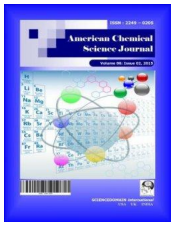

American Chemical Science Journal
8(3): 1-8, 2015, Article no.ACSj.18463
ISSN: 2249-0205

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In silico Studies on Nuclei Magnetic Resonance Spectroscopy, Molecular Orbitals and Geometry Optimization of Antidiabetic Drug, *N,N*-dimethylimidodicarbonimidic Diamide (Metformin)

I. E. Otuokere[1*] and C. O. Alisa[2]

[1]Department of Chemistry, Michael Okpara University of Agriculture, Nigeria.
[2]Department of Chemistry, Federal University of Technology, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author IEO designed the study, performed the in silico NMR analysis, molecular orbital studies, geometry optimization and wrote the first draft of the manuscript. Author COA managed the analyses of the study the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSj/2015/18463
Editor(s):
(1) Silvia Antonia Brandán, Inorganic Chemistry Institute, National University of Tucumán (UNT), Argentina.
Reviewers:
(1) Mohamed Ahmed Mohamed Nagy, Chemistry Department, Beni-Suef University, Egypt.
(2) S. Sheriff, Benghazi University, Benghazi, Libya.
Complete Peer review History: http://sciencedomain.org/review-history/9855

Original Research Article

Received 23rd April 2015
Accepted 3rd June 2015
Published 19th June 2015

ABSTRACT

N,N-dimethylimidodicarbonimidicdiamide (Metformin) is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. The *in silico* nuclear resonance magnetic spectroscopy of metformin was performed using ACD/I-lab. The chemical shifts of the methyl protons labelled 6 and 7 appeared as a singlet at 3.03 ppm while the chemical shifts of the amino protons labelled 3, 5, 10 and 11 appeared as a singlet at position 10.16, 6.66, 8.57 and 8.38 ppm respectively. The methyl carbons at 6 and 7 positions showed chemical shift at 38.77 ppm while the imine carbons showed chemical shifts at 158.20 and 159.10 ppm. All conformational analysis (geometry optimization) study was performed on a window based computer using Argus lab software. The metformin structure was generated by Argus lab, and minimization was performed with the semi-empirical Parametric Method 3 (PM3). The minimum

**Corresponding author: E-mail: ifeanyiotuokere@gmail.com;*

potential energy was calculated by geometry convergence function in Argus lab software. Surfaces were created to visualize highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO) and electrostatic potentials (ESP) mapped density. The minimum potential energy calculated using geometry convergence function in Argus lab software was found to be 41.537840 kcal/mol. This energy minimum is the most stable conformation of the molecule.

Keywords: Metformin; diabetes; geometry optimization; electrostatic potentials; energy.

1. INTRODUCTION

N,N-dimethylimidodicarbonimidicdiamide (Metformin) is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function [1,2,3]. Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period [4]. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications [5]. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma [6]. Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage to the eyes [5]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced [6]. There are three main types of diabetes mellitus: Type 1 DM results from the body's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown [5] Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly [5]. As the disease progresses a lack of insulin may also develop [6]. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise [5]. Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level [5]. Its use in gestational diabetes has been limited by safety concerns. It is also used in the treatment of polycystic ovary syndrome [7,8,9] and has been investigated for other diseases where insulin resistance may be an important factor. Metformin works by suppressing glucose production by the liver [10]. Recent studies indicate that metformin improves endothelial function in women with chest pain and normal coronary arteries [8]; is associated with reduced mortality in chronic heart failure [11,12]. Short term studies have demonstrated that metformin reduces total calorie intake, [13,14].

In view of biological importance of metformin, we report the computational study on nuclei magnetic resonance spectroscopy, molecular orbital's and geometry optimization of antidiabetic drug, *N,N*-dimethylimidodicarbonimidicdiamide (metformin). This work also describes the computer aided conformational analysis that is based on geometry optimization (active conformation) of metformin by ArgusLab software. Argus Lab is the electronic structure program that is based on the quantum mechanics, it predicts the potential energies, molecular structures; geometry optimization of structure, vibration frequencies of coordinates of atoms, bond length, bond angle and reactions pathway [15]. Conformational analysis of molecule is based on molecular mechanics, it is method for the calculation of molecular structures, conformational energies and other molecular properties using concept from classical mechanics. A molecule is considered as a collection of atoms held together by classical forces [16]. These forces are described by potential energy function of structural features like bond lengths, bond angles and torsion angles etc. The potential energy of the molecule was the sum of the following terms:

$$E = E_{str} + E_{ang} + E_{tor} + E_{vdw} + E_{opp} + E_{ele}$$

where all E's represent the energy values corresponding to the given types of interaction (kcal/mol). The subscripts str, ang, tor, vdw, oop and ele denote bond stretching, angle bonding, torsion deformation, van der waals interactions, out of plain bending and electronic interaction, respectively [16].

These terms are of importance for the accurate calculation of geometric properties of molecules. The set of energy functions and the corresponding parameters are called a force field [16]. The molecular mechanics method

calculates the energy as a function of the coordinates and energy minimization is an integral part of method. A molecular geometry is constructed by using computer graphics techniques and the atoms moved (without breaking bonds) using an energy minimization technique until the net forces on all atoms vanish and the total energy of the molecule reaches a minimum. The 3D (3 rotatable bonds) structure of molecule corresponding to this energy minimum is one of the stable conformations of molecule but not necessarily the most stable one [17].

2. EXPERIMENTAL DETAILS

The NMR predictions were performed using ACD/ I-Lab. All conformational analysis (geometry optimization) study was performed on a window based computer using Argus lab software [16]. The metformin structure is generated by Argus lab, and minimization was performed with the semi-empirical Parametric Method 3 (PM3) [17]. The minimum potential energy is calculated by using geometry convergence function in Argus lab software. Surfaces were created to visualize highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO) and electrostatic potentials (ESP) mapped density of metformin.

3. RESULTS AND DISCUSSION

The structure of metformin with atom label is shown Fig. 1. The ^{1}HNMR, ^{13}CNMR, optimized structure, electrostatic potential (ESP) mapped density, highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) of metformin are presented in Figs. 2 – 7 respectively. Atomic coordinates, bond lengths, bond angles, dihedral angles, improper torsions and energy components of metfromin are presented in Tables 1–6 respectively.

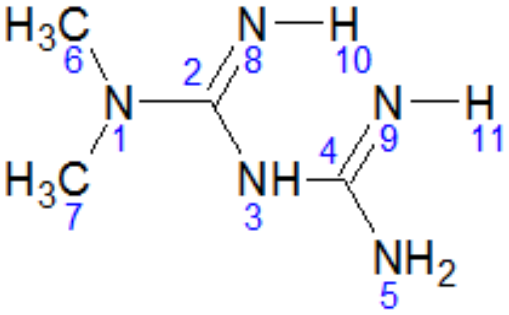
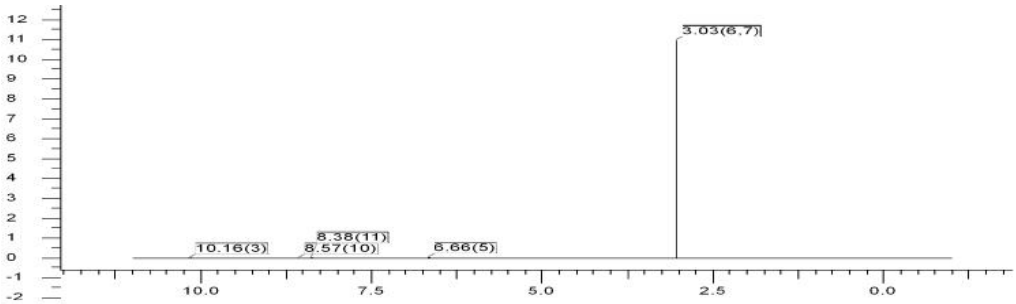


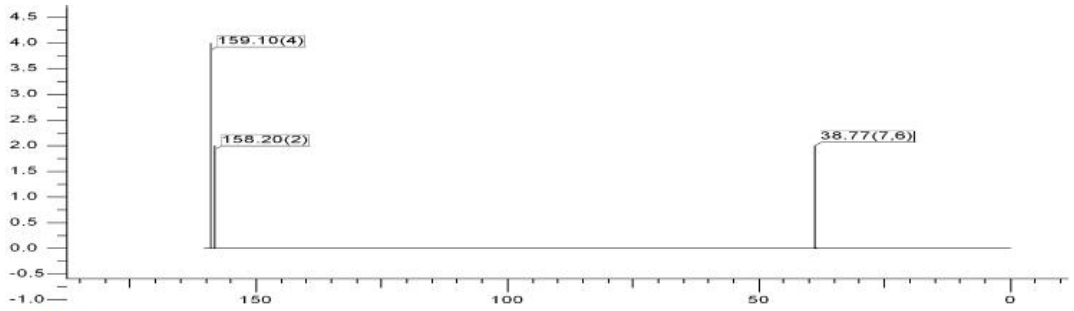
Fig. 1. Structure of N,N-dimethylimidodicarbonimidic diamide (metformin)



Shifts:

Atom	Shifts	Errors
6, 7	3.03	0.37
5	6.66	1.39
11	8.38	3.25
10	8.57	3.25
3	10.16	2.96

Fig. 2. ^{1}HNMR spectrum of metformin (ACD lab computed)



Shifts:

Atom	Shifts	Errors
6, 7	38.77	1.23
2	158.20	4.47
4	159.10	4.47

Fig. 3. ^{13}CNMR spectrum of metformin (ACD lab computed)

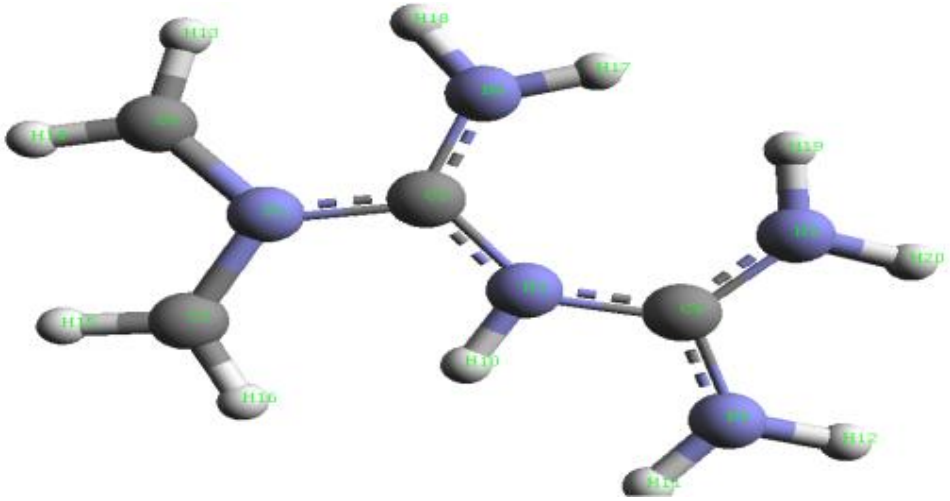


Fig. 4. Optimized structure of metformin

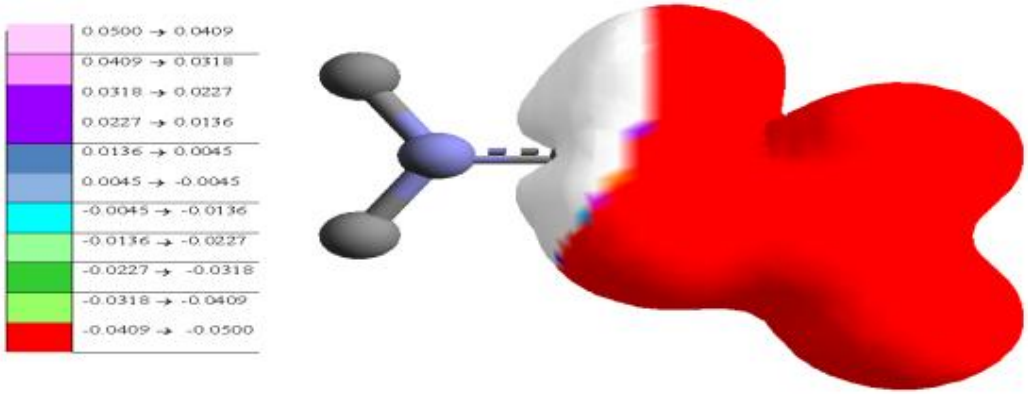


Fig. 5. Electrostatic potential (ESP) mapped density of metformin

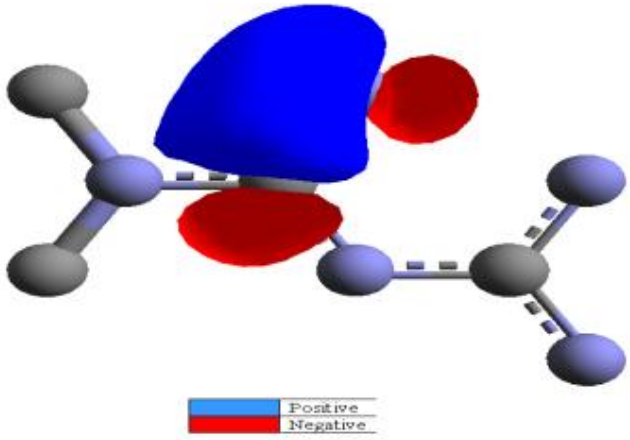


Fig. 6. Highest occupied molecular orbital (HOMO)of metformin

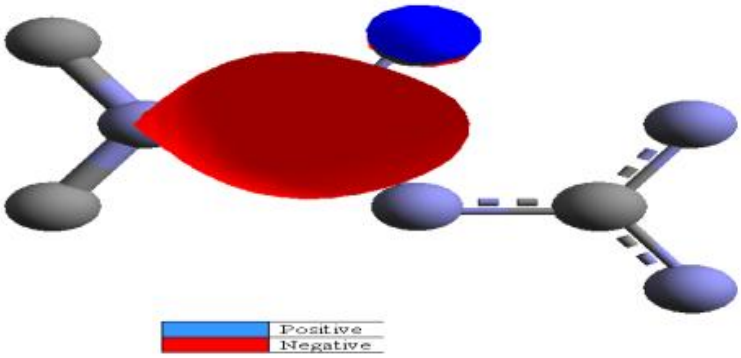


Fig. 7. Lowest unoccupied molecular orbital (LUMO) of metformin

The chemical shifts of the methyl protons labelled 6 and 7 appeared as a singlet at 3.03 ppm while the chemical shifts of the amino protons at position 3, 5, 10 and 11 appeared at 10.16, 6.66, 8.57 and 8.38 ppm respectively. ACD/HNMR Predictor used parameters gleaned from its internal database with over 1,384,000 experimental chemical shifts and 449,000 experimental coupling constants for 165,000 structures [18]. To quantitate intramolecular interactions in metformin structure and to predict its chemical shifts, HNMR Predictor module used a unique algorithm based on intramolecular interaction parameters for over 3,000 structural fragments, which have been carefully derived from experimental data using ACD/Labs' specially developed HNMR data processing approaches [18]. The methyl carbons at 6 and 7 positions showed chemical shift at 38.77 ppm while the imine carbons showed chemical shifts at 158.20 and 159.10 ppm. ACD/CNMR Predictor quickly and precisely calculated ^{13}C NMR spectra for almost any drawn organic structure. The calculated chemical shifts are always provided with the confidence limits, so that the calculated values could be trusted. In this case, the CNMR Predictor module calculated chemical shifts with an accuracy of 3 ppm or better. For this purpose, the module used a large internal data base with over 2,017,000 experimental chemical shifts and 81,400 coupling constants for 165,000 structures [18]. To quantitate intramolecular interactions in metformin structure and to predict its chemical shifts, CNMR Predictor module used a unique algorithm based on the intramolecular interaction parameters for over 3,000 structural fragments, which have been carefully derived from the experimental data using the specially developed CNMR data processing approaches. It calculated the spin-spin interaction of carbon nuclei with the magnetic nuclei of other elements, proceeding from the natural ratio of magnetic isotopes [18]. Computational advances have generated many tools which are widely used to construct models, minimization and representations of molecular

structure [13-15]. Computer assisted molecular design (CAMD) have been successfully used to detect three dimensional arrangements of atoms in metformin. Molecular mechanics was a mathematical formalism, which attempted to reproduce atomic coordinates (Table 1), bond length (Table 2), bond angles (Table 3), dihedral angles (Table 4), improper torsions (Table 5) molecular geometries, bond energies (Table 6).

Electrostatic Potential of metformin (Fig. 5): The electrostatic potential is a physical property of a molecule related to how a molecule is first "seen" or "felt" by another approaching species. A distribution of electric charge creates an electric potential in the surrounding space [19]. A positive electric potential means that a positive charge will be repelled in that region of space. A negative electric potential means that a positive charge will be attracted. A portion of a molecule that has a negative electrostatic potential will be susceptible to electrophilic attack – the more negative the better. Quick Plot ESP mapped density generated an electrostatic potential map on the total electron density contour of the molecule [19]. The electron density surface depicts locations around the molecule where the electron probability density is equal. This gives an idea of the size of the molecule and its susceptibility to electrophilic attack. The surface color reflects the magnitude and polarity of the electrostatic potential. The red end of the spectrum shows regions of highest stability for a positive test charge, magenta/ blue show the regions of least stability for a positive test charge [19]. These images show that the imino-end of the molecule is electron rich relative to the amino end. The Highest occupied molecular orbital, HOMO (Fig. 6) and lowest unoccupied molecular orbital, LUMO (Fig. 7) orbitals are commonly known as *Frontier Orbitals* and were found to be extremely useful in explaining chemical reactivity [19].

Table 1. Atomic coordinates of metformin

Atom no	x	y	z
1 N	25.814800	-7.258900	0.000000
2 C	27.144800	-7.258900	0.000000
3 N	27.809800	-8.410700	0.000000
4 C	29.139800	-8.410700	0.000000
5 N	29.804800	-9.562600	0.000000
6 C	25.149800	-6.107100	0.000000
7 C	25.149800	-8.410700	0.000000
8 N	27.809800	-6.107100	0.000000
9 N	29.804800	-7.258900	0.000000

Table 2. Bonds length of metformin

1 2 (N) (C)	1.346235	662.009133
1 6 (N) (C)	1.422764	560.825517
1 7 (N) (C)	1.422764	560.825517
2 3 (C) (N)	1.346235	662.009133
2 8 (C) (N)	1.346235	662.009133
3 4 (N) (C)	1.346235	662.009133
4 5 (C) (N)	1.346235	662.009133
4 9 (C) (N)	1.346235	662.009133

Table 3. Bond angles of metformin

Atom numbers	Bond angles	Alternate bond angles
2 1 6 (C) (N) (C)	120.000000	219.857183
2 1 7 (C) (N) (C)	120.000000	219.857183
1 2 3 (N) (C) (N)	120.000000	423.785655
1 2 8 (N) (C) (N)	120.000000	423.785655
6 1 7 (C) (N) (C)	120.000000	202.792364
3 2 8 (N) (C) (N)	120.000000	423.785655
2 3 4 (C) (N) (C)	120.000000	239.379973
3 4 5 (N) (C) (N)	120.000000	423.785655
3 4 9 (N (C) (N)	120.000000	423.785655
5 4 9 (N) (C) (N)	120.000000	423.785655

Table 4. Dihedral angles of metformin

Atom numbers		Dihedral angles
3 2 1 6	(N)-(C)-(N)-(C)	6.737110 2
8 2 1 6	(N)-(C)-(N)-(C)	6.737110 2
3 2 1 7	(N)-(C)-(N)-(C)	6.737110 2
8 2 1 7	(N)-(C)-(N)-(C)	6.737110 2
1 2 3 4	(N)-(C)-(N)-(C)	13.474221 2
4 3 2 8	(C)-(N)-(C)-(N)	13.474221 2
2 3 4 5	(C)-(N)-(C)-(N)	13.474221 2
2 3 4 9	(C)-(N)-(C)-(N)	13.474221 2

Electrophilic attacks were shown to correlate very well with atomic sites having high density of the HOMO orbital, whereas nucleophilic attack correlated very well with atomic sites having high density of the LUMO orbital. The positive and negative phases of the orbital are represented by the two colors, the blue regions represent an

increase in electron density and the red regions a decrease in electron density [19]. The minimum potential energy calculated using geometry convergence function in Argus lab software was found to be 41.537840 kcal/mol. This energy minimum is the stable conformation of the molecule.

Table 5. Improper torsions of metformin

Atom numbers	Improper torsions
6 7 1 2 (C)-(C)-(N)-(C)	2.000000
3 8 2 1 (N)-(N)-(C)-(N)	2.000000
5 9 4 3 (N)-(N)-(C)-(N)	2.000000

Table 6. Energy components (au) of metformin

Energy type	Energy (au)
Molecular mechanics bond length	0.017052
Molecular mechanics bond angle	0.000000
Molecular mechanics dihedral angle	0.000000
Molecular mechanics improper torsions	0.000000
Molecular mechanics vander waals	0.049142
Molecular mechanics coulomb	0.000000
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Total	0.066194 au
Total	41.537840 kcal/mol

4. CONCLUSION

The nuclear resonance magnetic spectroscopy of metformin was performed using ACD/I-lab. The in silico NMR chemical shifts can serve as a background knowledge for experimental NMR analysis. The minimum potential energy calculated using geometry convergence function in Argus lab software was found to be 41.537840 kcal/mol. This energy minimum is the most stable conformation of the molecule.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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