemical properties of compound Phenformin

Property	Value	Property	Value
Melting point	176.5 °C	pKa	11.97
Solubility in water	0.232 mg/mL	Polar Surface Area	97.78 Å^2
LogP	-0.72	Refractivity	80.72 m^3·mol^{-1}
LogS	-3	polarizability	22.14 Å^3

Source*: Virtual Repository Drugbank®* *[https://www.drugbank.ca/drugs/DB00914].*

Other physical and chemical properties (Table II) could be obtained through the virtual repository, ChemSpider® [http://www.chemspider.com], properties linked to the structural composition of the molecule, of which we can highlight its density (1.2 ± 0.1 g / cm3) and its surface tension (53.9 ± 7.0 dyne / cm), and its ability to form hydrogen bonds by determining atoms with the potential to receive or donate electrons in hydrogen bonds.

Table II
Physical-chemical properties of the drug Phenformin

Properties	Value	Properties	Value
Molecular Formula	$C_{10}H_{15}N_5$	Monoisotopic Mass	205.132751 Da
Density	1.2±0.1 g/cm^3	Refractive index	1.620
Boiling point	332.2±35.0 °C (760 mmHg)	Molar Refractivity	57.9±0.5 cm^3
Steampressure	0.0±0.7 mmHg (25°C)	Superficial tension	53.9±7.0 dyne/cm
EnthalpyofVaporization	57.5±3.0 kJ/mol	Molar Volume	164.8±7.0 cm^3
Receptors #H	5	Donors #H	6

Souce: Virtual RepositoryChemSpider® [http://www.chemspider.com/Chemical-Structure.7953.html?rid=b198ff5a-af1e-4fb2-b788-418501a8740f].

The two-dimensional structure of Phenformin [Fig. 1], obtained through Drugbank®, was then in its fundamental state, presenting only the molecular formula (C10H15N5) and the connectivity of the atoms, with an initial conformation of easy visualization, but with potential energy different from the molecule in its native form.

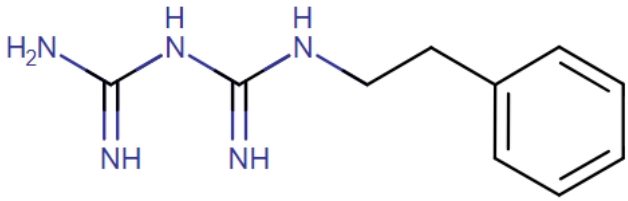

Fig.1. The two-dimensional structure of Phenformin
Souce: Virtual Repository Drugbank® https://www.drugbank.ca/drugs/DB00914].

The elemental analysis allows to determine which are the chemical elements and how much of each element in a compound that has been isolated, it can be done qualitatively or quantitatively, the Phenformin, presents displays molar mass in the value of 205.265, exact mass of 205.132745503, with molecular formula C10 H15N5, being composed of 58, 51% of Carbon, 34,12% of Nitrogen and 7.37% of Hydrogen, having a total of 30 atoms. When submitted to Mass spectrum (m / z abundance, it presented three peaks of 205,206 and 207 (Fig. 2).

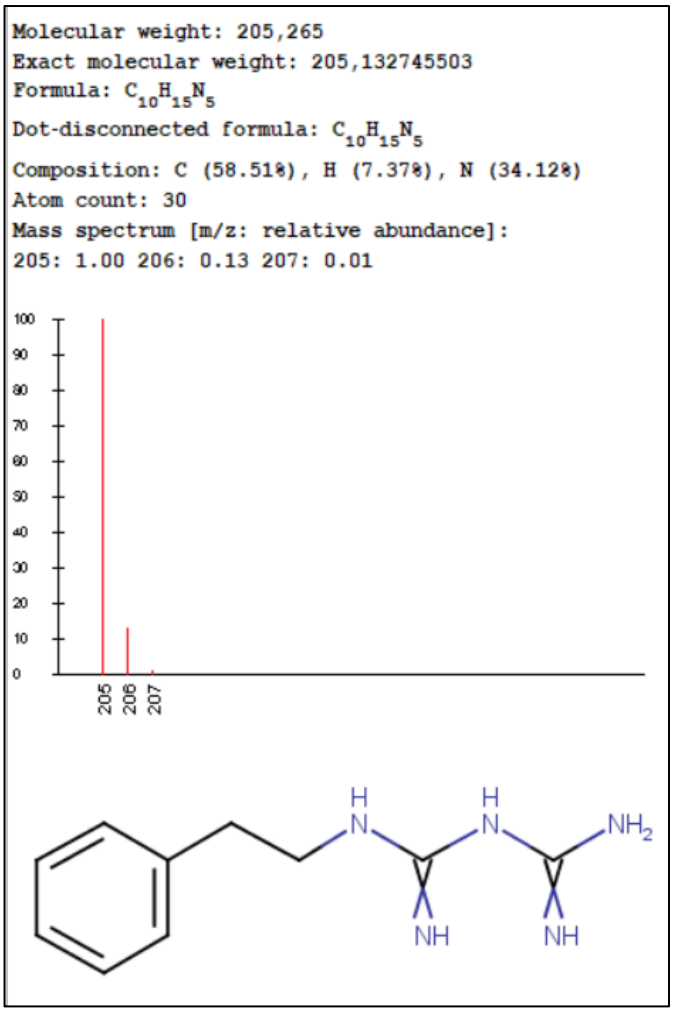

Fig.2.Elementary analysis of the basic descriptors of a structure.

When removing a molecule from an online repository or drawing it two-dimensionally, it is not in its most stable conformation and, to obtain more precise calculations about the molecule and its final more stable conformation, we need to perform a geometric optimization, which uses the energy minimization process [21]. This geometric optimization can be performed through Avogadro® open license software, configuring it to perform uninterrupted interaction cycles calculated through the MMFF94 force field, parameterized with the Steepest Descent algorithm. The obtained structure [fig. 3], considered theoretically more stable, behaved its atoms each in its place of least possible potential energy, making the integral potential energy of the molecule assume a value of [-794.4979 kJ · mol-1], no longer varying, reaching a stationary point of the energy surface [10].

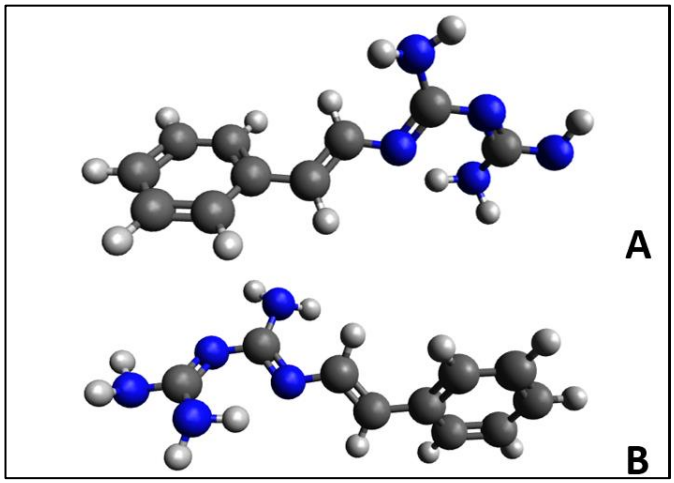

Fig. 3.Optimized structure of the compound Phenformin (A - Rotation of 0 ° / B Rotation of 180 °) optimizedusing the force field MMFF94

After the process of energy minimization and, consequently, geometric optimization, each atom occupies its lowest energy place in the structure, so with a theoretically more stable structure, it is also possible to calculate the formal and partial charges of each atom as well as its valence. These data [Table III], valence, correspond to the literature, which serves to validate the results obtained.

Among the calculated properties is the formal charge of each atom, which can be considered as the most reliable prediction of the real charges that each ion or molecule has [22] and considers that, unlike the ionic bond, where there is transfer in this case, the electron pair is divided equally between both atoms forming the covalent bond, being an electron sharing [23]. The neutrality of the formal charge, through the optimization, it is possible to observe in the results obtained [Table III] the existence of partial (residual) charges, these charges coming from the electrons are closer to or farther from one of the atoms of the bond, taking with them cargo [24].

Table III

Atomic properties of the compound Phenformin obtained after optimization using the field of force classic MMFF94

Atom	Elementtype		Valencia	Formal change	Partialchange	X (Å)	Y (Å)	Z (Å)
1	N	Ng+	2	0	-0.110	-0.47487	-1.27475	0.66390
2	N	Ng+	2	0	-0.004	-2.37144	-2.20982	1.55400
3	N	Ng+	3	0	-0.286	-2.18136	0.07265	1.58417
4	N	Ng+	3	0	-0.289	-2.60736	-4.43037	2.08629
5	N	Ng+	3	0	-0.289	-0.53099	-3.71551	1.80363
6	C	C3	4	0	0.007	0.50369	0.11699	-1.09244
7	C	Car	3	0	-0.044	1.16652	1.43875	-1.38636
8	C	C3	4	0	0.109	0.25226	-0.03607	0.39985
9	C	Car	3	0	-0.058	2.56134	1.52734	-1.48878
10	C	Car	3	0	-0.058	0.39931	2.60251	-1.53494
11	C	Car	3	0	-0.062	3.17609	2.75466	-1.74448
12	C	Car	3	0	-0.062	1.01588	3.82920	-1.78968
13	C	Car	3	0	-0.062	2.40342	3.90465	-1.89467
14	C	C+	3	0	0.425	-1.60927	-1.11665	1.25739
15	C	C+	3	0	0.387	-1.83639	-3.35776	1.78566
16	H	H	1	0	0.034	-0.44422	0.06345	-1.64286
17	H	H	1	0	0.034	1.11812	-0.71139	-1.46613
18	H	H	1	0	0.070	-0.48490	0.84770	0.08780
19	H	H	1	0	0.070	1.18439	-0.01807	0.97425
20	H	H	1	0	0.062	3.17809	0.63891	-1.37098
21	H	H	1	0	0.062	-0.68452	2.56012	-1.45955
22	H	H	1	0	0.062	4.25840	2.81283	-1.82564
23	H	H	1	0	0.062	0.41353	4.72624	-1.90728
24	H	H	1	0	0.062	2.88273	4.85948	-2.09384
25	H	H	1	0	0.255	-1.98497	0.82339	0.93951
26	H	H	1	0	0.255	-3.17271	-0.04917	1.75158
27	H	H	1	0	0.255	-3.56047	-4.14363	2.28114
28	H	H	1	0	0.255	-2.22266	-4.97016	2.85217
29	H	H	1	0	0.255	-0.37204	-4.57843	1.29874
30	H	H	1	0	0.255	0.02449	-2.96702	1.37767

As for the properties calculated using the Avogadro® software, one can calculate the dipole moment (μ) of the structure which, due to the difference in electronegativity between the atoms, is related to the way the electric charges are distributed by the molecule and to the polarization, separation between positive and negative charges [24]. Some other properties of the structure are directly linked to the dipole moment (μ), as the melting and boiling points and their solubility in water [23]. The compound Phenformin presented a dipole moment (μ) of estimated value in 0.000, that is, null, characterizing the molecule as apolar, thus not being possible its vectorial representation.

In the final geometry achieved by the optimization, all the connections (Table IV) analyzed

were characterized by a predominance of covalence, the covalent bonds being defined as having the homologous length to the average distance between the nuclei of the two atoms that carry the bond, native form(greater stability and lower energy)[22][23][24]. We can also analyze the bonds between the carbon-nitrogen (C9-N4) and (C10-N5) and carbon-carbon ((C1-C8), (C2-C4) and (C3-C5)) as second order, or double bonds, and the bond between carbons (C6 -C7) as the only one to have rotatability.

Table IV

Properties of Phenformin After OptimizationUsing Classical Force field MMFF94

Bond	Type	InitialAtom	Final Atom	OrderofBond	Rotability	Length(Å)
1	C – N	C7	N4	1	Não	1.38778
2	C – N	C9	N4	2	Não	1.28788
3	C – N	C9	N5	1	Não	1.36549
4	C – N	C10	N5	2	Não	1.2873
5	C – N	C9	N1	1	Não	1.35907
6	H – N	H1	N1	1	Não	1.00909
7	H – N	H2	N1	1	Não	1.01261
8	C – N	C10	N2	1	Não	1.35357
9	H – N	H3	N2	1	Não	1.01237
10	H – N	H4	N2	1	Não	1.02456
11	C – N	C10	N3	1	Não	1.35396
12	H – N	H5	N3	1	Não	1.01415
13	H – N	H6	N3	1	Não	1.01278
14	C – C	C6	C8	1	Não	1.50837
15	C – C	C6	C7	1	Sim	1.53045
16	C – H	C6	H7	1	Não	1.09782
17	C – H	C6	H8	1	Não	1.09785
18	C – C	C1	C8	2	Não	1.40124
19	C – C	C2	C8	1	Não	1.40125
20	C – H	C7	H9	1	Não	1.09703
21	C – H	C7	H10	1	Não	1.09733
22	C – C	C1	C3	1	Não	1.39568
23	C – H	C1	H11	1	Não	1.08778
24	C – C	C2	C4	2	Não	1.39569
25	C – H	C2	H12	1	Não	1.08788
26	C – C	C3	C5	2	Não	1.39367
27	C – H	C3	H13	1	Não	1.08678
28	C – C	C4	C5	1	Não	1.3937
29	C – H	C4	H14	1	Não	1.08681
30	C – H	C5	H15	1	Não	1.08669

With respect to the conformational characterization, all the angles between the connections and the torsion angles, called dihedral, could be calculated. Taking as examples of higher and lower angulation between bonds, the angles (N2 - C10 - N5) and (C6 - C7 - N4) with 129.8063 ° and 0.0000 ° respectively; (H14 - C4 -

C5 - C3) and (H13 - C3 - C5 - C4) with 179.9430 ° and - 179.9701 ° respectively, for example the largest and smallest dihedral angles. Finally, the software allowed the rendering and visualization of the van der Waals surface (Fig. 4).

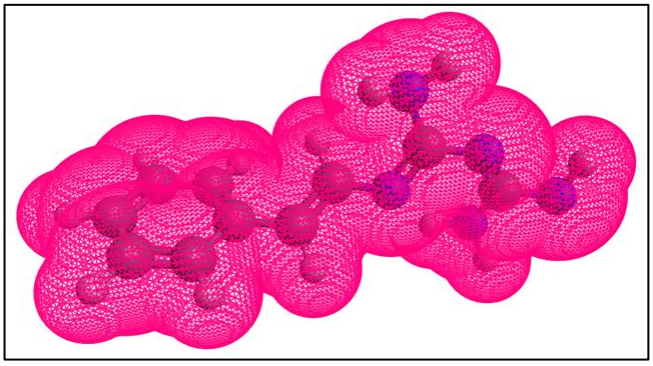

Fig. 4. Van der Waals surface of the drug Phenformin.

IV. CONCLUSIONS

According to the cited literature and results obtained using the Marvin Sketch ©, Arguslab® and Avogrado® computational freeware software, the calculations performed allowed to obtain basic properties of the treprostinil molecule, and the electronic and structural characterization of this drug, which identified a stable energy conformation, potential energy of -1081.3789 kcal.mol-1 and formation heat -57.388 Kcal.mol-1 through the semi-empirical method (PM3). The calculations also allowed to observe the contributions in the molecular structure of the Phenformin drug was geometrically optimized by means of classical force field calculations using the Avogadro® freeware set up at MMFF94 steepest descent until reaching the point of least potential energy reaching the theoretically more stable conformation of its native form, obtaining at the end of this process the energy of [-794.4979 kJ · mol-1]. Its mass [205.132751 Da] and null dipole moment were also characterized. With the optimized structure, it was possible to calculate the connection lengths, the angles between connections and the dihedral angles, with special emphasis on the connection (C6 - C7) because it is the only one to have rotatability. In addition to the calculations, the Van der Waals surface of the structure was rendered. The obtained data consist of an initial stage for future studies of molecular semi-empirical modeling and molecular docking, seeking to optimize this compound and its possible analogues in biological potential.

V. ACKNOWLEDGMENT

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