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Characterization in Silico of the Structural Parameters of the Antifungal Agent Ketoconazole

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Abstract: Computational methods of molecular modeling is a useful tool for planning of bioactive compounds such as drugs. This paper aims to describe initial aspects of approach in silico of the antifungal agent ketoconazole, using the ACD / ChemSketch® and Arguslab® software as a necessary step for future studies of structural change, hoping to optimize the action of this drug. The employed methodological steps were: (1) obtaining ketoconazole structure from Chemspide repository; (2) visualization of three-dimensional structure of this drug by ChemSketch® software, with representation of the electron density map; (3) performing semiempirical calculation Arguslab® and Avogrado® software (Austin Model 1-QM-AM1) to optimize the structure, determining the potential electrostatic and Mulliken atomic charges, generation of three- dimensional maps of the electrostatic potential, calculation of orbital border and the minimum potential energy of the molecule. The optimization of ketoconazole structure proved to be feasible. From the electrostatic potential map and load distribution of Mulliken, identified the oxygen atom of the acyl termination (O_3) as a preferred site for nucleophilic reactions. Energy ketoconazole minimum potential was estimated at -139841.4989 kcal/mol The now obtained descriptors will be key objects for future studies of relationships between three-dimensional structure of the drug and its biological activity.

Keywords: ketoconazole, semi-empirical calculation, molecular modeling

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1. Introduction

The discovery of new drugs has its foundation in the molecular design of new structures capable of presenting pharmacological effects desired safely adequate bioavailability to their therapeutic use and convenience dosage [1]. As a useful tool for this purpose, is the computational molecular modeling, both to allow the development of novel pharmacological agents and as they allow optimization of an existing drug prototype [2]. For the modeling, it becomes possible to enhance specific properties which influence the interaction of a molecule to its receptor [1].

In molecular modeling, quantum methods are commonly employed of which stem from the resolution of the Schrödinger equation for calculating the energy of molecular orbitals, and can be performed ab beginning or semiempiricamente, by a simplified Hamiltonian operator and parameters adjusted from experimental data . In this method, the quality will depend on the theoretical approaches that give it birth, as well as the breadth and quality of information used in its parameters [3].

Dewar et al [1] developed a series of programs in order to make available the semi-empirical calculations, providing chemically precise structural information at a reasonable cost calculation time. Among them stand out PM3 (Parametric Method 3 or Method Parametric 3) [1], and AM1 (Austin Model 1 or Model Austin 1) [4].

2. Historical Aspects of Agent Antifungal Ketoconazole

Fungal infections are among the most common causes of skin diseases, assuming more severe in elderly or immunocompromised due to organ transplants, cancer chemotherapy or AIDS [5, 19]. The cellular structural similarities between fungi and mammals restrict the possibility of a wide variety of agents anfúngicos, while justifying the high toxicity thereof [6].

Azoles (imidazoles and triazoles) are a group of synthetic entifúngicos agents with broad spectrum of activity [7]. These drugs inhibit the microsomal enzyme sterol 14-α-demethylase, affecting the biosynthesis of ergosterol in the plasma membrane, causing the accumulation of 14-α-metilesteróis; the latter can break down the compact arrangement of the acyl chains of the phospholipids, impairing the activity of enzyme systems connected to the membranes, thus inhibiting the growth of fungus [8]. Examples of azoles include fluconazole, itraconazole, ketoconazole or miconazole, among others. Ketoconazole was the first azole to be administered orally for systemic fungal infections [7]. Because of its hepatotoxicity has been replaced by itraconazole, except when the lower cost of ketoconazole overcome vantagesn latter [8].

For its historical importance, ketoconazole can be considered the prototype of the class of imidazoles. Known officially as oxide cis-1-acetil-4-[4-[2-(2,4-diclorofenil)-2-(1-H-imidazol-1-ilmetil)-1, 3-dioxalan-4 -il]metoxifenil]-piperazina), with molecular formula $C_{26}H_{28}Cl_2N_4O_4$ [9], It appears as a white powder, insoluble in water with melting point between 148°C and 152°C [10]. In a structure (Figure 1) are evident two basic groups, piperazine and imidazole, with their pKa values equal to 2.94 and 6.5, respectively. By law, the ketoconazole formulation should contain between 90.0% and 110.0% of the stated amount of the active principle [11].

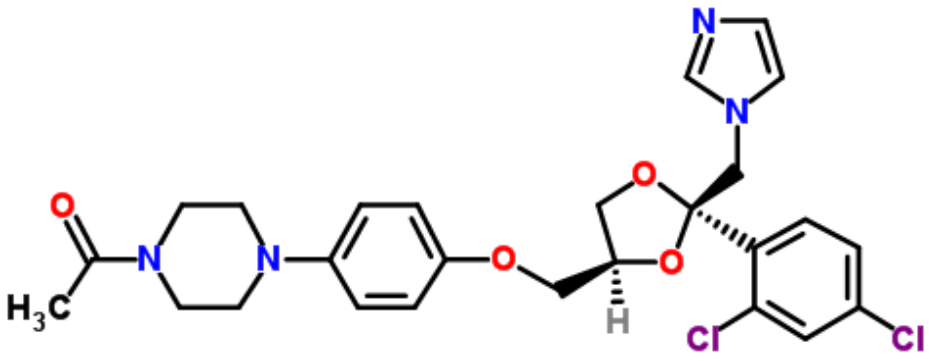

Figure 1. Ketoconazole chemical structure.

Source: Loch et al, 2011 [12]

In this perspective, this paper aims to describe aspects of the initial approach of ketoconazole in silico using ACD / ChemSketch®, Arguslab® and Avogrado® software as a necessary step for future studies of structural change, hoping to optimize the action of this drug.

3. Materials and Methods

This work was developed in software based on the Windows operating system, according to the following steps: (1) obtaining ketoconazole structure from ChemSpider repository (http://www.chemspider.com/); (2) visualization of three-dimensional structure by ChemSketch® software, with representation of the eletônica density map; (3) performing semiempirical calculation Arguslab® software (Austin Model 1-QM-AM1) to optimize the structure, determination of electrostatic potentials and

Mulliken atomic charges, generation of three- dimensional maps of the electrostatic potential, calculation of frontier orbitals and the minimum potential energy of the molecule [13].

4. Results and Discussion

Based on the energy minimization principle, the structure can be optimized so that the most stable conformations are identified. At this stage, the three-dimensional structure of the ketoconazole may be displayed (Figure 2), and the Cartesian coordinates of the constituent atoms in the lower power state may be determined (Table 1).

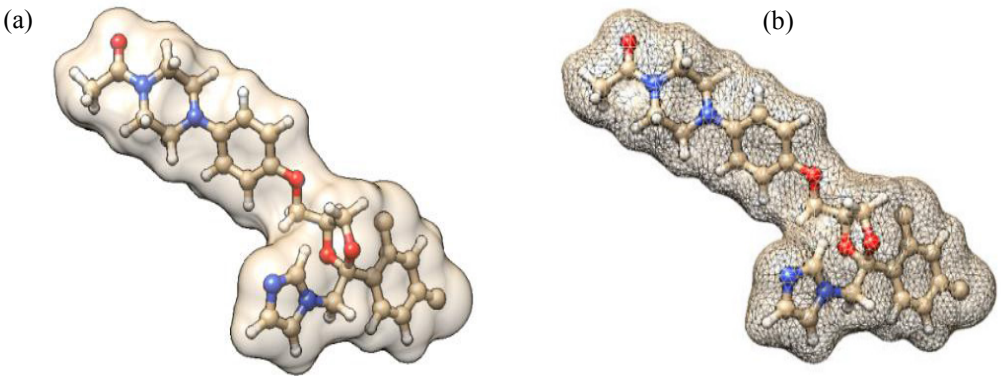

Figure 2. Three-dimensional visualization of ketoconazole molecule. (a) of the atomic volume visualization; (b) visualization of electron density.

Table 1. Atomic coordinates of ketoconazole molecule, obtained by *software* Arguslab®.

Atoms	x	y	z
1 C	-9.514143	7.139607	-0.198018
2 C	-8.017524	7.056339	-0.046951
3 O	-7.318610	7.941021	-0.620071
4 N	-7.413028	6.049729	0.662334
5 C	-8.223371	5.112066	1.458681
6 C	-7.516726	3.774719	1.585202
7 C	-6.184122	4.002254	2.203739
8 C	-5.370620	4.703879	1.175775
9 C	-5.954601	6.093992	0.887833
10 C	-5.513176	2.717640	2.413255
11 C	-4.532217	2.670098	3.415110
12 C	-3.745124	1.533576	3.592272
13 C	-3.950001	0.409429	2.794713

14 C	-4.922495	0.427615	1.798903
15 C	-5.696529	1.569484	1.594069
16 O	-3.114964	-0.717977	2.943316
17 C	-3.734173	-1.634014	3.884051
18 C	-2.648788	-2.618867	4.346411
19 C	-2.200056	-2.441041	5.801711
20 O	-2.307250	-3.740116	6.363510
21 C	-2.828031	-4.694762	5.449175
22 O	-3.074092	-3.967070	4.260318
23 C	-4.178293	-5.251745	5.964751
24 N	-5.251736	-4.250474	6.105200
25 C	-6.536559	-4.452715	5.710763
26 C	-7.345780	-3.554630	6.364357
27 N	-6.585870	-2.777329	7.181973
28 C	-5.315000	-3.233123	7.005005
29 C	-1.778060	-5.815436	5.191025
30 C	-0.442168	-5.526785	4.779237
31 C	0.484440	-6.560346	4.572441
32 C	0.116973	-7.894015	4.766268
33 C	-1.180325	-8.205960	5.161520
34 C	-2.111235	-7.187306	5.363626
35 C	-1.088396	8.966422	4.557373
36 C	0.050238	-4.165179	4.573477

Molecular electrostatic potential (molecular electrostatic potentials - MEP) are based on the properties calculated charge density directly from molecular wave function. The MEP measures the interaction of a positively charged point with nuclei and electrons of a molecule [14]. The interaction between the molecules is between regions of opposite electrostatic potential. Figure 3 shows the electrostatic potential map (in hartrees), in which areas of color, shade "in blue" suggest potential of positive values, ie electron deficiency; while the shades of color areas "red" indicates negative values of the electrostatic potential, that is, regions rich in electrons.

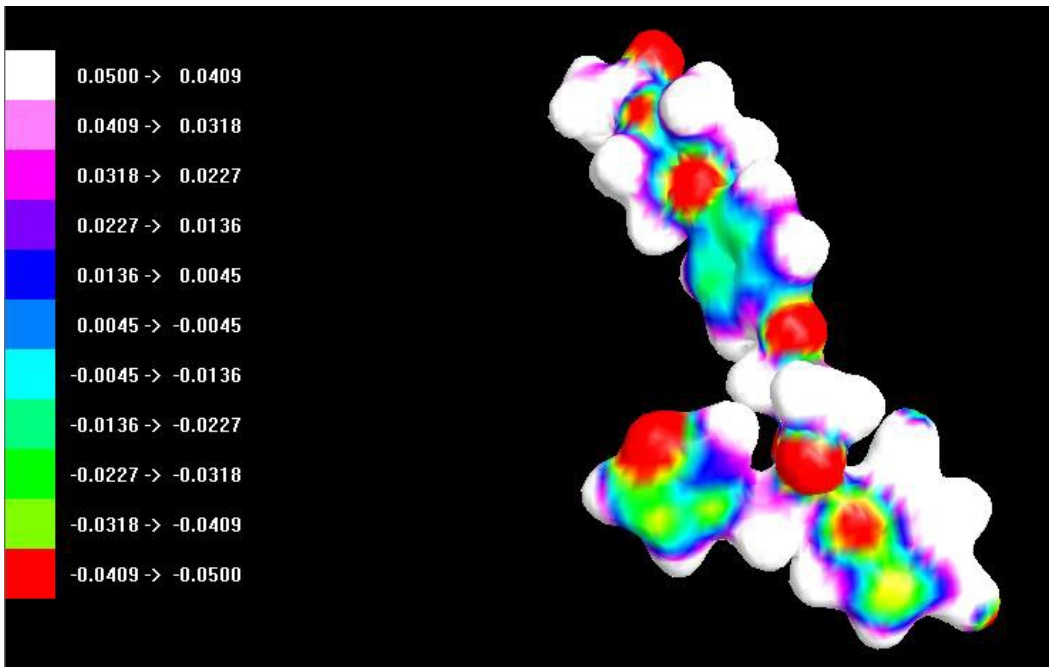

Figure 3. Electrostatic potential map of ketoconazole, built by Arguslab©.

By orbital border shall mean the occupied molecular orbital of higher energy (HOMO Highest Occupied Molecular Orbital-) and unoccupied molecular orbital of lower energy (LUMO Lowest Unoccupied Molecular Orbital-). These molecular orbitals are endowed with special importance, since much of the chemical reactions occur in a spatial orientation in which the overlap of the HOMO and LUMO of the respective reagents is maximum [15]. The interactions of atomic orbital and orbital border occur when these are closer to the orbital energy of the atomic orbital any pair of molecules. To ketoconazole, these orbitals can be viewed as shown in figure 4.

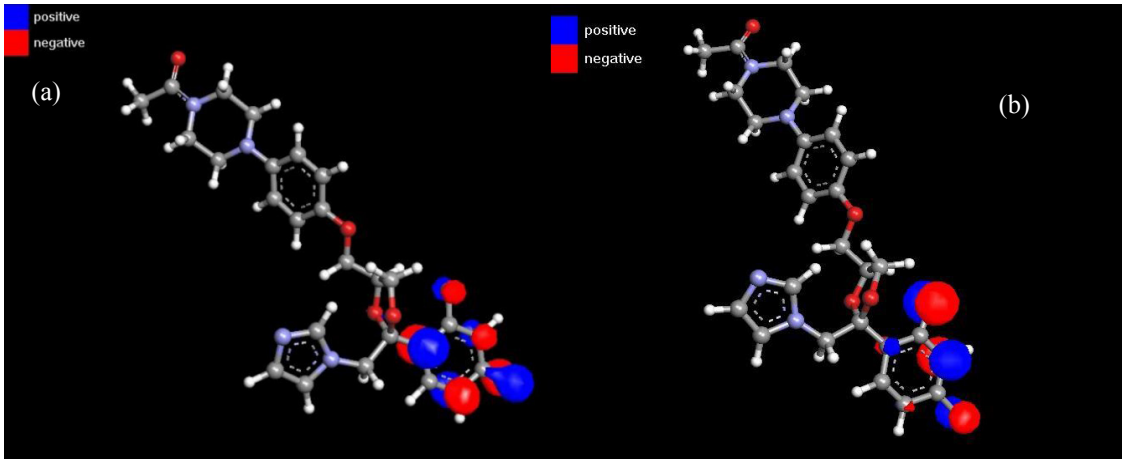

Figure 4. Molecular orbital border to ketoconazole, built by Arguslab©.; (a) HOMO Orbital; (b) LUMO Orbital.

The population analysis of Mulliken is a method used to dterminação of partial atomic charges, the results of which are heavily dependent on the basis set used. This is a partition scheme, based on the use of matrix density and coating, to distribute the electrons in a molecular entity of a fractional mode between its various parts (atoms, bonds, orbital) [16]. However, comparison of population analyzes for a range of molecules is useful for a quantitative description of the intramolecular interactions of chemical reactivity and structural regularities [15]. Analyzing the distribution Mulliken charges of ketoconazole to the molecule (Table 2) we can observe a very nucleophilic end in oxygen atom (O_3) of the acyl terminus, and this is the preferred place for reactions.

Minimization of energy is a mathematical procedure for finding stable conformations (minimum energy) of a molecule as determined by calculations of molecular mechanics or quantum-mechanical [15]. The molecular dynamics is capable of overcoming small obstacles and therefore is more efficient in finding a minimum deeper location than the simple minimization [17].

Table 2. Population analysis of Mulliken Mulliken atomic charges to the atoms of C, N and O of ketoconazole molecule.

Atomics charges of Muliken		
1	C	-0.3590
2	C	0.2889
3	O	-0.3741
4	N	-0.1126
5	C	-0.1910
6	C	-0.2150
7	N	-0.0346
8	C	-0.2171
9	C	-0.2006
10	C	-0.0717
11	C	-0.1623
12	C	-0.2046
13	C	0.0544
14	C	-0.1988
15	C	-0.2021
16	O	-0.2199
17	C	-0.0933
18	C	-0.0475
19	C	-0.1229
20	O	-0.2982
21	C	0.3459

22	O	-0.2815
23	C	-0.2736
24	N	0.2386
25	C	-0.3953
26	C	-0.2224
27	N	-0.1426
28	C	-0.3054
29	C	-0.3157
30	C	-0.3812
31	C	0.0423
32	C	-0.4432
33	C	-0.2634
34	C	-0.0545
35	C	0.2700
36	C	0.5197

The Hartree-Fock method makes simulations of experimental parameters such as solvation energy and dipole moment. This method presents an approach to the solution of the Schrödinger equation for the system of many bodies (atoms, molecules or solids), using the numerical and computational simulation. For the Hartree-Fock method, the basis set 3-21G, 6-31G, 6-311G and LANL2DZ are among the most widely used today in computational studies [18]. Using the Hartree-Fock method was obtained minimum potential energy state of ketoconazole in an amount equal to -139841.4989 kcal/mol.

5. Conclusion

By semi empirical approach, optimization of ketoconazole structure proves to be viable, and the conformation of lowest energy can be presented, with a minimum potential energy was equal to -139841.4989 kcal / mol. From the electrostatic potential map and load distribution of Mulliken, identified the oxygen atom of the acyl termination (O_3) as a preferred site for nucleophilic reactions. The now obtained descriptors will be key objects for future studies of relationships between three-dimensional structure of the drug and its biological activity.

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