**Theoretical Characterization of the Hydrogen Bonding Interaction Sites of Mycolactone C Using the ONIOM Method**

**Kadjo François KASSI 1, Sopi Thomas AFFI 1,3, N’guessan Yao Silvère DIKI 2,Mamadou Guy-Richard KONÉ 1,3\*, Georges Stéphane DEMBÉLÉ 1,3, Nahossé ZIAO 1,3**

*1Laboratoire de Thermodynamique et de Physico-Chimie du Milieu, UFR SFA, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, République de Côte-d’Ivoire.*

*2Laboratoire de Constitution et Réaction de la Matière, UFR SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d’Ivoire.*

*3Groupe Ivoirien de Recherches en Modélisation des Maladies (GIR2M)*

*\*Corresponding author:* [*guyrichardkone@gmail.com*](mailto:guyrichardkone@gmail.com)*,*

**ABSTRACT**

In this work, the ONIOM method, recognized for its effectiveness on large molecules, was used to determine the geometric, energetic and spectroscopic parameters of hydrogen bond interactions of mycolactone C. Mycolactone C; one of the most virulent forms of toxin, found in Africa and Australia. It has eight (08) oxygen heteroatoms which are all hybridized sp2 and sp3. Using quantum chemistry methods, at the ONIOM level (B3LYP/6-311+G (d, p): AM1), we have been able to determine the preferential binding sites of the hydrogen bonds in the eight mycolactone C oxygen heteroatoms studied. Analysis of the results revealed that the heteroatom O5sp2 is the most suitable site for creating a strong hydrogen bond based on the geometric, energetic (free enthalpy of complexation) and spectroscopic (vibration frequency shifts) parameters. The identification of this O5sp2 heteroatom is a significant step forward in the development of a methodology for annihilating the infection and the destructive effects of this toxin.

**Keywords:** *Mycobacterium ulcerans*; Mycolactone; ONIOM; Hydrogen bond, Quantum chemistry,Buruli ulcer.

1. **INTRODUCTION**

Buruli ulcer is a disease caused by *Mycobacterium ulcerans*, a bacteria belonging to the family of agents responsible for tuberculosis and leprosy[1]. Long neglected, this disease which is widely encountered in humid tropical and subtropical countries, moreover, it has experienced an upsurge in West Africa since 1980[2]. This situation has led the World Health Organization (WHO) to classify it as an emerging disease and to recognize it as a public health and development problem [3]. *Mycobacterium ulcerans* secretes a toxin called mycolactone into skin tissue, which causes extremely deep tissue damage due to its cytotoxic and immunosuppressive properties. Based on previous studies; six (06) different naturally occurring molecular structures of mycolactones have been isolated [4]. Mycolactones are the toxins produced by *Mycobacterium ulcerans*; they are the main responsible agent of Buruli ulcer. Buruli ulcer is a disease spread around the world with a higher infection rate in Africa. The cytotoxic and immunosuppressive properties of mycolactones are the basis of the destruction of the skin and subcutaneous tissue. The current treatments are mainly antibiotic therapies and reconstructive surgery in cases of severe infection. The mode of action of the toxin is so far unknown in medical circles. Sadly, the therapeutic arsenal against Buruli ulcer remains very limited despite the progress observed in the quality of the management and the medical care [5]. Antibiotic therapy and reconstructive surgery, with its high cost and numerous relapses (16 to 28%) in cases of serious infection remain the actual standard treatments [6]. The lack of knowledge of the mode of action of mycolactone in the literature and in the medical world hinders a better management of Buruli ulcer. Most of the research work in this area ~~is~~ are focused on the ecology of Mycobacterium ulcerans, the dermal part of the disease manifestation, synthesis and the structural characterization of mycolactones. The relationship between the mycolactones, produced by Mycobacterium ulcerans and the proteins which are indexed to be responsible for the development of Buruli ulcer is related to the conformation and the interactions between these two types of molecules. Research into the biological activities of mycolactones is essential for a good understanding of their mode of action and also for establishing a new therapeutic protocol as part of a preventive or even curative treatment for Buruli ulcer.

The hydrogen bond (H bond) is of utmost importance for the cohesion of matter[7]. Indeed, it is one of the most important inter-molecular interactions involved in supramolecular chemistry and in particular protein-ligand interactions[8] and crystal engineering [9], [10]. In general, the molecules are polyfunctional, as they contain several heteroatoms capable of receiving H bonds. Therefore, it appears important to be able to characterize the preferential site (s) of H-binding. There is, however, very limited work devoted to the study of competition between different sites within a single molecular structure. Etter and Reutzel [11] have been among the first research teams to attempt to classify the acceptor power of H binding (ALH) of different organic functionalities. More recently, some work were focused on the competition between nitrogen and oxygen atoms[12]–[16] or between amino and nitrile nitrogen atoms[17] in different structural units. This work, which is part of the Buruli ulcer control program, is interested in mycolactone C. It aims to determine, by the methods of Quantum Chemistry, some physicochemical properties of mycolactone C, in particular the geometric, energetic and spectroscopic parameters of hydrogen bonding established on heteroatoms; in order to indicate the probable site of intermolecular interaction of the toxin. The calculations are carried out at the ONIOM level (B3LYP / 6-311 + G (d, p): AM1). Based on these findings, it will be possible to propose a theoretical model of annihilation of the destructive effects of mycolactone C.

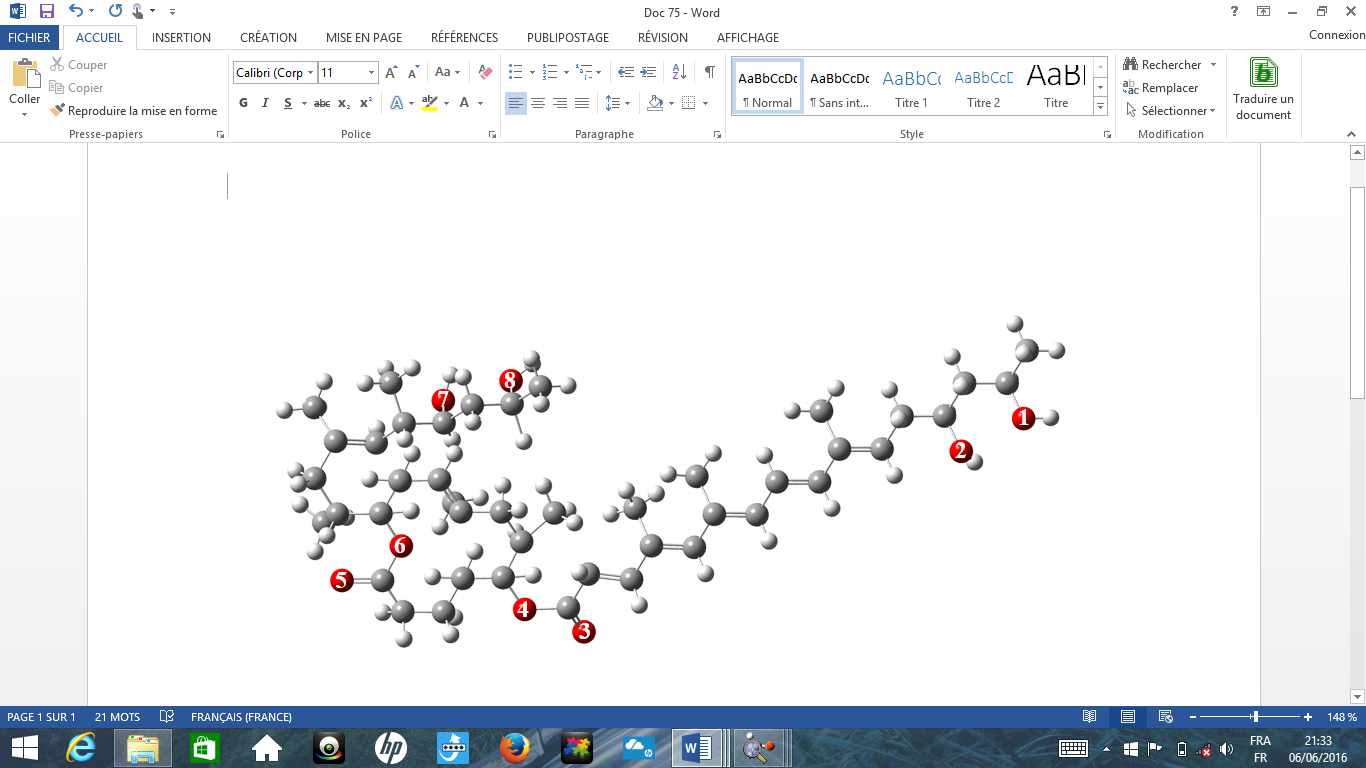
1. **MATERIALS AND METHODS**

**2.1 Method and Level of Calculation**

Mycolactone C heteroatom numbering is done from the longest side chain, from right to left through the lactone nucleus. Thus, the numbers 1, 2, 3, 4, 5, 6, 7 and 8 denote heteroatoms and correspond respectively to the names of the various complexes formed. Respectively; **Figures 1 and 2** show the 2D and 3D molecular structures of mycolactone C.

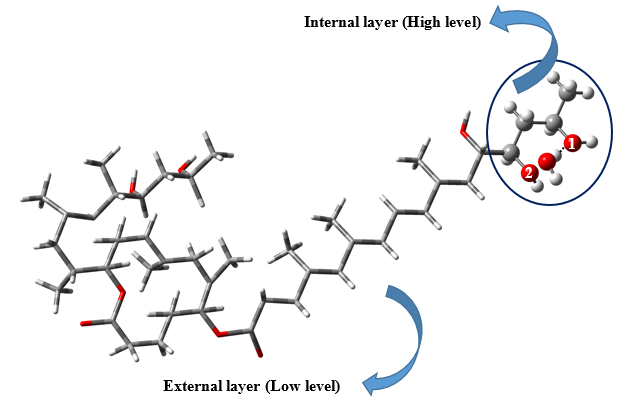
****

**Figure 1:** 2D structure of mycolactone C



**Figure 2:** Molecular structure of mycolactone C visualized with Gauss-View 03 software.

The ONIOM method, developed by Morokuma *et al.* [18] is used to determine some physicochemical properties of mycolactone C and its various hydrogen bond complexes. The ONIOM method has often been used with success on large molecules [18]. It consists in dividing the studied system into several layers, each of the layers being processed at a different level of calculation. It therefore makes it possible to describe precisely the part of the system that is of particular interest for the study; so called the internal layer or even the model system. However, it describes the rest of the system less precisely, called the external layer or environment. **Figure 3** presents the description of the cutting of a mycolactone complex with the ONIOM method.



**Figure 3:** Model for cutting a mycolactone complex with the ONIOM 2 method.

The ONIOM method makes it possible to obtain the energy of the real system at a high computational level. The total energy of the real system, determined by extrapolation, is obtained from three independent calculations. The theory of this method is based on the fact that the energy difference between a high level calculation () and another low level () for the real system is equal to the energy difference between a high level calculation (**)** and a low level calculation (**)** for the model system. The high and low level calculation methods being the same in both cases, allow the following relationship to be established:

Knowledge of three of these terms gives access to the fourth without the need to calculate it. We can therefore have the energy of the real system at a high level of computation from three less expensive computations. The total interaction energy obtained from the ONIOM 2 calculation [19]called extrapolated energy, is defined below :

All the calculations were carried out at the ONIOM level (B3LYP / 6-311 + G (d, p): AM1) with the Gaussian 03 software[20] . The choice of Functional Density (DFT / B3LYP) makes it possible to obtain results that are relatively less heav1y but deemed to be effective. The presence of diffuse and polarization functions is important for taking into account the free doublets of heteroatoms.

**2.2 Geometry Optimization**

All the complexes of mycolactone C were built on each of its heteroatoms, in particular the oxygen atoms, with a water molecule as hydrogen bond donor (DLH) (**Figure 4**).

. 

**Figure 4:** Geometric parameters d, D, α, β describing an H bond [21].

Before optimization, for all complexes, the angle of linearity α was set at 180 ° and the angle of direction β was set at 109.5 ° for sp3 hybridized oxygen and 120 ° for oxygen hybridized sp2, according to the Gillespie method or VSEPR method (Valence Schell Electronic Pair Repulsion) **figure 5**. The distance between an oxygen atom of mycolactone C and a hydrogen atom of the probe (H2O) is fixed at 2Å. These values correspond respectively to the angles and the minimum approach distance of the hydrogen bond[22].



**Figure 5**: Definition of the angles of linearity and direction describing the interactions by the hydrogen bond on the oxygen atoms sp2 and sp3

**2.3 Energy Parameters**

Knowledge of the variations in energy contributions to internal energy at 0 K and 298 K between the product and the reactants contributes to the energetic characterization of a chemical reaction. For a given energy parameter X, its variation is determined through the following relation:

The optimization of the geometries and the calculation of the frequencies of the free and interacting molecules make it possible to determine the variation of the internal energy at 0 K and at 298 K relative to the relationship studied. The variation of the internal energy at 298 K, is a sum of the various electronic contributions, of the translation, ~~of~~ rotation, ~~of~~ vibration and of the internal energy at 0K through the relation:

Calculations of the frequencies of the optimized molecules allow access to all the components of internal energy. In addition, E0K commonly called ZPVE (Zero Point Vibrational Energy) corresponds to the contribution of the vibration of molecules at 0 K. It reflects the vibrational energy at the zero point induced by the normal modes of vibration of frequency νi of the N nuclei at 0 K. It is defined by relation (5):

With 3N-6 defined as the number of normal modes of vibration of a non-linear molecule (3N-5 for a linear molecule); k the Boltzmann constant; h the Planck constant and R gas constant). This energy is corresponds to the internal energy at 0 K (). During a temperature rise from 0 K to T (K), it is necessary to take into account the additional energy term corresponding to the population of vibrational energy levels during the change of the temperature. This term is defined by:

As for the contributions of rotation and translation, they are taken from the ideal gas approximation by this equation:

The variation of the internal energy ΔE 298K at 298 K can then be written:

From this relation, is deduced the enthalpy of reaction at 298 K. It corresponds to the variation of the internal energy corrected by the term Δ (PV), and ΔnRT (Δn being the variation of the number of gaseous moles during the reaction).

The entropic contributions of , translation, rotation and vibration of a given species at 298 K are grouped together in the total entropy term S. The reaction entropy is determined according to the relation.

However; the Gibbs energy at 298 K, related to the reaction is obtained by the relation below:

**2.4. Spectroscopic Parameters**

Spectroscopic descriptors are generally considered to be spectroscopic scales of H bond basicity. Hydrogen bond causes elongation of the X-H bond formed between the donor atom X and the hydrogen atom H. This results in a decrease of a few hundred cm-1 of the wave number of the stretching vibration, as well as a significant increase in intensity. However, their implementation relates to vibrational spectroscopy where we measure the frequency shift of the elongation vibration of the H-bond donor during the formation of the complex. When is negative, it means that the frequency is shifting in the red for the complex and when ∆ν (X-H) is positive, we use the term "displacement in the blue"[23]. The shifts were calculated using asymmetric vibrators of the "free" water (H2O) molecule in the complex. The theoretical frequency shifts with water were respectively determined, for the complexes formed with the oxygen atoms sp2 and sp3 by the relations below (12) and (13)

At the level of ONIOM theory (B3LYP / 6-311 + G (d, p): AM1) the vibration frequency is 3923.88 cm-1

**3. RESULTS AND DISCUSSION**

**3.1 Geometric Parameters Analysis**

The various complexes formed were optimized in order to determine their geometry of the equilibrium. **Figure 5** gives an illustration of the geometries of the complexes formed with the hybridized oxygen atoms sp2 or sp3, before and after the optimization.

|  |
| --- |
|  |
| Initial (left) and optimized (right) structures of the H binding complex formed from mycolactone C on the  **O2sp3** heteroatom |
|  |
| Initial (left) and optimized (right) structures of the H binding complex formed from mycolactone C on the **O3sp2** heteroatom |
|  |
| Initial (left) and optimized (right) structures of the H binding complex formed from mycolactone C on the **O5sp2** heteroatom |

**Figure 6**: Example of initial and optimized structures of some H binding complexes formed from mycolactone C

Figure 6 was obtained from Gauss View implemented in Gaussian 03 software. The part presented in tube is the outer layer, optimized at the low level precisely in AM1. The part presented in "ball and stick" is the model system. It is optimized at the B3LYP / 6-311 + G (d, p) level.

The visualization of the different optimized geometries of the H binding complexes (**Figure 6**) made it possible to observe that the optimizations of the geometries of the complexes were successful for all the heteroatoms of mycolactone C. This phenomenon can be explained by the lack of mesomerism around the heteroatoms. This lack of mesomerism around these heteroatoms reinforces their basicity.

**Table 1:** Geometric parameters of mycolactone C hydrogen bond complexes calculated at the ONIOM level (B3LYP / 6-311 + G (d, p): AM1).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **MYCOLACTONE C** | | |
|  |  |  |  |  |
| **O1sp3** |  | 140.60 | 114.80 | 2.52 |
| **O2sp3** |  | 167.00 | 110.30 | 1.90 |
| **O3sp2** |  | 170.40 | 125.25 | 1.98 |
| **O4sp3** |  | 135.90 | 137.80 | 2.59 |
| **O5sp2** |  | 168.90 | 118.80 | **1.87** |
| **O6sp3** |  | 131.20 | 156.51 | 2.84 |
| **O7sp3** |  | 126.90 | 126.29 | 2.28 |
| **O8sp3** |  | 170.50 | 117.90 | 1.99 |

**Table 1** shows that, the heteroatoms **O2sp3** (α = 167.00 °; β = 110.30 °), **O3sp2** (α = 170.40 °; β = 125.25 °), **O5sp2** (α = 168.90 °, β = 118.80 °) and **O8sp3** (α = 170.50 °; β = 117.90 °), exhibiting high values of linearity angles of and direction are likely to create strong hydrogen bonds. On the other hand, the heteroatoms **O1sp3** (α = 140.60 °; β = 114.80 °), **O4sp3** (α = 135.90 °; β = 137.80 °), **O6sp3** (α = 131.20 °; β = 156.51 °) and **O7sp3** (α = 126.90 °; β = 126.29 °) are heteroatoms that do not make strong hydrogen bonds.

Regarding the lengths of the H bonds (distance d), the practice is to consider a contact as an H bond, if the distance d is less than the sum of the Van der Waals radii, taking respectively 1.52 Å, for oxygen atoms and 1.0 Å for hydrogen atoms; or d≤2.52 Å for contact with oxygen[24]. It is also known that the H bond is as strong as its distance is short. The heteroatoms **O1sp3** (2.52 Å), **O2sp3** (1.90 Å), **O3sp2** (1.98 Å), **O5sp2** (1.87 Å), **O7sp3** (2.28 Å) and **O8sp3** (1.99 Å) having some hydrogen bonds length less than the sum of the Van der Waals radii, are likely to be some sites of strong hydrogen bonding. Only the **O4sp3** (2.59 Å) and **O6sp3** (2.84 Å) heteroatoms, having distance values d greater than the sum of the Van der Waals radii, are not probable sites for the creation of a strong hydrogen bond. Therefore, the **O5sp2** heteroatom (**1.87 Å**), with the smallest d distance value, creates a very good affinity with the hydrogen of the probe.

The analysis of the geometric parameters clearly indicates that the heteroatom **O5sp2** is the most probable site of creation of a strong hydrogen bond because of the lower values of distance d and the good values of the angles of linearity and the direction observed.

**3.2 Analysis of Energy Parameters**

The energy parameters determined are the variations of the thermodynamic quantities linked to the complexation by H bond of the sites considered.

**Table 2:** Energy parameters of H-bond complexation of different heteroatoms.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **MYCOLACTONE C** | | | | |
|  |  |  |  |
| **O1sp3** | -15.57 | -0.12 | 20.04 |
| **O2sp3** | -29.94 | -0.06 | -09.40 |
| **O3sp2** | -35.96 | -0.07 | -14.76 |
| **O4sp3** | -02.77 | -0.09 | 25.29 |
| **O5sp2** | **-44.28** | -0.09 | **-16.77** |
| **O6sp3** | 19.32 | -0.14 | 22.51 |
| **O7sp3** | 15.75 | -0.12 | 21.75 |
| **O8sp3** | -30.53 | -0.06 | -10.08 |

The exothermicity of each of the complexation reactions of the water molecule on all the different sites of mycolactone C is reflected in the negative values of the complexation enthalpies. Only the heteroatoms **O1sp3** (-15.57 kJ⁄mol), **O2sp3** (-29.94 kJ⁄mol), **O3sp2** (-35.96 kJ⁄mol), **O4sp3** (-2.77 kJ⁄mol), **O5sp2** (- 44.28 kJ⁄mol) and **O8sp3** (-30.53 kJ⁄mol) show negative values of enthalpy of complexation. This shows that the complexation reactions with these sites are exothermic. On the other hand, on heteroatoms **O6sp3** (19.32 kJ⁄mol) and **O7sp3** (15.75 kJ⁄mol), the complexation reactions are not exothermic according to the positive values of enthalpy of complexation. Regarding the free enthalpy of complexation, only the heteroatoms **O2sp3** (-9.40 kJ⁄mol), **O3sp2** (-14.76 kJ⁄mol) **O5sp2** (-16.77 kJ⁄mol) and **O8sp3** (-10.08 kJ⁄mol) show negative values. These values show that the reactions evolve in the direction of the spontaneous formation of the complexes at a temperature of 298.15 K and at a pressure of 1 atm. It should be noted that the lowest value of free enthalpy of complexation is observed with the O5sp2 heteroatom (-16.77 kJ⁄mol). However, in contrast; the heteroatoms **O1sp3** (20.04 kJ⁄mol), **O4sp3** (25.29 kJ⁄mol), **O6sp3** (22.51 kJ⁄mol) and **O7sp3** (21.75 kJ⁄mol), present some positive free enthalpies values from complexation. This indicates that there is no possibility ~~of~~ for a spontaneous reaction between the water molecule and the different sites.

After examining the values of the energy complexation parameters, it comes out that the **O5sp2** heteroatom that has the lowest values of the free enthalpy of complexation (-16.77 kJ⁄mol), gives the most stable complex.

**3.3 Analysis of Spectroscopic Parameters**

**Table 3:** Variations of the theoretical frequencies ∆ν (O-H) (cm-1) calculated at the level of theory ONIOM (B3LYP / 6-311 + G (d, p): AM1) on mycolactone C.

|  |  |  |
| --- | --- | --- |
|  | | **Mycolactone C** |
| **O1sp3** |  | 212.54 |
| **O2sp3** |  | 212.64 |
| **O3sp2** |  | 222.47 |
| **O4sp3** |  | 191.05 |
| **O5sp2** |  | **223.10** |
| **O6sp3** |  | 222.02 |
| **O7sp3** |  | 218.25 |
| **O8sp3** |  | 198.93 |

Table 3 shows that the vibration frequency calculated at the ONIOM level (B3LYP / 6-311 + G (d, p): AM1) is 3923.88 cm-1. The strongest value of the variations in vibration frequencies is observed on the **O5sp2** heteroatom (223.10 cm-1). This behavior reflects the attraction effect exerted by the acceptor atom (O5sp2) of the monomer on the hydrogen of the probe. Moreover, it has been established that the greater the variation in vibration frequencies, the stronger the H bond. This observation shows that the **O5sp2** heteroatom is the preferred site for a stronger H binding.

The analysis of the values of the geometric, energetic and spectroscopic parameters clearly shows that the **O5sp2** heteroatom is the best site of interaction by hydrogen bonding of this toxin.

**4. CONCLUSION**

The values of the intermolecular descriptors were made possible thanks to the ONIOM method. The first part of this study was about the study of all the geometric and energetic descriptors of mycolactone C at the ONIOM level (B3LYP / 6-311 + G (d, p): AM1). We have been able to highlight through our findings that oxygen atom **O5sp2** is the probable center of interaction of mycolactone C. The analysis of the spectroscopic parameters of the hydrogen bond complexes, such as the shifts of the vibration frequency with water in the second part of our study, has also confirmed that the **O5sp2** heteroatom is the site that establishes the stronger attraction effect with the hydrogen of the probe. Based on these results, the **O5sp2** heteroatom was identified as the suitable and likely site of intermolecular interaction. These researches are real baselines and promising for researchers for overcoming the destructive of mycolactones. For future investigations; it will be interesting to undertake some researches which will mainly be focused on further understanding of the interaction of mycolactone C through the oxygen atom **O5sp2**; for a better treatment of Buruli ulcer. Moreover, it is now possible to improve these results on the theoretical characterization of the physico-chemical properties of mycolactone C by using other levels of calculation with more extensive bases. Thus, we are planning in a nearby future;

* to extend the study of hydrogen bonding interactions to other mycolactones in order to better understand their inhibitory effects as well as their biological activities;
* to characterize the protonation interactions of different mycolactones complexes in order to predict their stays in the fats of the subcutaneous tissues.

**REFERENCES**

[1] A. A. Kobina, « Burden and Historical Trend of Buruli Ulcer Prevalence in Selected Communities along the Offin River of Ghana », *PLoS Negl Trop Dis*, vol. 10, no 14, p. 1371‑1382, 2016.

[2] P. Abgueguen, E. Pichard, et J. Audry, « Buruli ulcer or Mycobacterium ulcerans infection », *Medecine et Maladies Infectieuses*, vol. 40, no 12, p. 60‑69, 2010.

[3] M. Beissner, « Loop-Mediated Isothermal Amplification for Laboratory Confirmation of Buruli Ulcer Disease-Towards a Point-of-Care Test. », *PLoS Negl Trop Dis*, vol. 9, no 111, p. 4219‑4230, 2015.

[4] M. Beissner, « Implementation of a national reference laboratory for Buruli ulcer disease in Togo », *PLoS Negl Trop Dis*, vol. 7, no 11, p. 1371‑1380, 2013.

[5] M. A. Kabiru, « Short Report: Buruli Ulcer Control in a Highly Endemic District in Ghana: Role of Community-Based Surveillance Volunteers », *Am. J. Trop. Med. Hyg*, vol. 92, no 11, p. 115‑117, 2015.

[6] E. Torrado, « Evidence for an intramacrophage growth phase of Mycobacterium ulcerans », *Infect Immun*, vol. 75, no 12, p. 977‑987, 2007.

[7] P. R. Rablen, J. W. Lockman, et W. L. Jorgensen, « Ab Initio Study of Hydrogen-Bonded complexes of Small Organics Molecules with Water », *J. Phys. Chem A*, vol. 102, no 21, p. 3782‑3797, 1998.

[8] G. A. Jeffrey et W. Saenger, *hydrogen bonding in biological structures*. Berlin, 1991.

[9] G. R. Desiraju, « Designer Crystal: intermolecular interactions, network structures and supramolecular synthons », *chemical Communications*, p. 1475‑1482, 1997.

[10] B. Moulton et M. J. Zaworotko, « From Molecules to crystal Engineering : Supramolecular Isomerim and Polymorphism in Network Solids », *Chem. Rev*, vol. 101, no 6, p. 1629‑1658, 2001.

[11] M. C. Etter et S. M. Reutzel, « Hydrogen bond directed cocrystallization and molecular recognition properties of acyclic imides », *J. Am. Chem. Soc*, vol. 113, p. 2586‑2598, 1991.

[12] H. J. Bohm, S. Brode, U. Hesse, et G. Klebe, « Oxygen and nitrogen in competitive situations: which is the hydrogen-bond acceptor? », *Chem. Eur. J*, vol. 2, p. 1509‑1513, 1996.

[13] M. G.-R. Koné, S. T. Affi, N. Ziao, K. Bamba, et E. F. Assanvo, « Hydrogen bonding sites in Benzimidazolyl-chalcones molecules: An ab initio and DFT investigation », *Journal of Chemical and Pharmaceutical Research*, vol. 7, no 12, p. 805‑812, 2015.

[14] S. T. Affi, N. Ziao, et K. Bamba, « Détermination, par des méthodes ab initio et dft, des sites et énergies de protonation d’une série de molécules d’imidazopyridinyl-chalcones substituées », *European Scientific Journal*, vol. 11, no 33, p. 138‑148, 2015.

[15] I. Nobeli, « Hydrogen bonding properties of oxygen and nitrogen acceptors in aromatic heterocycles », *J. Comput. Chem*, vol. 18, p. 2060‑2074, 1997.

[16] N. Ziao, C. Laurence, et J. Y. Le Questel, « Amino nitrogen and carbonyl oxygen in competitive situations », *Cryst. Eng. Comm*, vol. 4, no 59, p. 326‑335, 2002.

[17] N. Ziao, « Amino and Cyano N atoms in competitive situations », *Acta Cryst*, vol. 57, p. 850‑858, 2001.

[18] S. Maeda, « Intrinsic reaction coordinate : calculation, bifurcation, and automated search », *International Journal of Quantum Chemistry*, vol. 115, no 15, p. 258‑269, 2015.

[19] L. W. Chung, « The ONIOM method : its foundation and applications to metalloenzymes and photobiology », *Computational Molecular Science*, vol. 2, no 12, p. 327‑350, 2012.

[20] M. J. Frisch *et al.*, *Gaussian 09*. Wallingford CT: Gaussian, Inc, 2009.

[21] W. Piao, « Development of azo‐based fluorescent probes to detect different levels of hypoxia », *Angewandte Chemie International Edition*, vol. 59, no 149, p. 13028‑13032, 2013.

[22] A. E. Reed, « Natural population analysis », *The Journal of Chemical Physics*, vol. 83, no 12, p. 735‑782, 1985.

[23] S. Tothadi, « Designing ternary cocrystals with hydrogen bonds and halogen bonds », *Chemical Communications*, vol. 49, no 171, p. 7791‑7793, 2013.

[24] J. GRATON, « Basicité des Amines et de Nicotines: Liaison Hydrogène et Protonation », ÉCOLE DOCTORALE DE CHIMIE - BIOLOGIE, Nantes.