Deanship of Graduate Studies

Al-Quds University

Design of Novel 6-Aminocaproic acid Prodrugs by Computational Methods

Nidaa Mazein Salem Leqeanea

M. Sc. Thesis

Jerusalem-Palestine

1438/2017

Design of Novel 6-Aminocaproic acid Prodrugs by Computational Methods

Prepared By

Nidaa Mazein Salem Leqeanea

B. Sc., Pharmacy, Al-Quds University, Palestine

Supervisor

Prof. Dr. Rafik Karaman

A thesis submitted in partial fulfillment of requirements for the degree of Master of Pharmaceutical Science, Al-Quds University.

1438/2017

Al-Quds University

Deanship of Graduate Studies

Pharmaceutical Science Program

Thesis Approval

Design of Novel 6-Aminocaproic acid Prodrugs by Computational Methods

Prepared by: Nidaa Mazein Salem Leqeanea

Registration No.: 21213200

Supervisor: Prof. Dr. Rafik Karaman

Master thesis Submitted and Accepted, Date: .....

The names and signatures of the examining committee members are as follows:

1- **Head of Committee: Prof. Rafik Karaman** **Signature:**.....
2- **Internal Examiner: Dr. Hatem Hejaz** **Signature:**.....
3- **External Examiner: Dr. Naser Shraim** **Signature:**.....

...

Jerusalem–Palestine
1438/2017

Dedication

To my father and mother, My mentors and my heroes, Whom affection, love, encouragement and endless support made me what I am and guide me to such success and honor. “I'm only here today because of you”

To my Husband, Brothers and my Sisters, Who helped me all the way through this journey and supported me in every possible way and whom without this work would not be in the way it is now.

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: ……Nidaa……..

Nidaa Mazein Salem Leqeanea

Date: 26/8/2017

Acknowledgment

I would like to take this opportunity to express my deep gratitude to my adorable teacher and guide Dr. Rafik Karaman for all of his encouragement and sincere support throughout the work. This work would not be the way it is without you Dr., thank you.

Thanks to the examination committee members; for their valuable suggestions and comments, and for devoting some of their time to evaluate this work.

Finally, I thank anyone and everyone who has shown me friendship and kindness, encouragement, constructive criticism and time, anyone who ever believed in me.

Nidaa Leqeanea

Abstract

Background and objectives: Unraveling the mechanisms of a number of enzyme models has allowed for the design of efficient chemical devices having the potential to be utilized as prodrug linkers that can be covalently attached to commonly used drugs which can chemically, and not enzymatically, be converted to release the active drug in a programmable manner. For instance, exploring the mechanism for a proton transfer in Kirby‘s N-alkylmaleamic acids (enzyme model) has led to the design of a number of prodrugs such as tranexamic acid, acyclovir, atenolol.

Method: Based on density functional theory (DFT) calculations for the acid-catalyzed hydrolysis of several N-alkylmaleamic acid derivatives five 6-aminocapproic acid prodrugs were designed, aiming to provide a drug with potentially to have higher bioavailability than its parent drug.

Result and discussion: DFT calculations at B3LYP/6-31G(d,p) for intramolecular proton transfer in **1-7** and prodrugs of 6-aminocapproic acid, **ProD1-ProD5,** demonstrated that the reaction rate is dependent on the strain energy difference between the intermediate and the reactant ($E_{s\ INT\text{-}GM}$). This suggests that the reaction is governed by strain effect. Additionally, no correlation was found between the proton transfer efficiency and the distance between the two reactive centers (r_{GM}) and the attack angle (α).

Conclusion: Hence, the rate by which the prodrug releases the 6-aminocapproic acid drug can be determined according to the structural features of the promoiety (Kirby’s enzyme model).

Table of Contents

Declaration……i

Acknowledgment……ii

Abstract……...……...…iii

Table of contents……...iv

List of Tables……vii

List of Charts …….…….vii

List of Schemes ……...vii

List of Figures ……viii

List of Abbreviations …….…..ix

Chapter One: Introduction ……1-15

1.1 Prodrug ……..……...2

1.2 Computational method background…….….7

1.2.1 Ab nation method ……..7

1.2.2 Semi-empirical method ……...……..9

1.2.3 Density functional theory ……..……..10

1.2.4 Molecular mechanics ……,.…...…..11

1.3 6- Aminocapproic acid …….…..….12

1.4 Research problem ……..…….14

1.5 Thesis objectives ……..……..14

1.5.1 General objectives ……....15

1.5.2 Specific objectives ……..……15

1.6 Research questions …….……15

Chapter Two: Literature Review ……16-26

2.1 Intramolecular and intermolecular reaction……..…...17

2.2 Intramolecular processes used for design of potential prodrugs……...…...18

2.3 Computationally designed prodrug based on enzyme model…….…….19

2.4 Kirby's enzyme model based on the acid catalyzed hydrolysis of *N*-Alkylmaleamic acid …….……...22

Chapter Three: Computational Design Section…….……...…..27-33

3.1 Calculations programs ……...…….28

3.1.1 Arguslab……..…….28

3.1.2 Gaussian 2009 ……...……...29

3.1.3 Molden ……...……..30

3.2 Calculation methods ……31

3.2.1 6-Aminocapproic acid prodrugs calculation method……31

Chapter Four: Result and Discussion……...…...33-46

4.1 6-Aminocapproic acid prodrugs……..34

4.2 General consideration …….37

4.3 Optimized geometries of the entities involved in the proton transfer of 6-aminocapproic acid **ProD1-ProD5**.....39

4.3.1 Global minimum geometries ……40

4.3.2 Transition state geometries ……..41

4.3.3 Product geometries ……..42

4.4 Mechanistic investigation The DFT at B3LYP/6-31G (d,p) level of kinetic and thermodynamic properties for 6-aminocaproic acid prodrugs **ProD1–ProD5** ……..42

4.4.1 The role of the strain energy on the rate of the proton transfer in processes 6-aminocaproic acid **ProD1-ProD5**……..…….44

Chapter Five: Conclusion and Future Direction ……..…….47-49

5.1 Conclusion……..48

5.2 Future Direction …….48

References :…….49-55

Supplementary Material ……..……56-90

Abstract in Arabic……. 91

List of Tables

Table No.	Title	Page
Table 1	DFT (B3LYP) calculated properties for the proton transfer reactions of in 6-aminocaproic acid **ProD1-ProD5**.	43
Table 2	DFT (B3LYP/6-31 G (d, p)) calculated kinetic and thermodynamic properties for the proton transfers in 6-aminocaproic acid **ProD1-ProD5** and **1-7**.	44

List of Charts

Chart No.	Title	Page
Chart 1	Schematic representation of the reactants in the proton transfers of 6-aminocaproic acid **ProD1-ProD5**. GM is the global minimum structure , r_{GM} is the O-H distance in the GM, α is the angle of attack (Hydrogen bonding) O1H2O3in the GM.	38

List of Schemes

Scheme No.	Title	Page
Scheme1	Proposed mechanism for the acid-catalyzed hydrolysis of maleamic acids.	24

List of Figure:

Figure 1: Chemical structure of 6-aminocaproic acid……12

Figure 2: Chemical structure of tranxmic acid …….13

Figure 3: Acid-catalyzed hydrolysis of maleamic acids **1-7**……..23

Figure 4: Anhydrid linkers used in the five 6 aminocapproic acid prodrug……..25

Figure 5: Proposed 6-aminocapproic acid prodrugs…….....37

Figure 6a**:** DFT optimized structures for the global minimum (**GM**) structures in the intramolecular proton transfer reaction of 6-Aminocapproic acid **ProD1-ProD 5**…...…40

Figure 6b**:** DFT optimized structures for the transition state (**TS**) structures in the intramolecular proton transfer reaction of 6-Aminocapproic acid **ProD1-ProD5**……...41

Figure 6c: DFT optimized structures for the linker product **(P)** structures in the intramolecular proton transfer reaction of6-Aminocapproic acid **ProD1-ProD 5**…...…42

Figure 7a: Plot of the ES for intermediates of **1-7** N-alkylmaleamic acid vs. relative rate (log k rel) ……...…46

Figure 7b: Plot of the DFT calculated $\Delta G^{\ddagger}$ vs. relative rate (log krel) in **1-7** N-alkylmaleamic acid…….……...46

Figure 7c: Plot of the MM2 calculated difference in strain energies between intermediates and reactants (ES ($_{INT-GM}$)) vs. relative rate (log krel) in N-alkylmaleamic acids **1-7**…….....…46

Figure 7d: Plot of the DFT calculated $\Delta G^{\ddagger}$ vs. MM2 calculated difference in strain energies between intermediates and reactants (ES ($_{INT-GM}$)) in 6-aminocapproic acid **ProD 1- ProD 5**……..…….……46

List of Abbreviation:

Abbreviation	Definition
ADEPT	Antibody direct enzyme prodrug therapy
DFT	Density functional theory
GP	Gas phase
GM	Global minimum
H_2O	Water
HF	Hartree -fock method
HLB	Hydrophilic lipophilic balance
H	Enthalpy
MM	Molecular mechanic
P	Product
ProD	Prodrug
QM	Quantum mechanic
r_{GM}	Distance in global minimum
S	Entropy
T	Tempreture
UFF	Universal Force Field
$\Delta G^{\ddagger}$	Activation energy
A	Attack angel
TS	Transition state
INT	Intermediate

UV	Ultra violet
Vis	Visible
GC	Gradient corrected
VDEPT	Virus direct enzyme prodrug therapy

Chapter One

Introduction

Chapter one

Introduction

1.1 Prodrug

The term prodrug was first introduced by Albert, by using pharmacologically inactive chemical moiety which can be used to temporarily change the physicochemical properties of a drug, in order to increase its usefulness and decrease its associated toxicity. The prodrug design usually implies a covalent link between a drug and a chemical entity [1]. Generally, *in vivo*, prodrugs can be enzymatically or chemically degraded to furnish the parent active drug which exerts its therapeutic effect. Ideally the aim of prodrug usage that have to be achieved, is the conversion of the prodrug to the parent drug and a non-toxic moiety, followed by the subsequent rapid elimination of the released linker group [2, 3].

Rationale of a Prodrug Design

The optimization of absorption, distribution, metabolism, and excretion properties (ADME) are the rationale behind the use of prodrugs. In addition, prodrug could be used to increase the selectivity of drugs for their intended target. Development of a prodrug with improved properties may also represent a life-cycle management opportunity. In order to increase the utility of biologically active compounds, the prodrug approach can be used as a very versatile strategy, because one can optimize any of the ADME properties of potential drug candidates [4]. Generally, some prodrugs contain a promoiety (linker) that is removed by an enzymatic or chemical reaction, while other prodrugs undergo molecular modification such as an oxidation or reduction reaction to release their active drugs. In some cases, the first promoiety linked to the parent drug molecule is attached to a second linker to have a double prodrug. These linkers are usually different each from other and are cleaved by different mechanisms. At other case, two biologically active drugs can be linked together in a single molecule called a codrug. In a codrug, each drug acts as a promoiety for the other [5].

The Prodrug Design Can Be Utilized In The Following

(i) Improving the solubility and consequently bioavailability of the active drug; more than 30% of drug discovery compounds have poor aqueous solubility. The rate-limiting step to absorption may be the dissolution of the drug molecule from the dosage form [6]. In order to increase the aqueous solubility of the parent drug molecules, the prodrug approach can be used via attached ionizable or polar neutral functions, such as phosphates, amino acids, or sugar moieties to the active drug. So, the dissolution rate are will be improved [7]. These prodrugs can be used not only to enhance oral bioavailability but also to prepare parenteral or injectable drug delivery.

(ii) Increasing permeability and absorption; the efficacy of the drug is affected by membrane permeability [3]. Unfacilitated and largely nonspecific passive transport mechanisms are the most common absorption routes in oral drug delivery. The hydrocarbon moiety modification, can be used to enhance the lipophilicity of poorly permeable drugs. In such cases, the prodrug strategy can be an extremely valuable option. Improvement of the lipophilicity has been the most widely investigated and successful field in prodrug research. This improvement can be achieved by masking polar ionized or nonionized functional groups. So, oral or topical absorption is expected to be enhanced [8].

(iii) Distribution profile modification; before the drug reaches its physiological target and exerts the desired effect. Thus, several pharmaceutical and pharmacokinetic barriers have to be bypassed.

(iv) Fast metabolism and excretion prevention; the total amount of active drug that reaches the systemic circulation and consequently its target, may be greatly reduced by the first-pass effect in the gastrointestinal tract and liver. Sublingual or buccal administration or controlled release formulations could be used to overcome this problem. The prodrug strategy can also be used to prevent fast metabolic drug degradation . This is usually done by masking the metabolically labile but pharmacologically essential functional group(s) of the drug.

(v) Reduction of the toxicity; the structure or function of cells, tissues, and organs which can be detrimental to the organism can be changed by adverse drug reactions. Targeting drugs to desired cells and tissues via site-selective drug delivery is often used to reduce the toxicity; also in sometimes this can be accomplished by altering one or more of the ADME barriers. A successful site-selective prodrug must be precisely transported to the site of action, where it should be selectively and quantitatively transformed into the active drug, which is retained in the target tissue to produce its therapeutic effect [9]. The opportunities for selective drug delivery and targeting may be diminished by the ubiquitous distribution of most of the endogenous enzymes that are responsible for prodrugs bioactivation. Therefore, antibody-directed enzyme prodrug therapy or genes that encode prodrug activating enzymes are used to deliver exogenous enzymes selectively. This approach is particularly used with highly toxic compounds in order to reduce the toxicity of the drugs at other sites in the body such as anticancer drugs [10, 11].

The prodrug approach strategy is facing two major challenges

(1) Hydrolysis of prodrugs by esterases; prodrugs undergoing *in vivo* cleavage to the active drug by catalysis of hydrolases such as peptidases, phosphatases, and carboxylesterases. The hydrolysis is considered as the aim of most common prodrugs approaches [8]. The main challenge with prodrugs susceptible to esterase hydrolysis is the less than complete absorption which observed with several hydrolase-activated prodrugs of penicillins, cephalosporins, and angiotensin converting enzyme inhibitors. Due to their premature hydrolysis during the absorption process in the enterocytes of the gastrointestinal tract, these prodrugs typically have bioavailabilities around 50% [8]. The active drug upon hydrolysis is released inside the enterocytes, in most cases it is more polar and less permeable than the prodrug and is more likely to be effluxes by passive and carrier-mediated processes back into the lumen than to proceed into blood, therefore limiting oral bioavailability.

(2) Bioactivation of the prodrug by cytochrome P450 enzymes. The variability in prodrug activation and thus to the efficacy and safety of drugs using P450s bioactivation

pathwayis due to genetic polymorphisms of a prodrug activating P450s [12]. Because there are many intrinsic and extrinsic factors that can influence the process, bioconversion of prodrugs is perhaps the most vulnerable link in the chain. For example, genetic polymorphisms, age-related physiological changes, or drug interactions, which leads to adverse pharmacokinetic, pharmacodynamics, and clinical effects, may lead to decrease or increase in the activity of many prodrug activating enzymes. Besides that, there are wide interspecies variations in both the expression and function of the major enzyme systems activating prodrugs, and these can pose challenges in the preclinical optimization phase [13].

Nonetheless, searching for an entirely new therapeutically active agent with suitable ADMET properties is more difficult and complicated than developing a prodrug. Chemical and enzymatic stability, solubility, low clearance by the liver or kidney, permeation across biological membranes, potency, and safety, all these specific properties have to be found in an ideal drug candidate. So, prodrug development can still be a more feasible and faster strategy [14].

In the prodrug approach, the conversion of a prodrug to the parent drug at the target site is crucial, to be successful approach. Generally, metabolisms by enzymes that are distributed throughout the body are involved in the activation. These prodrugs have a major problem, which is the difficulty in predicting their bioconversion rates, and thus their pharmacological or toxicological effects. In addition, the rate of hydrolysis is not always predictable, and various factors such as age, health conditions and gender can affect the bioconversion [15].

In order to minimize or eliminate the undesirable drug physicochemical properties while maintaining the desirable pharmacological activity, there are two major prodrug design approaches that are considered as widely used among all other approaches [16]. The first 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 unwanted drug properties. Considerable knowledge of particular enzymes or carriers, including their molecular and functional characteristics are required for this type of "targeted-prodrug" [17]. Antibody-

directed enzyme prodrug therapy (ADEPT) or antibody-directed catalysis is an example for such approach. In this strategy, antigens expressed on tumors cells are utilized to target enzymes to the tumor site. At the beginning in such approach, an enzyme-antibody conjugate is administered and given sufficient time to interact with tumor cells and to be eliminated from the circulation. Subsequently, a prodrug is given and selectively activated extracellular at the tumor site [18].

Gene-directed enzyme prodrug therapy (GDEPT) and virus-directed enzyme prodrug therapy (VDEPT) are alternative approaches designed to overcome the limitations of (ADEPT) [19]. In these approaches, prodrug administration follows genes encoding prodrug-activating enzymes which are targeted to tumor cells. In GDEPT, for gene targeting non-viral vectors that contain gene-delivery agents, such as peptides, cationic lipids or naked DNA, are used [20]. In VDEPT, viral vectors are used for gene targeting, the most commonly used viruses are retroviruses and adenoviruses. For both GDEPT and VDEPT, the vector has to be taken up by the target cells, and the enzyme must be stably expressed in tumor cells. This process is called transduction [21]. Insufficient transduction of tumor cells in vivo has limited the effectiveness of GDEPT and VDEPT.

The chemical design approach is the second 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.

There are two main classes of prodrugs: (1) carrier linked prodrugs, and (2) bioprecursor prodrugs. In the carrier-linked prodrugs, the active molecule (the drug) is temporary linked to a carrier (also known as a promoiety) through a bio-reversible covalent linkage. When the carrier-linked prodrug is exposed to physiological environment, it undergoes biotransformation, releasing the parent drug and the carrier. Ideally, the carrier should be non-immunogenic, easy to synthesize at a low cost, stable under the conditions of prodrug administration, and undergo biodegradation to inactive metabolites [22].

Based on the site of conversion into the pharmacologically active agent, the prodrugs can be additionally classified into two groups:

• Type I – metabolized intracellularly. Type IA prodrugs (e.g., acyclovir, cyclophosphamide, 5-fluorouracil, L-DOPA, zidovudine) are metabolized at the cellular targets of their therapeutic actions. Type IB prodrugs (e.g., carbamazepine, captopril, molsidomine, primidone) are converted to parent drugs by metabolic tissues, namely by the liver.

• Type II – metabolized extracellularly. Type IIA – in the milieu of the gastrointestinal fluid (e.g., loperamide oxide, sulfsalazine). Type IIB – within the circulatory system and/or other extracellular fluid compartments (e.g., aspirin, bambuterol, fosphenytoin). Type IIC – near or inside therapeutic target/cells (ADEPT, GDEPT) [22].

Currently, 5–7% of the approved drugs worldwide can be classified as prodrugs, and approximately 15% of all new drugs approved in 2001 and 2002 were prodrugs.

1.2 Computational Methods Background

Few decades ago, computational methods for calculating molecular properties of ground and transition states has been used by organic, bioorganic and medicinal chemists, in order to solve chemical problems. These computational methods use principles of computer science, for calculating the structures, physical and chemical properties of molecules. Theoretical results emerged from these methods, incorporated into efficient computer programs.

Today, for predicting structure-energy calculations for drugs and prodrugs alike, quantum mechanics (QM) such as *ab initio*, semi-empirical, density functional theory (DFT), and molecular mechanics (MM) are commonly and increasingly being used, and broadly accepted as precise tools [23].

Both static and dynamic situations can be handled by the above mentioned computational methods. In all cases when the studied system‘s size increase, the computer time, memory and disk space increase drastically. [24]

1.2.1 Ab initio methods

Three decades ago, *ab initio* quantum chemistry has become important tool in the study of atoms, molecules and, increasingly, in modeling complex systems such as those arising in biology and materials science. The computational solution of the electronic Schrodinger equation is the underlying core technology. In this equation, by means of a well-defined, automated approximation, the positions of a collection of atomic nuclei, and the total number of electrons in the system can be determined, it is also can be used to calculate the electronic energy, electron density, and other properties. For systems containing tens, or even hundreds, of atoms, the ability to obtain "good-enough" solutions to the electronic Schrodinger equation has revolutionized the ability of theoretical chemistry to address essential problems in a wide range of disciplines; the Nobel Prize awarded to John Pople and Walter Kohn in 1998 is a reflection of this observation [25].

Ab initio methods are based entirely on theory from first principles. They are generally useful only for small systems. The ab initio molecular orbital methods, such as HF, G1, G2, G2MP2, MP2, MP3 and MP4 are based on rigorous use of the Schrodinger equation with a number of approximations. When all approximations are sufficiently small in magnitude and when the finite set of basis functions tends toward the limit of a complete set. Ab initio electronic structure methods have the advantage that they can be made to converge to the exact solution. The convergence is usually not monotonic, and sometimes the smallest calculation gives the best result for some properties. But The disadvantage of *ab initio* methods is their enormous computational cost, they take a significant amount of computer time, memory, and disk space [26].

Schrodinger equation: is a fundamental equation of physics for describing quantum mechanical behavior. It is also often called the Schrödinger wave equation, and it is a partial differential equation that describes how the wave-function of a physical system evolves over time.

$$\hat{H}\Phi = E\Phi$$

H is the *Hamiltonian Operator* of the system (a set of operations which enables one to calculate its energy), Φ is the *Wave-function* which describes the positions and motions of all the particles (nuclei and electrons) of the system, and *E* is the energy.

To solve this equation, one needs to make a few approximations. The first is called the Born-Oppenheimer approximation, and consists in assuming that the nuclei do not move on the timescale of electron motion. Therefore one can find wave-functions and energies for the electrons at a given, "frozen", nuclear configuration.

1.2.2. Semi-empirical methods

Semi-empirical method make many approximations, and obtain some parameters from empirical data, thus they are less accurate methods. The semi-empirical quantum methods depend on the Hartree–Fock formalism and they are very important in computational chemistry for treating large molecules. Semi-empirical calculations are much faster than their ab initio counterparts. If the molecule being computed is not close enough to the molecules in the data base used to parameterize the method, their results can be inaccurate. The most used semi-empirical methods are MINDO, MNDO, MINDO/3, AM1, PM3 and SAM1 [27].

Despite their limitations, semi-empirical methods allow a study of systems that are out of reach of more accurate methods, so they are often used in computational chemistry. For example, molecules consisting of thousands of atoms could be studied by modern semi-empirical programs, while ab initio calculations that obtain similar thermochemical accuracy are feasible on molecules consisting of less than 50-70 atoms. Semi empirical calculations may be useful in several situations, such as:

1. Computational modeling of structure-activity relationships to gain insight about reactivity or property trends for a group of similar compounds.

2. Development and testing of new methodologies and algorithms, for example development of hybrid quantum mechanics / molecular mechanics (QM/MM) methods for modeling of biochemical processes.

3. Checking for gross errors in experimental thermochemical (e.g. heat of formation) data.

4. In many applications where qualitative insight about electronic structure and properties is sufficient.

In general, the result of semi-empirical can be trusted only in situations when they are known to work well (e.g. systems similar to molecules in the parameterization set) and strong caution should be taken in cases where semi-empirical methods are known to fail (e.g. prediction of activation barriers).

1.2.3 Density functional theory

The density functional theory (DFT) is another commonly used quantum mechanical modeling method in physics and chemistry. It is used to investigate the electronic structure (principally the ground state) of many-body systems. With this theory, functionals can be used to determine the properties of many-electron systems. Hence, the name density functional theory comes from the use of functionals of the electron density. Unlike the *wave-function*, which is not a physical reality but a mathematical construct, electron density is a physical characteristic of all molecules. So, DFT is among the most popular and versatile methods available in condensed-matter physics, computational physics, and computational chemistry. The DFT method is used to calculate structures and energies for medium-sized systems (30–60 atoms) of biological and pharmaceutical interest and is not restricted to the second row of the periodic table [28].

A *functional* is defined as a function of a function, and the energy of the molecule is a functional of the electron density. The electron density is a function with three variables– x-, y-, and z-position of the electrons. Unlike the wave-function which becomes significantly more complicated as the number of electrons increases, the determination of the electron density is independent of the number of electrons.

DFT is widely used computational method, and can be applied to most systems. Like all computational methods, DFT methods are more useful for some types of calculations than others. DFT methods, unlike *ab initio* methods, can be used for calculations

involving metals. Hybrid methods, such as B3LYP, are often the method of choice for reaction calculations. Some DFT methods are specifically designed for specific applications, such as the MPW1K hybrid method, designed for determination of kinetics problems.

The most significant advantage to DFT methods is a significant increase in computational accuracy without the additional increase in computing time. DFT methods such as B3LYP/6-31G(d) are oftentimes considered to be a standard model chemistry for many applications [29].

The use of DFT method is significantly increasing but some difficulties still encountered when describing intermolecular interactions, especially van der Waals forces (dispersion); charge transfer excitations; transition states and some other strongly correlated systems. Incomplete treatment of dispersion can adversely affect the DFT degree of accuracy in the treatment of systems which are dominated by dispersion. Also, there is a challenge in determining the most appropriate method for a particular application. The practitioner should, prior to choosing a DFT method, consult the literature to determine the suitability of that choice for that particular problem or application. In general practice (including educational environments), the B3LYP/6-31g(d) model chemistry is considered by most to be a good general-purpose choice [28].

1.2.4 Molecular mechanics

Molecular mechanics is a mathematical approach used for the computation of structures, energy, dipole moment, and many other physical properties. Many diverse biological and chemical systems are widely calculated by this method such as proteins, large crystal structures, and relatively large solvated systems. However, determination of parameters such as the large number of unique torsion angles present in structurally diverse molecules make a limitation for this method [30].

Molecular mechanics can also be used to supply the potential energy for molecular dynamics computations for large molecules. But, they are inappropriate for bond-breaking reactions.

In order to investigate functional mechanisms of biological macromolecules based on their 3D and electronic structures, Ab initio methods have been utilized. The size of the system, which ab initio calculations can handle, is relatively small despite the large sizes of bio-macromolecules surrounding solvent water molecules. Accordingly, ab initio calculations can be used in isolated models of areas of proteins such as active sites. However, the regulation of electronic structures and geometries of the regions of interest have been affected by the disregarded proteins and solvent surrounding the catalytic centers.

Quantum mechanics/molecular mechanics (QM/MM) calculations are utilized to overcome the above discrepancies, in this type of calculations the system is divided into QM and MM regions, where QM regions correspond to active sites to be investigated and are described quantum mechanically. MM regions which are described molecular mechanically are correspond to the remainder of the system. Warshel and Levitt were the pioneer in this work of the QM/MM method [31], and since then, there has been much progress on the development of a QM/MM algorithm and applications to biological systems [32].

1.3 6-Aminocaproic acid

6-Aminohexanoic acid (Figure 1) is a synthetic lysine analog that suppresses fibrinolytic activity by fitting into plasminogen´s lysine-binding site and preventing the binding of plasminogen to fibrin [33].

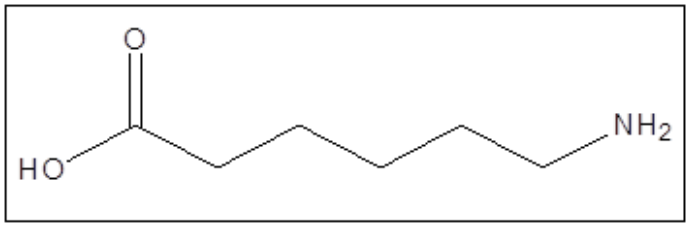


Figure 1: Chemical structure of 6-aminocaproic acid

Other anti-fibrinolytic drugs that are available are aprotinin, and tranexamic acid, which used intraoperatively, postoperatively, and during extracorporeal membrane oxygenation (ECMO) to reduce bleeding and minimize the need for exogenous blood products. However, because of risk of renal failure, cardiac failure, stroke, or encephalopathy that is associated with the use of aprotinin, the latter was removed from the market. Hence, 6-aminocaproic acid and tranexamic acid are potentially safer alternatives. In the UK, tranexamic acid, a tissue plasminogen and plasmin inhibitor, is most commonly used, with evidence for benefit in cardiac, orthopaedic, urological, gynaecological, and obstetric surgery. In the USA, 6-aminocaproic acid, which also inhibits plasmin, is commonly used [34].

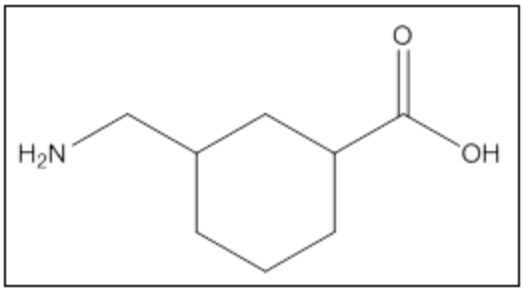


Figure 2: chemical structure of tranexamic acid

Studies on this drug have showed a clinical benefit in decreasing mortality and morbidity in many traumatic cases, and in different types of surgery, like coronary artery bypass grafting in which the administration of 6-aminocaproic acid resulted in a significant decrease in blood loss and blood transfusion requirements [35]. Also, the administration

of this drug can reduce blood loss and consequently transfusion and transfusion-related risk in patient who undergoes hip replacement [36] .

As well as, it can reduce the incidence of secondary hemorrhage following traumatic hyphema [37]. It plays an important role in dental extraction in patients with hemophilia [38], and in control hemorrhage in patients with amegakaryocytic thrombocytic thrombocytopenia [39] .

In adults, oral absorption appears to be a zero-order process. Renal excretion is the primary route of elimination, whether aminocaproic acid is administered orally or intravenously. Sixty-five percent of the dose is recovered in the urine as unchanged drug and 11% of the dose appears as the metabolite adipic acid. The terminal elimination half-life is approximately 2 hours.

1.4 Research problem

The bioavailability of 6-aminocaproic acid is only 24%. This low value is attributed to the amino acid nature of the drug. At physiological pH, 6-aminocaproic acid exists mainly in the ionized form; this ionization decreases the ability of 6-aminocaproic acid to be transferred to the systemic blood circulation. This limited permeation results in poor bioavailability of the anti-bleeding drug [40].

Thus, using the prodrug approach to increase 6-aminocaproic acid bioavailability can be utilized. This can be achieved by making a covalent linkage between 6-aminocaproic acid and a non-toxic promoiety that increases the lipophilicity of 6-aminocaproic acid.

The proposed prodrugs were designed such that the prodrug will be chemically intraconverted to 6-aminocaproic acid and a non-toxic moiety in a rate which is only dependent on the structural features of the inactive linker.

1.5 Thesis objective

1.5.1 General objective

To design 5 prodrugs of 6- aminocaproic acid which have better bioavailability than 6-aminocaproic acid, using a variety of different molecular orbital and molecular mechanics methods and correlations between experimental and calculated reactions rates.

1.5.2 Specific objective

►Calculations of Kirby's enzyme model mechanism for the design of 6-aminocaproic acid prodrugs which have the following properties:

1- Converted to 6-aminocaproic acid in a controlled manner.
2- The linker attached to the drug moiety and the whole 6-aminocaproic acid prodrug moiety should be non-toxic and safe.
3- Enhanced bioavailability compared to the naked 6-aminocaproic acid.

1.6 Research questions

- Would the DFT and ab inito methods be capable of predicting reaction rates similar to that obtained by Kirby?
- Would the DFT calculations be good methods for a design of 6-aminocaproic acid prodrugs that have the potential to increase the bioavailability of the active drug and be cleaved in physiological environments to furnish the active drug and a non-toxic moiety?

Chapter Two

Literature Review

Chapter Two

Literature Review

2.1 Inter- and Intramolecular Chemical Processes

There are two kinds of forces, or attractions that operate in molecule intramolecular and intermolecular forces. Intermolecular forces: The forces holding molecules together and play important roles in determining the properties of substances. Intermolecular forces are particularly important in terms of how molecules interact and form biological organisms or even life.

Bulk properties such as the melting points of solids and the boiling points of liquids are determined by intermolecular forces. When the molecules in liquids have enough thermal energy to overcome the intermolecular attractive forces that hold them together, the boiling happened, thereby forming bubbles of vapor within the liquid. Similarly, solids melt when the molecules acquire enough thermal energy to overcome the intermolecular forces that hold them into place in the solid.

Intermolecular forces arise from the interaction between positively and negatively charged species, so, they are electrostatic in nature. Like covalent and ionic bonds, intermolecular interactions are the sum of both attractive and repulsive components [41].

There are three major types of intermolecular interactions:

1- Dipole–dipole interactions: arise from the electrostatic interactions of the positive and negative parts of molecules with permanent dipole moments; their strength is proportional to the magnitude of the dipole moment and to $1/r^6$, where r is the distance between dipoles.
2- London dispersion forces: the formation of instantaneous dipole moments in polar or nonpolar molecules as a result of short-lived fluctuations of electron charge distribution is the main cause for London force formation. As a result temporary formation of an induced dipole in adjacent molecules occurred. The

outer electrons of larger atom are less tightly bound and are therefore more easily perturbed, so, larger atoms tend to be more polarizable than smaller ones.

3- Hydrogen bonds: are especially strong dipole–dipole interactions between molecules that have hydrogen bonded to a highly electronegative atom, such as O, N, or F. The resulting partially positively charged H atom on one molecule (the *hydrogen bond donor*) can interact strongly with a lone pair of electrons of a partially negatively charged O, N, or F atom on adjacent molecules (the *hydrogen bond acceptor*).

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 [42].

2.2 Intramolecular Processes Used for the Design of Potential Prodrugs

Many organic chemists and biochemists have been inspired in the striking efficiency of enzyme catalysis, 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 have the ability to replace natural enzymes in the conversion of prodrugs to their parent drugs.

Enzymes are mandatory for the interconversion of many prodrugs to their active parent drugs. In this bioconversion of the prodrugs, there are important enzymes involved which include amides, such as, trypsin, chymotrypsin, elastase, carboxypeptidase, and aminopeptidase, and esters, such as paraoxonase, carboxylesterase, cetylcholinesterase and cholinesterase. 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.

In intramolecular processes (enzyme models) approach, the intraconversion of a prodrug to its active parent drug does not require enzyme catalysis. The release rate of the active drug is determined only by the factors playing dominant role in the rate limiting step of the intraconversion process. All disadvantages associated with prodrug interconversion

by enzymes have the potential to be eliminated by using this approach. As discussed before, prodrug bioconversion is perhaps the most vulnerable link in the chain, since many intrinsic and extrinsic factors can affect the interconversion process.

2.3 Computationally Designed Prodrugs Based On Enzyme Models

Over the past sixty years, many pioneering studies have been vastly contributed in understanding how enzymes catalyze biochemical transformations. Today, the scientific community consensus that enzyme catalysis is based on the combination effects between the catalysis by functional groups and the ability to reroute intermolecular reactions through alternative pathways by which substrates can bind to pre organized active sites.

In general, enzymatic reaction rates are 10^{10} to 10^{18} fold more than their non-enzymatic bimolecular counterparts. The substrate binding within the confines of the enzyme active site is the main cause to the tremendous rate acceleration which manifested by enzyme. The main driving force and contributor to catalysis is the binding energy of the resulting enzyme substrate complex. It is assumed that this binding energy is used to overcome the physical and thermodynamic factors that make barriers to the reaction (free energy) in all bio-transformations catalyzed by enzymes [43-46].

The chemists are fascinated by the high rates of intramolecular reactions, because they remind them of the efficiency of enzyme catalysis and it is broadly believed that a common source is, at least for a significant part, responsible for both enthalpic and entropic effects. A number of chemists and biochemists have been prompted by the similarity between intramolecularity and enzymes in order to design chemical models based on intramolecular reactions consisting of two reacting centers to understand the mode and the mechanism by which enzymes exert their high catalytic activities [47-49] .

In the past five decades proposals have been made from attempts to interpret changes in reactivity versus structural variations in intramolecular systems. Among these proposals:

(i) Koshland ˝orbital steering˚ that suggests a rapid intramolecularity comes from a severe angular dependence of organic reactions, such as in the lactonization of rigid hydroxy acids [50]

(ii) ″proximity‟ in intramolecular processes (near attack conformation) model as proposed by Bruice and demonstrated in the lactonization of di-carboxylic acids semi-esters [51, 52]

(iii) ″stereopopulation control‟ based on the concept of freezing a molecule into a productive rotamer as advocated by Cohen [53-55]

(iv) Menger‘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 [56-58]

(v) 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 [49, 59, 60].

Investigation on intramolecularity have played essential role in elucidating the chemistry of functional groups involved in enzyme catalysis as well as in unraveling the mechanisms proposed for particular processes. Thus, it is highly believed that these investigations have the potential to provide an adequate understanding of how efficiency depends on structure in intramolecular catalysis which in turns could shed light on related problems in enzyme catalysis. In addition, understanding these intramolecular processes can provide a basis for a design of prodrugs that have the ability to release their active parent drugs in predicted manner.

At *Intramolecular reactions* the functional groups bring together on the same molecule, to model what goes on when an enzyme brings together the same functional groups in its active site [61]. The distance between the two reacting centers determine the nature of the reaction (intermolecular or Intramolecular). *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 [62].

Karaman's group advocate The mechanisms of some intramolecular processes to understand enzyme catalysis (enzyme models) in order to design novel prodrug linkers [63]. Among the studied intramolecular processes:

- Acid-catalyzed lactonization of hydroxy-acids as researched by Cohen [64] and Menger[65].
- S_N2-based ring closing reactions as studied by Brown, Bruice, and Mandolin [66].
- Proton transfer between two oxygens in Kirby's acetals [67, 68], and proton transfer between nitrogen and oxygen in Kirby's enzyme models [67, 68] and proton transfer from oxygen to carbon in some of Kirby's enol ethers[69].

The recent studies of Karaman group on intramolecularity have demonstrated that there is a necessity to further explore the reaction mechanisms in order to determine the factors affecting the reaction rate such as (1) The driving force for enhancements in rate for intramolecular processes are both entropy and enthalpy effects. In the cases by which enthalpic effects were predominant such as ring-cyclization and proton transfer reactions proximity or/and steric effects were the driving force for rate accelerations. (2) The distance between the two reactive centers determine the nature of the reaction being intermolecular or intramolecular. (3) In SN2-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. (4) Accelerations in the rate for intramolecular reactions are a result of both entropy and enthalpy effects, and (5) in Kirby's acetal systems, an efficient proton transfer between two oxygens and between nitrogen and oxygen were affordable when strong hydrogen bonds are developed in the products and the corresponding transition states leading to them [63, 65, 66]. Unraveling the reaction mechanism would allow for better design of an efficient chemical device to be utilized as a prodrug linker that can be covalently linked

to a drug which can chemically, but not enzymatically, be cleaved to release the active drug in a programmable manner.

2.3 Kirby's Enzyme Model Based on the Acid Catalyzed Hydrolysis of N-Alkylmaleamic Acids

Proton transfer reactions are the most common processes catalyzed by enzymes. Scientists have encouraged exploiting intramolecularity in modeling enzyme catalysis due to the fact that reactions of an enzyme active site and substrate are between functional groups held in a close proximity. Both, enzymes and intramolecularity are similar in that the reacting centers are held together, noncovalently with the enzymes, and covalently with the intramolecular process. The tremendous high efficiency of enzymes catalysis depends on a combination of some factors that most of them have been recognized but none of them was fully understood. Although the devoted research to the chemistry of enzyme catalysis is growing rapidly a number of several factors remain to be studied [70, 71].

The acid-catalyzed hydrolysis of **1-7** maleamic acid (Figure 3) was kinetically investigated by Kirby et al. The study demonstrated that the cleavage of amide bond is due to intramolecular nucleophilic catalysis by the adjacent carboxylic acid group and the tetrahedral intermediate breakdown is the rate-limiting step (scheme 1) [72]. In 1996, the reaction was computationally investigated by Katagi using AM1 semi-empirical calculations. In contrast to what was suggested by Kirby, Katagi's study demonstrated that the rate- limiting step is the formation of the tetrahedral intermediate and not its dissociation [73]. Later on, Kluger and Chin have experimentally researched the mechanism of the intramolecular hydrolysis process utilizing several *N*-alkylmaleamic acids derived from aliphatic amines with a wide range of basicity [74]. The study findings demonstrated that the identity of the rate-limiting step is function of both the basicity of the leaving group and the solution acidity [75].

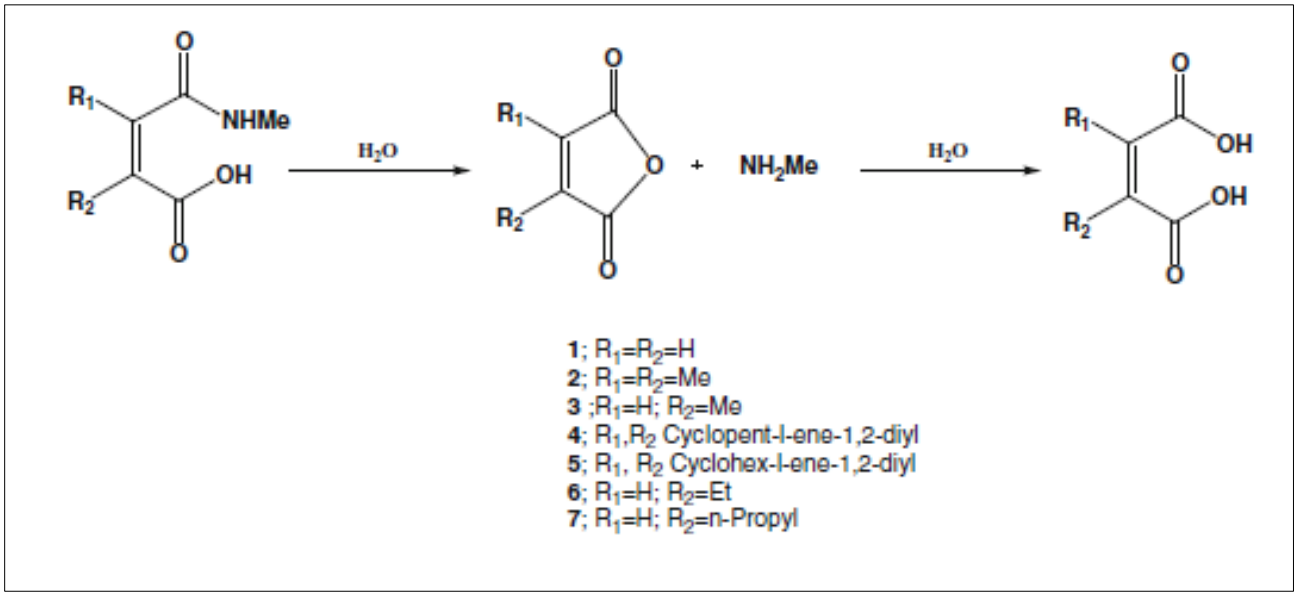
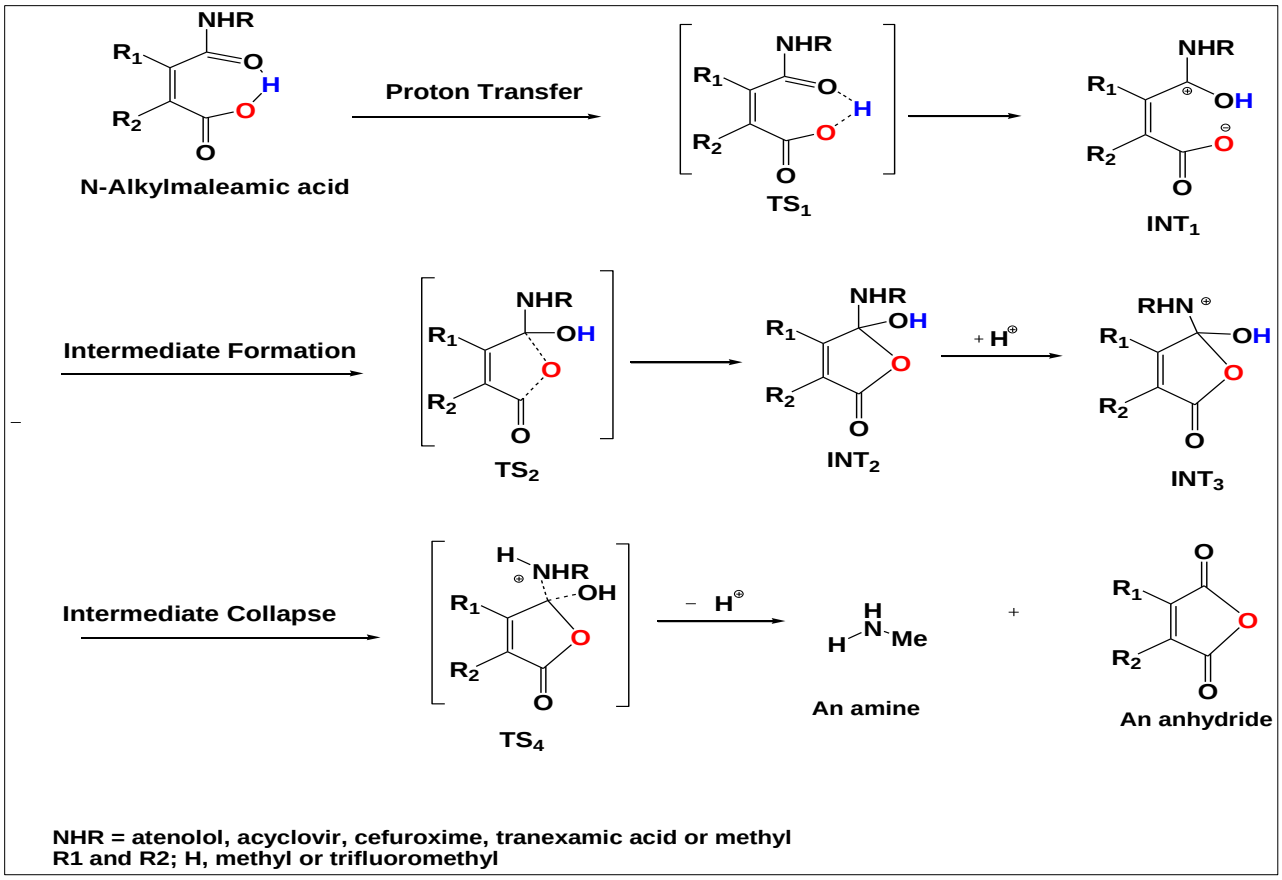


Figure (3): Acid-catalyzed hydrolysis of *N*-alkylmaleamic acids **1-7**



Scheme 1: Proposed mechanism for the acid-catalyzed hydrolysis of *N*-alkylmaleamic acids.

Moreover, the calculations demonstrate 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 rate-limiting step is the dissociation of the tetrahedral intermediate. When the leaving group (methylamine) in **1-7** was replaced with a group having a low pKa value the hydrolysis in water was the formation of the tetrahedral intermediate which is the rate-limiting step .

In addition, the calculations revealed that the efficiency of the intramolecular acid-catalyzed hydrolysis by the carboxyl group is remarkably sensitive to the pattern of substitution on the carbon–carbon double bond; 1) difference between strain energy

between intermediate and product and strain energy between intermediate and reactant; 2) distance between hydroxyl oxygen of the carboxylic group and amide carbonyl carbon and 3) the attack angle. 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 [49, 76-78].

In order to design novel prodrugs , Kirby's enzyme model have been utilized [72] , a mechanistic study using DFT calculation methods at B3LYP/6-31G (d,p), B3LYP/311+G (d,p) levels and hybrid GGA (MPW1k) on an intramolecular acid catalyzed hydrolysis of maleamic (4-amino-4-oxo-2- butenoic) acids (Kirby's *N*-alkylmaleamic 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; and (3) dissociation of the tetrahedral intermediate to provide products (scheme 1).

Based on the calculation results of Kirby`s model (proton transfer in *N*-alkylmaleamic acids) we propose five 6-aminocaproic acid prodrugs using 5 linkers (Figure 4)

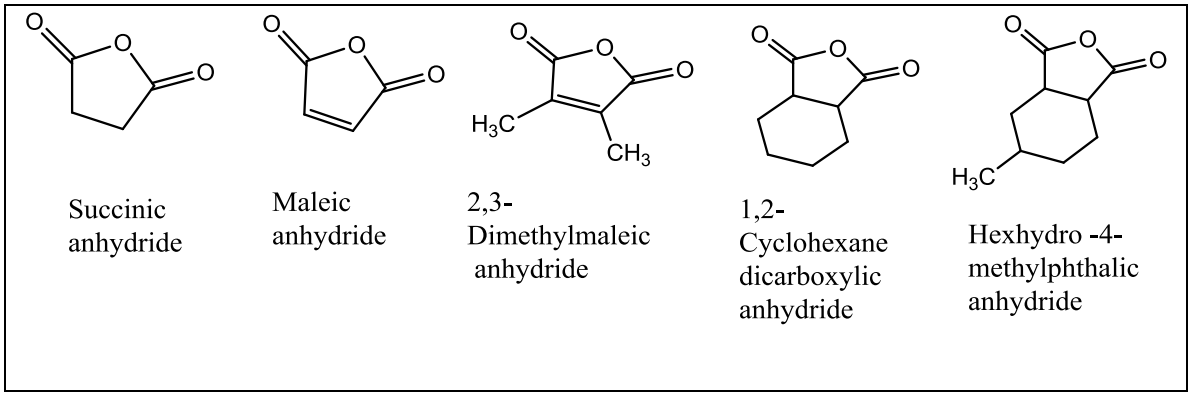
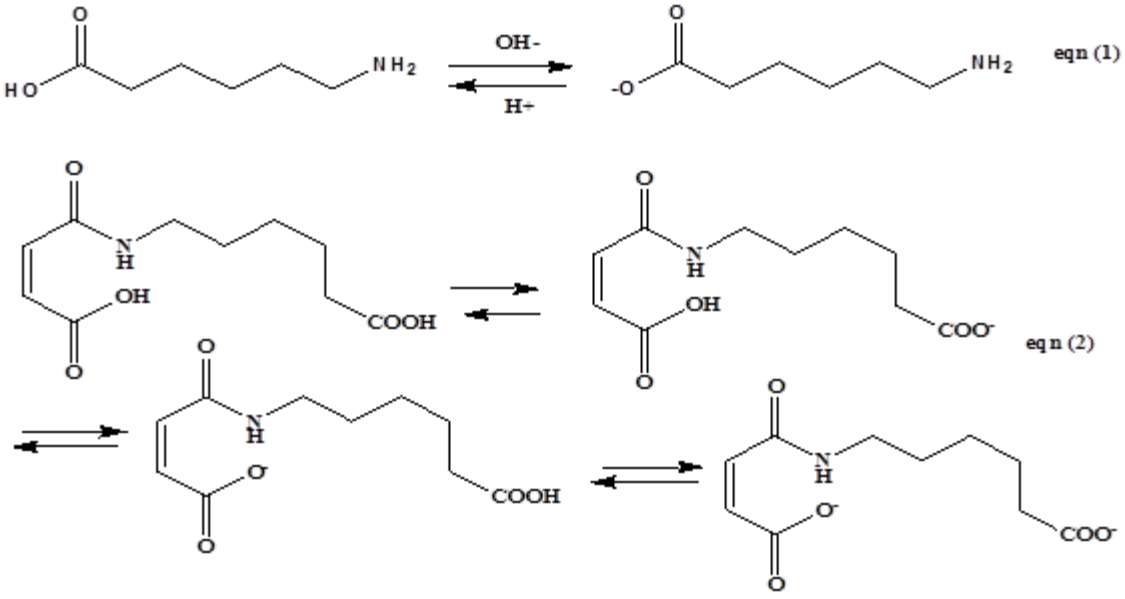


Figure 4: anhydride linkers used in the five 6 aminocaproic acid prodrug.

As shown in (equation 2), 6-aminocaproic acid **ProD1-ProD5** have a carboxylic group (hydrophilic moiety) and a lipophilic moiety (the rest of the prodrug), where the combination of both moieties secures a relatively modified HLB.



In most of the physiologic environments (pH 1- 8.0) 6-aminocaproic acid will exist primary in the ionized forms (equation 1) while its prodrugs, 6-aminocaproic acid **ProD1**-**ProD5**, will equilibrate between the ionic and the free acid forms (equation 2) especially in a physiological environment of pH 5.5-6.8 (intestine). Thus, it is expected that 6-aminocaproic acid **ProD1- ProD5** may have a better bioavailability than the parent drug due to neutralizing the ionized amine group which results in absorption improvement. In addition, these prodrugs may be used in different dosage forms (i.e. enteric coated tablets, topical use and etc.) because of their potential solubility in organic and aqueous media due to the ability of the carboxylic group to be converted to the corresponding carboxylate anion in a physiological pH of around 6.0.

Chapter Three

Methodology

Computational Design Section

Chapter Three

Computational (Design) section

Calculation Programs and method

3.1 Calculation Programs

The following calculation programs were used in the prodrugs design:

1-ArgusLab

2-Guassian 2009

3-Molden

3.1.1 Argus lab

Argus Lab is a very versatile freeware, This software was originally intended to perform only semi-empirical quantum mechanical (QM) calculations on small molecules, but has now grown to be used for several types of computational chemistry experiments as well as molecular-docking experiments which is useful for biological chemists.

Argus Lab can be used easily to do molecular mechanics (MM) (UFF/Amber force fields) and QM semi-empirical (MINDO, AMI, PM3) , it can be used also, for many calculations such as evaluating single-point energies for fixed geometry, geometry optimization in the ground state, and more importantly obtain UV-VIS spectra of delocalized systems in gas phase and in various dielectric environments [79].

There are two main modules: Molecule Building Option, and Calculation Option.

Molecule Building, Modifications and Visualization: from Builder Tool you choose Tools . Any molecule can be made from this toolbox by using the mouse. If you want to add atoms or molecular moieties (such as a benzene ring), select that, then right click on the visualizing panel to do so. Anything you want to do to the molecule that you are building can be done by left click. You can select an atom or a bond (should turn yellow),

and pressing the Ctrl-key allows you to select multiple atoms/bonds [80]. Double-Clicking on the molecule selects the whole molecule that was built. These operations can be performed only if the "selection" mode is clicked. Changing atoms and their state of hybridization or the nature of the bonds can be done by selecting them and doing a right-click to choose from the various available options. Hydrogen atoms in the molecule can be filled by just clicking "H" button.

In Arguslab many pre-made sample (molecules) are existed , and you may choose from them to use as starting points. SAVE the file as a xyz coordinate file [81] .

Many option are available in Arguslab , such as, rotations, translations, and zooming options through mouse clicks on icons on the upper left. In addition you can measure distances between atoms, and obtain angles/dihedral angle between three/four atoms in the molecule.

In computational Chemistry many calculation can be done using Argus lab, such as semi-empirical calculations like MINDO and AMI, a single point energy calculation, quantum chemistry (Hartree-Fock SCF) and molecular mechanics calculations. Geometry optimization using QM methods is slower, semi-empirical methods like MINDO, AMI and PM3 are pretty fast. After geometry optimization, you can do UV-Vis spectra calculation, which would be done using MINDO-CI(S). Save your calculation (.agl) after it is done. The outputs of the calculations can be found in the "molecule tree-view" option under "Tools" menu [82].

3.1.2 Gaussian 2009

Gaussian program is an electronic structure package capable of predicting many properties of atoms, molecules and reactive systems, such as ; molecular energies , structures , vibrational frequencies , electron densities , by utilizing ab initio, density functional theory, semi-empirical, molecular mechanics, and various hybrid methods.

Gaussian 09 which is used by chemists, chemical engineers, biochemists, physicists and other scientists worldwide is the latest version of the Gaussian series of electronic structure programs. By using the fundamental laws of quantum mechanics, Gaussian 09

can predict the energies, molecular structures, vibrational frequencies and molecular properties of molecules and reactions in a wide variety of chemical environments. Gaussian 09's models can be applied for both stable species and compounds which are difficult or impossible to observe experimentally (e.g., short-lived intermediates and transition structures) [83].

In Gaussian 09 you can find the most advanced modeling capabilities available today, so, it significantly expand the range of problems and systems which can be studied through it, many new features and enhancements which are available.

Gaussian 09 provide many calculation method or function which include MP2, HF, B3LYP, RHF that are found in basis set . In addition there is many calculation tool and Options , for example, SCF=QC, Freq=Raman, SP, Opt=(MaxCycle=N, TS) [84].

3.1.3 Molden

Molden is a general molecular and electronic structure processing program. So, it can be described as a package for displaying molecular density from the ab initio packages GAMESS-UK , GAMESS-US and GAUSSIAN, and the Semi-empirical packages Mopac/Ampac, in addition it supports a number of other programs via the molden Format.
All the information required from the GAMESS / GAUSSIAN output file can be read by molden. Also it is capable of displaying molecular orbitals, the electron density and the molecular minus atomic density [85].

Either the spherically averaged atomic density or the oriented ground state atomic density can be subtracted for a number of standard basis sets. Molden supports contour plots, 3-d grid plots with hidden lines and a combination of both.

It can write a variety of graphics instructions; postscript, XWindows, VRML, povray, OpenGL, tekronix4014, hpgl, hp2392 and Figure

Atom typing and firing optimisation jobs can be done automatically and interactively from within Molden. The powerful Z-matrix editor which is available in Molden give full control over the geometry and allows to build molecules from scratch, including polypeptides**.**

3.2 calculation method

3.2.1 Method type

There are roughly three types, or categories, of density functional methods.

Local density approximation (LDA) methods assume that the density of the molecule is uniform throughout the molecule, and is typically not a very popular or useful method.

Gradient corrected (GC) methods look to account for the non-uniformity of the electron density.

Hybrid methods, as the name suggests, attempt to incorporate some of the more useful features from *ab initio* methods (specifically Hartree-Fock methods) with some of the improvements of DFT mathematics. Hybrid methods, such as B3LYP, tend to be the most commonly used methods for computational chemistry practitioners.

3.2.2 6-Aminocaproic acid prodrug calculation method

The Becke three-parameter, hybrid functional combined with the Lee, Yang, and Parr correlation functional, denoted by B3LYP, were employed in the calculations using density functional theory (DFT). The DFT calculations at B3LYP/6-31G (d,p) and B3LYP/311+G (d,p) levels, MP2 calculations, and all other calculation were carried out using the quantum chemical package Gaussian-2009 [86-89].

The restricted Hartree-Fock method was used for all calculations were carried. The Argus Lab program was used to obtain the starting geometries of all calculated molecules [90]

and were initially optimized at the HF/6-31G level of theory, followed by optimization at the B3LYP/6-31G (d,p) level. All internal rotations were included in total geometry optimizations. Second derivatives were estimated for all 3N-6 geometrical parameters during optimization.

An energy minimum (a stable compound or a reactive intermediate) has no negative vibrational force constant. The global minimum search was achieved by 360 rotation of the carboxylic group about the C6–C7 bond (i.e. variation of the dihedral angle O1C7C6C5). A transition state is a saddle point which has only one negative vibrational force constant [91]. At first the normal reaction coordinate method was used to locate the transition states [92]. At this method the enthalpy changes were monitored by stepwise changing the interatomic distance between two specific atoms. Then the energy gradient method at the B3LYP/6-31G (d,p) level of theory was used to reoptimize the geometry at the highest point on the energy profile [92].

The "reaction coordinate method" was used to calculate the activation energy for 6-aminocaproic acid **ProD1-ProD5**. In this method, one bond length is constrained for the appropriate degree of freedom while all other variables are freely optimized.

The activation energy values for the proton transfer processes 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" [92].

The transition state structures were verified by their only one negative frequency. Full optimization of the transition states was accomplished after removing any constrains imposed while executing the energy profile. The activation energies obtained from DFT at B3LYP/6-31G (d,p) level of theory for 6- aminocaproic acid **ProD1-ProD5** were calculated in gas phase and water phase [93-96].

Chapter Four

Result and Discussion

Chapter Four

Results and Discussion

4.1 6-Aminocapproic acid prodrugs

In order to design efficient prodrugs, there is a crucial need to further explore mechanisms for many intramolecular processes. An accurate design of an efficient chemical device that can be used as a prodrug linker which covalently linked to a drug that can chemically convert into the active parent drug, would be provided by exploration of the reaction mechanism.

Many prodrugs were designed by using different prodrugs linkers by using the enzyme model of Kirby's *N*-alkylmaleamic acids, such as tranexamic acid (to treat bleeding conditions) [97], cefuroxime (antibacterial) [67] and acyclovir (anti-viral drug to treat Herpes Simplex) prodrugs [77] . Additionally, exploring the mechanism of Menger's Kemp acid enzyme model has led to the design of dopamine prodrugs for the treatment of Parkinson's disease [98]. In addition , prodrugs for masking the bitter taste of atenolol (anti-hypertensive) [99, 100] and paracetamol (pain killer) were designed, synthesized and their kinetics were studied [101] .

In addition the calculations study on Kirby's acetals mechanism led to a design of novel prodrug such as, aza-nucleoside (to treat myelodysplastic syndromes) [102], phenylephrine (decongestant) [103], atovaquone [104-106] and statins (to treat high cholesterol levels in the blood) [76]. In these prodrugs, the hydroxyl group of the active drug was linked to the acetal moiety, upon the exposure of such prodrug to physiological environment; it has the potential to convert to its parent active form with rates that are solely determined on the structural features of the linker (Kirby's acetal).

The results of DFT calculation on Kirby enzyme model of *N*-alkylmaleamic acids which done by Karamans group were found to be in accordance with that reports by Kirby and Lancaster [61] and Kluger and Chin [73]. The findings demonstrated that the hydrolysis

of *N*-alkylmaleamic acids occurs by one mechanism, but the rate-limiting step was found to be largely dependent on the medium solvent and on the nature of the amine leaving group. According to these results two different rate-limiting steps were proposed: one involves the formation of a tetrahedral intermediate [74], and the second involves the dissociation of the tetrahedral intermediate [61, 73].

The DFT calculations which were done in gas phase showed that the rate-limiting step for the hydrolysis of all maleamic acid amides studied is a tetrahedral formation regardless of the nature of the amine leaving group. On the other side, when the DFT calculations were done in a dielectric constant of 78.39 (water) the rate- limiting step for the hydrolysis of acid amides having primary amine as a leaving group was the dissociation of the tetrahedral intermediate, whereas the rate-limiting step for that having acyclovir or cefuroxime moieties was the tetrahedral intermediate formation.

Since the bioavailability of 6-aminocaproic acid is only 24% due to the amino acid nature of the drug. At physiological pH, 6-aminocaproic acid will exist mainly in the ionized form; this ionization will decrease the ability of 6-aminocaproic acid to be transferred to the systemic blood circulation. This limited permeation results in poor bioavailability of the anti-bleeding drug. In my thesis I proposed five 6-aminocaproic acid prodrugs (Figure.5) based on proton transfers in Kirby's N-alkylmaleamic acids enzyme model.

The 6-aminocaproic acid prodrugs, **ProD1- ProD5**, have a carboxylic acid group (hydrophilic moiety) and a lipophilic moiety (the rest of the molecule), where the combination of both groups ensures a modified HLB. It should be noted that the HLB value will be determined upon the physiologic environment by which the prodrug is dissolved. For example, for prodrugs intended to be given as solutions or syrups to children or pediatrics , prodrug will reach the stomach and it will primarily exist in the carboxylic acid form whereas in the blood circulation the carboxylate anion form will be predominant. Because the linker (Kirby's enzyme model) undergoes fast hydrolysis at low pH such as the stomach, it is planned that **ProD1- ProD5** 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). 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). The experimental determined pK_{a1} for 6-aminocaproic acid **ProD1- ProD5** linkers is in the range of 4-6. Therefore, it is expected that the pK_as of the corresponding prodrugs will have similar pK_a range as for the carboxylic linkers. Since the pH for the small intestine lies in the range of 5.5-6.8, the calculated unionized (acidic) /ionized ratio will be in the range of 10-50%. Although the percentage of the acidic form is not significantly high, we expect that these prodrugs to undergo an efficient proton transfer to yield the anti-fibrinolytic drug, 6-aminocaproic acid.

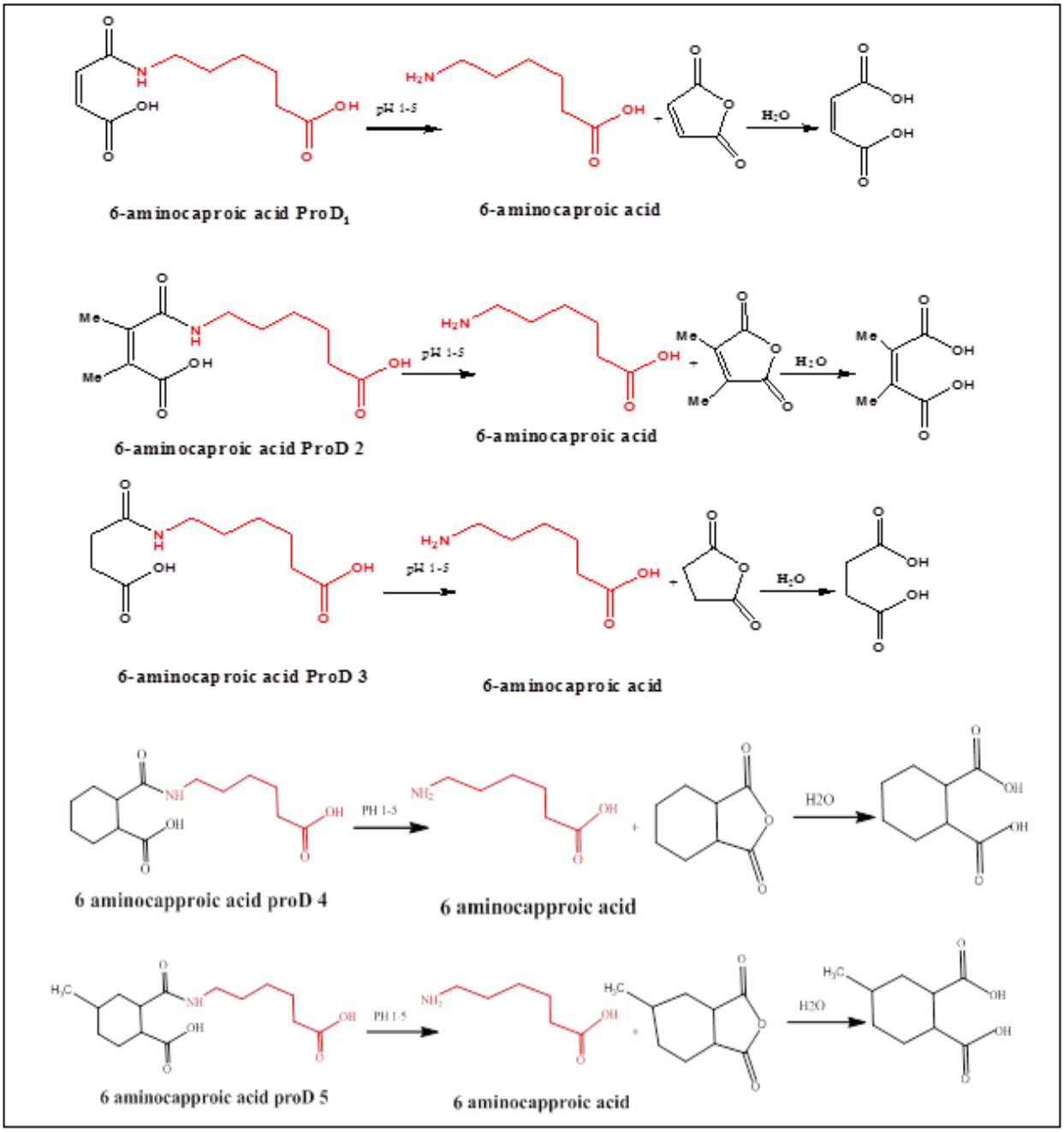


Figure 5: proposed 6-aminocaproic acid prodrugs

4.2 General consideration

The orientation of the carboxylic acid affects the ground state energy of the *N*-alkylmaleamic acid moiety especially when there is a possibility for engagement in

intramolecular hydrogen bonding. Therefore, the identification of the most stable conformer (global minimum) for each of Kirby's *N*-alkylmaleamic acids **1–7** and 6-aminocaproic acid prodrugs **ProD1–ProD5** are cruicially important. The global minimum search was achieved by 360 rotation of the carboxylic group about the C6–C7 bond (i.e. variation of the dihedral angle O1C7C6C5) and calculation of the energies of the resulting conformers.

In the DFT calculations of the starting geometries in 6-aminocapproic acid ProD1-**ProD5**, two different types of conformations were considered: one in which the carboxylic hydroxyl proton is *syn* to the amide group (Chart 1) and another in which it is *anti* (Chart 1). The global minimum search for 6-aminocapproic acid **ProD1**- **ProD5** revealed that all of them exist in the *syn* orientation (Figure 6a) except for **ProD3** which exists in anti-orientation.

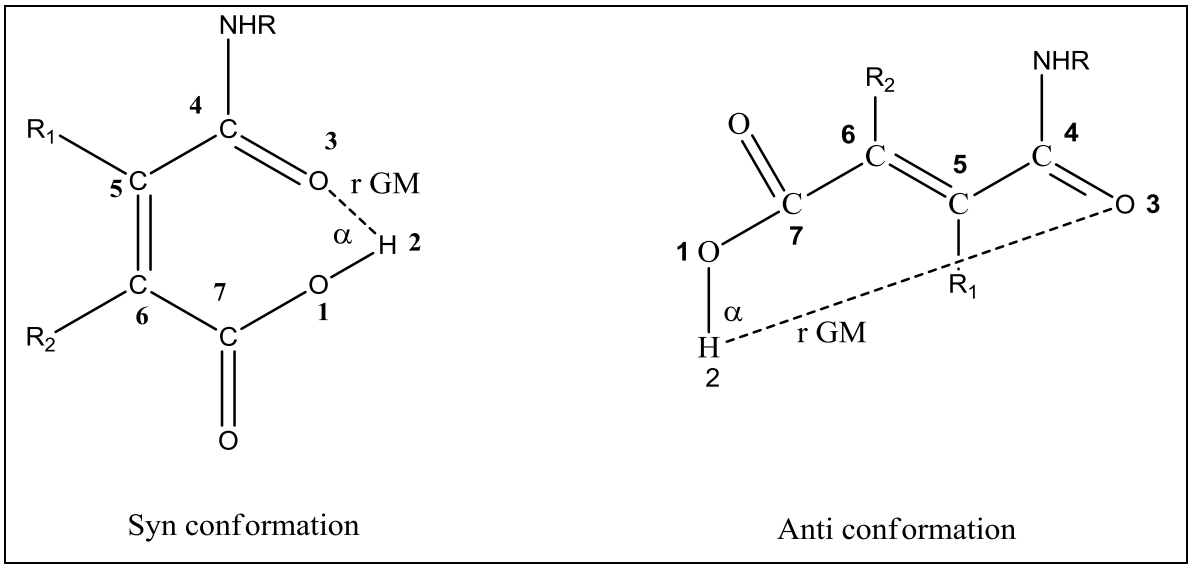


Chart 1**:** Schematic representation of the reactants in the proton transfers of 6-aminocaproic acid **ProD1-ProD 5**. GM is the global minimum structure, r_{GM} is the O—H distance in the GM. α, is the angle of attack (hydrogen bonding) O1-H2-O3 in the GM.

4.3 Optimized structures for the entities involved in the acid-catalyzed hydrolysis of 6-Aminocaproic acid prodrugs ProD1–ProD5

4.3.1 Global minimum geometries (GM)

Global minimum structures (GM): The global minimum structures for **ProD1 GM–ProD 5 GM** are illustrated in Figure.6. Careful inspection of the calculated structures in Figure.6a reveals that all the global minima of the reactants, except **ProD3 GM**, exist in conformation by which their carboxyl group is engaging intramolecularly in a hydrogen bonding net to form a seven-membered ring.

Further, the DFT calculated angle α values in the global minimum structures (**ProD1 GM–ProD5 GM**) were in the range of 58.4°, 51.6 °,13.6 °, 48.9 °, 48.8 °respectively whereas the DFT calculated hydrogen bonding length (r_{GM}) were in range of 5.3 Å , 5.6 Å, 5.7 Å, 4.8 Å, 4.5 Å, respectively.

4.3.2 Transition state geometries (TS)

The optimized DFT calculated transition state geometries for 6-aminocapproic acid **ProD1 TS –ProD5 TS** are illustrated in Figure 6b.

4.3.3 Product geometries (P)

The DFT calculated geometries for the products of 6- aminocapproic acid **ProD1 P – ProD5 P** are illustrated in Figure 6c.

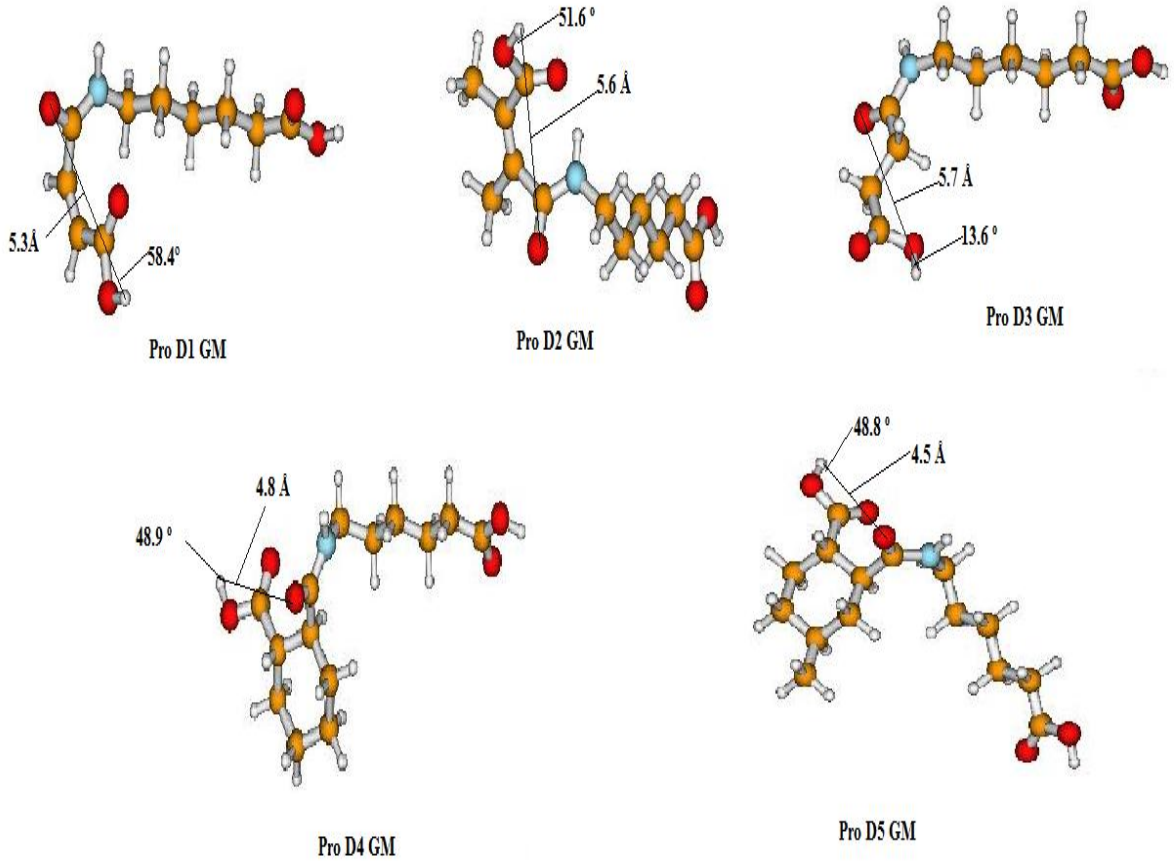


Figure 6a**:** DFT optimized structures for the global minimum (**GM**) structures in the intramolecular proton transfer reaction of 6-aminocaproic acid **ProD1-ProD5**.

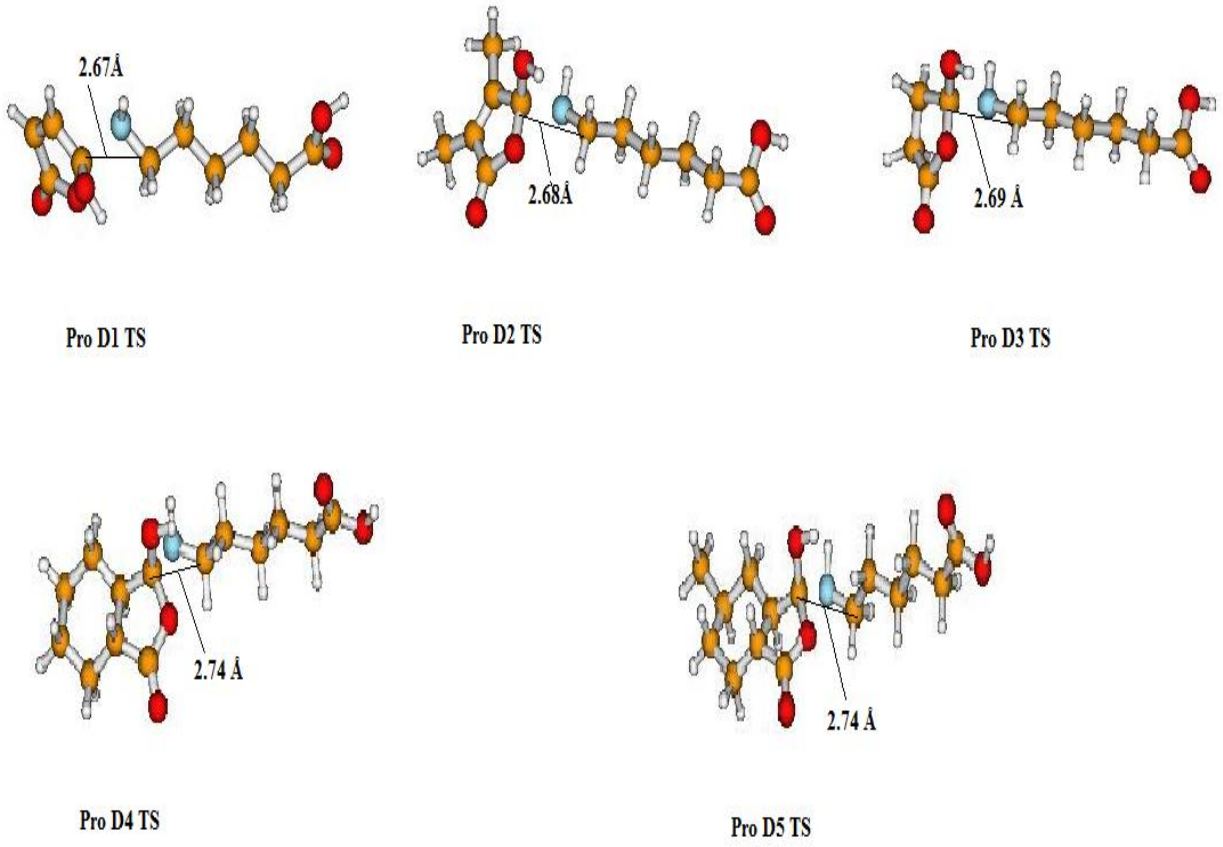


Figure 6b: DFT optimized structures for the transition state (**TS**) structures in the intramolecular proton transfer reaction of 6-aminocaproic acid **ProD1-ProD5**.

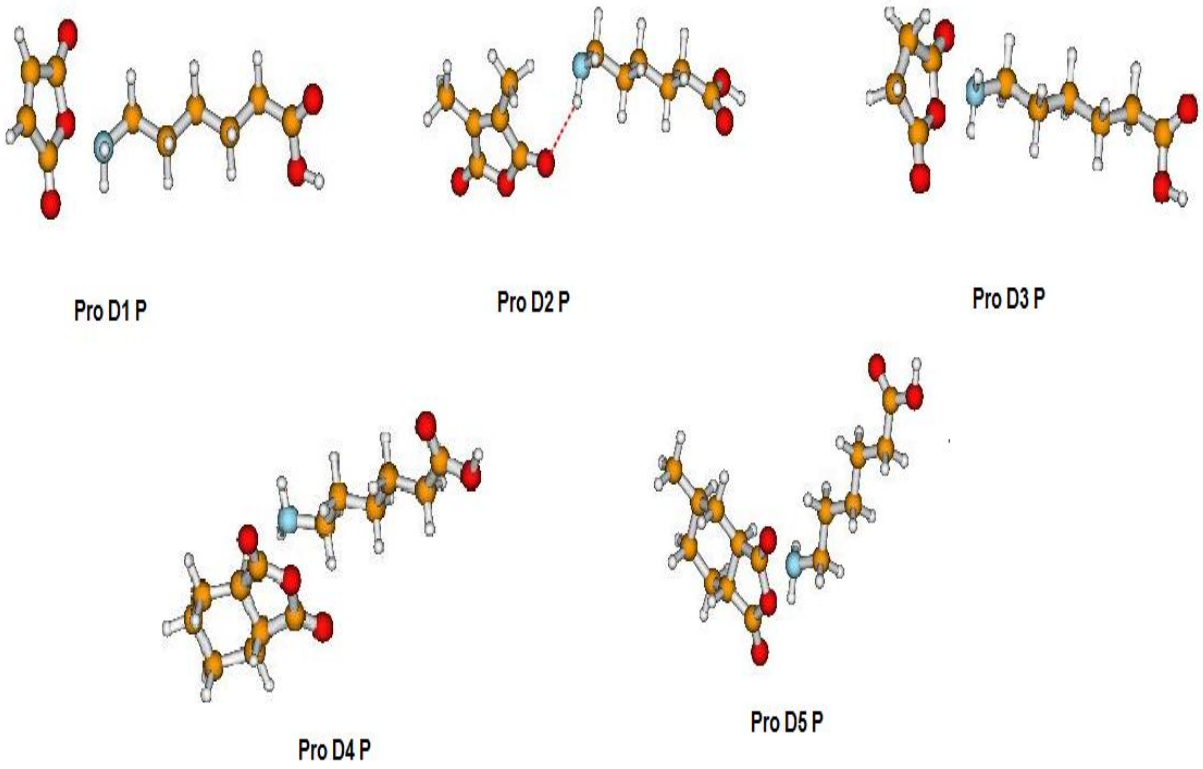


Figure 7c: DFT optimized structures for the linker product **(P)** structures in the intramolecular proton transfer reaction of 6-aminocaproic acid **ProD1-ProD5**.

4.4 Mechanistic investigation The DFT at B3LYP/6-31G (d,p) level of kinetic and thermodynamic properties for 6-aminocaproic acid prodrugs ProD1–ProD5

Kinetic and thermodynamic properties of 6-aminocaproic acid **ProD1- ProD5** were calculated using the quantum chemical package Gaussian 2009 [86]. The values of enthalpy and entropy for all structures involved in the acid-catalyzed hydrolysis, global minimum (GM), transition states (TS), intermediates (INT) and products (P), were calculated in the gas phase and water (Table 1).

Table 1: DFT (B3LYP) calculated properties for the proton transfer reactions of in 6-aminocaproic acid **ProD1- ProD5**

Structure	DFT enthalpy,H, In Hartree	DFT entropy, S, Cal/Mol-Kelvin	DFT frequency cm
6-amino proD1 GM	-821.0178119	145.005	--
6-amino proD1 TS	-820.9576831	136.482	-133.495
6-amino proD2 GM	-899.6624866	156.394	--
6-amino proD2 TS	-899.6097675	153.4	-103.549
6-amino proD3 GM	-822.2561339	150.233	---
6-amino proD3 TS	-822.1959113	137.617	-34.770
6-amino proD4 GM	-978.3185609	161.07	---
6-amino proD4 TS	-978.2508079	151.928	-32.369
6-amino proD5 GM	-1017.636522	167.313	---
6-amino proD5 TS	-1017.568501	158.812	-31.110

Using the calculated DFT enthalpy and entropy energy values for the global minimum structures, **1GM-7GM,** and 6-aminocaproic acid, **ProD1 GM- ProD5 GM**, and their derived transition states, **1TS-7TS,** and 6-aminocaproic acid, **ProD1 TS- ProD5 TS,** (Table 1) the enthalpy ($\Delta H^{\ddagger}$), the entropy ($T\Delta S^{\ddagger}$), and the activation energy ($\Delta G^{\ddagger}$) values for the proton transfers in these systems were calculated. The calculated values are listed in Table 2.

Table 2: DFT (B3LYP/6-31G (d,p) calculated kinetic and thermodynamic properties for the proton transfers in 6-aminocaproic acid **ProD1**-**ProD5** and in **1**-**7**

System	ΔES $ES_{(INT-GM)}$	log k (exp.)	$\Delta H^{\ddagger}$ (GP)	$T\Delta S^{\ddagger}$ (GP)	$\Delta G^{\ddagger}$ (GP)	$\Delta H^{\ddagger}$ (H_2O)	$\Delta G^{\ddagger}$ (H_2O)
6-amino proD1	3.2	--	37.73	-2.531	39.26	39.84	40.37
6-amino proD2	2.2	--	33.08	-0.889	33.97	29.83	35.72
6-amino proD3	11.8	--	37.78	-3.746	41.53	40.47	44.21
6-amino proD4	14.7	--	42.51	-2.715	45.23	43.92	46.63
6-amino proD5	14.0	--	42.68	-2.52	45.20	44.15	46.67
1	10.3	0	27.31		28.08	32.29	33.06
2	5.3	4.37	13.93		16.42	17.56	20.05
3	6.9	1.49	24.41		24.90	27.93	28.42
4	15.6	-4.3	34.42		36.77	35.76	38.11
5	10.1	2.73	13.25		17.41	18.96	23.12
6	12.5	1.51	23.83		23.92	27.19	27.28
7	12.4	1.64	24.86		25.03	27.38	27.55

4.4.1 The role of the strain energy on the rate of the proton transfer in processes 6-aminocaproic acid ProD1-ProD5

To test whether the discrepancy in the rates of **1–7** and 6-aminocapproic acid **ProD1**-**ProD5** is a result of proximity orientation (difference in the distance between the two reactive centers) or stems from steric effects (strain energy) we computed using Allinger's MM2 method [30] the strain energies for the reactants and intermediates in **1–7** and 6-aminocapproic **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 graphically in Figure 7a demonstrate a good correlation between the experimental log krel and the MM2-calculated intermediate strain energy values (Es) with a correlation coefficient (r) of 0.93. The correlation results imply that the rate of the reaction for systems having less strained intermediates such as **2** and **5** are higher than that having more strained intermediates. This might be attributed to the fact that the transition state structures resemble that of the corresponding intermediate.

In addition the difference in MM2 strain energies of the intermediates and reactants (ΔEs = Es ($_{INT-GM}$) are summarized in Table 2. The calculated MM2 (ΔEs) values for the reactions of **1-7** were examined for correlation with the experimental (log k_{rel}) values. The correlation results illustrated graphically in Figure 7c demonstrate a fair correlation between the experimental log krel and the MM2-calculated strain energy values (ΔEs) with a correlation coefficient (r) of 0.8. Examination of Figure 7c and Table 2 reveals that N-alkylmaleamic acids having small differences in the strain energies between their reactants and intermediates, such as system **2** the corresponding proton transfer rates are high and vice versa.

In order to further support this conclusion, activation energy values for **1-7** as calculated in dielectric constant of 78.39 (water) ($\Delta G^{\ddagger}$ H_2O, see Table 2) were examined for correlations with both log k_{rel} for system **1-7** and ΔEs for 6-aminoicapproic acid prodrugs.(Figures 7b and7d) with a correlation coefficient of 0.96 and 0.9, respectively.

It should be emphasized, that an attempt to correlate the distance O3–H 2 (r_{GM}) with $\Delta G^{\ddagger}$ for 6-aminiocapproic acid prodrugs failed to give any significant relationship between the two parameters.

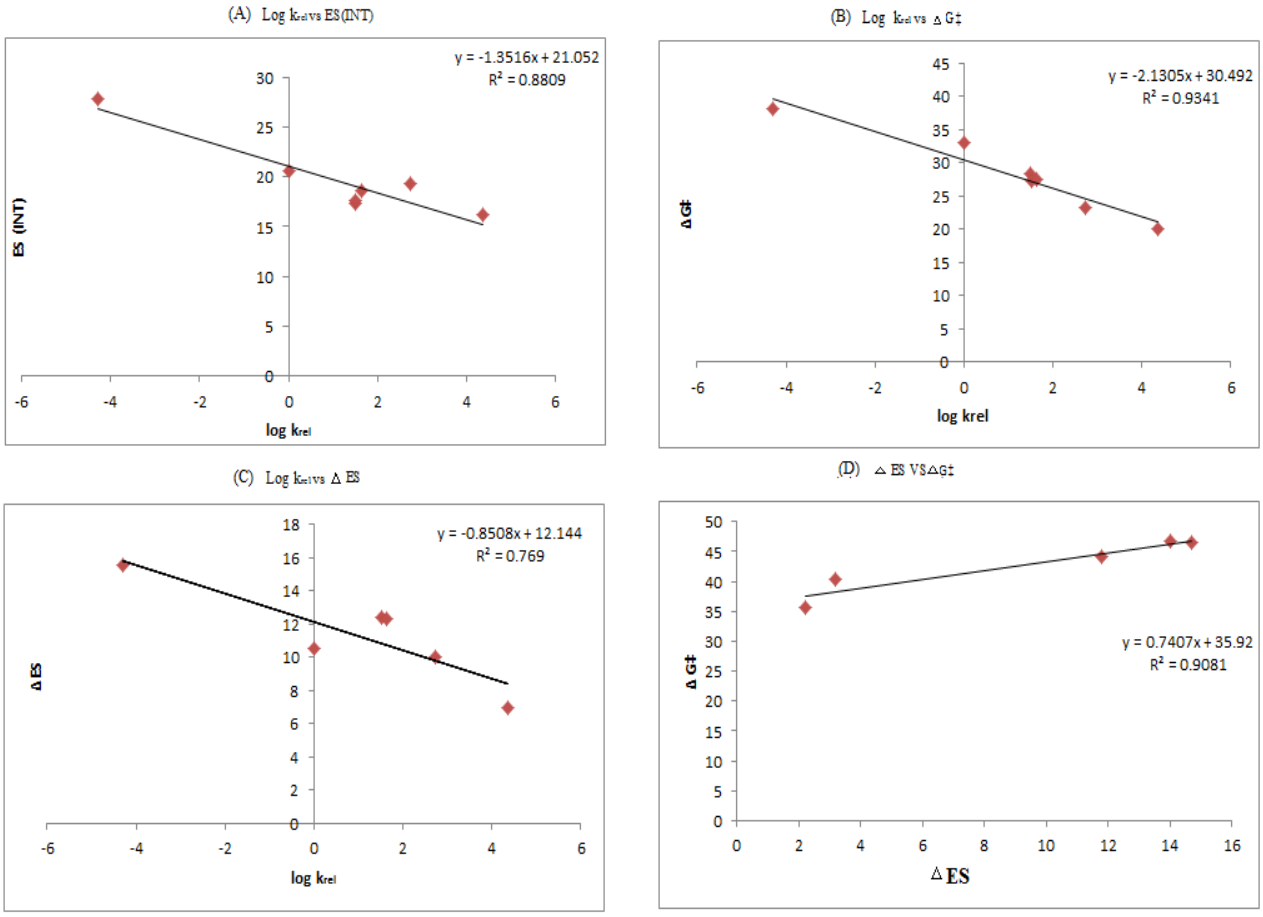


Figure 7: (a) plot of the ES for intermediates of **1-7** N-alkylmaleamic acid vs. relative rate (log k rel) .(b) Plot of the DFT calculated $\Delta G^{\ddagger}$ vs. relative rate (log krel) in **1-7** *N*-alkylmaleamic acid. (c) Plot of the MM2 calculated difference in strain energies between intermediates and reactants ($ES_{(INT-GM)}$) vs. relative rate (log krel) in N-alkylmaleamic acids **1-7**. (d) Plot of the DFT calculated $\Delta G^{\ddagger}$ vs. MM2 calculated difference in strain energies between intermediates and reactants ($ES_{(INT-GM)}$) in 6-aminocapproic acid **ProD 1- ProD5**.

Chapter Five:
Conclusion and Future Direction

Chapter Five

Conclusion and Future Direction

5.1 Conclusion

Based on Kirby`s enzyme model (Proton transfer in N-alkylmaleamic acids) five different 6-aminocaproic acid prodrugs were designed.

The DFT calculation results revealed that the rate of a proton transfer in processes of 6-aminocapprpic acid **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 ($E_{s\ INT\text{-}GM}$). Therefore it is expected that **ProD2** having the lowest difference in strain energy will undergo a fastest proton transfer (high rate cleavage).

5.2 Future Direction:

According to the DFT calculations done for systems **1-7** and the designed 6-aminocapproic acid prodrugs, it is recommended to synthesize 6-aminocapproic acid **ProD2** 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 6-aminocapproic acid **ProD2** to its parent drug, 6-aminocapproic acid. Further, another pharmacokinetic parameter values will be calculated including oral bioavailability, and other pharmacokinetic parameters as deemed necessary.

Reference

References:

1. Karaman, R., Using predrugs to optimize drug candidates. Expert Opin Drug Discov, 2014. **9**(12): p. 1405-19.
2. Sinkula, A.A. and S.H. Yalkowsky, Rationale for design of biologically reversible drug derivatives: prodrugs. J Pharm Sci, 1975. **64**(2): p. 181-210.
3. Stella, V.J., W.N. Charman, and V.H. Naringrekar, Prodrugs. Do they have advantages in clinical practice? Drugs, 1985. **29**(5): p. 455-73.
4. Huttunen, K.M., H. Raunio, and J. Rautio, Prodrugs--from serendipity to rational design. Pharmacol Rev, 2011. **63**(3): p. 750-71.
5. DI, L. and E.H. KERNS, Solubility Issues in Early Discovery and HTS, in Solvent Systems and Their Selection in Pharmaceutics and Biopharmaceutics, P. Augustijns and M.E. Brewster, Editors. 2007, Springer New York: New York, NY. p. 111-136.
6. Muller, C.E., Prodrug approaches for enhancing the bioavailability of drugs with low solubility. Chem Biodivers, 2009. **6**(11): p. 2071-83.
7. Fleisher, D., R. Bong, and B.H. Stewart, Improved oral drug delivery: solubility limitations overcome by the use of prodrugs. Advanced Drug Delivery Reviews, 1996. **19**(2): p. 115-130.
8. Beaumont, K., et al., Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. Curr Drug Metab, 2003. **4**(6): p. 461-85.
9. Ettmayer, P., et al., Lessons learned from marketed and investigational prodrugs. J Med Chem, 2004. **47**(10): p. 2393-404.
10. Denny, W.A., Hypoxia-activated prodrugs in cancer therapy: progress to the clinic. Future Oncol, 2010. **6**(3): p. 419-28.
11. Shanghag, A., et al., Prodrugs as drug delivery systems. Design of controlled release drug delivery systems, 2006: p. 75-106.
12. Testa, B. and S.D. Kramer, The biochemistry of drug metabolism--an swwdffdcc introduction: part 3. Reactions of hydrolysis and their enzymes. Chem Biodivers, 2007. **4**(9): p. 2031-122.
13. R, T.B., et al., Improvement of oral peptide bioavailability: Peptidomimetics and prodrug strategies. Adv Drug Deliv Rev, 1997. **27**(2-3): p. 235-256.
14. Wang, W., et al., Prodrug approaches to the improved delivery of peptide drugs. Current pharmaceutical design, 1999. **5**(4): p. 265-287.
15. Draganov, D. and B.N. La Du, Pharmacogenetics of paraoxonases: a brief review. Naunyn-Schmiedeberg's archives of pharmacology, 2004. **369**(1): p. 78-88.
16. Higuchi, T. and V.J. Stella, Pro-drugs as novel drug delivery systems1975: The Society.

17. Amidon, G., G. Leesman, and R. Elliott, Improving intestinal absorption of water-insoluble compounds: A membrane metabolism strategy. J Pharm Sci, 1980. **69**(12): p. 1363-1368.
18. Fleisher, D., B.H. Stewart, and G.L. Amidon, [28] Design of prodrugs for improved gastrointestinal absorption by intestinal enzyme targeting. Methods in enzymology, 1985. **112**: p. 360-381.
19. Stella, V.J. and K.J. Himmelstein, Prodrugs and site-specific drug delivery. J Med Chem, 1980. **23**(12): p. 1275-1282.
20. Aghi, M., F. Hochberg, and X.O. Breakefield, Prodrug activation enzymes in cancer gene therapy. The Journal of Gene Medicine, 2000. **2**(3): p. 148-164.
21. Greco, O. and G.U. Dachs, Gene directed enzyme/prodrug therapy of cancer: historical appraisal and future prospectives. Journal of cellular physiology, 2001. **187**(1): p. 22-36.
22. Zawilska, J.B., J. Wojcieszak, and A.B. Olejniczak, Prodrugs: a challenge for the drug development. Pharmacological reports, 2013. **65**(1): p. 1-14.
23. Reddy, M.R. and M.D. Erion, Free energy calculations in rational drug design2001: Springer Science & Business Media.
24. Parr, R.G., D.P. Craig, and I.G. Ross, Molecular orbital calculations of the lower excited electronic levels of benzene, configuration interaction included. The Journal of Chemical Physics, 1950. **18**(12): p. 1561-1563.
25. Friesner, R.A., Ab initio quantum chemistry: methodology and applications. Proc Natl Acad Sci U S A, 2005. **102**(19): p. 6648-6653.
26. Parr, R.G., On the genesis of a theory. International Journal of Quantum Chemistry, 1990. **37**(4): p. 327-347.
27. Dewar, M.J. and W. Thiel, Ground states of molecules. 38. The MNDO method. Approximations and parameters. J Am Chem Soc, 1977. **99**(15): p. 4899-4907.
28. Parr, R.G. and W. Yang, Density-functional theory of the electronic structure of molecules. Annual Review of Physical Chemistry, 1995. **46**(1): p. 701-728.
29. Van Mourik, T. and R.J. Gdanitz, A critical note on density functional theory studies on rare-gas dimers. J Chem Phys, 2002. **116**(22): p. 9620-9623.
30. Burkert, U. and N.L. Allinger, Molecular mechanics. Vol. 177. 1982: American Chemical Society Washington, DC.
31. Warshel, A. and M. Levitt, Theoretical studies of enzymic reactions: dielectric, electrostatic and steric stabilization of the carbonium ion in the reaction of lysozyme. J Mol Biol, 1976. **103**(2): p. 227-249.
32. Field, M.J., Simulating enzyme reactions: challenges and perspectives. Journal of computational chemistry, 2002. **23**(1): p. 48-58.
33. Ortmann, E., M. Besser, and A. Klein, Antifibrinolytic agents in current anaesthetic practice. British journal of anaesthesia, 2013. **111**(4): p. 549-563.

34. Daily, P.O., et al., Effect of prophylactic epsilon-aminocaproic acid on blood loss and transfusion requirements in patients undergoing first-time coronary artery bypass grafting. J Thorac Cardiovasc Surg, 1994. **108**(1): p. 99-108.
35. Harley, B.J., et al., The effect of epsilon aminocaproic acid on blood loss in patients who undergo primary total hip replacement: a pilot study. Canadian journal of surgery, 2002. **45**(3): p. 185.
36. Kutner, B., et al., Aminocaproic acid reduces the risk of secondary hemorrhage in patients with traumatic hyphema. Archives of ophthalmology, 1987. **105**(2): p. 206-208.
37. Walsh, P.N., et al., Epsilon-Aminocaproic acid therapy for dental extractions in haemophilia and Christmas disease: a double blind controlled trial. British journal of haematology, 1971. **20**(5): p. 463-475.
38. Gardner, F.H. and R.E. Helmer, Aminocaproic acid: use in control of hemorrhage in patients with amegakaryocytic thrombocytopenia. JAMA, 1980. **243**(1): p. 35-37.
39. Nilsson, I.M., Clinical pharmacology of aminocaproic and tranexamic acids. J Clin Pathol, 1980. **33**(Suppl 14): p. 41-47.
40. Pilbrant, Å., M. Schannong, and J. Vessman, Pharmacokinetics and bioavailability of tranexamic acid. European journal of clinical pharmacology, 1981. **20**(1): p. 65-72.
41. Hirschfelder, J.O. and W.J. Meath, The nature of intermolecular forces. 1967.
42. Pitzer, K.S., Inter-and Intramolecular Forces and Molecular Polarizability. Advances in Chemical Physics, Volume 2, 1959: p. 59-83.
43. Hanson, K.R. and E.A. Havir, 3 The Enzymic Elimination of Ammonia. The enzymes, 1972. **7**: p. 75-166.
44. Swiegers, G., Mechanical catalysis: methods of enzymatic, homogeneous, and heterogeneous catalysis2008: John Wiley & Sons.
45. Fersht, A., Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding1999: Macmillan.
46. White, A., et al., Principles of biochemistry. Principles of Biochemistry., 1959(Edn 2).
47. Menger, F.M. and R. Karaman, A singularity model for chemical reactivity. Chemistry–A European Journal, 2010. **16**(5): p. 1420-1427.
48. Jencks, W.P., Catalysis in chemistry and enzymology1987: Courier Corporation.
49. Kirby, A.J. and F. Hollfelder, From enzyme models to model enzymes2009: Royal Society of Chemistry.
50. Dafforn, A. and D. Koshland, Proximity, entropy and orbital steering. Biochemical and biophysical research communications, 1973. **52**(3): p. 779-785.
51. Lightstone, F.C. and T.C. Bruice, Separation of ground state and transition state effects in intramolecular and enzymatic reactions. 2. A theoretical study of the formation of transition states in cyclic anhydride formation. J Am Chem Soc, 1997. **119**(39): p. 9103-9113.

52. Bruice, T.C. and U.K. Pandit, The effect of geminal substitution ring size and rotamer distribution on the intramolecular nucleophilic catalysis of the hydrolysis of monophenyl esters of dibasic acids and the solvolysis of the intermediate anhydrides. J Am Chem Soc, 1960. **82**(22): p. 5858-5865.
53. Milstien, S. and L.A. Cohen, Concurrent general-acid and general-base catalysis of esterification. J Am Chem Soc, 1970. **92**(14): p. 4377-4382.
54. Milstien, S. and L.A. Cohen, Rate acceleration by stereopopulation control: models for enzyme action. Proceedings of the National Academy of Sciences, 1970. **67**(3): p. 1143-1147.
55. Milstien, S. and L.A. Cohen, Stereopopulation control. I. Rate enhancement in the lactonizations of o-hydroxyhydrocinnamic acids. J Am Chem Soc, 1972. **94**(26): p. 9158-9165.
56. Menger, F. and M. Ladika, Fast hydrolysis of an aliphatic amide at neutral pH and ambient temperature. A peptidase model. J Am Chem Soc, 1988. **110**(20): p. 6794-6796.
57. Menger, F., On the source of intramolecular and enzymatic reactivity. Accounts of chemical research, 1985. **18**(5): p. 128-134.
58. Menger, F., et al., Directionality of proton transfer in solution. Three systems of known angularity. J Am Chem Soc, 1983. **105**(15): p. 4996-5002.
59. Barber, S.E., K.E. Dean, and A.J. Kirby, A mechanism for efficient proton-transfer catalysis. Intramolecular general acid catalysis of the hydrolysis of 1-arylethyl ethers of salicylic acid. Canadian journal of chemistry, 1999. **77**(5-6): p. 792-801.
60. Kirby, A. and P. Lancaster, Structure and efficiency in intramolecular and enzymic catalysis. Catalysis of amide hydrolysis by the carboxy-group of substituted maleamic acids. Journal of the Chemical Society, Perkin Transactions 2, 1972(9): p. 1206-1214.
61. Kirby, A.J., Efficiency of proton transfer catalysis in models and enzymes. Accounts of chemical Research, 1997. **30**(7): p. 290-296.
62. Karaman, R., Reevaluation of Bruice's proximity orientation. Tetrahedron Letters, 2009. **50**(4): p. 452-456.
63. Karaman, R., Analysis of Menger's 'spatiotemporal hypothesis'. Tetrahedron Letters, 2008. **49**(41): p. 5998-6002.
64. Karaman, R., The effective molarity (EM) puzzle in proton transfer reactions. Bioorganic chemistry, 2009. **37**(4): p. 106-110.
65. Karaman, R., Cleavage of Menger's aliphatic amide: a model for peptidase enzyme solely explained by proximity orientation in intramolecular proton transfer. Journal of Molecular Structure: THEOCHEM, 2009. **910**(1): p. 27-33.
66. Karaman, R., A general equation correlating intramolecular rates with 'attack'parameters: distance and angle. Tetrahedron Letters, 2010. **51**(39): p. 5185-5190.
67. Karaman, R., Prodrugs for masking bitter taste of antibacterial drugs—a computational approach. Journal of molecular modeling, 2013. **19**(6): p. 2399-2412.

68. Karaman, R. and S. Alfalah, Multi Transition States for SN2 Reaction in Intramolecular Processes. International Review of Biophysical Chemistry (IREBIC), 2010. **1**(1): p. 14-23.
69. Karaman, R., B. Fattash, and A. Qtait, The future of prodrugs–design by quantum mechanics methods. Expert opinion on drug delivery, 2013. **10**(5): p. 713-729.
70. Kraut, D.A., K.S. Carroll, and D. Herschlag, Challenges in enzyme mechanism and energetics. Annual review of biochemistry, 2003. **72**(1): p. 517-571.
71. Kallarakal, A.T., et al., Mechanism of the reaction catalyzed by mandelate racemase: structure and mechanistic properties of the K166R mutant. Biochemistry, 1995. **34**(9): p. 2788-2797.
72. Kirby, A. and P. Lancaster, Structure and efficiency in intramolecular and enzymic catalysis. Catalysis of amide hydrolysis by the carboxy-group of substituted maleamic acids. J. Chem. Soc., Perkin Trans. 2, 1972(9): p. 1206-1214.
73. Katagi, T., AM1 study of acid-catalyzed hydrolysis of maleamic (4-amino-4-oxo-2-butenoic) acids. Journal of computational chemistry, 1990. **11**(9): p. 1094-1100.
74. Kluger, R. and J. Chin, Carboxylic acid participation in amide hydrolysis. Evidence that separation of a nonbonded complex can be rate determining. Journal of the American Chemical Society, 1982. **104**(10): p. 2891-2897.
75. Karaman, R., Prodrugs Design Based on Inter-and Intramolecular Chemical Processes. Chemical biology & drug design, 2013. **82**(6): p. 643-668.
76. Karaman, R., et al., Computationally designed prodrugs of statins based on Kirby's enzyme model. Journal of molecular modeling, 2013. **19**(9): p. 3969-3982.
77. Karaman, R., et al., Prodrugs of acyclovir–a computational approach. Chemical biology & drug design, 2012. **79**(5): p. 819-834.
78. Karaman, R., Analyzing the efficiency in intramolecular amide hydrolysis of Kirby's N-alkylmaleamic acids–A computational approach. Computational and Theoretical Chemistry, 2011. **974**(1): p. 133-142.
79. Naz, A., et al., Conformational analysis (geometry optimization) of nucleosidic antitumor antibiotic showdomycin by arguslab 4 software. Pak. J. Pharm. Sci, 2009. **22**(1): p. 78-82.
80. Allen, M.P. and D.J. Tildesley, Computer simulation of liquids1989: Oxford university press.
81. Levine, I.N., D.H. Busch, and H. Shull, Quantum chemistry. Vol. 6. 2009: Pearson Prentice Hall Upper Saddle River, NJ.
82. Hafeez, A., et al., Computational study on the geometry optimization and excited–State properties of Riboflavin by ArgusLab 4.0. Pak. J. Pharm. Sci, 2013. **26**(3): p. 487-493.
83. Frisch, M., et al., Gaussian 09, rev. A. 02. Gaussian Inc., Wallingford, CT, 2009.

84. Frisch, M., et al., Gaussian 09, revision A. 02, Gaussian, Inc., Wallingford, CT, 2009 Search PubMed;(b) C. Lee, W. Yang and RG Parr. Phys. Rev. B: Condens. Matter Mater. Phys, 1988. **37**: p. 785.
85. Schaftenaar, G. and J.H. Noordik, Molden: a pre-and post-processing program for molecular and electronic structures. Journal of computer-aided molecular design, 2000. **14**(2): p. 123-134.
86. Zhao, Y. and D.G. Truhlar, Density functionals with broad applicability in chemistry. Accounts of chemical research, 2008. **41**(2): p. 157-167.
87. Zhao, Y. and D.G. Truhlar, Exploring the limit of accuracy of the global hybrid meta density functional for main-group thermochemistry, kinetics, and noncovalent interactions. Journal of Chemical Theory and Computation, 2008. **4**(11): p. 1849-1868.
88. Zheng, J., Y. Zhao, and D.G. Truhlar, The DBH24/08 database and its use to assess electronic structure model chemistries for chemical reaction barrier heights. Journal of Chemical Theory and Computation, 2009. **5**(4): p. 808-821.
89. Frisch, M., et al., Gaussian 09, revision D. 01, 2009, Gaussian, Inc., Wallingford CT.
90. Casewit, C., K. Colwell, and A. Rappé, Application of a universal force field to main group compounds. J Am Chem Soc, 1992. **114**(25): p. 10046-10053.
91. Murrell, J. and K.J. Laidler, Symmetries of activated complexes. Transactions of the Faraday Society, 1968. **64**: p. 371-377.
92. Müller, K., Reaction paths on multidimensional energy hypersurfaces. Angewandte Chemie International Edition in English, 1980. **19**(1): p. 1-13.
93. Cances, E., B. Mennucci, and J. Tomasi, A new integral equation formalism for the polarizable continuum model: Theoretical background and applications to isotropic and anisotropic dielectrics. J Chem Phys, 1997. **107**(8): p. 3032-3041.
94. Mennucci, B. and J. Tomasi, Continuum solvation models: a new approach to the problem of solute's charge distribution and cavity boundaries. J Chem Phys, 1997. **106**(12): p. 5151-5158.
95. Mennucci, B., E. Cances, and J. Tomasi, Evaluation of solvent effects in isotropic and anisotropic dielectrics and in ionic solutions with a unified integral equation method: theoretical bases, computational implementation, and numerical applications. The Journal of Physical Chemistry B, 1997. **101**(49): p. 10506-10517.
96. Tomasi, J., B. Mennucci, and E. Cances, The IEF version of the PCM solvation method: an overview of a new method addressed to study molecular solutes at the QM ab initio level. Journal of Molecular Structure: THEOCHEM, 1999. **464**(1): p. 211-226.
97. Karaman, R., et al., Design, synthesis and in vitro kinetic study of tranexamic acid prodrugs for the treatment of bleeding conditions. Journal of computer-aided molecular design, 2013. **27**(7): p. 615-635.
98. Karaman, R., Computational-Aided Design for Dopamine Prodrugs Based on Novel Chemical Approach. Chemical biology & drug design, 2011. **78**(5): p. 853-863.

99. Karaman, R., K. Dajani, and H. Hallak, Computer-assisted design for atenolol prodrugs for the use in aqueous formulations. Journal of molecular modeling, 2012. **18**(4): p. 1523-1540.
100. Karaman, R., et al., Design, synthesis, and in vitro kinetics study of atenolol prodrugs for the use in aqueous formulations. The Scientific World Journal, 2014. **2014**.
101. Hejaz, H., R. Karaman, and M. Khamis, Computer-assisted design for paracetamol masking bitter taste prodrugs. Journal of molecular modeling, 2012. **18**(1): p. 103-114.
102. Karaman, R., Prodrugs of aza nucleosides based on proton transfer reaction. Journal of computer-aided molecular design, 2010. **24**(12): p. 961-970.
103. Karaman, R., D. Karaman, and I. Zeiadeh, Computationally-designed phenylephrine prodrugs–a model for enhancing bioavailability. Molecular Physics, 2013. **111**(21): p. 3249-3264.
104. Karaman, R. and H. Hallak, Computer-Assisted Design of Pro-drugs for Antimalarial Atovaquone. Chemical biology & drug design, 2010. **76**(4): p. 350-360.
105. Karaman, R., et al., Computationally designed atovaquone prodrugs based on Bruice‘s enzyme model. Curr Comput Aided Drug Des, 2014. **10**(1): p. 15-27.
106. Karaman, R., Antimalarial Atovaquone Prodrugs Based on Enzyme Models-Molecular Orbital Calculations Approach. Antimalarial Drug Research and Development, Banet, A C. & Brasier, P. Ed, 2013: p. 1-67.

Supplementary material

Supplementary Material

Content

1.Xyz Cartesian coordinates for the DFT optimized GM, TS, TET and P in 6-aminocapproic acid **ProD1- ProD5**

ProD1 GM

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.532931
C	1.424338	0.000000	2.048619
C	1.449022	0.001607	3.564280
C	2.876657	0.023894	4.075430
C	2.914382	-0.105294	5.565064
O	3.846606	0.687959	6.163661
N	-1.305412	0.016177	-0.603066
C	-1.924858	-1.102842	-1.129567
C	-1.104794	-2.304605	-1.462468
C	-1.010999	-3.413685	-0.727501
C	-1.621380	-3.592690	0.595791
O	-1.868998	-4.894764	0.910427
O	2.246259	-0.824106	6.310587
O	-3.133858	-1.060584	-1.424881
O	-1.902644	-2.755685	1.457945
H	0.546439	0.919180	-0.359705
H	0.568080	-0.900755	-0.365212
H	-1.910204	0.754440	-0.317499
H	-0.547100	-0.901593	1.916484
H	-0.538491	0.905050	1.914513
H	1.967700	0.901068	1.662387
H	1.964685	-0.904079	1.665400
H	0.924218	-0.907668	3.958435

H	0.897285	0.896907	3.950628
H	3.391190	0.970538	3.767023
H	3.455137	-0.838743	3.650119
H	3.830175	0.548433	7.123062
H	-0.613622	-2.237091	-2.449300
H	-0.455860	-4.296262	-1.090341
H	-2.276827	-4.943045	1.789658

ProD1 TET

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.458441
C	1.494603	0.000000	1.856934
C	2.235045	-0.051218	0.736887
C	1.324115	-0.060426	-0.440583
N	-0.724226	-1.151066	1.984793
C	-2.015131	-1.384524	1.360315
C	-2.796131	-2.521473	2.020613
C	-4.127935	-2.701742	1.319532
C	-4.915401	-3.839198	1.938897
C	-6.267103	-3.980594	1.270643
C	-7.093289	-5.095557	1.825863
O	-6.624325	-5.669460	2.968171
O	-0.550779	1.260562	1.777925
O	1.487493	-0.092766	-1.647923
O	-8.149637	-5.552060	1.381285

H	-6.860971	-3.035228	1.390584
H	-6.142320	-4.166501	0.170737
H	-4.345025	-4.798455	1.838985
H	-5.057469	-3.656567	3.035596
H	-4.721141	-1.753077	1.386338
H	-3.955431	-2.910436	0.231869
H	-2.206905	-3.472355	1.964747
H	-2.968949	-2.295859	3.104279
H	-2.653531	-0.453430	1.375880
H	-1.821335	-1.641813	0.280904
H	-7.228107	-6.374945	3.249061
H	-0.808989	-1.051399	2.977954
H	1.792975	0.041066	2.901788
H	3.317584	-0.073507	0.615181
H	-1.258186	1.451377	1.147762

ProD1 TS

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.441703
C	1.476184	0.000000	1.803496
C	2.210401	-0.013647	0.693377
C	1.294887	-0.041256	-0.477748
N	-0.830099	-1.526639	2.052663
C	-2.244939	-1.693660	1.679897
C	-2.744904	-3.067452	2.127311

C	-4.201603	-3.328628	1.727334
C	-4.710244	-4.700483	2.185605
C	-6.167708	-4.958730	1.761479
C	-6.688309	-6.310675	2.194674
O	-6.640433	-6.461897	3.544688
O	-0.670081	1.156445	1.890960
O	1.550555	-0.077346	-1.652041
O	-7.105209	-7.180856	1.463114
H	-6.816667	-4.190834	2.201806
H	-6.275355	-4.907796	0.675402
H	-4.068090	-5.486212	1.767255
H	-4.633738	-4.781712	3.275652
H	-4.845631	-2.543785	2.148331
H	-4.297790	-3.249556	0.635798
H	-2.091984	-3.836734	1.697588
H	-2.648797	-3.153806	3.219891
H	-2.881617	-0.900248	2.098774
H	-2.302827	-1.612689	0.590681
H	-6.985021	-7.353085	3.724103
H	-0.719777	-1.579059	3.062192
H	1.810668	0.020601	2.834007
H	3.286265	-0.013023	0.582675
H	-0.349401	1.913499	1.379053

ProD1 product

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.399247
C	1.425669	0.000000	1.829791
C	2.192707	-0.090291	0.742070
C	1.315940	-0.111665	-0.454867
N	-1.048598	-2.706060	2.159301
C	-2.419872	-2.802630	1.652480
C	-3.052829	-4.130616	2.089751
C	-4.466799	-4.331874	1.530541
C	-5.101935	-5.654572	1.975379
C	-6.518187	-5.848489	1.401886
C	-7.159477	-7.151037	1.824139
O	-7.292690	-7.233118	3.174387
O	-0.756303	0.998624	1.939957
O	1.583591	-0.196902	-1.620865
O	-7.521561	-8.039212	1.085125
H	-7.163245	-5.028067	1.741027
H	-6.503829	-5.837978	0.309394
H	-4.465891	-6.490541	1.657284
H	-5.149428	-5.696091	3.069291
H	-5.109844	-3.497858	1.845881
H	-4.432581	-4.293876	0.433123
H	-2.398197	-4.948295	1.766133
H	-3.084781	-4.173392	3.187738

H	-3.082041	-1.976862	1.981411
H	-2.375657	-2.763755	0.558347
H	-7.700612	-8.098130	3.348971
H	-1.106791	-2.577984	3.174798
H	1.721516	0.044022	2.869578
H	3.269514	-0.148147	0.667635
H	-0.548119	-3.552534	1.937787

ProD2 GM

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.484713
C	1.143386	0.000000	2.194444
C	2.480211	0.027264	1.545318
C	-1.374847	0.050331	2.087854
O	-2.184596	0.931338	1.737269
N	-1.713788	-0.937432	2.982212
C	-3.003393	-0.989530	3.620950
C	-3.617075	-2.391232	3.667959
C	-2.860105	-3.323506	4.590889
C	-3.533526	-4.680505	4.655313
C	-2.776248	-5.611636	5.581083
C	-3.349633	-6.992200	5.549921
O	-3.055301	-7.733304	6.655411
O	-4.023473	-7.550464	4.681842
C	1.183329	-0.023107	3.669979

O	1.797690	1.049757	4.236733
O	0.784979	-0.897788	4.446246
H	-3.706766	-0.304511	3.066662
H	-2.904117	-0.604732	4.676995
H	-1.011023	-1.553063	3.321799
H	-3.662781	-2.830460	2.638999
H	-4.671570	-2.280765	4.032305
H	-2.809482	-2.877590	5.618088
H	-1.805721	-3.450993	4.232999
H	-3.585193	-5.132997	3.630566
H	-4.587527	-4.566113	5.017775
H	-2.797990	-5.226975	6.633821
H	-1.700577	-5.685908	5.268325
H	-3.439597	-8.619060	6.568004
H	-0.986805	0.345895	-0.395877
H	0.199824	-1.034132	-0.376686
H	0.797009	0.677502	-0.395105
H	2.622093	0.999332	1.009153
H	2.563284	-0.801750	0.799803
H	3.298336	-0.079643	2.297293
H	1.796854	0.950499	5.202651

ProD2 TET

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.456499

C	1.496767	0.000000	1.860246
C	2.241139	-0.048847	0.731961
C	1.322559	-0.056771	-0.441933
N	-0.728577	-1.150236	1.978721
C	-2.054166	-1.326133	1.411421
C	-2.824107	-2.483033	2.049613
C	-4.196058	-2.596577	1.414827
C	-4.957303	-3.777959	1.982566
C	-6.340447	-3.867946	1.372373
C	-7.157508	-4.998765	1.908687
O	-6.621292	-5.670337	2.964902
O	-0.557373	1.259820	1.773642
C	1.888385	0.057899	3.267452
C	3.696525	-0.078666	0.559032
O	1.480356	-0.080298	-1.651032
O	-8.259332	-5.391790	1.516805
H	-6.913832	-2.922136	1.566182
H	-6.269156	-4.001069	0.260016
H	-4.396462	-4.726975	1.781264
H	-5.044002	-3.679970	3.095747
H	-4.774538	-1.653599	1.595131
H	-4.089786	-2.714069	0.305239
H	-2.260411	-3.440578	1.907889
H	-2.932917	-2.316466	3.152121
H	-2.671791	-0.385918	1.509165

H	-1.919926	-1.528646	0.311459
H	-7.225007	-6.377913	3.240584
H	-0.759857	-1.084305	2.977720
H	-1.244060	1.459226	1.123543
H	4.213547	0.376135	1.439097
H	3.988998	0.482689	-0.363120
H	4.048526	-1.135690	0.449308
H	2.988427	0.223893	3.374273
H	1.622420	-0.900776	3.781291
H	1.344627	0.894248	3.776058

ProD2 TS

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.436163
C	1.478051	0.000000	1.821800
C	2.219472	-0.017000	0.704627
C	1.298540	-0.044935	-0.461603
N	-0.858891	-1.512491	2.042498
C	-2.269437	-1.657094	1.644565
C	-2.823735	-2.987261	2.154832
C	-4.279682	-3.222724	1.736288
C	-4.852162	-4.538601	2.275616
C	-6.310034	-4.768890	1.836007
C	-6.895132	-6.061762	2.357539
O	-6.900277	-6.101723	3.716393

O	-0.668724	1.165010	1.883178
C	1.889905	0.041532	3.255257
C	3.698829	-0.021330	0.496536
O	1.565226	-0.080501	-1.636036
O	-7.316860	-6.975772	1.684491
H	-6.934289	-3.943686	2.201576
H	-6.391617	-4.796944	0.746777
H	-4.233766	-5.376521	1.928603
H	-4.802521	-4.545600	3.370279
H	-4.901927	-2.386773	2.085633
H	-4.347748	-3.216395	0.639802
H	-2.188541	-3.801415	1.785082
H	-2.755224	-3.014299	3.252753
H	-2.889912	-0.823385	2.005186
H	-2.301597	-1.634038	0.551381
H	-7.282927	-6.963014	3.954876
H	-0.779451	-1.546231	3.055763
H	-0.343773	1.914232	1.362803
H	4.244804	0.075050	1.437651
H	3.996582	0.795681	-0.168684
H	4.012768	-0.950559	0.008566
H	2.961381	0.221029	3.366267
H	1.658922	-0.906836	3.757300
H	1.342358	0.830261	3.782455

ProD2 product

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.436163
C	1.478051	0.000000	1.821800
C	2.219472	-0.017000	0.704627
C	1.298540	-0.044935	-0.461603
N	-1.398845	-2.463340	2.423680
C	-2.809392	-2.607943	2.025746
C	-3.363689	-3.938110	2.536013
C	-4.819637	-4.173573	2.117469
C	-5.392116	-5.489450	2.656798
C	-6.849988	-5.719739	2.217188
C	-7.435086	-7.012611	2.738721
O	-7.440231	-7.052573	4.097574
O	-0.668724	1.165010	1.883178
C	1.889905	0.041532	3.255257
C	3.698829	-0.021330	0.496536
O	1.565226	-0.080501	-1.636036
O	-7.856814	-7.926621	2.065673
H	-7.474243	-4.894536	2.582757
H	-6.931572	-5.747793	1.127959
H	-4.773721	-6.327370	2.309784
H	-5.342475	-5.496449	3.751460
H	-5.441881	-3.337622	2.466814
H	-4.887702	-4.167244	1.020983

H	-2.728496	-4.752264	2.166264
H	-3.295178	-3.965148	3.633934
H	-3.429866	-1.774234	2.386367
H	-2.841552	-2.584887	0.932562
H	-7.822881	-7.913863	4.336057
H	-1.319405	-2.497080	3.436944
H	4.244804	0.075050	1.437651
H	3.996582	0.795681	-0.168684
H	4.012768	-0.950559	0.008566
H	2.961381	0.221029	3.366267
H	1.658922	-0.906836	3.757300
H	1.342358	0.830261	3.782455
H	-0.858044	-3.213638	2.022868

ProD3 GM

C	0.000000	0.000000	0.000000
N	0.000000	0.000000	1.435094
C	1.122513	0.000000	2.239060
O	0.970609	-0.140771	3.469139
C	-0.678186	-1.226103	-0.624865
C	-0.682332	-1.099325	-2.134957
C	-1.435622	-2.251175	-2.769721
C	-1.393807	-2.153759	-4.281489
C	-2.210718	-3.232932	-4.917023
O	-2.258737	-3.151810	-6.276693

O	-2.832994	-4.163041	-4.400978
C	2.494134	0.205715	1.625820
C	3.542995	0.319358	2.713088
C	4.925008	0.488039	2.173696
O	5.065668	0.275732	0.833878
O	5.952606	0.786216	2.785787
H	-0.546715	0.924418	-0.348977
H	1.054388	0.063800	-0.387078
H	-1.730886	-1.314726	-0.251640
H	-0.138054	-2.160046	-0.325046
H	0.371797	-1.081053	-2.515317
H	-1.160115	-0.129404	-2.431315
H	-2.502073	-2.248067	-2.424100
H	-0.990971	-3.227392	-2.445246
H	-0.339978	-2.245365	-4.654788
H	-1.789195	-1.162046	-4.625690
H	-0.871247	-0.179412	1.880323
H	2.498241	1.134945	0.999195
H	2.735426	-0.660103	0.954852
H	3.531111	-0.600057	3.359439
H	3.316114	1.191183	3.383716
H	5.994799	0.393746	0.581614
H	-2.799950	-3.875396	-6.628465

ProD3 TET

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.448002
C	1.491705	0.000000	1.919394
C	2.303160	-0.150964	0.639050
C	1.294328	-0.104835	-0.475834
N	-0.767897	-1.133914	1.956966
C	-2.042402	-1.353107	1.296883
C	-2.848641	-2.486334	1.932897
C	-4.141766	-2.691635	1.169216
C	-4.924299	-3.856160	1.743016
C	-6.243467	-4.028320	1.019640
C	-7.046682	-5.187372	1.515417
O	-6.567105	-5.808958	2.628058
O	-0.543217	1.271002	1.751540
O	1.407647	-0.132760	-1.691856
O	-8.091325	-5.644637	1.044642
H	-6.876322	-3.108502	1.138185
H	-6.070664	-4.182726	-0.078690
H	-4.324728	-4.799102	1.656881
H	-5.115495	-3.690146	2.834829
H	-4.761261	-1.758956	1.217475
H	-3.915398	-2.885160	0.088594
H	-2.250076	-3.433108	1.922874
H	-3.078169	-2.244782	3.002461

H	-2.674781	-0.417909	1.296676
H	-1.822240	-1.609244	0.222360
H	-7.155646	-6.541051	2.870326
H	-0.881356	-1.026790	2.946205
H	1.693191	-0.833488	2.631341
H	1.703549	0.970128	2.430308
H	2.850788	-1.125303	0.605515
H	3.045732	0.674699	0.512894
H	-1.281348	1.442975	1.153379

ProD3 TS

N	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.842000
O	1.383176	0.000000	2.265672
C	1.807401	-1.270532	2.561618
O	2.943021	-1.509068	2.870830
C	0.633881	-2.231433	2.422310
C	-0.586650	-1.309805	2.387586
O	-0.641233	1.084857	2.452449
C	0.654208	1.138025	-0.656646
C	0.677669	0.942429	-2.173572
C	1.398271	2.076820	-2.911131
C	1.405790	1.890046	-4.432810
C	2.147386	3.026399	-5.160970
C	2.171682	2.855880	-6.663330

O	3.161888	2.747683	-7.351502
O	0.918216	2.831595	-7.188308
H	1.033296	2.711351	-8.146162
H	3.186455	3.092032	-4.829530
H	1.658374	3.982883	-4.936799
H	0.377251	1.835671	-4.806760
H	1.880737	0.932180	-4.681106
H	2.432256	2.147218	-2.546636
H	0.921671	3.036781	-2.666829
H	-0.353901	0.863747	-2.546698
H	1.160589	-0.017385	-2.392700
H	1.678084	1.194379	-0.274552
H	0.166469	2.103133	-0.425126
H	-0.965792	-0.070584	-0.313260
H	-0.151260	1.889362	2.230243
H	-1.404786	-1.678509	1.765263
H	-0.970396	-1.116847	3.392057
H	0.643185	-2.957097	3.237382
H	0.760507	-2.776150	1.480984

ProD3 product

N	0.000000	0.000000	0.000000
C	0.000000	0.000000	3.000000
O	1.383176	0.000000	3.423672
C	1.807401	-1.270532	3.719618

O	2.943021	-1.509068	4.028830
C	0.633881	-2.231433	3.580310
C	-0.586650	-1.309805	3.545586
O	-0.641233	1.084857	3.610449
C	0.654208	1.138025	-0.656646
C	0.677669	0.942429	-2.173572
C	1.398271	2.076820	-2.911131
C	1.405790	1.890046	-4.432810
C	2.147386	3.026399	-5.160970
C	2.171682	2.855880	-6.663330
O	3.161888	2.747683	-7.351502
O	0.918216	2.831595	-7.188308
H	1.033296	2.711351	-8.146162
H	3.186455	3.092032	-4.829530
H	1.658374	3.982883	-4.936799
H	0.377251	1.835671	-4.806760
H	1.880737	0.932180	-4.681106
H	2.432256	2.147218	-2.546636
H	0.921671	3.036781	-2.666829
H	-0.353901	0.863747	-2.546698
H	1.160589	-0.017385	-2.392700
H	1.678084	1.194379	-0.274552
H	0.166469	2.103133	-0.425126
H	-0.965792	-0.070584	-0.313260
H	-1.404786	-1.678509	2.923263

H	-0.970396	-1.116847	4.550057
H	0.643185	-2.957097	4.395382
H	0.760507	-2.776150	2.638984
H	0.482162	-0.851045	-0.243531

ProD4 GM

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.527914
C	1.426194	0.000000	2.087121
C	2.247814	-1.141045	1.491725
C	2.232305	-1.112185	-0.022453
C	0.811317	-1.155690	-0.548507
C	1.374132	-0.210269	3.601382
O	0.965153	-1.290149	4.076405
C	-0.744347	1.226321	1.988575
O	-2.073278	1.023987	2.191472
N	1.756849	0.785292	4.474296
C	2.317257	2.053966	4.104526
C	3.803029	2.039007	3.728209
C	4.690725	1.721807	4.913155
C	6.154582	1.775332	4.522812
C	7.039563	1.449630	5.709495
C	8.486547	1.593777	5.361018
O	9.321406	0.913837	6.196467
O	9.022088	2.234147	4.454167

O	-0.326246	2.371867	2.178578
H	1.728328	2.479911	3.240560
H	2.179790	2.750637	4.980399
H	3.986064	1.295690	2.909953
H	4.060950	3.052888	3.326279
H	4.446196	0.702976	5.310737
H	4.495326	2.456241	5.737266
H	6.355310	1.047088	3.695191
H	6.412504	2.794574	4.133250
H	6.850872	0.404818	6.069550
H	6.824905	2.146973	6.562393
H	10.242042	1.057301	5.928223
H	1.787239	0.542261	5.437973
H	1.912773	0.977527	1.821887
H	-0.537427	-0.915420	1.905412
H	-2.491374	1.850693	2.479413
H	1.835312	-2.117760	1.858907
H	3.302183	-1.056306	1.860746
H	2.740147	-0.183444	-0.389658
H	2.807574	-1.989218	-0.415190
H	0.331355	-2.125401	-0.257185
H	0.819623	-1.103620	-1.667120
H	0.426015	0.968560	-0.369882
H	-1.057902	-0.071156	-0.361862

ProD4 TET

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.508790
C	1.456209	0.000000	2.016665
C	2.095967	1.323716	1.678583
C	2.075119	1.477528	0.160549
C	0.700718	1.275035	-0.460745
C	-0.618075	-1.121938	2.300407
O	0.076438	-1.300629	3.483531
C	1.271868	-0.423409	3.508554
N	2.406601	-1.118686	4.086854
C	2.290517	-1.494385	5.485138
C	2.883335	-0.451195	6.438787
C	2.757638	-0.937616	7.868760
C	3.374008	0.053544	8.835677
C	3.323443	-0.483843	10.252532
C	3.904361	0.493330	11.223775
O	4.445658	-0.090443	12.330077
O	0.810442	0.680036	4.241110
O	-1.588350	-1.845002	2.132639
O	3.952935	1.723772	11.174702
H	1.214869	-1.676997	5.772038
H	2.845679	-2.462452	5.638048
H	3.961239	-0.276916	6.189641
H	2.353886	0.528610	6.318746

H	3.267812	-1.930386	7.974220
H	1.677201	-1.090846	8.124593
H	4.438303	0.253898	8.547819
H	2.829404	1.032095	8.786015
H	3.880517	-1.453933	10.327165
H	2.261150	-0.675037	10.560094
H	4.791743	0.591898	12.925565
H	2.637555	-1.907566	3.517521
H	2.030022	-0.818168	1.500874
H	-0.515998	0.937773	1.870426
H	1.536408	2.159282	2.170386
H	3.150489	1.354503	2.051144
H	2.788157	0.736275	-0.285811
H	2.444648	2.501046	-0.104856
H	0.052146	2.153154	-0.205711
H	0.806358	1.248094	-1.575417
H	0.533055	-0.901202	-0.394770
H	-1.047899	-0.025098	-0.391656
H	0.515767	0.371785	5.109253

ProD4 TS

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.522230
C	1.423702	0.000000	2.094786
C	2.179636	1.259935	1.695350

C	2.207690	1.354504	0.149006
C	0.793346	1.238409	-0.469706
C	-0.643761	-1.128262	2.299657
O	0.037458	-1.233821	3.515515
C	1.141021	-0.341599	3.545038
N	2.558471	-1.314696	4.206009
C	2.109684	-2.387918	5.094718
C	1.376534	-1.992732	6.387722
C	1.090894	-3.187727	7.306433
C	0.353830	-2.798572	8.592659
C	0.068413	-3.998258	9.496701
C	-0.648026	-3.622841	10.774826
O	-0.896969	-4.712929	11.544037
O	0.900845	0.765556	4.319759
O	-1.572609	-1.838597	2.038735
O	-0.974799	-2.505052	11.108462
H	1.478323	-3.065922	4.510844
H	3.010975	-2.961196	5.362062
H	0.421013	-1.513993	6.131892
H	1.977152	-1.249455	6.933481
H	2.039281	-3.681739	7.561655
H	0.499394	-3.931569	6.754768
H	-0.593007	-2.303853	8.346153
H	0.939907	-2.060187	9.152455
H	0.994388	-4.517467	9.776405

H	-0.542030	-4.751094	8.981686
H	-1.354249	-4.383062	12.335999
H	3.001664	-0.585859	4.771273
H	1.954253	-0.872842	1.695969
H	-0.504844	0.913850	1.875992
H	3.203974	1.243126	2.084582
H	1.686301	2.144062	2.119395
H	2.794171	0.496045	-0.213166
H	0.878268	1.232847	-1.562663
H	0.221468	2.141592	-0.210392
H	0.470974	-0.919454	-0.369970
H	-1.020493	0.018597	-0.395745
H	0.555962	0.467878	5.174497
H	2.651277	2.303479	-0.148648

ProD4 product

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.522230
C	1.423702	0.000000	2.094786
C	2.179636	1.259935	1.695350
C	2.207690	1.354504	0.149006
C	0.793346	1.238409	-0.469706
C	-0.643761	-1.128262	2.299657
O	0.037458	-1.233821	3.515515
C	1.141021	-0.341599	3.545038

N	3.449571	-1.926448	4.621538
C	3.000784	-2.999670	5.510247
C	2.267634	-2.604484	6.803251
C	1.981995	-3.799479	7.721962
C	1.244930	-3.410323	9.008188
C	0.959513	-4.610010	9.912230
C	0.243074	-4.234592	11.190355
O	-0.005868	-5.324681	11.959566
O	0.900845	0.765556	4.319759
O	-1.572609	-1.838597	2.038735
O	-0.083698	-3.116803	11.523991
H	2.369423	-3.677674	4.926373
H	3.902075	-3.572947	5.777591
H	1.312113	-2.125745	6.547421
H	2.868253	-1.861207	7.349010
H	2.930382	-4.293490	7.977184
H	1.390494	-4.543320	7.170297
H	0.298093	-2.915604	8.761682
H	1.831007	-2.671939	9.567984
H	1.885488	-5.129219	10.191933
H	0.349070	-5.362846	9.397215
H	-0.463149	-4.994814	12.751528
H	3.892764	-1.197611	5.186802
H	1.954253	-0.872842	1.695969
H	-0.504844	0.913850	1.875992

H	3.203974	1.243126	2.084582
H	1.686301	2.144062	2.119395
H	2.794171	0.496045	-0.213166
H	0.878268	1.232847	-1.562663
H	0.221468	2.141592	-0.210392
H	0.470974	-0.919454	-0.369970
H	-1.020493	0.018597	-0.395745
H	2.651277	2.303479	-0.148648
H	4.111833	-2.295259	3.957120

ProD5 GM

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.527262
C	1.426344	0.000000	2.086585
C	2.253713	-1.129221	1.481012
C	2.252862	-1.078975	-0.039829
C	0.822046	-1.142705	-0.556974
C	1.396268	-0.209146	3.600550
O	0.462389	-0.830318	4.147719
C	-0.742898	1.227907	1.987462
O	-2.090961	1.061691	2.058581
C	3.079572	-2.215038	-0.606123
N	2.397522	0.295345	4.405445
C	3.643126	0.854223	3.967709
C	4.861114	-0.064991	4.124804

C	5.164818	-0.374737	5.575585
C	6.450482	-1.168619	5.699097
C	6.733515	-1.510750	7.148185
C	8.064573	-2.175546	7.297439
O	8.215455	-2.840409	8.477333
O	9.028145	-2.198452	6.529068
O	-0.308596	2.346429	2.272719
H	3.570319	1.153494	2.885752
H	3.827593	1.791424	4.569341
H	5.738341	0.456989	3.661767
H	4.703141	-1.019917	3.561283
H	4.323062	-0.960134	6.028435
H	5.255747	0.581140	6.153943
H	7.308643	-0.581655	5.279344
H	6.373377	-2.112109	5.099609
H	5.938850	-2.186964	7.558390
H	6.745683	-0.580823	7.776477
H	9.099343	-3.236918	8.519248
H	2.329914	0.084306	5.376001
H	1.886691	0.994779	1.834817
H	-0.544353	-0.908901	1.910664
H	-2.504714	1.881188	2.371580
H	1.840650	-2.114172	1.821874
H	3.307056	-1.050962	1.856960
H	2.707852	-0.103040	-0.366887

H	0.361141	-2.121481	-0.266161
H	0.824316	-1.087871	-1.675547
H	-1.058360	-0.082719	-0.358853
H	0.412880	0.974150	-0.370684
H	4.129040	-2.155449	-0.232564
H	3.095048	-2.161664	-1.720239
H	2.652005	-3.200493	-0.304898

ProD5 TET

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.508657
C	1.456191	0.000000	2.013250
C	2.091409	1.325632	1.678004
C	2.081885	1.478735	0.152292
C	0.694560	1.278566	-0.459038
C	-0.614449	-1.117702	2.306071
O	0.080062	-1.280884	3.501294
C	1.272817	-0.437366	3.502232
N	2.412117	-1.171310	4.037324
C	2.220267	-1.856189	5.297244
C	2.465214	-1.030755	6.564528
C	2.331885	-1.903857	7.795498
C	2.548710	-1.093399	9.057913
C	2.478392	-1.977814	10.287045
C	2.544547	-1.164604	11.540410

O	2.957887	-1.869471	12.631029
O	0.854504	0.681358	4.260656
O	-1.585754	-1.841808	2.160498
C	2.624812	2.840260	-0.232279
O	2.272621	0.022963	11.727730
H	1.178016	-2.280395	5.326642
H	2.942980	-2.721604	5.292252
H	1.732810	-0.184766	6.620688
H	3.490705	-0.579776	6.535993
H	3.080561	-2.736708	7.748053
H	1.313956	-2.372232	7.817584
H	1.774549	-0.285708	9.134794
H	3.547880	-0.587751	9.018151
H	3.314978	-2.724082	10.287756
H	1.512452	-2.549318	10.305194
H	2.964375	-1.292523	13.410344
H	3.201857	-0.556418	4.062659
H	2.029921	-0.819502	1.497802
H	-0.517067	0.937946	1.869304
H	3.144626	1.363127	2.053107
H	1.527291	2.162525	2.161824
H	2.761393	0.687989	-0.273251
H	0.793432	1.258065	-1.574452
H	0.050222	2.156793	-0.194220
H	0.537961	-0.898441	-0.394425

H	-1.047313	-0.029218	-0.392894
H	0.398053	0.361502	5.049470
H	1.976158	3.651688	0.174382
H	2.664116	2.942252	-1.342392
H	3.654877	2.974424	0.174260

ProD5 TS

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.522633
C	1.417506	0.000000	2.096984
C	2.166841	1.267090	1.697318
C	2.198000	1.364952	0.151005
C	0.785628	1.242245	-0.469414
C	-0.657344	-1.125821	2.296074
O	0.014341	-1.266669	3.493351
C	1.136087	-0.341212	3.566622
N	2.528324	-1.228990	4.383009
C	2.388592	-1.607850	5.794068
C	2.814100	-0.543729	6.819391
C	2.679390	-1.029416	8.267433
C	3.141450	0.009116	9.295786
C	2.997509	-0.481798	10.736739
C	3.461295	0.531789	11.759445
O	3.292794	0.072950	13.025388
O	0.667077	0.832298	4.216147

O	-1.605667	-1.808218	2.014055
C	2.893549	2.650560	-0.309934
O	3.930183	1.622871	11.521014
H	1.346659	-1.907120	5.953378
H	2.985049	-2.515010	5.955709
H	2.229016	0.371037	6.671259
H	3.860713	-0.266173	6.626574
H	3.258190	-1.955341	8.395953
H	1.631959	-1.299976	8.464694
H	2.570252	0.936834	9.173726
H	4.188091	0.278095	9.111877
H	3.568621	-1.404529	10.903224
H	1.956575	-0.739082	10.971073
H	3.615201	0.778385	13.611578
H	3.394797	-0.726092	4.217154
H	1.949807	-0.872202	1.694368
H	-0.499105	0.915670	1.875721
H	3.194977	1.269332	2.082099
H	1.663758	2.143466	2.124675
H	2.790021	0.510696	-0.212797
H	0.873078	1.239538	-1.562308
H	0.209579	2.142379	-0.208881
H	0.473813	-0.916936	-0.373154
H	-1.021487	0.013657	-0.393191
H	0.110961	0.549260	4.955675

H	2.341324	3.536944	0.024243
H	2.964005	2.695147	-1.401909
H	3.909396	2.720321	0.093801

ProD5 product

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.522633
C	1.417506	0.000000	2.096984
C	2.166841	1.267090	1.697318
C	2.198000	1.364952	0.151005
C	0.785628	1.242245	-0.469414
C	-0.657344	-1.125821	2.296074
O	0.014341	-1.266669	3.493351
C	1.136087	-0.341212	3.566622
N	3.403575	-1.787105	4.896243
C	3.263843	-2.165965	6.307301
C	3.689350	-1.101844	7.332624
C	3.554641	-1.587531	8.780666
C	4.016701	-0.548999	9.809019
C	3.872759	-1.039912	11.249972
C	4.336546	-0.026326	12.272678
O	4.168045	-0.485164	13.538622
O	0.667077	0.832298	4.216147
O	-1.605667	-1.808218	2.014055
C	2.893549	2.650560	-0.309934
O	4.805433	1.064756	12.034247

H	2.221910	-2.465235	6.466611
H	3.860299	-3.073125	6.468942
H	3.104267	-0.187078	7.184493
H	4.735963	-0.824288	7.139808
H	4.133440	-2.513456	8.909187
H	2.507210	-1.858091	8.977927
H	3.445502	0.378719	9.686959
H	5.063341	-0.280020	9.625110
H	4.443872	-1.962644	11.416458
H	2.831825	-1.297197	11.484306
H	4.490451	0.220270	14.124811
H	4.270047	-1.284207	4.730387
H	1.949807	-0.872202	1.694368
H	-0.499105	0.915670	1.875721
H	3.194977	1.269332	2.082099
H	1.663758	2.143466	2.124675
H	2.790021	0.510696	-0.212797
H	0.873078	1.239538	-1.562308
H	0.209579	2.142379	-0.208881
H	0.473813	-0.916936	-0.373154
H	-1.021487	0.013657	-0.393191
H	2.341324	3.536944	0.024243
H	2.964005	2.695147	-1.401909
H	3.909396	2.720321	0.093801
H	3.401469	-2.616775	4.323786

تصميم ادوية مساعدة مبتكرة من حمض امينوكابروك باستخدام الطرق الحسابية

اعداد : نداء مازن سالم لقيانية

اشراف : البروفسور رفيق قرمان

الملخص:

أدى توضيح الية عمل عدد من نماذج الانزيم إلى تصميم ادوات كيميائية فعالة لها القابلية ان تستخدم كروابط لتصميم ادوية مساعدة .هذه الروابط ترتبط مع الدواء الام برابطة تساهمية لتكوين (prodrug) . بحيث يتحول (prodrug) الى الدواء النشط عن طريق روابط داخل الجزيء نفسه دون أي تدخل من الإنزيمات. وتكون عملية التحول من (prodrug) الى الدواء النشط بطريقة مبرمجة. على سبيل المثال توضيح الية انتقال البوتونات في نموذج انزيم العالم كاربي ادى الى تصميم عدد من (prodrug) مثل tranxmic acid, acyclovir ,atenolol

باستخدام الطرق الحسابية DFT على مستويات مختلفة لعملية نقل البروتون ضمن جزئي من نموذج الانزيم للعالم كاربي حيث ثم استغلال هذا النموذج للعالم كاربي لتصميم خمسة ادوية مساعدة من 6-Aminocaproic acid

استنادا الى الطرق الحسابية DFT لعملية نقل البروتون ل 1-7 وخمسة Prodrug من 6-aminocaproic acid تبين ان سرعة انتقال البروتونات تعتمد على قوة strain وليس هناك علاقة بين سرعة الانتقال والمسافة بين المركزين اللذين يحدث بينهما انتقال البروتونات وزاوية الهجوم

وبالتالي فان سرعة تحويل الدواء المساعد الى الدواء النشط تعتمد على الخواص الشكلية للرابط المستخدم .

عمادة الدراسات العليا

جامعة القدس

تصميم أدوية مساعدة مبتكرة من حمض امينو كابروك بالطرق الحسابية

إعداد

نداء مازن سالم لقيانية

رسالة ماجستير

فلسطين ـ القدس

1438/2017

تصميم أدوية مساعدة مبتكرة من حمض امينو كابروك بالطرق الحسابية

إعداد

نداء مازن سالم لقيانية

بكالوريوس صيدلة-جامعة القدس، فلسطين

المشرف الرئيسي: بروفيسور رفيق قرمان

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الصيدلانية من كلية الدراسات العليا جامعة القدس-فلسطين.

1438/2017