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Computational study of amiloride – a WADA banned molecule

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Abstract

Amiloride (AMI) is a representative of diuretics. Among other purposes, it is utilized for high blood pressure or swelling induced by heart failure. While it is identified as one of the safest medicines available, it is also listed in the World Anti-doping Agency's list of substances banned in sport, because it is considered a masking agent. Since it has been abused in sports, methods have been developed to be able to detect it during doping controls. We have computationally investigated selected structural and reactive properties of the AMI molecule in this work, employing density functional theory calculations.

Keywords: Amiloride; DFT; reactivity; structure

1. INTRODUCTION

Amiloride (AMI) is a molecule that belongs to a group of diuretics. The compounds from this group are very important, as they increase the volume of urine that flows, and they also improve the excretion of water and electrolytes (Souza et al. 2021). In particular, AMI blocks the excretion of potassium and absorption of sodium ions (Chen et al. 2019; Souza et al. 2021). When it comes to the clinical aspects, it is essential to mention that AMI is used for hypertension, edema, heart failure, etc. (Chen et al. 2019; Pereira, da Silva, Marra, Muñoz, & Richter 2016; Souza et al. 2021). In sports activities, diuretics can be abused from two aspects. First, they can lead to significantly faster weight loss. This refer to judo, karate and other martial arts, where category classifications are made according to weight (Idris & Alnajjar 2013). Secondly, since they increase the urine flow, they can practically cover other forbidden substances consumed to achieve better sport results (Pereira et al. 2016). Due to AMI's narrow therapeutic range, it is necessary to apply a sensitive analytical approach to efficient detection. Very low amounts of AMI lead to intensive urine excretion, increasing its potential for abuse and making it difficult to detect (Peralta, Fernández, & Masi 2011). For these reasons, only several appropriate HPLC methods have been reported so far (Alliegro, Dyer, Cragoe, Glaser, & Alliegro 1992; Bechgaard 1989; Yip, Coates, & Thiessen 1984). On the other side, Chen et al. (2019) developed a technique based on UV-LED induced fluorescence for rapid determination of amiloride in tablet and human serum. Pereira et al. (2016) developed an amiloride detection system based on multiple pulse amperometric detections. Just

recently, Souza et al. (2021) have developed a method for amiloride detection in urine by square-wave cathodic stripping voltammetry. The potential of amiloride to be abused served as a motivation to study its reactive properties via density functional theory (DFT) calculations. This approach has proven to be very useful for understanding the intrinsic reactive properties of middle-sized organic molecules and how they might interact with other molecules or nanostructures, which is essential for developing novel methods for their detection.

2. COMPUTATIONAL DETAILS

DFT calculations were performed using the Jaguar (Bochevarov et al. 2013; Jacobson et al. 2017) program, as implemented in Schrödinger Materials Science Suite 2021-1 (SMSS). The main GUI of SMSS, Maestro program, was used to prepare input files, perform visualization, and post-process the results. A B3LYP (Becke 1993; Lee, Yang, & Parr 1988; Stephens, Devlin, Chabalowski, & Frisch 1994; Vosko, Wilk, & Nusair 1980) exchange-correlation functional was for DFT calculations, together with 6-31G(d,p) basis sets. Ground state geometry of the amiloride molecule was obtained by geometrical optimization. To confirm that the true ground state of amiloride was achieved, a vibrational analysis was performed, which yielded only positive frequencies. Information about the properties of amiloride was obtained after single-point energy calculations.

3. RESULTS AND DISCUSSION

3.1. Structural properties and frontier molecular orbitals

Firstly, the AMI molecule's geometrical optimization has been performed at the B3LYP/6-31G(d,p) level of theory, followed by the vibrational analysis. The vibrational analysis yielded only positive frequencies, indicating that the true ground state was identified. The optimized geometry of AMI molecule is presented in Figure 1, together with some typical bond lengths angles.

For validation of the obtained structure, the experimental and computational IR spectra were compared, and it was found that mentioned level of theory yielded excellent agreement with the experiment. As indicated in Figure 1, the highest bond length has been calculated for the C−Cl bond, equal to 1.78 Å. The lowest bond length was calculated in the case of the C=O bond, equal to 1.26 Å.

The reactive properties of the AMI molecule have been first addressed through the analysis of the frontier molecular orbitals. Namely, the locations of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbitals (LUMO) principally indicate

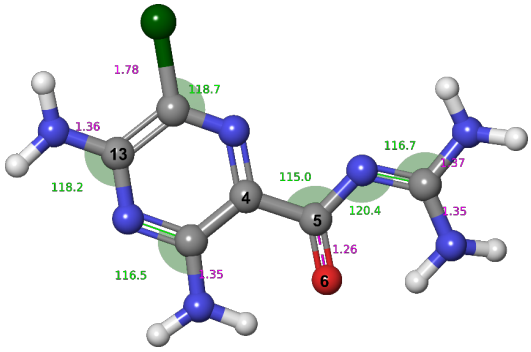

Figure 1. Optimized geometry of AMI molecule with typical bond lengths (in Åin pink color) and angles (green color) and numeration of the selected atoms (black color).

the molecular sites that are abundant or deficient in electrons, respectively. In this work, the HOMO and LUMO orbitals were mapped to the electron density surface, as presented in Figure 2.

As it can be seen in Figure 2, the projection of HOMO orbital to electron density surface shows that the extreme values are located above the carbon atom C4, located within the benzene ring, and nearby nitrogen atoms (one of them belonging to NH_2 group). The mentioned sites are rich with electrons and, therefore, prone to interact with other molecules' electron-deficient sites. On the other side, LUMO orbital's projection shows two sites with extreme values, carbon atoms C5 and C13, meaning that these two sites are electron-deficient and prone to interact with other molecules' electron-rich sites.

3.2. Charge distribution

To obtain a clearer picture of the AMI's reactivity, we have also performed the charge distribution analysis. Charge distribution principally determines the local reactivity of a molecule. In this work, charge distribution has been addressed through electrostatic potential charges, and these results are presented in Figure 3.

According to the results presented in Figure 3, the extreme values of atomic charges indicate that the most reactive atoms might be nitrogen atoms of the NH_2 groups and the oxygen atom. The nitrogen atoms belonging to the NH_2 groups are highly negatively charged. Of all four nitrogen atoms belonging to the NH_2 groups, the one closest to the chlorine atom has the lowest magnitude of charge. However, it still has a higher charge magnitude in comparison to the oxygen atom. The oxygen atom has a negative charge equal to −0.70e. The carbon atom C5 is highly positively charged. The chlorine atom is characterized by a low amount of negative charge (−0.13e). Finally, the highest positive charge has been calculated for the carbon atom connected with two NH_2 groups.

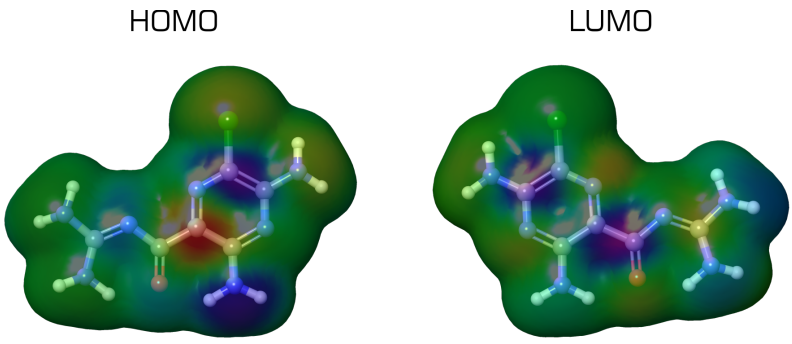

Figure 2. Projections of HOMO and LUMO to electron density surface (color legend: extreme values are presented by the red, blue and purple color).

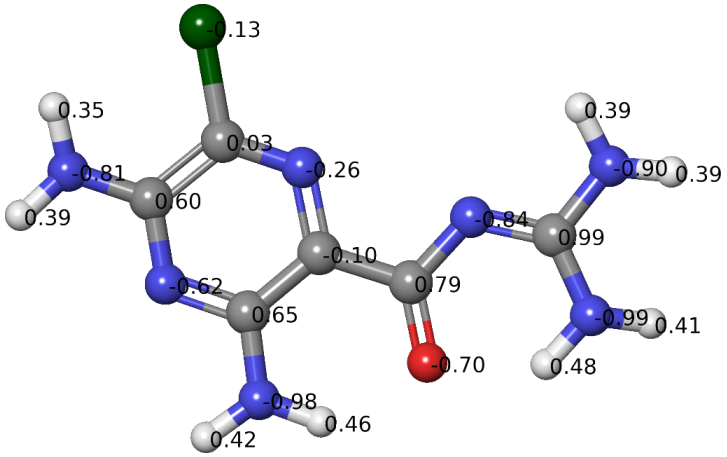

Figure 3. ESP atomic charges of AMI molecule.

4. CONCLUSIONS

DFT calculations have been performed on the AMI molecule, which is an important active component of medications used for blood pressure problems and heart failures. Since AMI can be abused in sports activities, it was essential to understand its reactive properties. Geometrical optimization was performed, and the typical bond lengths and angles have been summarized. Frontier molecular orbitals have been projected to the electron density surface to identify the electron-rich and electron-deficient molecular sites. Charge distribution was also performed to obtain insights into the local reactivity properties.

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