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# Design of Novel Amoxicillin Prodrugs by Computational Methods

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# Design of Novel Amoxicillin Prodrugs by Computational Methods

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**Design of Novel Amoxicillin Prodrugs by Computational Methods**

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## Dedication

This thesis is dedicated to my parents who sacrificed a lot for me to be what I am now. I am very grateful for their love, support and prayers.

*Thanks a lot.*

## Declaration

I certify that the thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signed: ……Waa'd……..

Waa'd Mohammad Saleh Hurani

Date: 26/7/2017

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**Abstract**

It is believed that the bitter taste of amoxicillin, an antibiotic drug is due to its amino group. Hence, it is expected that blocking the amino group with a suitable linker could inhibit the interaction between amoxicillin and its bitter taste receptor/s and hence masking the bitter taste of the drug. Part of this thesis will shed light on the use of molecular orbital methods such as Using Density Functional Theory, and *ab initio* for the design of novel prodrugs. This novel prodrug approach implies prodrug design based on enzyme models that were utilized for mimicking enzyme catalysis. The computational approach exploited for the prodrug design involves molecular orbital and molecular mechanics calculations and correlations between experimental and calculated values of intramolecular processes that were experimentally studied to assign the factors affecting the reaction rates in certain processes for better understanding on how enzymes might exert their extraordinary catalysis.

Using Density Functional Theory and *ab initio* calculations, intramolecular proton transfer reaction in Kirby's enzyme models on an intramolecular acid catalyzed hydrolysis of N-alkylmaleamic (4-amino-4-oxo-2-butenoic) acids (Kirby's N-alkylmaleamic acids) revealed that the reaction rate is largely dependent on the distance between the two reactive centers ($r_{GM}$), the attack angle (α) and the strain energy. The rate of the reaction is linearly correlated with $r_{GM}^2$, sin (180°-α), and the strain energy. The calculations demonstrated that the amide bond cleavage is due to intramolecular nucleophilic catalysis by the adjacent carboxylic acid group and the rate-limiting step is determined based on the nature of the amine leaving group.

Hence, the calculations provide a conceivable basis for designing amoxicillin prodrugs for masking bitter taste of the corresponding parent drugs, which have the potential to release the drug in a controlled release fashion. In addition, a linear correlation of the calculated values has drawn credible basis for designing amoxicillin prodrugs that are bitterless, and stable in neutral aqueous solutions, also the intra-conversion rates of the amoxicillin prodrugs to amoxicillin can be programmed according to the nature of the prodrug linker. Based on the calculated B3LYP/6-31 G (d,p) rates, high rates were predicted for **ProD4** and **ProD5** compared with **ProD1**, **ProD2** and **ProD3**.

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# List of Abbreviations

| Abbreviation | Definition |
|---|---|
| DFT | Density Functional Theory |
| GP | Gas Phase |
| GM | Global Minimum |
| H2O | Water |
| HF | Hartree -Fock method |
| HLB | Hydrophilic Lipophilic Balance |
| H | Enthalpy |
| MM | Molecular Mechanic |
| P | Product |
| ProD | Prodrug |
| QM | Quantum Mechanic |
| rGM | Distance in global minimum |
| S | Entropy |
| TS | Transition State |
| T | Temperature |
| UFF | Universal Force Field |
| ΔG‡ | Activation Energy |
| α | Attack angel |

# Chapter One

# Introduction

# Chapter one

# Introduction

## 1.1 Background

The acceptable taste of the active ingredient of a drug is a significant obstacle in developing a patient friendly dosage form. It is a key issue for doctors and pharmacists administering the drugs especially for the pediatric and geriatric populations. Organoleptic properties, such as taste, are an important factor when selecting a certain drug from the generic products available on the market that have the same active ingredient [1].

The past sixty years have seen an increasing number of chemists, biochemists, biologists and other researchers in various fields who use computational methods for better understanding of the mechanism of organic reactions and biochemical processes for predicting active biological molecules and for calculating the molecular properties of a new drug candidate [2].

Computational chemistry is the application of chemical, mathematical and computing skills to assist in solving chemical problems. The term computational chemistry is mainly used when developing a mathematical method so it can be automated for implementation on a computer. It uses computers to generate information such as properties of molecules or simulated experimental results. Recently, computational chemistry was utilized to improve solubility, stability and bioavailability of drugs and also to mask their bitter taste [3].

Most of the computational chemistry scientists' use based on Schrodinger equation; this is because the Schrodinger equation models the atoms and molecules with mathematics and it gives information about:

- Geometry optimizations.
- Frequency calculations.
- Protein calculations, i.e. docking.
- Electron and charge distributions.
- Potential energy surfaces (PES).
- Rate constants for chemical reactions (kinetics).
- Thermodynamic calculations- heat of reactions, energy of activation.
- Electronic structure.

- Transition structures.

## 1.2 The use of computational chemistry in drug design

Computational chemistry is used in many different ways. One particularly important way is in understanding physical or chemical problems more completely. There are some properties of a molecule that can be obtained computationally and more easily than by experiment. It also gives information about molecular bonding that cannot be obtained from any experimental method. A second use of computational chemistry is to model a molecular system before synthesizing that molecule in the laboratory, and helps chemists to make predictions before running the actual experiments so that they can be better prepared for making observations [4]. It uses the theoretical chemistry results, links them into efficient computer programs, and calculates the structures physical and chemical properties of molecules [3]. This is very useful information because synthesizing a single compound could require months of labor and raw materials, and generate toxic waste. The design and synthesis of prodrugs were based on intramolecular processes utilizing molecular orbital methods and correlations between experimental and calculated values, thus many experimental chemists are now using computational design to gain additional understanding of the compounds being examined in the laboratory [1].
Currently, there are two ways to approach chemistry problems: computational quantum chemistry and non-computational quantum chemistry.

- Computational quantum chemistry is mainly concerned with the numerical computation of molecular electronic structures by *ab initio* and semi-empirical techniques.
- Non-computational quantum chemistry concerned with the analytical expressions for the properties of molecules and their reactions.

Today the quantum mechanics (QM) such as *ab initio*, semi-empirical and density functional theory (DFT), and molecular mechanics (MM) are mainly being used and broadly accepted as precise tool for providing structure – energy calculations for drugs and prodrugs [3]. Definitions of these terms are useful in understanding the use of computational techniques for chemistry.

### 1.2.1 Quantum mechanics (QM)

QM is the correct mathematical description of the behavior of electrons and thus of chemistry. In reactions, quantum chemistry studies the ground state of individual atoms and molecules, the excited states, and the transition states that occur during chemical reactions.

It involves heavy interplay of experimental and theoretical methods:

- In theory, QM can predict the property of an individual atom or molecule in an exact manner.
- In practice, the QM equations have only been solved exactly for one electron systems. A myriad collection of methods has been developed for approximating the solution for multiple electron systems.

The quantum mechanics includes: (1) *Ab-initio* (2) Semi-empirical (3) DFT methods.

#### 1.2.1.1 *Ab initio* Methods

The term *ab initio* is Latin for ``from the beginning". *Ab initio* quantum chemistry has become very important tool in the study of atoms and molecules and in modeling complex systems such as those arising in biology [1]. *Ab initio* methods typically are adequate only for small systems that are based entirely on theory from first principles. The *ab initio* molecular orbital methods (QM) are based mainly on the Schrodinger equation, given the positions of a collection of atomic nuclei, and the total number of electrons in the system, energy, electron density, and calculates the electronic properties by means of a well-defined, automated approximation. The advantage of *ab initio* electronic structure methods that they can be made to converge to the exact solution, when all approximations are sufficiently small in magnitude. The disadvantage of *ab initio* methods is their enormous computational cost; they take a significant amount of computer memory, time, and disk space [5].

#### 1.2.1.2 Semi-empirical Methods

The semi-empirical quantum chemistry method is based on the Hartree–Fock formalism. Within this framework, certain pieces of information are approximated or completely excluded. Usually, the core electrons are not included in the calculation and only a minimal basis set is used. In order to correct for the errors introduced by omitting part of the calculation, the method is parameterized. Parameters help to estimate the omitted values and they are obtained by comparing the results to *ab initio* calculations or experimental data. Often, these parameters replace some of the integrals that are excluded [6].

The advantage of the semi empirical calculations is that they are much faster than *ab initio* calculations and their disadvantage is that the results can be unpredictable and fewer properties can be predicted reliably. If the molecule being computed is similar to molecules in the database used to parameterize the method, and then the results may be very good. If the molecule being computed is significantly different from anything in the parameterization set, the answers may be very poor [1].

Semi-empirical methods are MINDO, MNDO, MINDO/3, AM1, PM3 and SAM1. Calculations of molecules containing up to100 atoms can be done using semi-empirical methods [6].

#### 1.2.1.3 Density functional theory (DFT)

Density functional theory (DFT) is a computational quantum mechanical modeling method used in chemistry to investigate the electronic structure (principally the ground state) of many-body systems, in particular atoms, molecules. DFT has become very popular in recent years, this is justified based on the pragmatic observation that it is less computationally intensive than other methods with similar accuracy [7].

Using DFT method the energy of a molecule can be obtained from the electron density using functions that is functions of another function. This theory raised up with a theorem by Hoe burg and Kohn [8].

The DFT method is suitable for calculating energies and structures for medium-sized systems (30-60 atoms) of biological, medicinal, and pharmaceutical interest.

The use of DFT method become larger, however some difficulties when describing intermolecular interactions still encountered, especially transition states, van deer Waals forces (dispersion); charge transfer excitations;, global potential energy surfaces, and incomplete treatment of dispersion can adversely affect the DFT degree of accuracy in the treatment of systems which are govern by dispersion [7].

### 1.2.2 Molecular Mechanics

Molecular mechanics is a mathematical approach used for the computation of energy, structures, dipole moment, and other physical properties. It is mainly used in calculating many diverse biological and chemical systems such as large crystal structures, proteins, and relatively large solvated systems. However, this method is limited by the determination of parameters such as the large number of unique torsion angles [9].

Molecular mechanics simulations, for example, use a single classical expression for the energy of a compound. The database of compounds used for parameterization, i.e., the resulting set of parameters and functions is called the force field. A force field parameterized against a specific class of molecules, for example proteins, would be expected to only have relevance when describing other molecules of the same class. These methods can be applied to large biological molecules and proteins, and allow studies of the approach and docking of potential drug molecules. The treatment of large, condensed-phase systems (e.g., proteins in aqueous solution) entirely by *ab initio* methods is very expensive computationally and if a molecule is so big that a semi-empirical treatment cannot be used efficiently, it is still possible to model its behavior avoiding quantum mechanics totally by using molecular mechanics. However, it is often the case that a relatively small region of the system can be modeled at the *ab initio* quantum chemical level, whereas the rest of the system can be treated more approximately by means of molecular mechanics (MM). The technologies for coupling quantum chemical methods to these alternative types of models, mixed quantum mechanics (QM)/MM) have become an important component of the theoretical arsenal, enabling realistic modeling of the most complex molecular structures [1].

## 1.3 Amoxicillin

Amoxicillin is (2S,5R,6R)-6-{(E)-[(2R)-2-Amino-1-hydroxy-2-(4-hydroxyphenyl)ethylidene]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, As shown in (Fig. 1) with a molecular weight of 365.4 g/mol. and molecular formula of $C_{16}H_{19}N_3O_5S$.

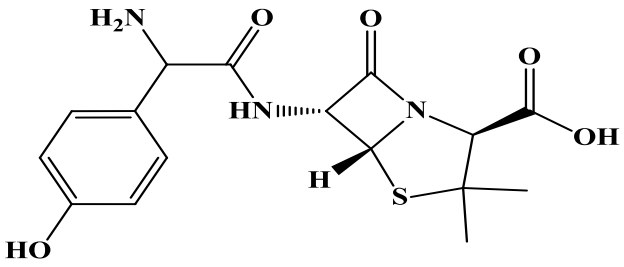



Figure 1: Chemical structure of amoxicillin.

It is the drug of choice within its class because it is well absorbed following oral administration. Amoxicillin is the mostly common antibiotic prescribed for children. It has high absorption after oral administration, which is not altered and affected by the presence of food. Amoxicillin dose reaches $C_{max}$ about 2 hours after administration and is quickly distributed, and eliminated by excretion in urine (about 60%- 75%). The antibacterial effect of amoxicillin is prolonged by the presence of a benzyl ring in the side chain. Because amoxicillin is susceptible to degradation by β-lactamases-producing bacteria, which are resistant to a broad spectrum of β-lactam antibiotics, such as penicillin, for this reason, it is often combined with clavulanic acid, a β-lactamase inhibitor. This increases effectiveness by reducing its susceptibility to β-lactamase resistance. Amoxicillin has two ionizable groups in the physiological range (the amino group in α-position to the amide carbonyl group and the carboxyl group). Amoxicillin has a good pharmacokinetics profile with bioavailability of 95% if taken orally, its half-life is 61.3 minutes and it is excreted by the renal and less than 30 % bio-transformed in the liver [9-12].

## 1.4 Research problem

Amoxicillin have extremely unpleasant and bitter taste, which is difficult to mask. This creates a serious problem in pediatric and geriatric patients. Even though, the strategies that were used for masking bitter taste by the use of sweeteners and flavors may cause a serious problem in diabetic pediatrics and geriatrics patients. Amoxicillin bitter taste sensation is the result of the hydrogen bonding between the free amino groups of the drug with the active site of the bitter taste receptors on the tongue. Designing a prodrug promoiety with a suitable linker could reduce or eliminate their bitterness by altering the ability of the drug to interact with their bitter taste receptors; this could be obtained by an appropriate modification of the structure and the size of the bitter compound. We believe that blocking the amine group in amoxicillin by making the proposed prodrugs may result in inhibition of the interaction between the amine group of the antibacterial agent and the bitter taste receptors and hence masking its bitterness.

The major problems in the administration of amoxicillin antibacterial drug:

1) Their bitter taste, which leads to lack of patient compliance and might create a serious challenge to the pharmacist in pediatric and geriatric formulations.
2) Synthesis of prodrugs, which have the potential to release the parental drugs in a controlled manner, have a good chance to overcome the frequent dosing problem.

## 1.5 Thesis Objectives

### 1.5.1 General objectives

The main goal of this research was to design prodrugs lacking any bitter or unpleasant sensation and having the potential to release their parent drugs in a controlled manner, using a variety of different molecular orbital and molecular mechanics methods and correlations between experimental and calculated reactions rates, five novel antibacterial prodrugs of amoxicillin were designed.

1.5.2 Specific objectives

►Calculations of Kirby's enzyme model mechanism for the design of amoxicillin prodrugs, which should have the following properties:

- Lack of bitter taste.
- Have a modified hydrophilic-lipophilic balance (HLB) value.
- To furnish upon cleavage a safe and non-toxic by-products.
- To release the parental drug in controlled manner.

## 1.6 Research questions

- Would the DFT calculations be good methods for the design of amoxicillin prodrugs that have the potential to mask the bitterness of the active drugs and be cleaved in physiological environments to furnish the active drugs in a programmable manner and a non-toxic moiety?
- Would the DFT and *ab initio* methods are capable of producing reaction rates similar to that obtained by Kirby?

# Chapter Two

# Literature review

# Chapter two

## 2.1 Literature Review

Bitter or unpleasant taste is a major problem in the food and medicine industries; amoxicillin has an extremely unpleasant and bitter taste which is difficult to mask. Taste become altered as a function of the aging process, which explains why most children find certain flavors to be too strong when adults do not, and they have larger number of taste buds than adults, which are responsible for sensitivity toward taste. Different approaches are commonly utilized to overcome bitter and unpleasant taste of drugs. This includes reduction of drug solubility in saliva, where a balance between bioavailability and reduced solubility must be achieved. But these approaches could not overcome the problem of bitterness [13].

Bitter molecules bind to G-protein coupled receptor-type T2R on the apical membrane of the taste receptor cells located in the taste buds on the tongue to give bitter, sweet or other taste sensations. Altering the ability of the drug to interact with taste receptors could reduce or eliminate their bitterness. This could be achieved by a suitable modification of the size and the structure of a bitter compound [14,15].

Amoxicillin generally suffers bitter taste sensation. Through many research approaches, the prodrug approach has been widely used for the development of drugs delivery to their site of action by physicochemical modulation that affect absorption or by targeting to specific enzymes or membrane transporters. Among these, various approaches that are used in order to minimize the undesirable properties of the drug while retaining the desirable therapeutic activity, the prodrug approach. This approach can be useful in the optimization of the clinical application of most of the drugs [16,17].

The new novel chemical approach involves the design of prodrugs for masking bitter taste based on intramolecular processes using DFT and *ab initio* methods and correlations of experimental and calculated reactions rates. In this approach, no enzyme is needed to catalyze the interconversion of a prodrug to its corresponding drug. The rate of drug release is controlled by the nature of the linker bound to the bitter drug. The role of the linker is to block the free amine

group in the corresponding parental drug and to convert it into the more stable amide group; the former is believed to be responsible for the bitterness of the drug [18].

Using computational chemistry numerous enzyme models were established for determining the reaction mechanism in order to investigate the driving force affecting the reaction rate for design an efficient prodrug capable of releasing the parental drug in a controlled manner. In which the promoiety can be covalently linked to a parent drug to result in chemically and not enzymatically cleavage upon exposure to physiological environment [19].

## 2.2 Enzyme catalysis

Enzymes are essential for the interconversion of many prodrugs to their active parent drugs, there are about 40,000 different enzymes in human cells, each controlling a different chemical reaction. They increase the rate of reactions by a factor of $10^{10}$ to $10^{18}$ fold than the non-enzymatic ones; among the most important enzymes involved in the bioconversion of prodrugs are those for amides, such as, trypsin, chymotrypsin, elastase,carboxy-peptidase, and amino-peptidase and for esters, such as paraoxnase, carboxyl-esterase, acetylcholine-esterase and choline-esterase. Most of these enzymes are hydrolytic enzymes, however, non-hydrolytic ones, including all cytochrome P450 enzymes, are also capable of catalyzing the bioconversion of ester and amide-based prodrugs. The significant rate of acceleration obtained by enzymes is brought about by the binding of the substrate within the confines of the enzyme pocket called the active site. The binding energy of the resulting enzyme-substrate complex is the main driving force and the major contributor to catalysis. It is believed that in all enzymatic reactions, binding energy is used to overcome prominent physical and thermodynamic factors that create barriers for the reaction ($\Delta G$) [20].

Enzyme catalysis maybe the most unpredictable approach, because there are many intrinsic and extrinsic factors can affect the bioconversion mechanism. For example there are genetic polymorphism of many enzymes can affect the bioconversion of the prodrug mechanism, drug interactions or age related physiological change can induce adverse clinical pharmacodynamic and pharmacokinetic effects, also there are a wide variation in expression and function of most of

enzymes activating the conversion of prodrugs, which could lead to serious challenges in the preclinical optimization phase [21].

Generally, enzymatic catalysis is required for most of prodrugs that are in clinical use in order to be converted into the parent drug. This is mainly for those prodrugs designed to furnish the parent drug in the blood stream following gastro-intestinal absorption. These prodrugs are mainly ester derivatives of drugs containing carboxyl or hydroxyl groups, which are converted into the parent drug by esterase-catalyzed hydrolysis, also non-enzymatic pathways for some prodrugs that can regenerate the parent drug, have emerged as an alternative approach by which prodrug activation is not influenced by inter- and intra-individual variability that affects the enzymatic activity [20].

### 2.1.1 Intramolecular vs. Intermolecular reactions

In some reactions, two pathways present themselves: one via intramolecular reaction and the other via intermolecular reaction.

- Intramolecular forces: is any force that holds the atoms together making up a molecule or compound, they contain all types of chemical bond and are stronger than intermolecular forces[22]. The nature of the reaction (intermolecular or Intramolecular) is mainly dependent on the distance between the two reacting centers. *Ab initio* calculations done by Karaman and Menger demonstrated that when the distance between the two reacting centers is about 2.4Å, the reaction is intramolecular, whereas when the distance is 3Å and more, the reaction prefers the intermolecular process [23].
- Intermolecular forces: The forces holding molecules together and play important roles in determining the properties of substances [24].

In general, intermolecular forces can be divided into several categories:

1. Strong ionic attraction, the more ionic, the higher the lattice energy.
2. Intermediate dipole-dipole forces; substances whose molecules have dipole moment, have higher melting point or boiling point than those of similar molecular mass, but their molecules have no dipole moment.

3. Hydrogen bonding; certain substances such as $H_2O$, HF, $NH_3$ form hydrogen bonds, and the formation of which affects properties of substance. Other compounds containing OH and $NH_2$ groups also form hydrogen bonds. Molecules of many organic compounds such as alcohols, acids, amines, and amino acids contain these groups, and thus hydrogen bonding plays an important role in biological science.
4. Metallic bonding; forces between atoms in metallic solids belong to another category. Valence electrons in metals are rampant. They are not restricted to certain atoms or bonds. Rather they run freely in the entire solid, providing good conductivity for heat and electric energy.
5. Weak London dispersion forces or van der Waal's force. These forces always operate in any substance.
6. Covalent bonding; is intramolecular force rather than intermolecular force. Covalent bonding holds atoms tighter than ionic attraction.

## 2.3 Prodrugs

The most important chemical tool among the past few decades is known as prodrug, a modified physicochemical, pharmacokinetic and pharmacodynamic characteristics of a drug molecule. Many therapeutic drugs have undesirable properties in clinical drug application; among the various approaches to minimize the undesirable drug properties is the chemical approach using prodrug.

Historically, the term prodrug was first introduced in 1958 by Albert [25]. Prodrugs are pharmacologically inactive chemical derivatives of a drug molecule that converted to its active form by enzymatic and/or chemical transformation within the body [26], and can be used to temporarily alter the physicochemical properties of a drug in order to increase its usefulness, by overcoming pharmaceutical, pharmacokinetic, or pharmacological barriers, such as poor solubility, low absorption, toxicity, lack of site specificity, insufficient chemical stability, and unacceptable odor/taste. Ideally, the prodrug should be converted to the parent drug and non-toxic moiety as soon as its goal is achieved, followed by rapid elimination of the released linker moiety [28]. The use of the term usually implies a covalent link between a drug and a chemical moiety. Prodrugs can be classified according to two major criteria:

- Chemical classes (carrier-linked prodrugs, bioprecursors, site-specific chemical delivery systems, etc.).
- Mechanism of activation (enzymatic versus nonenzymatic, activation by oxidation, reduction or hydrolysis, catabolic versus anabolic reaction).

### 2.3.1 Prodrug activation

Prodrugs that are designed to be activated by natural enzymes such as esterases and amidases may be susceptible to premature hydrolysis during the absorption phase in enterocytes of gastrointestinal tract, this might produce more polar and less permeable prodrug which lead to decrease in the bioavailability [29], while if the prodrug is activated by cytochrome P450 enzymes which are responsible for 75% of the enzymatic metabolism of prodrugs, a genetic polymorphisms might persist, which lead to variability in prodrug activation and thus affect the efficacy and safety of designed prodrugs [30]. Thus, it might be difficult to predict the bioconversion rate of the enzymatic hydrolysis of the prodrug and hence a difficulty in predicting their pharmacological or toxicological effects. Moreover, the rate of hydrolysis is not always predictable [31-35].

To overcome these problems the novel prodrug approach for drugs that contain hydroxyl, amine, or phenol groups can be designed based on intramolecular processes (enzyme models). The design of prodrugs is based on computational calculations using different molecular orbital and molecular mechanics methods, and correlations between experimental and calculated rate values for some intramolecular processes. The impressive efficiency of enzyme catalysis has encouraged many organic chemists' and biochemists to explore enzyme mechanisms by investigating particular intramolecular processes such as enzyme models, which proceed faster than their intermolecular counterparts. This research brings about the important question of whether enzyme models will replace natural enzymes in the conversion of prodrugs to their parent drugs.

There are two major prodrug design approaches that are considered as widely used among all other approaches to minimize or eliminate the undesirable drug properties.

- The first approach is the chemical design approach by which the drug is linked to inactive organic moiety, which upon exposure to physiological environment releases the parent

drug and a non-toxic linker, which should be eliminated without affecting the clinical profile.

- The second approach is the targeted drug design approach by which prodrugs can be designed to target specific enzymes or carriers by considering enzyme-substrate specificity or carrier-substrate specificity in order to overcome various undesirable drug properties. This type of "targeted-prodrug" design requires considerable knowledge of particular enzymes or carriers, including their molecular and functional characteristics [36-37].

In the past five decades, proposals have been made from attempts to interpret changes in reactivity versus structural variations in intramolecular systems.S cholarly studies have been done by, such as Bender, Jencks, Bruice,Menger, Kirby and Walesh have extensively studied a variety of intramolecular systems (enzyme models) for understanding how enzymes catalyze biochemical reactions. The similarity between intramolecularity and enzymes has excited a number of chemists and biochemists to design chemical models based on intramolecular reactions consisting of two reactive centers in order to gain additional information about the mode and the mechanism by which enzymes exert their high catalytic activities. Over the past 50 years, suggestions have been made to interpret changes in reactivity versus structural variations in intramolecular systems [39].

Among the proposals and hypothesis advocated by the above mentioned scientists and others to explain enzyme catalysis are:

- Koshland ‘‘orbital steering’’ which suggests a rapid intramolecularity arises from a severe angular dependence of organic reactions, such as in the lactonization of rigid hydroxy acids [40].
- ‘‘Proximity orientation’’ in intramolecular processes (near attack conformation) as proposed by Bruice and demonstrated in the lactonization of di-carboxylic acids semi-esters [41-43].
- ‘‘Stereopopulation control’’ based on the concept of freezing a molecule into a productive rotamer as advocated by Cohen [44-46].
- Manger’s ‘‘spatiotemporal hypothesis’’ which postulates that the rate of reaction between two reactive centers is proportional to the time that the two centers reside within a critical distance [47-49].

- Kirby's proton transfer models on the acid-catalyzed hydrolysis of acetals and N-alkylmaleamic acids which demonstrated the importance of hydrogen bonding formation in the products and transition states leading to them [50].

Recently Karaman's group has been researching the mechanistic pathways of some intramolecular processes that mentioned above which used as enzyme models. Utilizing DFT and *ab initio* molecular orbital calculation methods, Karaman's group studied the following intramolecular processes (enzyme models):

(a) Acid-catalyzed lactonization of hydroxy-acidsand proton transfer between two oxygens in rigid systems as investigated by Menger and Cohen, (b)$S_N2$-based-cyclization reactions of di-carboxylic semi-esters to yield anhydrides as studied by Bruice,(c)intramolecular $S_N2$-based ring-closing reactions as researched by Brown's and Mandolini's groups, (d) proton transfer between two oxygen's in Kirby's acetals and proton transfer between nitrogen and oxygen in Kirby's N-alkylmaleamic acids[2,50].

The conclusions raised up from these studies are:

- The nature of the reaction being intermolecular or intramolecular is determined on the distance between the two reactive centers. The distance between the two reacting centers is the main factor in determining whether the reaction type is intermolecular or intramolecular. When the distance exceeded 3 Å, an intermolecular engagement was preferred because of the engagement with a water molecule (solvent). When the distance between the electrophile and nucleophile was <3 Å, an intramolecular reaction was dominant [50,51].
- The driving forces for enhancements in rate for intramolecular processes are both entropy and enthalpy effects. In the cases by which enthalpy effects were predominant such as ring closing and proton transfer reactions proximity or/and steric effects were the driving force for the rate accelerations [50].
- In $S_N2$-based ring-closing reactions leading to three-, four- and five-membered rings the gem-dialkyl effect is more dominant in processes involving the formation of an

unstrained five-membered ring, and the need for directional flexibility decreases as the size of the ring being formed increases [50].

- Accelerations in the rate for intramolecular reactions are a result of both entropy and enthalpy effects [51].
- An efficient proton transfer between two oxygens and between nitrogen and oxygen in Kirby's acetal systems were affordable when a strong hydrogen bonding was developed in the products and the corresponding transition states leading to them [50].

# Chapter Three

# Computational (Design) section

# Chapter Three

# Computational (Design) section

## 3.1 Computational Design

The cost of developing a new drug can run into several billion dollars. The traditional process will usually take 10 or more years and result in the synthesis, purification and biological evaluation of ten thousand or more new compounds in order to produce a single potential drug candidate. By using computational design, it is possible for the user to create a set of new viable drugs. The output data raised from the calculation programs allows the user to explore each potential candidate and chose the best for production and further testing. By evaluating a large number of the most promising models and selecting, only the most promising cases it is possible to minimize production and testing costs [52].

Molecular modeling software allows the user to select atoms from the periodic table and to place them in a three dimensional workspace, the following programs were exploited in the design calculations:

3.1.1 Arguslab.
3.1.2 Gaussian2009.
3.1.3 Molden.

### 3.1.1 Arguslab:

Arguslab, a free docking software program, is a molecular modeling, graphics, and drug design program that offers quite good on-screen molecule-building facilities, with a moderate library of useful molecules. This program can do geometry optimizations using the UFF force field. The resulting energies are clearly distinguishable from those obtained using some of the more conventional force fields, and wherever possible one needs to re-optimize at a higher level. For this, Arguslab offers geometry optimization using the MNDO, AM1 or PM3 semi-empirical methods, as well as single point calculations. Version 3.1 of Arguslab has good facilities for calculating electron density or orbital surfaces at the semi-empirical levels, and displaying

them. There are also single point semi-empirical calculations using Extended Huckel (for a bigger element coverage) or ZINDO (for excited states for UV/visible absorption prediction) [52].

Argus lab writes its own format of molecule file, like .xml, but it can also write xyz files for input to other programs, e.g. Molden. It creates (and leaves behind) a lot of temporary files, which need to be managed. To start work using Arguslab, we press the 'New' button (top left) to get a new molecule screen, or press the 'Open' button to read in a molecule which have saved previously in the your Argus directory.

In Arguslab, we need to save our molecule with whatever name we want before doing geometry optimizations well as afterwards. If we forget to change the file name before modifying a molecule, files will be auto-saved with the name used previously, possibly damaging data which we wanted to keep. It is best not to maximize the molecule window, because then its title bar will display the name by which we are currently saving the files. Just drag its bottom right corner so that it fills most of the Arguslab worktop. To stop using Argus lab, click File Exit, if we have molecule windows open, this will just close one of these. We need to do it repeatedly to close all the windows (if we have several open) and then stop the program.

### 3.1.2 Gaussian 2009

Gaussian has implemented almost all the quantum mechanical methods, which include Hartree-Fock and post Hartree-Fock method and DFT method. The current version Gaussian 09 is the latest version in the Gaussian series of computer for chemistry designed to model a broad range of molecular systems under a variety of conditions, performing its computations starting from the basic laws of quantum mechanics.

The Gaussian program has been continuously developed by many researchers and today it is a software product that is used by thousands of chemists. It can be used to calculate the:

- Electronic properties, charges, molecular energy, IR, Raman, UV, NMR and other spectroscopic properties of small to media molecules.
- Comprehensive investigation of molecules and their reactions.
- Predicting optical spectra including hyperfine spectra.

- Investigate thermo chemistry and excited state processes.
- Gaussian_09 allows solvent effects to be taken into account when optimizing structures and predicting most molecular properties.
- Prediction of the vibration frequencies and numerous molecular properties for systems in the gas phase and in solution.

Traditionally, proteins and other large biological molecules have been out of reach of electronic structure methods. Theoretical study of molecules, their properties, and how they act together in chemical reactions can be done by the use Gaussian program. Experimental chemists can use it to study molecules and reactions of definite or potential interest, including both stable species and those compounds which are difficult or impossible to observe experimentally (short-lived intermediates, transition structures and so on) [53].

►Creating the first input file: input file scan be created in two ways:

•By hand: using local editor (VI, emacs and nedit).

• Using Molden.

►Viewing output files from files run in Gaussian 09. Further, input files for use in Gaussian 09 can be generated using Molden program.

►Dissecting the output file:the Z-matrix represents how the software knows the molecular geometry (structure).

### 3.1.3 Molden

Molden is a computational program package made for displaying molecular densities from the ab-initio packages, Games-US, Games-UK and Gaussian, as well as Mopac/Ampac. Molden reads all the required information from the GAMESS GAUSSIAN output file. Molden is capable of displaying Molecular Orbital, the electron density and the Molecular minus Atomic density.

The benefit of using this programs format is simple. Molden can interpret and convert information from all these programs into its own format, thereby providing a standardizing tool. The Molden program has a powerful Z-matrix editor which gives full control over the geometry

and allows you to build molecules from scratch, thereby allowing users to create the molecule of their choice and being able to save the geometry in the Molden format [54]**.**

Molden format:

- Incorporates numerous data stores in a text file.
- Molden has a powerful Z-matrix editor which gives full control over the geometry and allows building molecules from scratch, including polypeptides.
- Supports contour plots, 3-d grid plots with hidden lines and a combination of both. It can write a variety of graphics instructions; postscript, X-Windows, VRML, povray, OpenGL, tekronix4014 and hpgl, hp2392, also can animate reaction paths and molecular vibrations.
- Molden can optimize geometries with the combined Amber (protein) and GAFF (small molecules) force fields.

## 3.2 Calculation methods

### 3.2.1 Amoxicillin prodrugs

The Becke three-parameter, hybrid functional combined with the Lee, Yang, and Parr correlation functional, denoted B3LYP, were employed in the calculations using density functional theory (DFT).Calculations were carried out based on the restricted Hartree-Fock method [54]. All calculated molecule have a starting geometry that were calculated using Arguslab program, and were initially optimized at the HF/6-31G level of theory, followed by optimization at the B3LYP/6-31G(d,p) also, the calculations were carried out using the quantum chemical package Gaussian-2009 [55]. Total geometry optimizations included all internal rotations. Second derivatives were estimated for all 3N-6 geometrical parameters during optimization. Energy minimization (also called energy optimization, geometry minimization, or geometry optimization) is the process of finding an arrangement in space of a collection of atoms, according to some computational model of chemical bonding, the net inter-atomic force on each atom is acceptably close to zero, which refer to stable compound or a reactive intermediate exhibit the minimum energy with no negative vibrational force constant called energy minimum.

A transition state correspond to saddle points on the potential energy surface which has only one negative vibrational force constant [54]. Transition states were located first by the normal reaction coordinate method [56], where the enthalpy changes was monitored by changing the interatomic distance between two specific atoms, The vibrational frequencies are related to second derivatives; a minimum will have only positive frequencies while transition state should have one negative frequency. The vibrational analysis must be performed at the optimized geometry. Gaussian saves the optimized geometry into a checkpoint file, and the geometry can be recalled from this file before performing the vibrational analysis. The geometry at the highest point on the energy profile was re-optimized by using the energy gradient method at the B3LYP/6-31G(d, p) level of theory [54]. The activation energy values for the proton transfer processes (transfer of H7 from O6 into O1, Chart 1) were calculated from the difference in energies of the global minimum structures (GM) and the derived transition states.

Verification of the desired reactants and products was accomplished using the “intrinsic coordinate method” [56]. The transition state structures were verified by their only one negative

frequency. The activation energies obtained from the DFT at B3LYP/6-31G (d,p) level of theory for all molecules were calculated with and without the inclusion of solvent (water). The calculations with the incorporation of a solvent were performed using the integral equation formalism model of the Polarizable Continuum Model (PCM) [59-62].

In this model, the cavity is created via a series of overlapping spheres. The search for the global minimum structure in each of the systems studied was accomplished by 36 rotations of the carboxyl group about the bond C4-C6 in increments of 10° (i.e. variation of the dihedral angle O5C4C6C7, see Chart 1) and calculation of the energies of the resulting conformers.

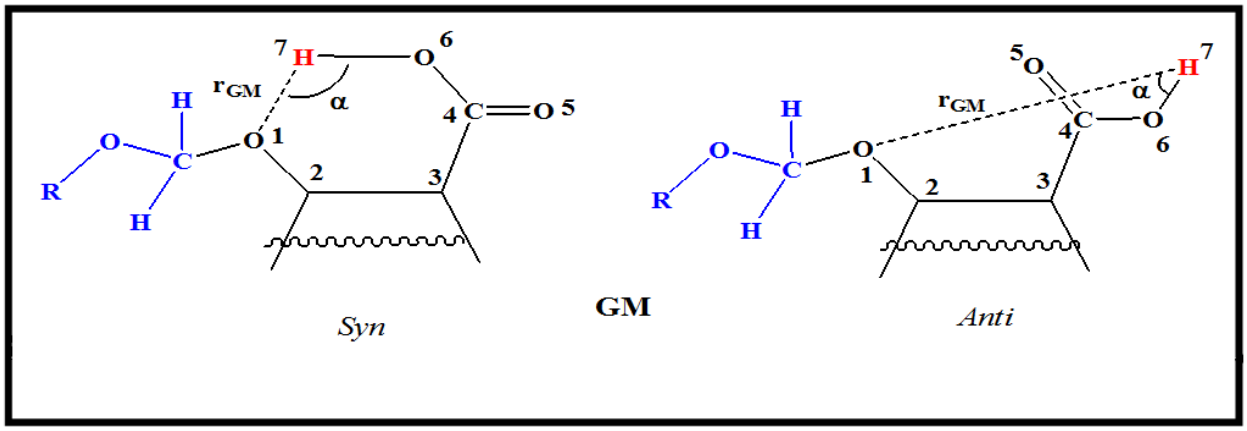



Chart 1: Schematic representation of the reactants in the proton transfers of amoxicillin. GM is the global minimum structure, $r_{GM}$ is the O—H distance in the GM. α, is the angle of attack (hydrogen bonding) O1-H7-O6 in the GM.

# Chapter Four

# Results and Discussion

# Chapter Four

## Results and Discussion

### 4.1 Bitterless amoxicillin prodrugs based on Kirby's maleamic acids enzyme model

The Mechanistic study of the acid-catalyzed hydrolysis of maleamic acids**1**-**7** (Fig.2) used for the design of amoxicillin prodrugs was kinetically investigated by Kirby. The study demonstrated that the amide bond cleavage is due to intramolecular nucleophilic catalysis by the adjacent carboxylic acid group and the rate-limiting step is the tetrahedral intermediate breakdown.

In order to utilize Kirby's enzyme model for the design of amoxicillin prodrugs, a mechanistic study using DFT calculation methods on an intramolecular acid catalyzed hydrolysis of maleamic (4-amino-4-oxo-2-butenoic) acids (Kirby's N-alkyl maleamic acids) **1**-**7** was conducted.

The calculations confirmed that the reaction involves three steps:

(1) Proton transfer from the carboxylic group to the adjacent amide carbonyl oxygen.

(2) Nucleophilic attack of the carboxylate anion onto the protonated carbonyl carbon.

(3) Dissociation of the tetrahedral intermediate to provide products.

The calculations indicated that the rate-limiting step is dependent on the reaction medium.

When the calculations were run in the gas phase the rate-limiting step was the tetrahedral intermediate formation. Whereas when the calculations were conducted in the presence of a cluster of water, the dissociation of the tetrahedral intermediate was the rate-limiting step.

In addition, when the leaving group (methylamine) in **1**-**7** was replaced with a group having a low pKa value the rate-limiting step of the hydrolysis in water was the formation of the tetrahedral intermediate and the rate of hydrolysis was found to be linearly correlated with the strain energy of the tetrahedral intermediate or the product. Systems having strained tetrahedral intermediates or products experience low rates and vice versa.

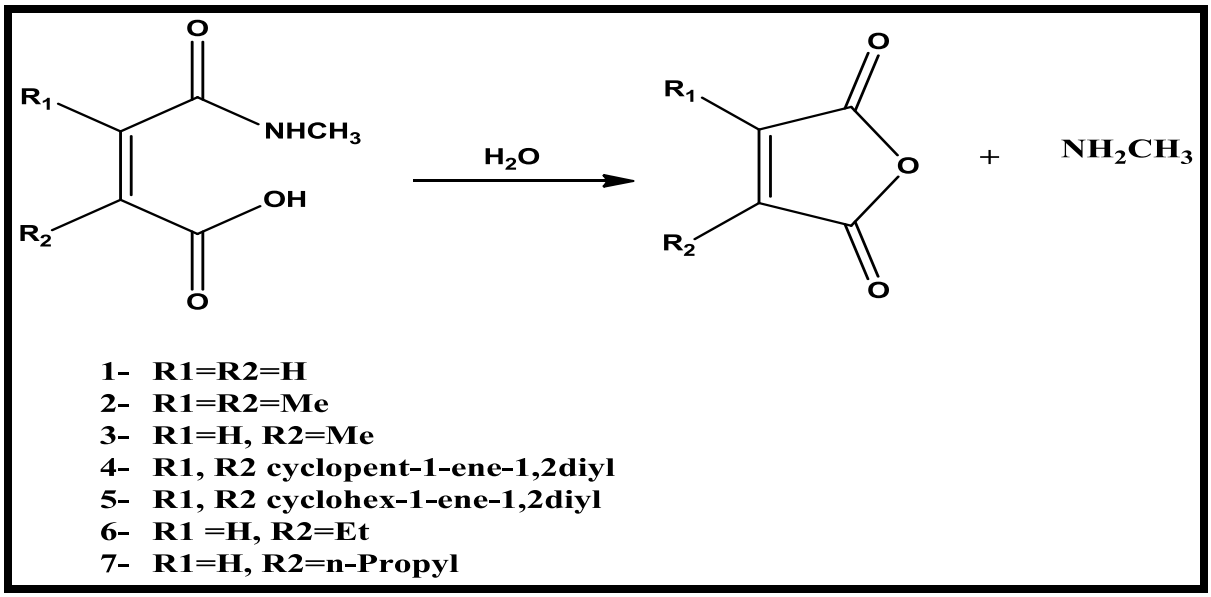



Figure 2: Acid catalyzed hydrolysis *of N*-alkylmaleamic acid **1**-**7**.

Furthermore, Karaman's calculations revealed a correlation between the acid-catalyzed hydrolysis efficiency and the following parameters:

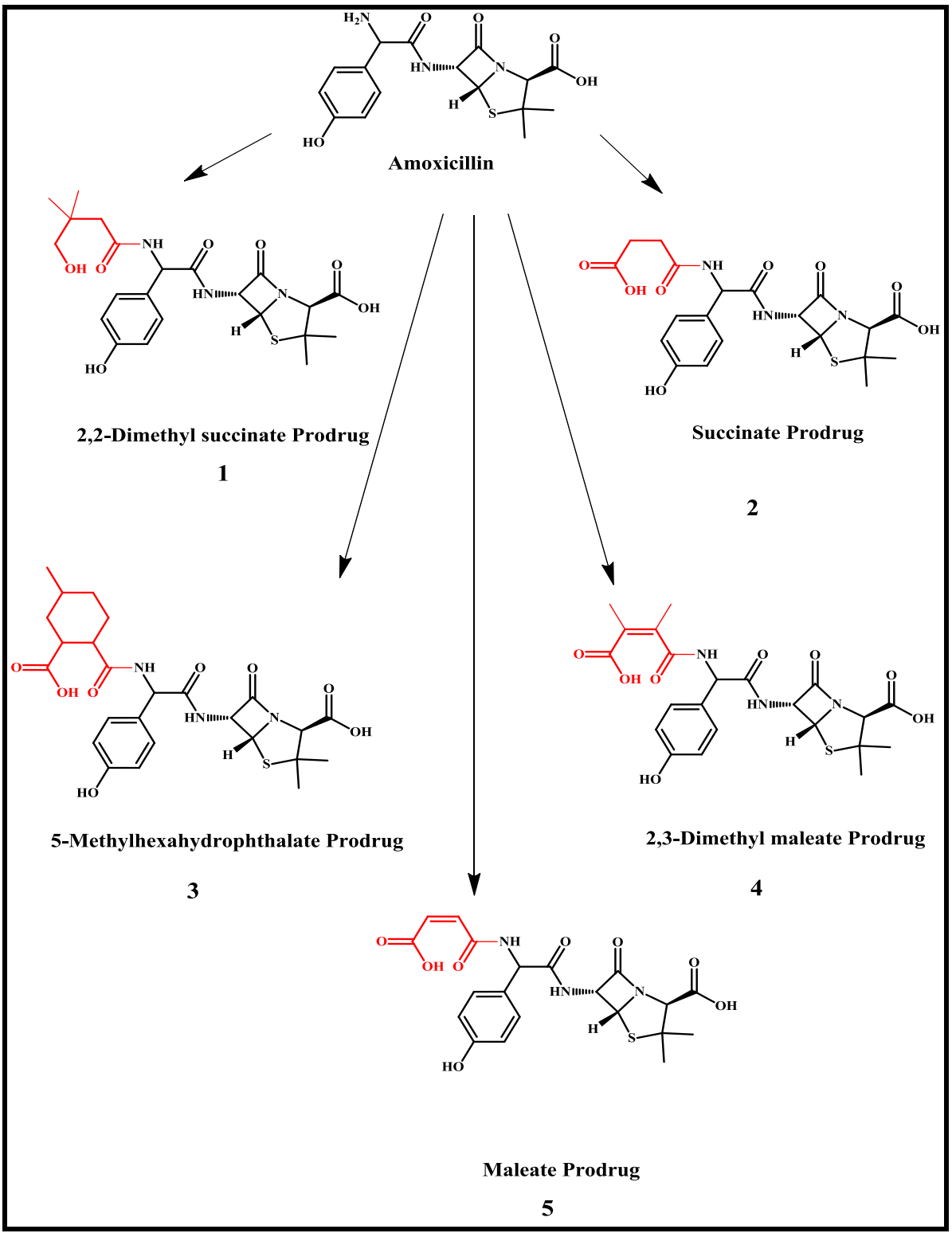
**1.** The distance between the hydroxyl oxygen of the carboxylic group and the amide carbonyl carbon.

**2.** The attack angle.

**3.**The difference between the strain energies of intermediate, product, intermediate, and reactant.

The aim of this work was to design various amoxicillin prodrugs that lack the bitterness of their parent drug and have the capability to chemically undergo hydrolysis in the intestine to give the active drug. Scheme 1 illustrates possible approaches for the design of amoxicillin prodrugs.

In this section, we report DFT at B3LYP 6-31G (d,p) level calculations of ground state and transition state structures, vibrational frequencies, and reaction trajectories for intramolecular proton transfer in amoxicillin prodrugs **ProD1**-**ProD5**.




Scheme 1: Proposed amoxicillin prodrugs.

As shown in (Scheme.1) the combination of both, the hydrophilic and lipophilic groups provides a prodrug entity with a potential to be with a high permeability (a modified HLB). The amoxicillin prodrugs **ProD1-5** have a carboxylic acid group (hydrophilic moiety) and a lipophilic moiety (the rest of the molecule), it should be noted that the HLB value will be determined upon the physiologic environment by which the prodrug is dissolved.

In the stomach where the pH is in the range 1-2, it is expected that amoxicillin **ProD1-5** will be in a free carboxylic acid form (a relatively high hydrophobicity) whereas in the blood stream circulation where the pH is 7.4 a carboxylate anion (a relatively low hydrophobicity) is expected to be predominant form. At pH 5, the hydrolysis of **ProD1-5** was too slow. This is because the pKa of amoxicillin prodrugs is in the range of 3-4, it is expected that at pH 5, the anionic form of the prodrug will be dominant and the percentage of the free acidic form that undergoes an acid-catalyzed hydrolysis will be relatively low.

Our strategy was to prepare amoxicillin prodrugs as sodium or potassium carboxylates due to their high stability in neutral aqueous medium. It should be indicated that compounds **1-7** undergo a relatively fast hydrolysis in acidic aqueous medium whereas they are quite stable at neutral pH. For example, for prodrugs intended to be given as solutions or syrups to children or pediatrics (for masking the bitterness of the parent drug) the prodrug will reach the stomach and it will primarily exist in the carboxylic acid form whereas in the blood circulation (in the cases of IV injection dosage form) the carboxylate anion form will be predominant. It is planned that the prodrugs will be obtained as sodium or potassium salts and will be given to adults in the form of enteric coated tablets in order to assure release of the parent drug in the intestine (pH 6-8) and not in the stomach (pH 1). This is because the linkers (Kirby's enzyme model) undergo fast hydrolysis at low pH such as the stomach. On the other hand, the prodrugs when dissolved in the intestine they can exist in both the carboxylate and free carboxylic acid forms (the ratio between the two forms will be determined on the $pk_a$ value of the prodrug).

Karaman found that the proton transfer is the rate-limiting step in the hydrolysis. Also the driving force for the proton transfer efficiency is the proximity of the two reactive centers ($r_{GM}$) and the attack angle (α); and the rate of the reaction is linearly correlated with $r_{GM}{}^2$ and sin (180°- α) short $r_{GM}$ values and with α values close to 180° (forming a linear H-bond) are more reactive due to the development of a strong hydrogen bonds in their transition state and product structures [50,55,63-96].

4.1.1 General Consideration:

The carboxylic acid moiety could be engaged in intermolecular or intramolecular hydrogen bonding. Therefore, the free energy of the reactant is strongly dependent on its conformation. We were concerned with the identification of the most stable conformation (Global Minimum, GM) for each prodrug in this study. The search for the global minimum structures for all prodrugs studied was accomplished by $360^0$ rotations of the carboxyl group about the bond C3-C4 in increments of 10° (i.e. variation of the dihedral angle O5C4C3C2, see Chart 1) and calculation of the energies of the resulting conformers.

Two different types of conformations were considered in the DFT calculations of the starting geometries in amoxicillin **ProD1-5**:

- One in which the carboxylic hydroxyl proton is syn to the alkoxy group in the β position of the carboxylic acid moiety (dihedral angle O5C4C3C2 = 0, Chart 1).
- Another in which it is anti (dihedral angle O5C4C3C2 = 180, Chart 1).

The global minimum search for amoxicillin **ProD1-5** revealed that **ProD4** and **ProD5** exist in the syn orientation and **ProD1**, **ProD2** and **ProD3** exist in the anti-orientation.

**4.1.2 Optimized geometries of the entities involved in the proton transfers of amoxicillin ProD1-5.**

Using the quantum chemical package Gaussian-98 [53], I have calculated the DFT B3LYP/6-31G (d,p) kinetic and thermodynamic parameters for the proposed amoxicillin prodrugs. (Figure.3a) and (Table.1) illustrates the DFT calculated properties for the global minimum structures of amoxicillin **ProD1-ProD5**, (Table.2) illustrates the DFT (B3LYP/6-31G (d,p) calculated kinetic and thermodynamic properties for the proton transfers in amoxicillin **ProD1-ProD5**.

The DFT calculated properties for the global minimum structures of amoxicillin **ProD1-5 (ProD1GM- ProD5GM)** indicates that amoxicillin **ProD1GM, ProD2GM** and **ProD3GM** exist in conformation by which the carboxylic hydroxyl group forms hydrogen bond with a molecule of water rather than intramolecularly. The preference of the carboxyl group in amoxicillin **ProD1GM**, **ProD2GM** and **ProD3GM** to be engaged intermolecularly with the solvent and not

intramolecularly is due to the fact that the latter is energetically expensive due to a high-energy barrier for the rotation of the carboxyl group around the C3-C4 bond.

On the other hand, the optimized geometries of amoxicillin **ProD3GM** and **ProD4GM** exist in conformation by which the carboxylic hydroxyl proton is engaged intramolecularly *via* hydrogen bonding with the neighboring alkoxy oxygen. Examination of the optimized global minimum structures in (Fig.3a) indicates that the DFT calculated hydrogen bonding length ($r_{GM}$) in the reactants engaged intermolecularly with a water molecule for **ProD1GM, ProD2GM** and **ProD3GM** was in the range of 4.08 Å-5.97 Å and the attack angle α (the hydrogen bond angle, O1H7O6) in the range of 27°-30°. On the other hand, the $r_{GM}$ and α value for amoxicillin **ProD3GM** and **ProD4GM** were 2.21Å-2.30Å and 142.09°-156.41°, respectively. It should be indicated that the hydrogen bonding length, $r_{GM}$ (O1-H7), varies according to the structural features of the reactant geometry. The optimized DFT calculated transition state geometries for amoxicillin **ProD1-5** are illustrated in (Fig.3b).

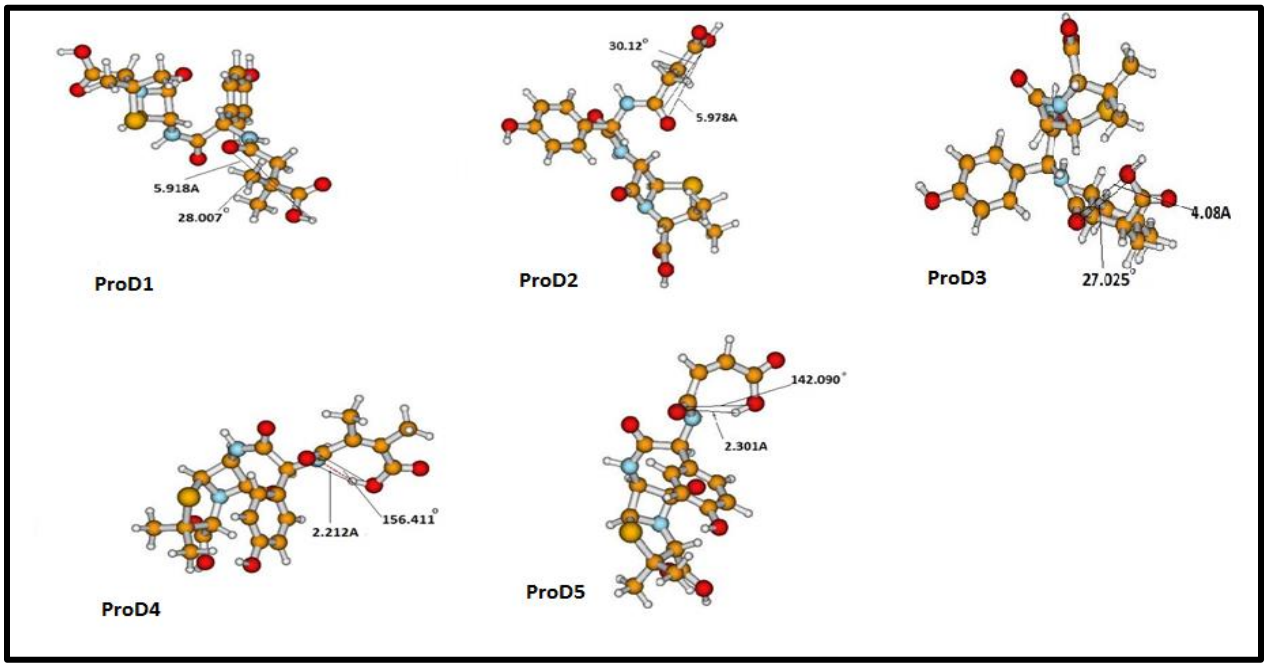



Figure 3: DFT optimized structures for the global minimum (GM) structures in the intramolecular proton transfer reaction of amoxicillin **ProD1- 5**.

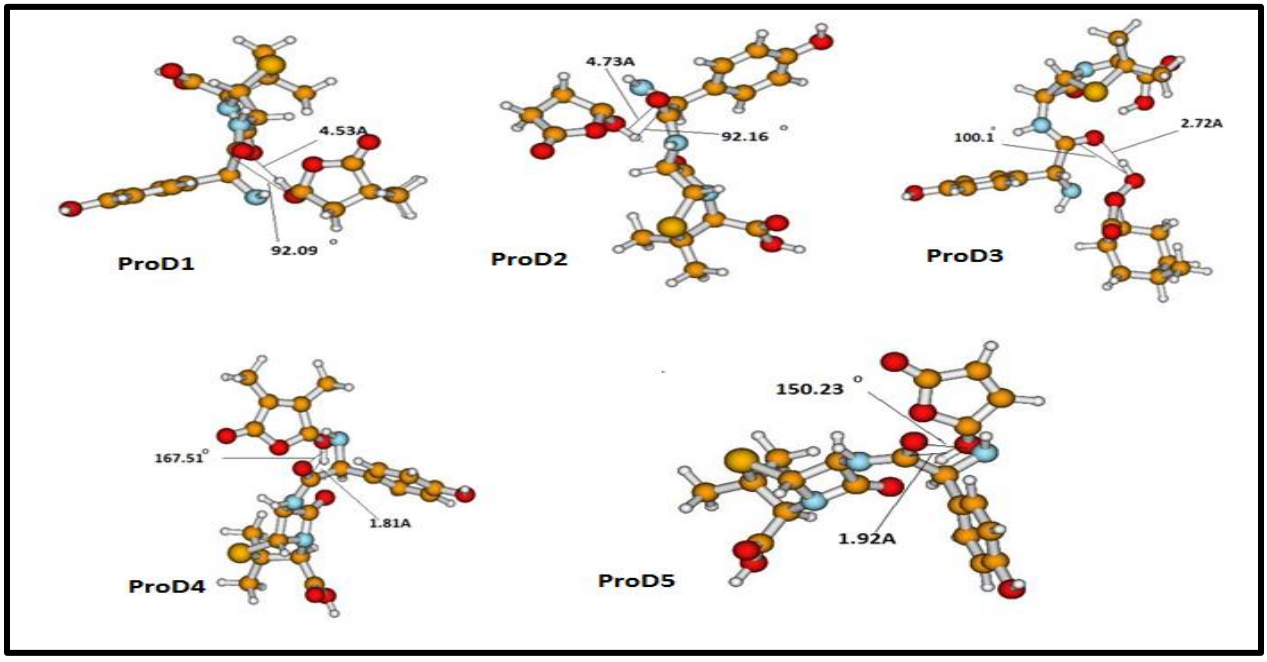



Figure 4: DFT optimized structures for the transition state (TS) structures in the intramolecular proton transfer reaction of amoxicillin **ProD1-5**.

Table 1: DFT (B3LYP) calculated properties for the proton transfer reactions of in amoxicillin **ProD1-5**.

| Compound | B3LYP, Enthalpy, H (gas phase) in Hartree | B3LYP (gas phase) Entropy, S, Cal/Mol-Kelvin | B3LYP Frequency $Cm^{-1}$ |
|---|---|---|---|
| Amoxicillin **ProD1GM** | -2018.925608 | 221.224 | ------- |
| Amoxicillin **ProD1TS** | -2018.866062 | 208.552 | 44.561i |
| Amoxicillin **ProD2GM** | -1940.29406 | 212.567 | ------- |
| Amoxicillin **ProD2TS** | -1940.229544 | 196.496 | 59.346i |
| Amoxicillin **ProD3GM** | -2135.662449 | 232.801 | ------- |
| Amoxicillin **ProD3TS** | -2135.609573 | 214.596 | 30.094i |
| Amoxicillin **ProD4GM** | -2017.664638 | 219.198 | ------- |
| Amoxicillin **ProD4TS** | -2017.649627 | 210.773 | 101.143i |
| Amoxicillin **ProD5GM** | -1939.024531 | 203.732 | ------- |
| Amoxicillin **ProD5TS** | -1938.996431 | 194.085 | 132.041i |

Table 2: DFT (B3LYP/6-31G (d,p) calculated kinetic and thermodynamic properties for the proton transfers in amoxicillin **ProD1-5**.

| System | ΔES | $\Delta H^{‡}$ (GP) | $T\Delta S^{‡}$ (GP) | $\Delta G^{‡}$ (GP) | $\Delta H^{‡}$ ($H_2O$) | $\Delta G^{‡}$ ($H_2O$) |
|---|---|---|---|---|---|---|
| **ProD1** | 15.7 | 37.36 | -3.76 | 41.13 | 42.75 | 46.51 |
| **ProD2** | 14.0 | 40.48 | -4.77 | 45.26 | 47.15 | 51.92 |
| **ProD3** | 14.5 | 33.18 | -5.41 | 38.58 | 40.73 | 46.13 |
| **ProD4** | 2.3 | 9.42 | -2.50 | 11.92 | 16.40 | 18.90 |
| **ProD5** | 3.1 | 17.63 | -2.86 | 20.49 | 24.80 | 27.66 |
| 1 | 0.3 | 27.31 | 28.08 | 32.29 | 33.06 | 0 |
| 2 | 5.3 | 13.93 | 16.42 | 17.56 | 20.05 | 4.37 |
| 3 | 6.9 | 24.41 | 24.90 | 27.93 | 28.42 | 1.49 |
| 4 | 15.6 | 34.42 | 36.77 | 35.76 | 38.11 | -4.3 |
| 5 | 10.1 | 13.25 | 17.41 | 18.96 | 23.12 | 2.73 |
| 6 | 12.5 | 23.83 | 23.92 | 27.19 | 27.28 | 1.51 |
| 7 | 12.4 | 24.86 | 25.03 | 27.38 | 27.55 | 1.64 |

$\Delta H^{‡}$ is the activation enthalpy energy (kcal/mol). $T\Delta S^{‡}$ is the activation entropy energy in kcal/mol. $\Delta G^{‡}$ is the activation free energy (kcal/mol).GP and H2O calculated in the gas phase and water, respectively.

### 4.1.3 The role of the distance O1-H7 ($r_{GM}$) and the angle O1H7O6 (α) on the rate of the proton transfer in processes amoxicillin ProD1-5

Careful inspection of the optimized structures for the global minimum (**GM**) structures in the intramolecular proton transfer reaction of amoxicillin **ProD1-5** in (Fig.3a) indicates that the distance between the two reactive centers $r_{GM}$ (O1-H7) varies according to the conformation of the global minimum structure (GM).

Short $r_{GM}$ distance values were achieved when the values of the attack angle (α) in the GM conformations were high and close to 180°, whereas small values of α resulted in longer $r_{GM}$ distances. In fact when the $r_{GM}$ values were plotted against the corresponding α values linear correlation was obtained with r = 0.996 (Fig.4). In addition, examination of the activation energy values ($\Delta G^{\ddagger}$) listed in (Table.2) reveals that the energy needed to execute proton transfer in amoxicillin **ProD1-5** is largely affected by both the distance between the two reactive centers $r_{GM}$ (O1-H7), and the attack angle α (O1H7O6).

When $r_{GM}$ and α values were examined for correlation with the calculated DFT enthalpy energies ($\Delta H^{\ddagger}$) a linear correlation was found between $\Delta H^{\ddagger}$ and $r_{GM}^2$ x sin (180-α) with a correlation coefficient of r = 0.983(Fig.5), and the activation free energies ($\Delta G^{\ddagger}$) with $r_{GM}^2$ x sin (180-α) gave an r = 0.987(Fig.6).

Systems with low $r_{GM}$ and high α values in their global minimum structures, such as **ProD4** and **ProD5** exhibit much higher rates (lower $\Delta G^{\ddagger}$) than these with high $r_{GM}$ and low α values, such as **ProD1, ProD2 and ProD3.** In the case **ProD1**, **ProD2 and ProD3** the interatomic distance between the nucleophile (OH) and electrophile (C=O) is too high to make the nucleophile attack accessible.

According to structural feature of 2,3-dimethylmaleatmoiety it contains two methyl groups on the C-C double bond (strained system) which results in a decrease of the distance between the two reactive centers (hydroxyl oxygen of the carboxylic group and the amide carbonyl carbon).

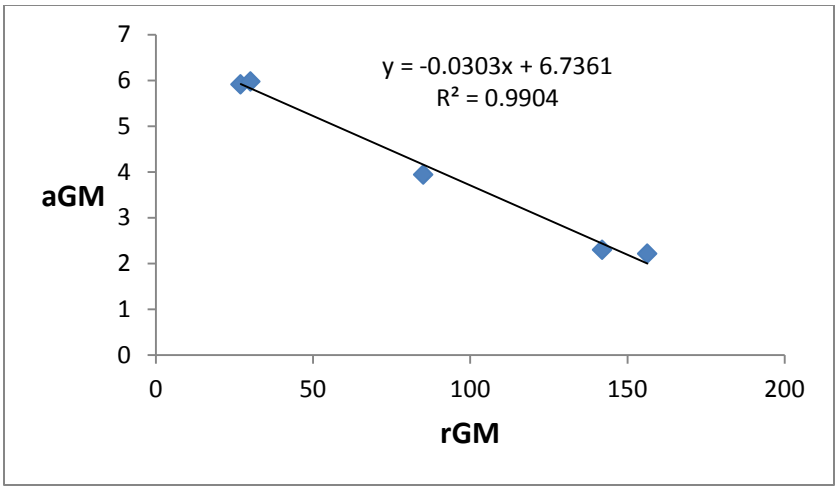



Figure 5: Plot of the DFT calculated $r_{GM}$ (Å) vs. angle α (°) in amoxicillin **ProD1-5**, where ($r_{GM}$) and (α) are the distance between the two reactive centers and the attack (hydrogen bond) angle in the GM structure, respectively.

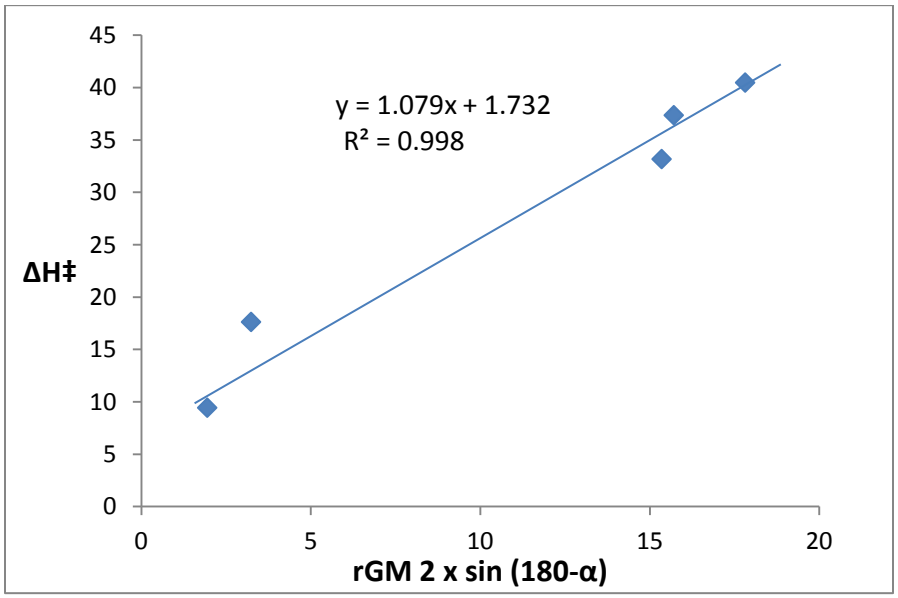



Figure 6: Plot of the DFT calculated $\Delta H^{\ddagger}$ vs. $r_{GM}{}^{2}$ x sin (180-α) in amoxicillin **ProD1-5**

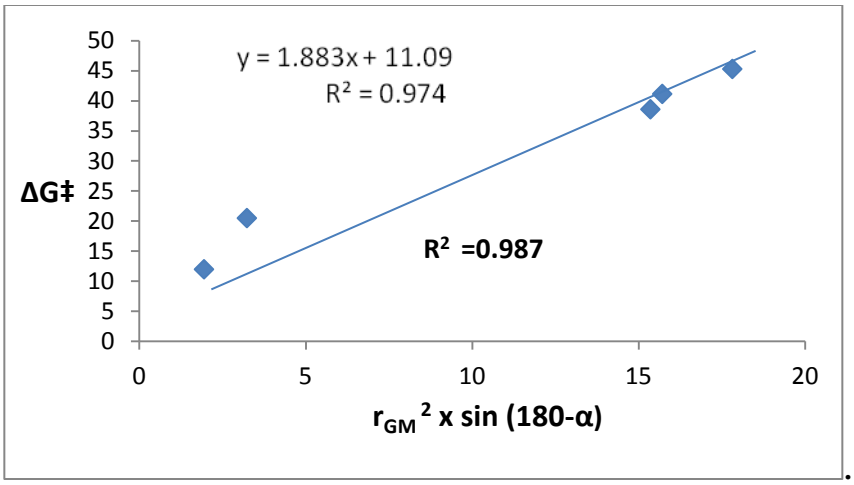



Figure 7: Plot of the DFT calculated $\Delta G^{\ddagger}$vs. $r_{GM}{}^{2}$ x sin (180-α) in amoxicillin **ProD1-5**.

### 4.1.4 The role of the strain energy on the rate of the proton transfer in amoxicillin ProD1-5 :

To investigate the role of the steric effects we computed using Allinger's MM2 method. The strain energies for the reactants, intermediates in **1–7** and amoxicillin **ProD1-ProD5**. The rate of hydrolysis for **1-7** was found to be linearly correlated with the strain energy of the tetrahedral intermediate. The correlation results illustrated in (Fig.7) demonstrate a good correlation between the experimental log krel and the MM2 calculated intermediate strain energy values (Es) with a correlation coefficient (r) of 0.88.

The DFT calculation results revealed that the rate of a proton transfer in processes of amoxicillin **ProD1-ProD5** and **1-7** is governed by strain effect. The rate of hydrolysis was found to be linearly correlated with the strain energy difference between the intermediate and the reactant ($E_s$ INT-GM). Therefore **ProD4** has lowest difference in strain energy so; it has the highest rate of proton transfer.

In order to further support this conclusion, activation energy values for **1-7** as calculated in dielectric constant of 78.39 (water) ($\Delta G^{\ddagger}$ H2O, see Table 2) were examined for correlations with both log krel for system 1-7 and ΔEs for amoxicillin prodrugs (Fig.8) and (Fig.9) with a correlation coefficient of 0.93 and 0.92 respectively.

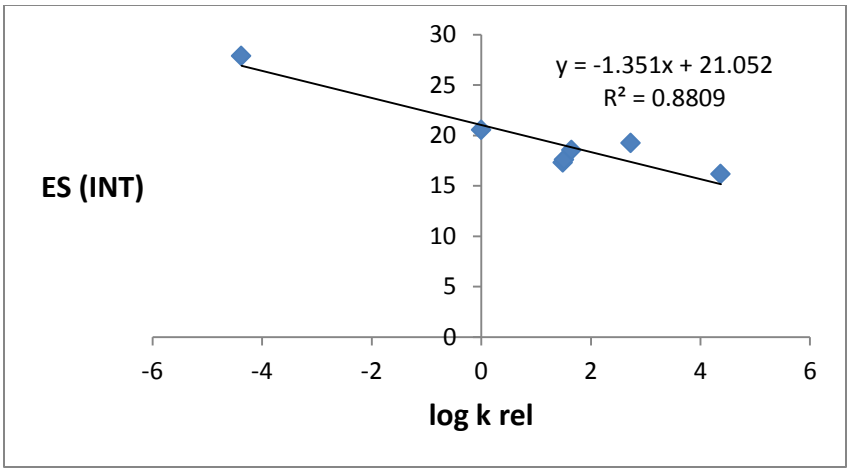



Figure 8: Plot of the ES for intermediates of **1-7** N-alkylmaleamic acid vs. relative rate (log krel).

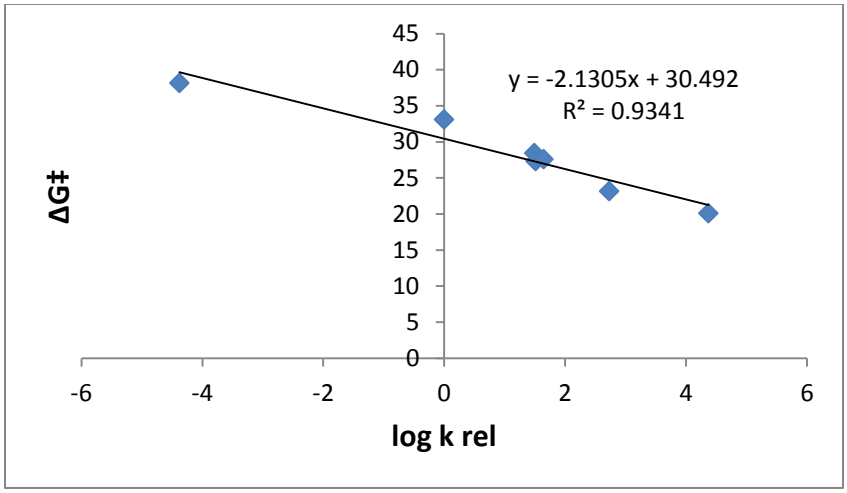



Figure 9: Plot of the DFT calculated ΔG‡ vs. relative rate (log krel) in **1-7** N-alkylmaleamic acid.

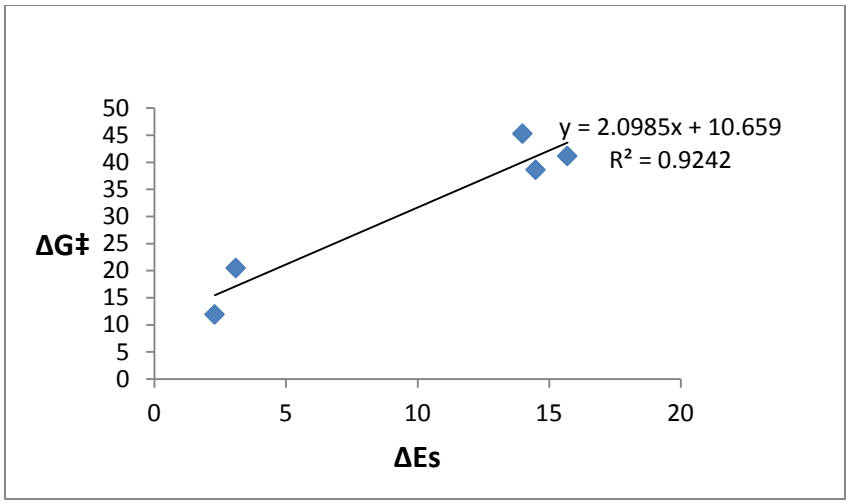



Figure 10: Plot of the DFT calculated ΔG‡ vs. MM2 calculated difference in strain energies between intermediates and reactants ES (INT-GM)) in amoxicillin **ProD1- ProD5**.

# Chapter Five

# Conclusions and future directions

# Chapter Five

## Conclusions and future directions

### 5.1 Conclusions and Future directions

Amoxicillin as mentioned before suffer bitter taste sensation and low stability. Several attempts were made in order to enhance their aqueous solubility and bioavailability. Among several research approaches, the prodrug approach has been widely used for an improvement of drugs delivery to their site of action by physicochemical modulation that affect absorption or by targeting to specific enzymes or membrane transporters.

The DFT calculation results revealed that the rate of a proton transfer in processes amoxicillin **ProD1**-**ProD5** is largely dependent on the geometric variations of the reactant (GM), mainly the distance between the two reactive centers, $r_{GM}$, and the angle of attack α. It was found that reactants with short $r_{GM}$ and α values close to 180º strong intramolecular hydrogen bonding provide stable transition states that lead to acceleration in rate. Also the rate of hydrolysis was found to be linearly correlated with the strain energy difference between the intermediate and the reactant ($E_s$ INT-GM).

In addition, the calculations indicated that the nature of the mechanism rely on the reaction solvent (medium). In aqueous medium the reaction rate-limiting step is the collapse of the tetrahedral intermediate whereas in the gas phase the tetrahedral intermediate formation is the rate-limiting step.

According to the DFT calculations and the designed amoxicillin prodrugs, it is recommended to synthesize amoxicillin **ProD4** and **ProD5** using Kirby's synthetic procedure. In *vitro* kinetic studies at different pH values should be made in order to be utilized for the in *vivo* pharmacokinetic studies, which should be followed to determine the $t_{1/2}$ values for the conversion of the amoxicillin **ProD4** and **ProD5** to its parent drug, amoxicillin. Bitter sensation studies should be conducted for amoxicillin prodrugs to determine if the designed prodrugs have or lack any bitter taste.

In the in *vivo* studies, the prodrug should be administered to animals by intravenous injection and oral administration, blood and urine samples should be collected at different times. The concentration of amoxicillin should be determined using a reliable bio-analytical method. Further, pharmacokinetic parameter values should be calculated including oral bioavailability, terminal elimination half-life and other pharmacokinetic parameters as deemed necessary.

## 5.2 Future directions

Future strategy to achieve more efficient amoxicillin prodrugs capable of eliminating amoxicillin bitterness, and releasing the parent drug in a programmable manner is (a) synthesis of amoxicillin prodrugs having around pH 6 (intestine pH) such as amoxicillin **ProD4** and **ProD5**; (ii) in *vitro* kinetic studies of these prodrugs performed at pH 6.5 (intestine) and pH 7.4 (blood circulation system); and (iii) in *vivo* pharmacokinetic studies should be done in order to determine the bioavailability and the duration of action of the tested prodrug. Furthermore, based on the in *vivo* pharmacokinetics characteristics of amoxicillin **ProD4** and **ProD5**, new prodrugs may be design and synthesized.

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# Supplementary Material

**ProD1GM**

| | | | |
|---|---|---|---|
| C | -2.117941 | -1.102334 | 2.329747 |
| C | -1.142765 | -1.132309 | 1.330078 |
| C | -1.538090 | -0.915967 | 0.008759 |
| C | -2.867830 | -0.650729 | -0.315647 |
| C | -3.833581 | -0.613255 | 0.689405 |
| C | -3.451025 | -0.850667 | 2.016207 |
| O | -0.658651 | -0.927723 | -1.069879 |
| C | 0.554163 | -1.569335 | -0.933688 |
| O | 1.475704 | -0.666483 | -0.288333 |
| C | 2.828919 | -0.920310 | -0.331505 |
| C | 3.700631 | 0.153049 | -0.034300 |
| C | 5.081419 | -0.095484 | -0.045550 |
| C | 5.587882 | -1.355074 | -0.353749 |
| C | 4.712481 | -2.398136 | -0.648018 |
| C | 3.333665 | -2.187674 | -0.634449 |
| C | 3.254450 | 1.555722 | 0.290928 |
| O | 1.951144 | 1.848050 | 0.215352 |
| C | -5.277044 | -0.286322 | 0.368055 |
| O | -5.585936 | -0.826658 | -0.917483 |
| O | 4.040769 | 2.427258 | 0.620215 |
| C | -5.570025 | 1.231241 | 0.440120 |
| N | -4.773280 | 2.034896 | -0.473782 |
| C | -5.329893 | 2.199535 | -1.812460 |
| O | 6.845393 | 2.753823 | 0.233223 |
| H | 5.760856 | 0.723359 | 0.169327 |
| H | 6.660479 | -1.516914 | -0.365829 |
| H | 2.663607 | -3.012349 | -0.846051 |
| H | 5.094702 | -3.386177 | -0.886785 |
| H | 1.440085 | 1.055809 | -0.042229 |
| H | 0.924363 | -1.791641 | -1.938919 |
| H | 0.468799 | -2.482985 | -0.331771 |
| H | -3.141348 | -0.488176 | -1.349890 |
| H | -0.102251 | -1.293534 | 1.585972 |
| H | -4.198664 | -0.838136 | 2.804964 |
| H | -1.826132 | -1.275257 | 3.361282 |
| H | -5.910902 | -0.761872 | 1.135880 |
| H | -5.360905 | 1.553488 | 1.466480 |
| H | -6.660796 | 1.368403 | 0.284933 |
| H | -6.503753 | -0.597723 | -1.116156 |
| H | -4.601138 | 2.944972 | -0.061900 |
| H | -4.713725 | 2.906565 | -2.375421 |
| H | -6.372346 | 2.571480 | -1.821023 |
| H | -5.309942 | 1.243389 | -2.339903 |
| H | 5.874233 | 2.735534 | 0.304675 |

| | | | |
|---|---|---|---|
| H | 7.127864 | 2.859649 | 1.150481 |

**ProD 1TS**

| | | | |
|---|---|---|---|
| C | -2.617666 | 1.440983 | 0.296984 |
| O | -1.571318 | -0.598029 | 0.002354 |
| C | -0.434249 | -1.143428 | 0.847965 |
| O | 0.312908 | -0.093580 | 1.265102 |
| C | 1.192628 | 0.477070 | 0.315412 |
| C | 0.819620 | 1.672666 | -0.291556 |
| C | 1.710479 | 2.248042 | -1.197834 |
| C | 2.930414 | 1.630506 | -1.478742 |
| C | 3.292834 | 0.434631 | -0.849631 |
| C | 2.409008 | -0.145301 | 0.066049 |
| C | 4.644305 | -0.190854 | -1.122342 |
| O | 4.497311 | -1.611217 | -1.055689 |
| N | 5.455645 | 0.049445 | 1.248410 |
| C | 5.946157 | -1.230281 | 1.749731 |
| C | 5.737851 | 0.319438 | -0.154240 |
| C | -3.829558 | 1.024110 | 0.138055 |
| O | -4.863669 | 1.694585 | 0.143469 |
| C | -3.947665 | -0.487032 | -0.067796 |
| C | -2.811910 | -1.298805 | -0.110334 |
| C | -2.843309 | -2.674279 | -0.280461 |
| C | -4.097679 | -3.278250 | -0.409044 |
| C | -5.261100 | -2.505913 | -0.379649 |
| C | -5.183588 | -1.120308 | -0.209347 |
| O | -3.992219 | 4.412701 | 0.615037 |
| H | -1.837564 | 0.319031 | 0.199174 |
| H | 6.705526 | -0.112756 | -0.481988 |
| H | 5.818793 | 1.402737 | -0.298039 |
| H | 7.026721 | -1.394665 | 1.578728 |
| H | 5.760486 | -1.292567 | 2.825967 |
| H | 5.363396 | -2.001977 | -1.233272 |
| H | 5.822274 | 0.802465 | 1.819436 |
| H | 3.607281 | 2.083860 | -2.198508 |
| H | 2.677441 | -1.063221 | 0.574170 |
| H | 1.443305 | 3.174573 | -1.696025 |
| H | -6.067369 | -0.491816 | -0.176062 |
| H | -6.228992 | -2.985571 | -0.485520 |
| H | -4.157098 | -4.353327 | -0.545611 |
| H | -1.933479 | -3.263208 | -0.324190 |
| H | -0.897887 | -1.633859 | 1.705014 |
| H | -0.142669 | 2.121834 | -0.067213 |
| H | 4.958005 | 0.106655 | -2.136986 |
| H | 0.086706 | -1.835359 | 0.181969 |

| | | | |
|---|---|---|---|
| H | 5.401714 | -2.045611 | 1.267975 |
| H | -4.309262 | 3.507740 | 0.434240 |
| H | -3.055033 | 4.253305 | 0.782406 |

**ProD2GM**

| | | | |
|---|---|---|---|
| C | 1.438231 | 1.522745 | 1.445555 |
| C | 0.408264 | 0.584462 | 1.328142 |
| C | 0.625634 | -0.562173 | 0.553960 |
| C | 1.849700 | -0.748698 | -0.096668 |
| C | 2.873343 | 0.192323 | 0.024637 |
| C | 2.661884 | 1.338050 | 0.805211 |
| N | -0.300601 | -1.561353 | 0.357432 |
| C | -1.435284 | -1.620309 | 1.204548 |
| O | -2.398796 | -0.641938 | 0.940111 |
| C | -3.105560 | -0.788427 | -0.310104 |
| C | -3.653182 | 0.616177 | -0.673787 |
| C | -4.982078 | 0.880943 | 0.040154 |
| C | -6.076048 | -0.075394 | -0.491547 |
| C | -5.492790 | -1.424953 | -0.974248 |
| C | -4.250599 | -1.820099 | -0.162888 |
| C | -2.599924 | 1.667294 | -0.375211 |
| O | -1.510682 | 1.504709 | -1.166032 |
| C | 4.219550 | -0.052227 | -0.637904 |
| O | 4.843302 | 1.165811 | -1.044176 |
| O | -2.684773 | 2.547038 | 0.451595 |
| C | 5.194790 | -0.772030 | 0.325187 |
| N | 6.444417 | -1.275278 | -0.219504 |
| C | 7.449390 | -0.289915 | -0.613966 |
| H | -4.826937 | 0.736163 | 1.114079 |
| H | -6.813165 | -0.251927 | 0.299920 |
| H | -4.512317 | -1.869829 | 0.900871 |
| H | -6.250638 | -2.212567 | -0.907379 |
| H | -0.841831 | 2.134247 | -0.847420 |
| H | -1.141386 | -1.477876 | 2.249817 |
| H | -1.828675 | -2.631980 | 1.053974 |
| H | -3.807135 | 0.628058 | -1.760724 |
| H | -5.279780 | 1.925429 | -0.083539 |
| H | -6.620129 | 0.397844 | -1.317067 |
| H | -2.388428 | -1.095570 | -1.077907 |
| H | -3.904099 | -2.817757 | -0.453524 |
| H | -5.221588 | -1.359588 | -2.035909 |
| H | 1.981958 | -1.644223 | -0.697474 |
| H | -0.550059 | 0.767416 | 1.797480 |

| | | | |
|---|---|---|---|
| H | 3.453548 | 2.074585 | 0.890890 |
| H | 1.269654 | 2.414062 | 2.043521 |
| H | 4.066357 | -0.711109 | -1.510474 |
| H | 4.648896 | -1.616579 | 0.763392 |
| H | 5.423853 | -0.080145 | 1.145194 |
| H | 4.225467 | 1.632319 | -1.623326 |
| H | 6.259681 | -1.916680 | -0.986047 |
| H | 7.666455 | 0.356423 | 0.243443 |
| H | 7.164640 | 0.361222 | -1.451250 |
| H | 8.375220 | -0.815747 | -0.871882 |

**ProD 2TS**

| | | | |
|---|---|---|---|
| C | 2.867289 | 2.131705 | -0.317291 |
| O | 2.219834 | 0.193506 | -0.924681 |
| C | 3.008421 | -0.429133 | 0.232046 |
| C | 3.259218 | -1.900390 | -0.084338 |
| C | 4.344959 | -2.430251 | 0.870048 |
| C | 5.703460 | -1.703573 | 0.657358 |
| C | 5.531861 | -0.365928 | -0.090690 |
| C | 4.293546 | 0.384697 | 0.404432 |
| C | 4.111294 | 1.806227 | -0.202731 |
| O | 5.098625 | 2.463670 | -0.500004 |
| C | 1.059111 | -0.554455 | -1.423103 |
| O | 0.199949 | -0.974237 | -0.421332 |
| C | -0.823596 | -0.106854 | -0.018208 |
| C | -2.102474 | -0.657878 | 0.039364 |
| C | -3.185035 | 0.124736 | 0.450518 |
| C | -2.959414 | 1.460966 | 0.809422 |
| C | -1.675110 | 1.997460 | 0.755336 |
| C | -0.588008 | 1.220358 | 0.345270 |
| C | -4.593747 | -0.447596 | 0.459080 |
| O | -5.381825 | 0.103159 | 1.512115 |
| C | -5.323939 | -0.139372 | -0.872620 |
| N | -6.593104 | -0.795981 | -1.123150 |
| C | -7.741058 | -0.383361 | -0.318307 |
| H | 2.040416 | 1.140198 | -0.775654 |
| H | 0.581973 | 0.143876 | -2.121298 |
| H | 1.441958 | -1.435914 | -1.932514 |
| H | 2.338136 | -0.317562 | 1.085677 |
| H | 2.338457 | -2.482411 | 0.015068 |
| H | 3.613518 | -1.999394 | -1.117415 |
| H | 3.998789 | -2.296900 | 1.902513 |
| H | 4.450191 | -3.509972 | 0.725365 |

| | | | |
|---|---|---|---|
| H | 6.394405 | -2.348264 | 0.102872 |
| H | 6.168655 | -1.521706 | 1.632725 |
| H | 6.397405 | 0.286178 | 0.042265 |
| H | 4.404562 | 0.542855 | 1.486736 |
| H | -2.235837 | -1.698165 | -0.243137 |
| H | -3.797009 | 2.062481 | 1.145860 |
| H | -1.504537 | 3.030044 | 1.043788 |
| H | 0.409158 | 1.648868 | 0.350289 |
| H | -4.982581 | -0.157613 | 2.352955 |
| H | -4.528569 | -1.544547 | 0.558535 |
| H | -4.637300 | -0.401908 | -1.687035 |
| H | -5.476326 | 0.946188 | -0.922485 |
| H | -6.483251 | -1.805760 | -1.096516 |
| H | -8.642351 | -0.854559 | -0.724983 |
| H | -7.866318 | 0.700748 | -0.410897 |
| H | -7.671560 | -0.614210 | 0.752976 |
| H | 5.451842 | -0.537018 | -1.171355 |

**ProD 3GM**

| | | | |
|---|---|---|---|
| C | 3.296172 | -2.178864 | -0.297354 |
| C | 4.004272 | -1.312677 | 0.783789 |
| C | 4.027755 | 0.106299 | 0.271541 |
| C | 2.832201 | 0.650990 | -0.026323 |
| C | 1.665973 | -0.312430 | 0.198130 |
| C | 1.908439 | -1.584369 | -0.654319 |
| N | 3.076104 | -1.312999 | 2.040543 |
| C | 1.682033 | -0.734800 | 1.686680 |
| O | 0.366849 | 0.296208 | -0.025216 |
| C | -0.154236 | 0.347578 | -1.346511 |
| O | -0.772245 | -0.844444 | -1.754709 |
| C | -2.024489 | -1.152849 | -1.261909 |
| C | -2.612984 | -0.517538 | -0.165806 |
| C | -3.900144 | -0.893727 | 0.245566 |
| C | -4.572967 | -1.916982 | -0.428472 |
| C | -3.966520 | -2.556709 | -1.511926 |
| C | -2.695146 | -2.180333 | -1.934121 |
| C | -4.554504 | -0.169673 | 1.415196 |
| C | -5.361168 | 1.065688 | 0.977084 |
| N | -4.594959 | 1.997953 | 0.164979 |
| C | -5.311498 | 3.237929 | -0.105290 |
| C | 2.726263 | 2.096505 | -0.390692 |
| O | 3.660345 | 2.764812 | -0.799056 |
| C | 5.398210 | -1.849085 | 1.113377 |

| | | | |
|---|---|---|---|
| O | 1.526018 | 2.677653 | -0.213101 |
| O | -3.604708 | 0.268422 | 2.380693 |
| O | 6.415590 | 2.064675 | -0.987069 |
| H | 4.960569 | 0.656850 | 0.171332 |
| H | 1.860939 | -1.342517 | -1.718034 |
| H | 3.931166 | -2.233957 | -1.186513 |
| H | 0.893364 | 2.001111 | 0.108583 |
| H | -0.872174 | 1.173906 | -1.341034 |
| H | 0.633970 | 0.535941 | -2.079706 |
| H | 1.101997 | -2.294175 | -0.452838 |
| H | 5.880957 | -1.242466 | 1.886841 |
| H | 6.043286 | -1.835959 | 0.229005 |
| H | 5.344816 | -2.880131 | 1.479031 |
| H | 3.192497 | -3.201086 | 0.084347 |
| H | 1.440758 | 0.136519 | 2.302633 |
| H | 0.886079 | -1.470301 | 1.840638 |
| H | 2.988946 | -2.341335 | 2.409394 |
| H | 3.548403 | -0.728832 | 2.836170 |
| H | -2.093500 | 0.247657 | 0.396182 |
| H | -5.563631 | -2.221054 | -0.101175 |
| H | -4.487097 | -3.356396 | -2.030648 |
| H | -2.209367 | -2.661107 | -2.776507 |
| H | -5.277708 | -0.859974 | 1.880491 |
| H | -5.670126 | 1.583353 | 1.893055 |
| H | -6.285963 | 0.709937 | 0.482299 |
| H | -3.081681 | -0.501165 | 2.642811 |
| H | -4.351346 | 1.549557 | -0.714231 |
| H | -4.715200 | 3.866420 | -0.773484 |
| H | -5.452690 | 3.790670 | 0.830289 |
| H | -6.309136 | 3.101646 | -0.563291 |
| H | 5.472942 | 2.313080 | -0.955132 |
| H | 6.836252 | 2.707803 | -0.402793 |

**ProD 3TS**

| | | | |
|---|---|---|---|
| C | -2.858698 | -2.576873 | -1.350178 |
| C | -2.135969 | -1.433870 | -1.001878 |
| C | -2.617167 | -0.532333 | -0.055867 |
| C | -3.865442 | -0.765797 | 0.542019 |
| C | -4.594328 | -1.907178 | 0.198012 |
| C | -4.089634 | -2.807622 | -0.743267 |
| N | -0.914242 | -1.290286 | -1.659111 |
| C | -0.305060 | -0.061927 | -1.704347 |
| O | 0.415336 | 0.239112 | -0.453469 |
| C | 1.740683 | -0.411051 | -0.080957 |

| | | | |
|---|---|---|---|
| C | 2.815522 | 0.658818 | -0.070380 |
| C | 3.995464 | 0.169613 | 0.341115 |
| C | 4.016723 | -1.301985 | 0.699772 |
| C | 3.518245 | -2.087947 | -0.551494 |
| C | 2.147432 | -1.538922 | -1.046297 |
| C | 1.559158 | -0.988501 | 1.332685 |
| C | 2.943920 | -1.511152 | 1.814202 |
| C | 2.539600 | 2.112007 | -0.435823 |
| O | 3.480578 | 2.908106 | -0.484675 |
| C | 5.393747 | -1.789699 | 1.151215 |
| C | -4.401868 | 0.237525 | 1.552972 |
| C | -5.163242 | 1.404249 | 0.895149 |
| N | -4.420715 | 2.042579 | -0.181933 |
| C | -4.958534 | 3.344755 | -0.561535 |
| O | 1.294793 | 2.378262 | -0.648738 |
| O | -3.362507 | 0.811419 | 2.337510 |
| O | 6.245001 | 2.658714 | -0.084710 |
| H | 0.640657 | 1.187535 | -0.472147 |
| H | 5.275287 | 2.726565 | -0.197439 |
| H | 6.565564 | 2.459769 | -0.971777 |
| H | -4.391068 | 3.739156 | -1.409492 |
| H | -4.837183 | 4.045098 | 0.271554 |
| H | -6.028520 | 3.336614 | -0.839052 |
| H | -5.327602 | 2.147070 | 1.683709 |
| H | -6.160373 | 1.037474 | 0.586906 |
| H | -5.126594 | -0.282535 | 2.200718 |
| H | -2.879541 | 0.088368 | 2.759981 |
| H | -2.057150 | 0.341154 | 0.252828 |
| H | -5.552892 | -2.098874 | 0.672609 |
| H | -4.656695 | -3.696066 | -1.003954 |
| H | -2.447951 | -3.259380 | -2.086268 |
| H | -1.004463 | 0.774218 | -1.787735 |
| H | 0.409744 | -0.070761 | -2.524918 |
| H | 0.810652 | -1.787647 | 1.302802 |
| H | 1.178433 | -0.201682 | 1.989087 |
| H | 2.251181 | -1.148614 | -2.061877 |
| H | 1.382871 | -2.319802 | -1.061451 |
| H | 3.429346 | -3.149791 | -0.295327 |
| H | 4.263372 | -2.010153 | -1.347912 |
| H | 2.887510 | -2.577291 | 2.062145 |
| H | 4.880749 | 0.797036 | 0.418028 |
| H | 5.732685 | -1.237427 | 2.033446 |
| H | 6.138933 | -1.643667 | 0.362595 |
| H | 5.373210 | -2.855242 | 1.405086 |
| H | -4.418531 | 1.430707 | -0.993496 |
| H | 3.258386 | -0.985165 | 2.719699 |

**ProD 4GM**

| | | | |
|---|---|---|---|
| C | -1.880370 | -1.209324 | 0.078365 |
| H | -0.913044 | -1.314117 | -0.042036 |
| O | 0.788793 | -1.221505 | -0.294902 |
| C | 1.226641 | 0.078996 | -0.221335 |
| C | 0.229510 | 1.060389 | -0.041819 |
| C | -1.210209 | 1.126186 | 0.078114 |
| C | -2.279271 | 0.065939 | 0.092415 |
| O | -3.454932 | 0.351844 | 0.132987 |
| H | -4.615533 | -1.278718 | -0.050697 |
| O | -5.133517 | -2.093576 | -0.144202 |
| H | -4.514117 | -2.768131 | 0.156603 |
| N | -1.612326 | 2.365697 | 0.223332 |
| O | -0.483165 | 3.176177 | 0.207210 |
| C | 0.616914 | 2.400368 | 0.048999 |
| C | 1.935254 | 2.847550 | -0.026638 |
| C | 2.889713 | 1.857608 | -0.197687 |
| C | 2.552706 | 0.490761 | -0.289416 |
| C | 1.596001 | -2.302410 | -0.836163 |
| O | 1.997992 | -3.190976 | 0.132486 |
| C | 3.071444 | -2.757887 | 0.965575 |
| H | 3.960127 | -2.513916 | 0.368872 |
| H | 2.788624 | -1.890935 | 1.571113 |
| H | 3.305818 | -3.592996 | 1.624699 |
| H | 2.427419 | -1.863405 | -1.398910 |
| H | 0.924796 | -2.843832 | -1.501679 |
| H | 3.349728 | -0.230482 | -0.404897 |
| H | 3.936770 | 2.132840 | -0.258986 |
| H | 2.185062 | 3.897687 | 0.044780 |

**ProD4TS**

| | | | |
|---|---|---|---|
| C | -1.739320 | -0.503634 | -0.371521 |
| N | -0.936692 | 0.590886 | -0.557836 |
| O | -0.334437 | 1.338724 | -0.388568 |
| C | -0.953206 | 2.105419 | 0.817524 |
| O | -1.793107 | 3.041296 | 0.355738 |
| C | -1.183004 | 4.205127 | -0.221571 |
| C | -1.274527 | -1.683629 | -0.121428 |
| O | -1.913512 | -2.716785 | 0.043983 |
| C | 0.246518 | -1.720502 | -0.017062 |
| N | 0.942058 | -2.816516 | 0.136484 |

| | | | |
|---|---|---|---|
| O | 2.309129 | -2.446133 | 0.190073 |
| C | 2.399606 | -1.103760 | 0.067428 |
| C | 1.114414 | -0.573227 | -0.062841 |
| C | 0.977819 | 0.806245 | -0.212569 |
| C | 2.088030 | 1.628942 | -0.231617 |
| C | 3.368875 | 1.053453 | -0.082441 |
| C | 3.553832 | -0.317245 | 0.069264 |
| H | 4.538592 | -0.755074 | 0.178094 |
| H | 4.234460 | 1.706966 | -0.097007 |
| H | 1.979165 | 2.698352 | -0.374430 |
| H | -0.068703 | 2.470385 | 1.348696 |
| H | -1.510430 | 1.332691 | 1.345667 |
| H | -0.699603 | 3.953084 | -1.169966 |
| H | -1.989779 | 4.914633 | -0.398997 |
| H | -0.452035 | 4.641707 | 0.469596 |
| H | -3.778163 | -2.172196 | 0.018566 |
| O | -4.698030 | -1.850747 | 0.000041 |
| H | -4.587137 | -0.972517 | -0.383875 |

**ProD5GM**

| | | | |
|---|---|---|---|
| O | 5.062547 | 0.513757 | -0.001538 |
| N | 4.277273 | -0.077442 | -0.002733 |
| O | 2.746450 | -1.109107 | -0.000949 |
| C | 1.817280 | -0.285661 | 0.000952 |
| O | 1.918318 | 0.989328 | 0.002799 |
| C | 0.383205 | -0.811877 | 0.000361 |
| N | 0.123827 | -2.096166 | 0.000410 |
| O | -1.295085 | -2.229044 | 0.000178 |
| C | -1.845696 | -0.987690 | 0.000040 |
| C | -0.827852 | -0.031135 | 0.000383 |
| C | -1.141255 | 1.353010 | -0.000218 |
| O | -0.207796 | 2.295770 | 0.000008 |
| C | -2.508651 | 1.682000 | -0.000701 |
| C | -3.500007 | 0.689466 | -0.000670 |
| C | -3.206497 | -0.675624 | -0.000463 |
| H | -3.973538 | -1.441007 | -0.000624 |
| H | -4.542487 | 1.000887 | -0.001077 |
| H | -2.779867 | 2.732908 | -0.001070 |
| H | 4.612903 | 1.369165 | -0.000318 |
| H | 0.720290 | 1.808080 | 0.000867 |

**ProD5TS**

| | | | |
|---|---|---|---|
| C | 1.443039 | 0.000000 | 1.932871 |
| N | 2.694185 | -0.047871 | 1.107438 |
| C | 3.797090 | 0.020118 | 1.622302 |
| C | 5.271532 | -0.033664 | 0.307483 |
| O | 5.962196 | -0.062548 | -0.376524 |
| C | 5.449521 | -0.206269 | -1.181356 |
| C | 2.569017 | -0.182739 | -0.218770 |
| H | 1.612240 | -0.204424 | -0.450488 |
| C | -0.685984 | 1.272605 | 2.003378 |
| C | -0.559777 | 1.285488 | 3.548972 |
| C | 1.537690 | 0.026681 | 3.273734 |
| C | 0.240203 | 0.041098 | 4.040995 |
| C | 0.436788 | 0.062620 | 5.557575 |
| C | -0.556101 | -1.220495 | 3.584993 |
| C | -0.700276 | -1.247414 | 2.039983 |
| H | -1.548329 | 1.274787 | 4.022224 |
| H | -0.208052 | 2.147642 | 1.554721 |
| H | -1.739101 | 1.286252 | 1.703170 |
| H | -0.247525 | -2.147420 | 1.613600 |
| H | -1.758044 | -1.246004 | 1.755024 |
| H | -0.040983 | -2.118326 | 3.939045 |
| H | -1.540479 | -1.207161 | 4.066599 |
| H | -0.526956 | 0.073316 | 6.077637 |
| H | 0.991137 | -0.818970 | 5.896302 |
| H | 0.996132 | 0.950900 | 5.869487 |
| H | 2.506727 | 0.027442 | 3.763974 |
| C | -1.212584 | -0.264586 | -0.716933 |
| O | -1.027845 | 0.090633 | -2.037615 |
| C | -0.937868 | 1.496431 | -2.262335 |
| H | -0.875034 | 1.634010 | -3.342554 |
| H | -0.048061 | 1.923614 | -1.788434 |
| H | -1.831280 | 2.013371 | -1.883793 |
| H | -2.037835 | 0.296389 | -0.250096 |
| H | -0.055270 | 2.195496 | 3.887043 |
| H | -1.432481 | -1.337140 | -0.70746 |

# تصميم أدوية مساعدة مبتكرة من لأموكسيسلن بالطرق الحسابية

***إعداد: وعد محمد صالح حوراني***

***إشراف: البروفيسور رفيق قرمان***

**الملخص**

تواجه العقاقير والأدوية المضادة للبكتيريا التي يتم تسويقها العديد من المشاكل مثل: المذاق المرّ وقلة الثبات مما يؤدي إلى عدم امتثال المريض للعلاج. وقد تم إيجاد تكنولوجيا طليعة الأدوية التي تساهم في حلّ مثل تلك المشاكل. وبناء على حسابات نظرية الكثافة الوظيفية التي تم تقديمها سابقاً، فقد تم تصميم طليعة الدواء ل الأموكسيلين 5-1. ومن المُعتقد بأن إزالة المذاق المُرّ الذي تتسم به الأدوية الأولية ينتج عن تبديل قدرة الدواء على التفاعل مع مستقبلات المذاق المرّ.

باستعمال الطرق الحسابية DFT molecular orbital على مستويات مختلفة من B3LYP 6-31G (d, p) وB3LYP/311+G (d, p) لعملية نقل البروتون ضمن جزئي في نموذج الإنزيم ل العالم كاربي واستغلال هذاالنموذج لتصميم طليعة الأموكسيسلن من دون طعم مر و ذو فعالية عالية للوصول إلى الدورة الدموية في الجسم مقارنة مع الدواء الأم.

لقد وجد أن معدل التحويل الداخلي ل الأموكسيسلن يتأثر بعملية نقل البروتون وان القوة الدافعة هي المسافة بين المركزين المتفاعلين و الزاوية بينهم و معدل حصول التفاعل يتناسب بشكل خطي مع كل منهما. وبالتالي فان معدل التحويل الداخلي ل طليعة الاموكسيسلن إلى الدواء الأم يمكن أن يجدول بالاعتماد على طبيعة الرابط الذي يتم ربطه بالدواء لعمل طليعة الدواء.