

**Deanship of Graduate Studies
Al-Quds University**



**Synthesis, Characterization and *in Vitro* Kinetics of Novel
Atenolol Prodrugs**

Ala' Ahmad Mohammad Qtait

M.Sc. Thesis

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Atenolol Prodrugs

Prepared By:
Ala' Ahmad Mohammad Qtait

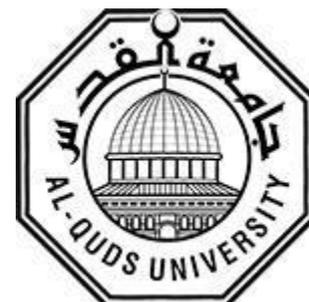
B.Sc., Pharmacy, Al-Quds University, Palestine.

Supervisor: Prof. Dr. Rafik Karaman

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University.

1438/2016

Al-Quds University
Deanship of Graduate Studies
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Thesis Approval

**Synthesis, Characterization and *in Vitro* Kinetics of Novel
Atenolol Prodrugs**

Prepared by: Ala' Ahmad Qtait

Registration No.: 21112815

Supervisor: Prof. Dr. Rafik Karaman

Master thesis Submitted and Accepted, Date: 21/12/2016

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

1-Head of Committee: Prof. Dr. Rafik Karaman

Signature:

2- Internal Examiner: Dr. Hatem Hejaz

Signature:

3- External Examiner: Dr. Nasr Shraim

Signature:

Jerusalem–Palestine
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الإهداء

إلى الأيادي البيضاء التي إمتدت لتنهضني من كل كبوة , وتسندني بعد كل عثرة , وتتشلني بكل حنان من
براشن اليأس لترفعني عاليا على معارج المجد !!!

إلى من كلت أنامله لينسج لحظات سعاديإلى من تجرع من كؤوس المر ليسقيني قطرات الحب والحنان
....إلى من علمني العطاء والصبر والجد دون إنتظار إلى من أحمل أسمه بكل إفتخار إلى القلب الرحب
.....أبي العزيز

إلى سيدة على سجادة صلاتها يعبق فوها بعير كلمات تناجي الرحمن وتدعوه ليأخذ بيدي ويوقفي.....إلى من
نسجت أحلامي بخيوط كدها وجمدها ...إلى ملاكي الذي يرتعش قلبي بذكرهأمي الغالية

إلى ملجئي وملاذيإلى البسمة الدافئة ..إلى من أرى نفسي وحلمي ومجدي بعينه ..إلى من شاركي
الدرب خطوة بخطوة ...معك أكون أنا ,وبدونك أكون مثل أي شيء ...زوجي الحبيب

إلى توؤم روحيونصفي الآخر ..إلى القلب الطيب ..أختي الحنونة ولاء

إلى من تحلو الحياة بوجودهم و عبق كلماتهم إخوتي أجبائي المهندس ضياء و الدكتور علاء والمحامي بهاء
وزوجاتهم.....

إلى قطعة من روحي....إلى الزهرة التي تفتحت لتزهر وتزين حياتيطفلاتي الغالية جنى

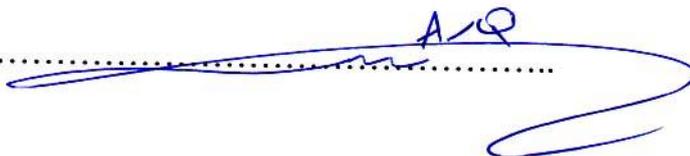
إلى عائلتي الثانيةالتي أحضنتني بكل الحب والحنان وطالما شجعنتني ودعمتني ... والد ووالدة زوجي
الغالين

إليكم جميعا أهدي هذا البحث المتواضع

الآء قطييط

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:.....A handwritten signature in blue ink, consisting of a long horizontal stroke with a loop at the end and the initials 'A/Q' written above it.

Ala' Ahmad Qtait

Date: 21/12/2016

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Abstract

Prodrugs are substances administered in an inactive form that is then metabolized in the body into the corresponding active drug. The rationale behind administering prodrugs is to optimize the drug absorption, distribution, metabolism, and excretion. Since first described in the 1950s, prodrugs continue to be a fertile area of research. There are a number of small pharmaceutical/biotech companies dedicated to use prodrugs for the delivery of older but problematic drugs as well as to develop broad-based prodrug technologies for application to new and future drugs

Recently Drug design become increasingly relies on computer modeling techniques. In the past few decades computational chemistry methods have been utilized in calculating physicochemical and molecular properties of compounds. This tool can be used to design prodrugs that chemically (intramolecular processes) interconvert to their parent drugs without any involvement of enzyme catalysis. The release of the active drug is solely dependent on the rate limiting step of the intramolecular process. Based on DFT calculations three different atenolol prodrugs were designed, synthesized and characterized using FT-IR, $^1\text{H-NMR}$ LC-MS and their *in vitro* intra-conversion to their parent drug, atenolol, revealed that the $t_{1/2}$ was largely affected by the pH of the medium. For atenolol ProD **1** *in vitro* kinetic study demonstrated that the $t_{1/2}$ was largely affected by the pH of the medium. The determined $t_{1/2}$ values in 1N HCl, buffer pH 3, and buffer pH 5 were 1.66, 3.73, and 168 hours, respectively. And at pH 7.4 no intraconversion was observed. On the other hand, atenolol **ProD 2** was found to be readily intraconverted, but In the case of atenolol **ProD 3** no reaction was observed.

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List of Abbreviations

Abbreviations	Definition
A°	Angstrom
ATE	Atenolol
BCS	Biopharmaceutics Classification system
CADD	computer-aided drug design
CAMD	computer-aided molecular design
CAMM	computer-aided molecular modeling
DFT	Density functional theory.
DMF	Dimethyl formamide
FAD	Food and Drug administration
FT-IR	Fourier Transform Infra- Red
HCl	Hydrogen Chloride
HPLC	High-performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
K _{obs}	The observed hydrolysis rate constant.
LC-MS	Liquid chromatography-Mass spectrometry
LC-MS	Liquid chromatography–mass spectrometry
M.P	Melting point
m/z	Mass-to-Charge ratio
MgSO ₄	Magnesium Sulfate
MI	Myocardial Infarction
MM	Molecular Mechanics
NaOH	Sodium Hydroxide
NMR	Nuclear magnetic resonance

PDA	Photodiode Array
pH	Power of Hydrogen
PK	pharmacokinetic
ppm	Part per million
ProD1	Atenolol prodrug
QM	Quantum mechanics
Q-TOF	Quadrupole Time-of-Flight
R _f	Retention Factor
rpm	Round per Minutes
t _{1/2}	Half life
TLC	Thin layer chromatography
US	United states
UV	Ultraviolet

Introduction

Chapter One:

Introduction

1.1 Background

Many therapeutic drugs have undesirable properties that may become pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug application[1]. Among the various approaches to minimize these undesirable drug properties while retaining the desirable therapeutic activity is the development of new chemical entities with desirable efficacy and safety. However, this is an expensive and time consuming process that needs a screening of thousands of molecules for biological activity (Figure 1.1). So that, it becomes much more feasible to modify and improve the properties of already existing drugs through exploring the prodrug approach[2].in order to get rid of their undesirable features and to increase their commercial life cycle and patentability [3] .

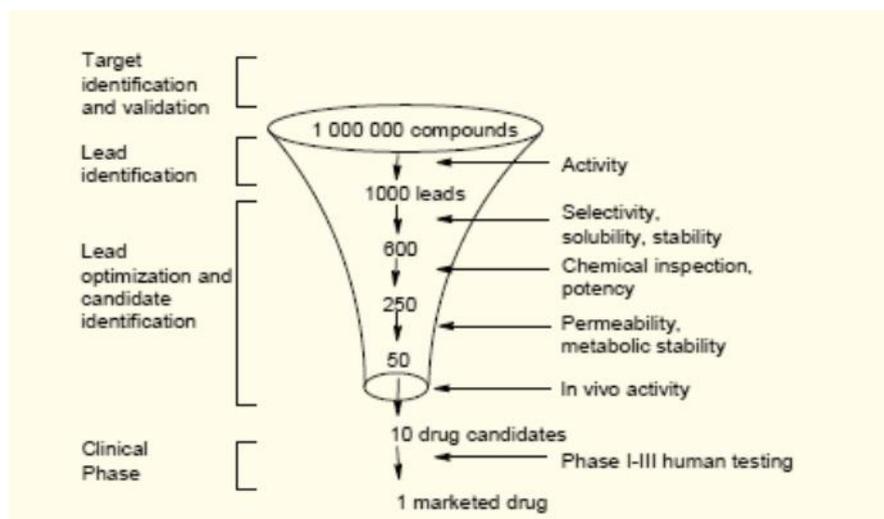


Figure (1.1) : The drug development funnel .

A very good indication of the success of the prodrug approach can be obtained by examining the prevalence of prodrugs on the market. It might come as a surprise to many people that currently approximately 10% of all globally marketed medicines can be classified as prodrugs, and in 2008 alone, 33% of all approved small-molecular-weight drugs were prodrugs [3, 4].

Recently drug design become increasingly relies on computer modeling techniques. This type of modeling is often referred to as computer-aided drug design using computational chemistry to discover, enhance, or study drugs and related biologically active molecules [5], ideally the computational method should be able to predict affinity before a compound is synthesized and hence in theory only one or two compounds need to be synthesized. These modern computational methods can be used for an intelligent design of innovative prodrugs. For example, mechanisms of some intramolecular processes that have been utilized for better understanding of enzyme catalysis have been recently investigated and exploited for the design of novel prodrug linkers. In this approach, no enzyme is needed for the catalysis of the intra conversion of a prodrug to its parent drug. The release of the drug from the corresponding prodrug is solely dependent on the rate limiting step for the intra conversion reaction [6, 7].

1.2 Prodrug

During the past few decade, the pharmaceutical sciences have undergone a molecular revolution which greatly impacted the field of drug design and delivery in general, and the utilization of the prodrug approach in particular [8]. Today, drug discovery teams in pharmaceutical companies, big and small, are multidisciplinary, because of this, the idea of implementing a potential prodrug solution to a problematic drug is more likely to be put in to action early [9].

1.2.1 The prodrug concept

The terms “pro-drug” and “pro-agent” were first used by Albert in 1958 in his article in Nature to signify a pharmacologically inactive chemical moiety that can be used to temporarily alter the physicochemical properties of a drug to increase its usefulness and decrease its associated toxicity. Others such as Harper also promoted the concept but used the term drug latention. The use of the term generally implies a chemical device by which a drug is linked to a chemical promoiety *via* a covalent bond [10-17].

According to the International Union of Pure and Applied Chemistry (IUPAC), the term prodrug is defined as “any compound that undergoes biotransformation before exhibiting its pharmacological effects (Figure 1.2) [18].

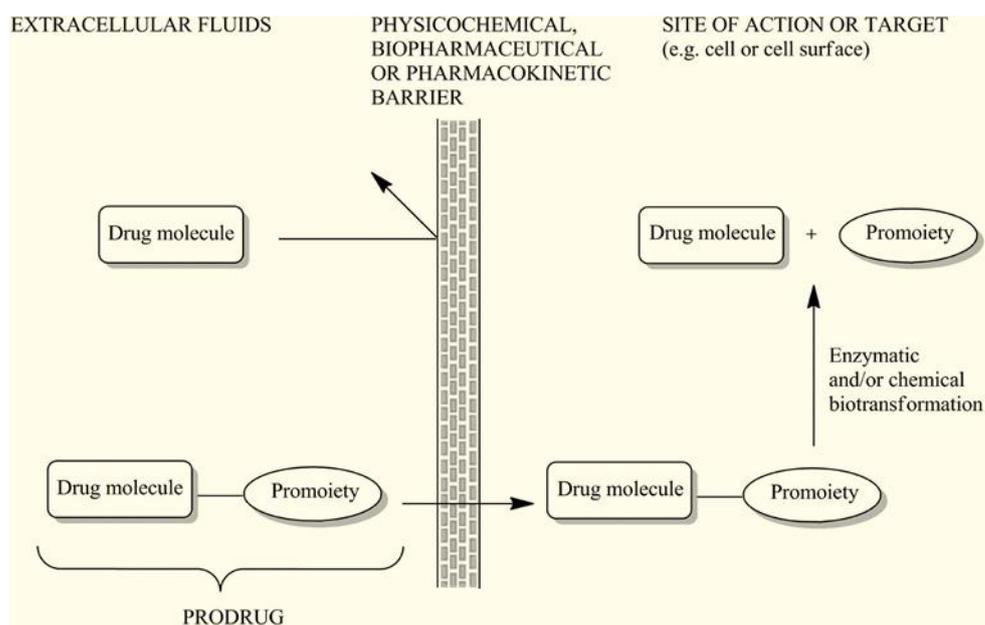


Figure 1.2: A simplified illustration of the prodrug concept.

In most cases, prodrugs are simple chemical derivatives that require only one to two chemical or enzymatic transformation steps to yield the active parent drug. In some cases, a prodrug may consist of two pharmacologically active drugs that are coupled together in a single molecule so that each drug acts as a promoiety for the other; such derivatives are called co-drugs [19, 20].

An ideal prodrug should be non-toxic, and has optimal physicochemical properties, such as lipophilicity, solubility, and stability in GI tract or in its desired dosage form, it must be stable in storage and resistant to degradation in different body fluids until that point when it reaches its site of action. Finally after reaching the specific site of action, there should be a quantitative release of the drug. It is important that the inactive moiety should be non-toxic, releases the active drug at an appropriate rate *in vivo* and preferably rapidly eliminated from the body [1, 21, 22].

The physicochemical properties and bioproperties of drugs that allow their formulation and delivery have recently been defined by the terms “drugable” or “drug-like” properties. Therefore, if a drug candidate does not have drugable properties while an appropriate prodrug of that molecule does, use of this prodrug can result in effective, safe, and efficient delivery of the parent drug to the systemic circulation and to the desired site of action [12].

1.2.2 History of prodrug design

Adrien Albert first introduced the term “pro-drug” in 1958 [10]. A few decades later, he apologized for having invented such an inaccurate term, because “predrug” would have been a more descriptive term. However, by that time, the original version was used too widely to be changed. Nonetheless, the prodrug concept has been invented long before Albert's publication. The first intentionally designed prodrug is most probably methenamine (or hexamine), which was introduced 1899 by Schering [12, 21, 23]. At the same time, Bayer introduced aspirin (acetylsalicylic acid) as a less irritating form of the anti-inflammatory agent sodium salicylate [23]. Many decades elapsed until Bayer introduced their next prodrug, the antibiotic prontosil, in 1935 [24]. In a similar way, Roche discovered the prodrug activity of the anti-tuberculosis drug isoniazid more than 40 years after its introduction in 1952 [25].

Since the 1960s there has been an explosive increase in the use of prodrugs in drug discovery and development. At the beginning of 21st century, the property-based drug design became an essential part of the drug discovery and development process [26]. This can be seen in published journal articles, reviews, and patents, as well as in numbers of clinically studied and marketed medicines. Moreover, dozens of prodrugs are currently undergoing clinical trials. To emphasize the extent of the successful implementation of the prodrug approach, almost 15% of the 100 best-selling small-molecular-weight drugs in 2009 were prodrugs [27].

1.2.3 Applications of prodrug approaches

In both drug discovery and drug development, prodrugs have become an established tool for improving physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically active agents that overcome barriers to a drug's usefulness, thus the prodrug approach has been extensively studied amongst the drug design scientist for a wide range of applications.

1.2.3.1 Pharmaceutical application

The formulation of a new chemical entity with suspected therapeutic benefits requires that the drug be formulated into a delivery form that is chemically stable, free from taste and odor problems (particularly if it is for pediatric use or intended for parenteral administration), and the drug formulation must be relatively free of irritation on administration. For intravenous usage, the drug should have adequate water solubility and remain in solution for sufficient time to permit administration of the complete dose [13].

The undesirable organoleptic properties and physicochemical problems associated with drug formulation can be resolved by prodrug approaches.

1.2.3.1.1 Improvement of Taste

Taste is an important parameter in case of drugs administering orally. Taste masking becomes a prerequisite for bitter drugs to improve the patient compliance especially in the pediatric and geriatric population. The problem of bitter taste of drug in pediatric formulations is a challenge to the formulators in the present scenario. Masking the bitter taste of drugs is a potential tool for the improvement of patient compliance which in turn decides the commercial success of the product [28]

Different approaches are commonly utilized to overcome bitter and an unpleasant taste of drugs. This includes reduction of drug solubility in saliva, where a balance between reduced solubility and bioavailability must be achieved, but these approaches were found to be limited and could not overcome the problem of bitterness [29].

Drugs or molecules interact with taste receptors on the tongue to give bitter, sweet or other taste sensations. So altering the ability of the drug to interact with taste receptors could reduce or eliminate their bitterness. This could be achieved by an appropriate modification of the structure and the size of a bitter compound.

1.2.3.1.2 Enhancement of chemical stability

A very important requirement of all drug products is that they must be chemically stable over a reasonable period, if a drug is chemically very unstable and the instability problem cannot be resolved by formulation means, it is sometimes possible to develop a prodrug with enhanced stability over the parent drug. This usually takes the form of chemical modification of the functional group responsible for the instability.

1.2.3.2 Pharmacokinetic applications

Drawbacks in pharmacokinetic parameters which affect the bioavailability and mean residence time of drug in body can be modulated by the prodrug approach. Following are the goals achieved by the prodrug approach.

1.2.3.2.1 Enhancement of bioavailability (lipophilicity)

For agents that are highly polar in nature, it is often the transport of the drug across the gastrointestinal mucosal cell membrane limits its absorption. Since most drugs are absorbed by passive diffusion, a degree of lipophilicity is necessary for efficient absorption through the gastrointestinal barrier.

Prodrugs are most frequently applied to mask polar and ionizable groups of a drug molecule with the aim to improve the membrane permeability and oral absorption. Two reasons can be attributed to the enhanced oral bioavailability of lipophilic compound :

1. The lipophilic form of a drug has enhanced membrane /water partition coefficient as compared to the hydrophilic form thus favoring passive diffusion.
2. The lipophilic prodrugs have poor solubility in gastric fluids and thus greater stability and absorption [30-32].

1.2.3.2.2 Prolongation of duration of action

Frequent dosing is required for drugs having short biological half-lives. This can be overcome by use of controlled release devices as well as the prodrug approach which can be employed for delayed and controlled drug release in systemic circulation.

Prolonged release drugs are quite important in the treatment of psychoses because these patients require medication for extended periods of time and often show high patient noncompliance rates [32, 33].

1.2.4 Amide prodrug

Amide prodrug approach can be used to enhance the stability of drugs, provide targeted drug delivery and to change lipophilicity of drugs like acids and acid chlorides[34]. Drugs that have carboxylic acid or amine group can be converted into amide prodrugs, generally they are used to a limited extent due to high *in vivo* stability. However, Prodrugs using facile intramolecular cyclization reactions have been exploited to get rid of this problem[35].

Amide prodrugs can be converted back to the parent drugs either by nonspecific amidases or specific enzymatic activation such as renal γ -glutamyltranspeptidase.

1.2.5 Prodrug design by computation methods

Since drug discovery and development is time and resources consuming process, there is an ever growing effort to apply computational power to the combined chemical and biological space in order to streamline drug discovery, design, development and optimization. Fast expansion in computation methods have been made possible by advances in software and hardware computational power and sophistication, identification of molecular targets, and an increasing database of publicly available target protein structures. Different terms are being applied to this area, including

computer-aided drug design (CADD), computational drug design, computer-aided molecular design (CAMD), computer-aided molecular modeling (Camm), rational drug design, in silico drug design and computer-aided rational drug design.

Computer-aided drug design is being utilized to identify active drug candidates, select the most likely candidates for further evaluation, and transform the biologically active compounds into suitable drugs by improving their physicochemical, pharmaceutical, ADMET/PK (pharmacokinetic) properties. Numerous compounds that were discovered and/or optimized using CADD methods have reached the level of clinical studies or have even gained US FDA approval [36-38].

Computational methods have accelerated discovery by reducing the number of iterations required and have often provided novel structures. Ideally the computational method should be able to predict affinity before a compound is synthesized and hence in theory only one compound needs to be synthesized [39, 40]. These computational methods considered as an effective tool that can be used for the design of innovative prodrugs for drugs that contain hydroxyl, phenol, or amine groups [41-44]. Nowadays, researchers have shifted from their way in synthesizing classical prodrugs into designing prodrugs for specific targeting of enzymes and transporters, thus increasing bioavailability and reducing toxicity, and achieving a better therapeutic drug's profile [45].

Targeted prodrugs can be divided into two kinds of approaches: the first by which a chemical moiety is linked to the parent active drug to selectively target an activating enzyme and the second by which the prodrug moiety is interconverted by intramolecular process to give the corresponding parent drug and a non-toxic linker without an involvement of enzymes [46-48]. Several prodrugs were designed by Karaman's group such prodrugs will undergo cleavage reactions in physiological environments like stomach at pH 1.5, intestine at pH 6.5 or/and blood circulation at pH 7.4, with rates that are solely dependent on the structural features of the pharmacologically inactive linker. Different linkers were investigated for the design of large number of prodrugs such as anti-Parkinson (dopamine), anti-viral (acyclovir) and anti-malarial (atovaquone) that might be efficient in releasing the parent drugs in various rates that are dependent on the nature or the structural features of the linkers and provide new novel prodrugs that might have the potential to be with enhanced dissolution and membrane penetration and hence better bioavailability [49-51].

Quantum mechanics (QM) such as ab initio, semi-empirical and Density Functional Theory (DFT), and Molecular Mechanics (MM) are increasingly being utilized and widely accepted as tools that provide structure-energy calculations for the prediction of potential drugs and prodrugs[52] . The ab initio molecular orbital methods (quantum mechanics) are based on rigorous utilization of the Schrodinger equation with a number of approximations. The use of this method is restricted to small systems that do not have more than thirty atoms due to the extreme cost of computation time. Semi-empirical methods are based on the Schrodinger equation with the addition of terms and parameters to fit experimental data and have afforded vast information for practical application. Calculations of molecules exceeding 50 atoms can be done using such methods. Density functional theory (DFT) is a quantum method used to calculate structures and energies for medium-sized systems of biological and pharmaceutical interest and is not restricted to the second row of the periodic table. Contrary to quantum mechanics, molecular mechanics is a mathematical approach used for the computation of structures, energy, dipole moment, and other physical properties, and is widely used in calculating many diverse biological and chemical systems [53-57].

1.3 Atenolol

Atenolol (ATE), 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzene acetamide, with a molecular weight of 266.3. Figure 1.3 is a selective β_1 -adrenoceptor antagonist, applied in the treatment of numerous cardiovascular disorders including: hypertension, angina, acute myocardial infarction, supraventricular tachycardia, ventricular tachycardia, and the symptoms of alcohol withdrawal. Atenolol does so by restricting certain nerve impulses, thereby controlling the rate and force of contraction, consequently reducing blood pressure in addition to its use in the treatment of Angina Pectoris.

On the outset, atenolol actively reduces the heart rate, in turn decreasing systolic and diastolic blood pressures. The net effect of both the heart rate and blood pressure being controlled is the reduction in myocardial work and oxygen requirement which reduce cardiovascular stress, thereby preventing arrhythmia and anginal attacks. Atenolol is currently available as tablets in strengths of 25 mg, 50 mg and 100 mg and an injectable formulation (in a strength of 5 mg/ml) supplied in 10-ml ampoules [58]

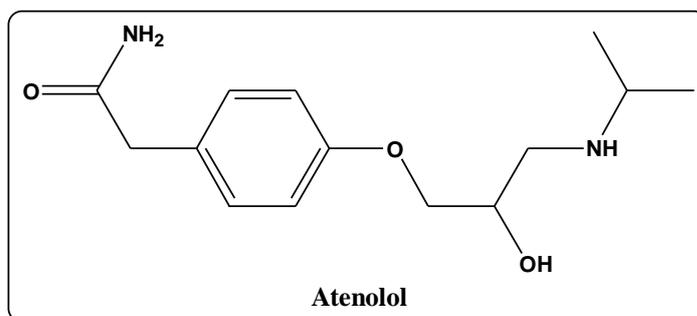


Figure (1.3): Chemical structure of atenolol.

1.4 Research problem

1. Atenolol is a very hydrophilic molecule with a pKa of 9.6, so it undergoes ionization in the stomach and intestine thus its oral bioavailability is low due to inefficient absorption through membranes. The bioavailability of atenolol is between 45% and 55% of the given dose and is not increased by administration of the drug in a solution form [59-61].
2. On the other hand, atenolol is one of the most important medicines used for prevention of several types of arrhythmias in childhood, but it is unlicensed [62]. Atenolol is also indicated as a first-step therapy for hypertension in elderly patients, who have difficulty in swallowing and, thus, tablets and capsules are frequently avoided. The ease of administration makes a liquid formulation an ideal dosage form for such patients [63]. As a result, extemporaneous compounding (off label), involves preparing an oral liquid from the pure drug powder, but formulations compounded from tablets and pure drug were stable only for less than one week [64, 65].
3. Furthermore, atenolol bitterness is considered as a great challenge to health sector when used among children and geriatrics. The main problem in oral administration of bitter drugs such as atenolol is non-compliance by the patients; this can be overcome by masking the bitterness of a drug either by decreasing its oral solubility on ingestion or eliminating the interaction of drug particles to taste buds [66-68].

Hence, it is expected that design and synthesis of a relatively more lipophilic prodrugs by masking amine group of atenolol will lead to an increase in the stability of its aqueous solutions since it will be more resistant to heat or/oxidation when

standing in aqueous solutions. In addition, it can provide the parent drug in a sustained release manner that might result in a better clinical outcome, more convenient dosing regimens and potentially less side effects [69].

1.5 Thesis objectives

Only few atenolol prodrugs have been synthesized to date, and scientists still try to find new options to enhance atenolol bioavailability and stability as well. In this research we tried to utilize the prodrug approach to synthesis a new entity that will perform better therapeutic profile than the parent drug, atenolol .

1.5.1 General objective

The main goal of this thesis was to synthesize novel prodrugs for atenolol that possess superior drug profile over atenolol parent drug and will be able to liberate atenolol in a chemically driven controlled manner once the prodrug reaches the blood circulation. Thus, introducing an alternative treatment option to the medical community that may help in addressing the critical need in hypertension treatment.

1.5.2 Specific Objectives

To achieve our thesis goal, the following specific objectives have to be accomplished:

1. To synthesize proposed atenolol prodrugs.
2. To purify and characterize the proposed prodrugs using several purification and characterization techniques.
3. To perform kinetic study of the interconversion of the synthesized atenolol prodrugs in different media 1 N HCl, buffer pH 3, buffer pH 5 and buffer pH 7.4.
4. The synthesized prodrugs should be readily dissolved in physiological media.
5. The synthesized prodrugs should increase the bioavailability profile of atenolol.
6. The synthesized prodrugs should have moderate hydrophilic lipophilic balance (HLB) value.
7. The synthesized prodrugs should be converted to atenolol in a programmable manner.

Literature review

Chapter Two:

2. Literature Review

2.1. Introduction

The most common linkage used in prodrug design is the ester linkage since it is easy to be synthesized and its function groups, hydroxyl and carboxyl acid, are widely available in most parent active drugs [70]. Amide bond is another commonly used linkage in prodrug design. It is derived from amine and a carboxyl group. The amide bond has higher enzymatic stability than the ester bond. Several other types of linkers including oximes, imines, disulfide and uncleavable thioether bond have also been used in the prodrug design [34, 71-75].

Previous studies on the synthesis of atenolol prodrugs were focused on obtaining an ester linkage for transdermal preparations only. Stability studies on these ester derivatives of atenolol have showed that the ester derivatives are much more stable than the corresponding alcohol, atenolol, when they are formulated in aqueous solutions [66, 76]. The only atenolol prodrug intended to be used for oral dosage form was atenolol aspirinate prodrug (aspirinm). It was described as antihypertensive therapy to reduce cardiovascular death, stroke, and myocardial infarction (MI), but studies showed that the coupling of atenolol and acetyl salicylic acid by means of an ester linkage did not produce good pharmacological results, neither *in vitro* nor *in vivo* [77].

2.2 Bioavailability

Atenolol is a water-soluble, ionizable drug and suffers from poor bioavailability when given by oral route due to its incomplete intestinal absorption and first-pass metabolism. Being a BCS class III drug, atenolol has a low log P (0.23) value, which also may be the reason for its poor intestinal absorption [78].

The combined effect of iontophoresis and esterification techniques was investigated by Anroop and coworkers by conducting a study on the transdermal delivery of a series of atenolol ester prodrugs. A number of ester prodrugs were synthesized, characterized and studied for physicochemical properties and stability. An evaluation

of the physicochemical parameters of the ester prodrugs showed significant increase in lipophilicity and slight reduction in the pKa value compared to that of the parent drug. In the prodrug series, permeation rate increased with an increase in the length of alkyl side chain up to the addition of 5 carbon units, but thereafter no specific pattern was recorded [66].

2.3 Stability

Since Atenolol is unstable in solutions and are not formulated for easy or accurate administration to children and elderly patients, the development of a liquid dosage form is a significant challenge. Several studies showed that the degradation rate of atenolol is dependent on the temperature, indicating higher stability at 4 °C. Atenolol syrup is stable for 9 days, with acceptable appearance [64].

Stability of atenolol in extemporaneously compounded liquid formulations was also studied in various vehicles. Atenolol powder or tablets was prepared at a concentration of 2 mg/ml in vehicles of Ora-Sweet, Ora-Sweet SF and Ora-Plus; as well as Roxane®: diluent consisting of 1% ethanol, 0.05% saccharin in a cherry-flavored, 33% polyethylene glycol 8000 and purified water. Atenolol was found to be not stable in Ora-Sweet. The authors recommend atenolol in Ora-Sweet SF as the vehicle where it was most stable for at least 90 days or in the Roxane Diluent where it was stable for up to 40 days [79].

Another study was conducted by Patel and coworkers by using simple syrup, a methylcellulose-based vehicle, Ora-Sweet, Ora-Plus, and Ora-Sweet SF vehicles. The concentration of atenolol in the formulations was 2 mg/mL. The HPLC results showed that all formulations prepared using the methylcellulose-based vehicle and Ora-Sweet SF were stable (ie, greater than 90% of atenolol remained unchanged) for more than 28 days. On the other hand, formulations prepared with simple syrup and Ora-Sweet were stable only for 14 days [65].

On the other hand, in a research study that was conducted by krzek to investigate the relationship between the polarity of atenolol, acebutolol, and propranolol described by their log P values and kinetic and thermodynamic parameters characterizing their degradation process in acidic solution. it has been found that the stability of drugs increases toward lipophilic propranolol in the assumed experimental model. The stability was found to be increased from the most hydrophilic atenolol, through

acebutolol, of lower polarity, to the most lipophilic propranolol. This study demonstrated that the stability of the chosen beta-adrenergic blocking agents increases with their lipophilicity [80].

2.4 Masking a bitter sensation

Many taste masking approaches have been used to improve the taste of strongly bad tasting medications and enhance the patient compliance. Among the approaches used to mask bitter taste of pharmaceuticals are: (1) taste masking using flavors, sweeteners and amino acids, (2) taste masking with lipophilic vehicle, (3) coating which is one of the most efficient and commonly used taste masking technique, (4) microencapsulation is a technique applicable to protect materials from oxidation, volatilizing as well as to mask their bitter tastes, (5) taste suppressants which are used for masking bitter taste of various compounding by competing on binding to the G-protein coupled receptor sites, (6) ion exchange resins, (7) inclusion complexes in which the drug molecule fits into the cavity of a complexing agent forming a stable complex, (8) pH modifiers, (9) adsorbates, (10) chemicals; the solubility and absorption of drugs can be modified by the formation of molecular complexes, (11) solid dispersions, (12) multiple emulsions, (13) liposomes and (14) prodrugs.

The problem of the bitter taste of drugs in pediatric and geriatric formulations still creates a serious challenge to pharmacists. Thus, different strategies should be developed in order to overcome this serious problem. The novel chemical approach utilized in this research involves the design of prodrugs for masking the bitter taste of pharmaceuticals based on intramolecular processes using density functional theory (DFT) and ab initio calculation methods and correlations of experimental and calculated reactions rates supposed by professor Karaman [81]. For example, paracetamol, a widely used pain killer and fever reducer found in the urine of patients who had taken phenacetin, has a very unpleasant bitter taste [82]. Phenacetin, on the other hand, lacks or has very slight bitterness. The difference in the structural features of both drugs is only in the nature of the group in the para position of the benzene ring. While in the case of paracetamol the group is hydroxyl, in phenacetin, it is ethoxy. Acetanilide has a chemical structure similar to that of paracetamol and phenacetin but lacks the group in the para position of the benzene ring, making it lacks the bitter taste characteristics of paracetamol (Figure 2.1).

These combined facts suggest that the presence of the hydroxy group on the para position is the major contributor for the bitter taste of paracetamol. It is believed that paracetamol interacts with the bitter taste receptors *via* hydrogen bonding which involves its phenolic group. In fact, recent study published by professor Karaman on binding of paracetamol and its prodrugs to bitter taste receptors has confirmed that prodrugs lacking the hydroxyl group are not bitter. Similarly, it is expected that blocking the amine group in atenolol with a suitable linker might inhibit the interactions between the amine group in atenolol and its bitter taste receptor/s and hence mask its bitterness.

The nature of the bitter taste activation with either paracetamol (*via* the phenolic group) or atenolol (*via* the amine group) is likely to be as a result of hydrogen bonding between the substrate (drug) and its receptor/s [83].

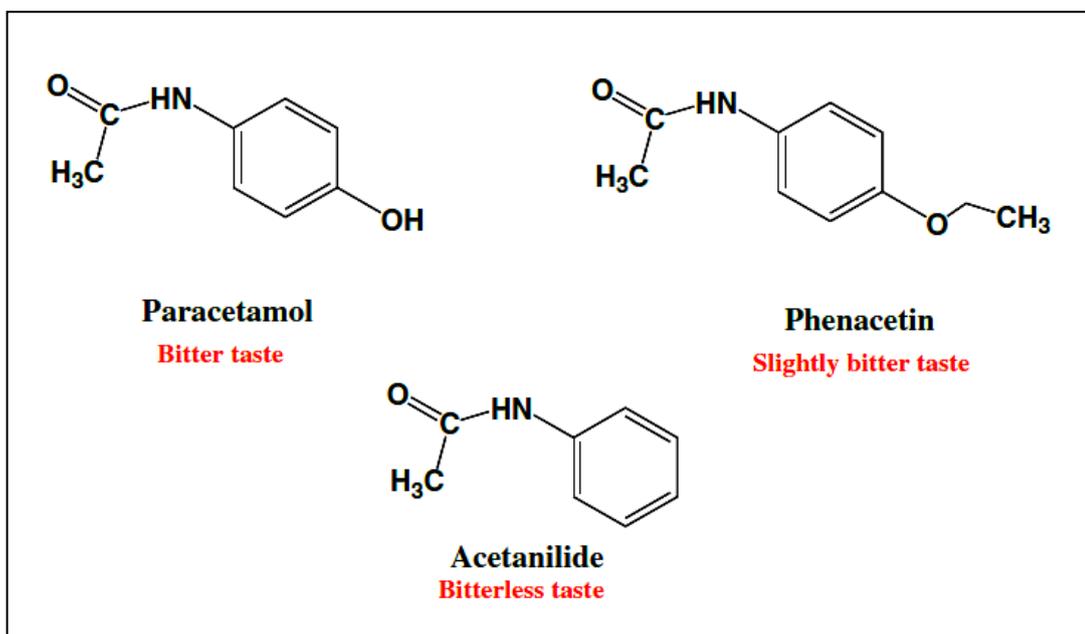


Figure 2.1: Chemical structures of paracetamol, phenacetin and acetanilide.

Experimental

Chapter Three:

3. Experimental Part

This chapter consists of two parts. The first one concerns with the reagents, instruments and reactions involved in atenolol **ProD 1**, **ProD 2** and **ProD 3** synthesis. The second one is the Kinetic studies part which describes the specific preparations and analysis used to investigate atenolol prodrugs hydrolysis in different pH solutions using an HPLC instrument.

1.1 Part one

1.1.1 Materials and Instrumentation

1.1.1.1 Materials:

Pure atenolol standard was commercially available, maleic anhydride, 2,3-dimethyl maleic anhydride, 2,2-dimethyl succinic anhydride, anhydrous sodium dihydrogen phosphate, sodium hydroxide, concentrated hydrochloric acid and magnesium sulfate anhydrous were commercially obtained from sigma Aldrich. HPLC grade solvents of methanol, acetonitrile and water were purchased from Sigma Aldrich. High purity chloroform, DMF and diethyl ether (> 99%) were purchased from Biolab .

1.1.1.2 Instrumentation

The melting points were determined in open capillaries on electrothermal stuart SMP3 advanced melting point apparatus, IR spectra were obtained from a KBr matrix (4000–400 cm^{-1}) using a Perkin-Elmer Precisely, Spectrum 100, FT-IR spectrometer.

The LC/ESI-MS/MS system used was Agilent 1200 series liquid chromatography coupled with a 6520 accurate mass quadrupole-time of flight mass spectrometer (Q-TOF LC/MS). The analysis was performed in the positive electrospray ionization mode. The capillary voltage was 4.0 kV, the scanned mass range was 200-540 m/z (MS). The high pressure liquid chromatography (HPLC) system consisted of an Alliance 2695 module equipped with 2996 Photodiode array detector from Waters (Germany). Data acquisition and control were carried out using Empower 2 TM software (Waters, Germany). Analytes

were separated on a 4.6 mm x 150 mm XBridge® C18 column (5 µm particle size) used in conjunction with a 4.6 x 20 mm, XBridge® C18 guard column. Microfilters of 0.45 µm porosity were normally used (Acrodisc® GHP, Waters).

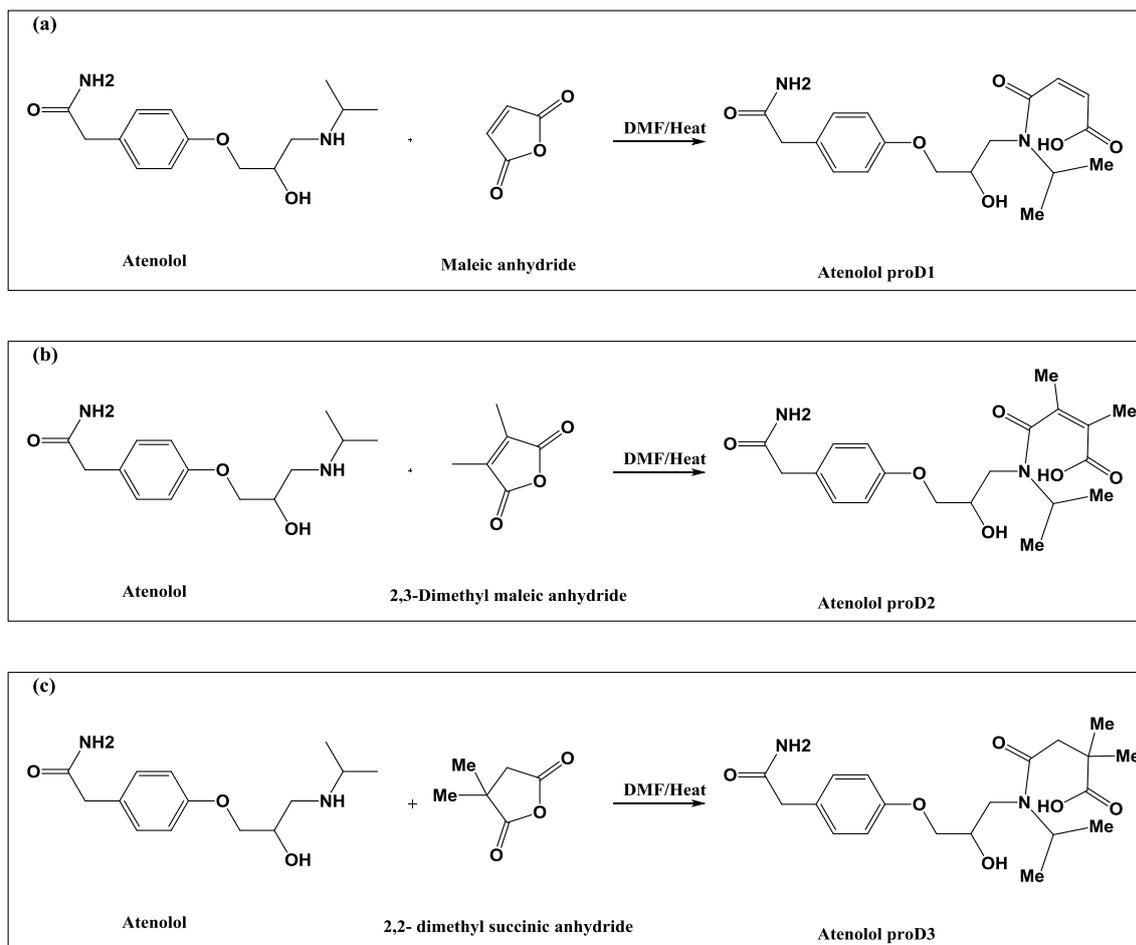
pH values were recorded on pH meter model HM-30G: TOA electronics™ was used in this study to measure the pH value for each sample, thin-layer chromatography (TLC) was carried out on TLC plastic sheets silica gel, 20 X 20 cm, layer thickness 0.2 mm, the spots on the chromatograms were localized by UV light.

UV-Spectrophotometer: the concentrations of samples were determined spectrophotometrically (UV-spectrophotometer, Model: UV-1601, Shimadzu, Japan) by monitoring the absorbance at λ_{\max} for each drug. Centrifuge: Labofuge®200 Centrifuge was used, 230 V 50/60 Hz. CAT. No. 284811. Made in Germany. ¹H-NMR: Data were collected using Varian Unity Inova 500 MHz spectrometer equipped with a 5-mm switchable and data were processed using the VNMR software. For ¹H-NMR, chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Spin multiplicities are described as s (singlet), brs (broad singlet), t (triplet), q (quartet), and m (multiplet).

1.1.2 Chemical synthesis of atenolol Prodrugs

Synthesis of the atenolol prodrugs was accomplished using Kirby's procedure [84]. (Scheme 1); in a 250 ml round bottom flask equipped with a stirrer and a condenser, 2.66 gm of standard atenolol (10 mmol) was dissolved in DMF (100 ml), then 1 equivalent maleic anhydride (0.98 gm (10 mmol)), 2,3-dimethyl maleic anhydride (1.26 gm (10 mmol)) or 2,2-dimethyl succinic anhydride (1.28 gm (10 mmol)) was added, the reaction mixture was refluxed for four hours, cooled, the DMF was removed by evaporation, the resulting residue was dissolved in 20 ml 1 N NaOH, extracted with 3 x 30 ml of ether, the organic layer was dried over MgSO₄ anhydrous and the dry residue was crystallized.

Reaction of atenolol with maleic anhydride provided atenolol **ProD 1** (2.95 gm) with yield of 81% , it was a white precipitate with M.P. of 185 °C, while reaction of atenolol with 2,3-dimethyl maleic anhydride provided atenolol **ProD 2** (2.75 gm) with yield of 70% , it was a white precipitate with M.P. of 205 °C, finally , reaction of atenolol with 2,3-dimethyl maleic anhydride provided atenolol **ProD 3** (3.03 gm) with yield of 77% , it was a yellowish greasy product .



Scheme 1: Synthetic schemes for the preparation of (a) atenolol **ProD 1**, (b) atenolol **ProD 2** and (c) atenolol **ProD 3** from atenolol and the corresponding anhydride.

1.2 Part two

1.2.1 Determination of the partition coefficient

The partition coefficients ($\log P$) were determined at room temperature with an organic phase (1-octanol)/aqueous phase (water). Before use, 1-octanol was saturated with water by stirring vigorously for 24 h. A known concentration of each compound was dissolved in water. The solution was shaken with a suitable volume of 1-octanol for 60 minutes. After shaking; the phases were separated by centrifugation at 10000 rpm for 10 minutes. The concentrations of the compounds in the water phase before and after the partitioning were determined by UV-visible spectrophotometer.

1.2.2 Kinetic studies on the stability of atenolol prodrugs at different buffer media

In this part, the conversion time of atenolol **ProD 1**, **ProD 2** and **ProD 3** into their parent drug in aqueous media were evaluated. This was accomplished by evaluating chemical hydrolysis of the prodrugs at pH 1, 3, 5 and 7.4 solutions which corresponding to different physiological media within the human body.

In order to determine the hydrolytic rate of the prodrugs at different pH media, certain preparations were performed as follows.

3.2.1.1 Buffer preparation

potassium dihydrogen phosphate (6.8 g) were dissolved in 900 ml water for HPLC, the pH of buffers pH 2 and pH 3 was adjusted by diluted o-phosphoric acid and water was added to a final volume of 1000 ml (0.05M). The same procedure was done for the preparation of buffers pH 5 and 7.4, however, the required pH was adjusted using 1 N NaOH [85].

3.2.1.2 Calibration curve for atenolol and atenolol Prodrugs

To construct a calibration curve for atenolol prodrugs and the parent drug, atenolol, several concentrations (10, 50, 100, 200, 300 and 500 ppm) were prepared. All samples were injected into HPLC-PDA. The optimal HPLC conditions used for the analysis of atenolol: 4.6 mm x 150 mm, 5 μ m, XBridge $\text{\textcircled{R}}$ C18 column, a mixture of buffer (pH 3.4):acetonitrile (85:15 v/v) as a mobile phase, a flow rate of 0.5 ml/minute and a UV detection at a wavelength of 230 nm. Peak area vs. concentration of the atenolol and atenolol prodrugs (ppm) was then plotted, and R^2 value of the plot was recorded.

3.2.1.3 Preparation of standard atenolol and atenolol prodrugs solutions.

A 500 ppm of standard atenolol was prepared by dissolving 50 mg of atenolol in 100 ml of 1N HCl, buffer pH 3, buffer pH 5 or buffer pH 7.4, and then each sample was injected into HPLC to detect the retention time of atenolol.

A 500 ppm of standard linker (maleic anhydride, 2,3-dimethyl maleic anhydride and 2,2-dimethyl succinic anhydride) was prepared by dissolving 50 mg of each linker in 100 ml of 1N HCl, buffer pH 3, buffer pH 5 or buffer pH 7.4, then each sample was injected into HPLC to determine the retention time for the linker.

A 500 ppm of atenolol **ProD 1-3** was prepared by dissolving 50 mg of the atenolol **ProD 1-3** in 100 ml of 1N HCl, buffer pH 3, buffer pH 5 or buffer pH 7.4 then each sample was injected into the HPLC to determine the retention time.

The progression of reaction was followed by monitoring the disappearance of the prodrug and appearance of atenolol and linker with time.

3.2.2 Hydrolysis of atenolol Prodrugs

Atenolol **ProD 1-3** rate hydrolysis was studied at 37 °C in buffer solutions at different pHs (1N HCl, pH 3, pH 5 and pH 7.4); samples of the reaction mixtures were analyzed directly by HPLC, quantitative analysis of atenolol prodrugs and their hydrolysis product, atenolol, was obtained using a calibration curve.

Results and Discussion

Chapter 4:

4. Results and discussion

4.1 Introduction:

Atenolol **ProD 1-3** were successfully synthesized, characterized and kinetically studied in different buffers with pH values corresponding to different physiological media inside the body.

As shown in Figure 4.1, the atenolol prodrugs have a carboxylic acid group (hydrophilic moiety) and a lipophilic moiety (the rest of the molecule), where the combination of both groups ensures a moderate HLB. It should be noted that the HLB value of the prodrug moiety will be determined upon the physiologic environment by which the prodrug is dissolved in. For example, in the stomach, the atenolol prodrugs will primarily exist in the carboxylic acid form whereas in the blood circulation the carboxylate anion form will be predominant.

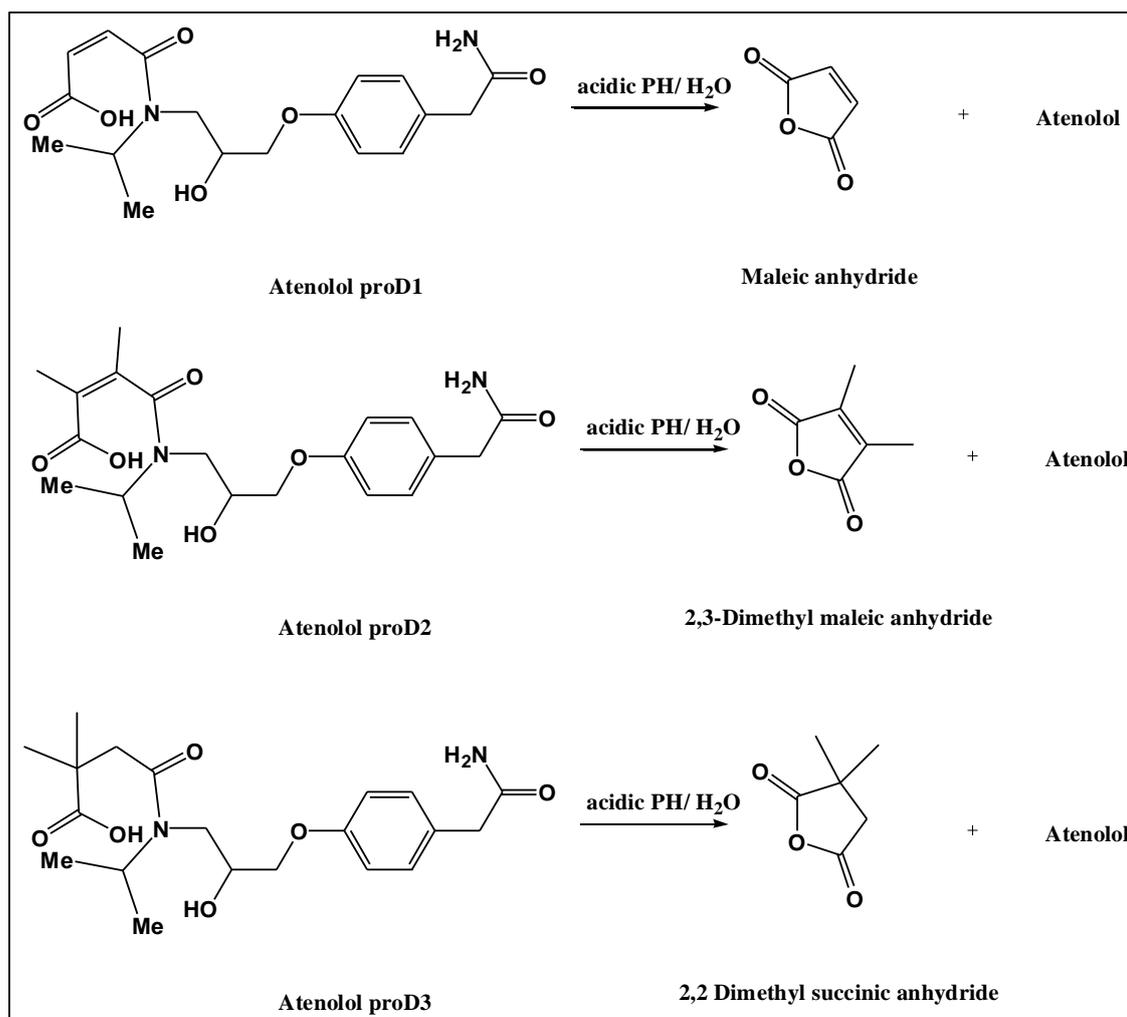


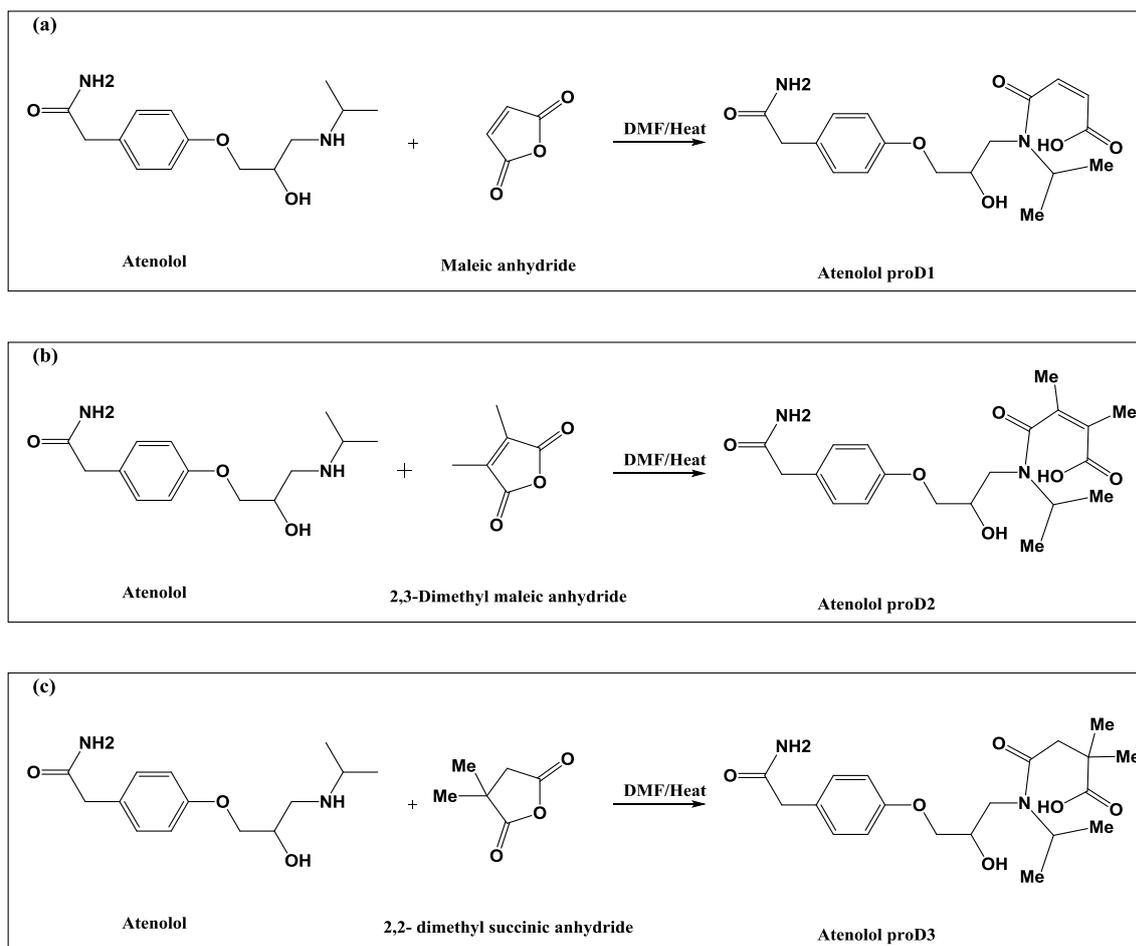
Figure 4.1: Acid-catalyzed hydrolysis for atenolol **ProD 1-3**

4.2 Results

4.2.1 Chemical synthesis:

Synthesis of the atenolol **ProD 1-3** were accomplished using Kirby's procedure [84]. The scheme demonstrates a conjugation reaction in which a nucleophilic attack (S_N2 reaction) takes place between the NH group of atenolol and the electrophile center of (maleic anhydride, 2,3-dimethyl maleic anhydride and 2,2-dimethyl succinic anhydride). This attack results in ring opening of (maleic anhydride, 2,3-dimethyl maleic anhydride and 2,2-dimethyl succinic anhydride) and the formation of an amide in the given prodrug, which is supposed to be cleaved once is exposed to a physiological environment within the body. Moreover, 1 N NaOH was added to dissolve the final

product in order to obtain sodium carboxylate salt since this form is expected to be stable in aqueous medium .



Scheme 1: Synthetic schemes for the preparation of (a) atenolol **ProD 1** , (b) atenolol **ProD 2** and (c) atenolol **ProD 3** from its parent drug, atenolol.

Reaction of atenolol with maleic anhydride, 2,3-dimethyl maleic anhydride or 2,2-dimethyl succinic anhydride provided atenolol **ProD 1**, **ProD 2** and **ProD 3**, respectively with yields of 81% , 70% and 77%, M.P. for **ProD 1** and **ProD 2** were 185 °C, 205 °C respectively and **ProD 3** provide yellowish greasy product (table 4.1).

Table 4.1: results of atenolol prodrugs chemical synthesis in the lab

Prodrug	Weight of product	Melting point	Appearance of product	% of yield
ProD 1	2.95 g	185 °C	White precipitate	81%
ProD 2	2.75 g	205 °C	White precipitate	70%
ProD 3	3.03 g	-	yellowish greasy product	77%

- Atenolol **ProD 1**, $^1\text{H-NMR}$ δ (ppm) CD_3OD 1.06 (q, 2H, $J = 7$ Hz, $\text{CH-CH}_2\text{-CH}_2$), 1.40 (q, 2H, $J = 7$ Hz, $\text{CH-CH}_2\text{-CH}_2$), 1.54 (m, 1H, $\text{CH}_2\text{-CH-CH}_2\text{-CH}_2$), 1.84 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-CH}$), 2.02 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-CH}$), 2.22 (m, 1H, $\text{CH}_2\text{-CH-CH}_2\text{-CH}_2$), 3.17 (d, 2H, $J = 6.5$ Hz, $\text{CH}_2\text{-N}$), 6.25 (d, 1H, $J = 14$ Hz, HC=CH), 6.45 (d, 1H, $J = 14$ Hz, HC=CH). IR ($\text{KBr}/\nu_{\text{max}}$ cm^{-1}) 1712 (C=O), 1643 (C=O), 1587 (C=C), 1420, 1280, 1200, 1132, 1058. LC-MS m/z 365 ($\text{M}+1$)⁺.
- Atenolol **ProD 2**, this prodrug was unstable and readily cleaved to its parent drug, atenolol.
- Atenolol **ProD 3**, $^1\text{H-NMR}$ δ (ppm) CD_3OD 1.26(d,6H), 2.99(s,6H), 3.28(s,2H), 3.45(s,2H), 3.96(m, 2H), 4.10(m, 1H), 4.23(m,1H), 4.3(m, 2H), 6.92(m, 2H), 7.22(m, 2H).

4.2.2 Characterizations:

Atenolol and atenolol prodrugs were characterized by TLC, melting point, partition coefficient, HPLC, FT-IR, LC_MS and H-NMR spectroscopy.

4.2.2.1 Thin layer chromatography:

TLC was performed continually as the fractions were obtained, to ensure product elution. Mobile phase was chloroform: methanol (50%:50%), TLC shows that a pure product was obtained, prodrugs shows the highest retention time values which indicate that they are more lipophilic than atenolol and linkers.

4.2.2.2 Melting point:

The melting point was performed for both atenolol and the proposed atenolol prodrugs as a preliminary analysis of the material, melting point was determined by using SMP3 advanced melting point apparatus.

It was given that the melting point of atenolol was 145 ° C, while that for atenolol **ProD 1** and **ProD 2** were 185 °C, 205 °C respectively. **ProD 3** provide a yellowish greasy product . As shown, products melting points were different from that of atenolol. This gives a preliminary indication that these compounds are not atenolol.

4.2.2.3 Water partition (log P)

Lipophilicity refers to the ability of the drug molecule to partition between two immiscible solutions, such as water and lipid like solutions (as 1- octanol). In a similar manner to solubility, lipophilicity is the sum of different physicochemical characteristics, such as molecular weight, intra and intermolecular H- bonding, polar surface area, ionic charge, pKa and counter ion [86].

Lipophilicity increases with the elevation in the molecular weight and decreases with the increase of H- bonding capacity and polar surface area [87]. Also lipophilicity, again similar to solubility, is dependent on the pH in an inverse manner; ionization of the compound usually leads to decreased lipophilicity. Lipophilicity of drug molecules is most often estimated by 1- octanol/water partition (log P) and distribution (log D) coefficients. The term log P is used when all the solute is in the neutral state and the log D is used when pH causes part or all of the solute to be ionized. To achieve sufficient membrane permeation by a passive transcellular mechanism, the log D values are generally between 0-3 and molecular weight < 550. On the other hand, good permeation of the drug molecule with a negative log P value points to the involvement of carrier mediated transport [87].

log p values of atenolol **ProD 1-3** were estimated by 1- octanol/water partition, they were 0.44, 0.48, 0.51 respectively, while it is 0.23 for atenolol, this means that that the prodrugs have significant increase in lipophilicity over their parent drug, atenolol.

4.2.2.4 FT-IR Analysis:

The following figures show the FT-IR spectra of atenolol, atenolol **ProD 1** and atenolol **ProD 3**. The spectrum of the prodrug shows that an aliphatic stretching of C=O group at 1712 is present, which is most likely an indication of the presence of the carbonyl group of the maleic linker, in addition to the presence of signals of common groups in both the parent drug and the prodrug.

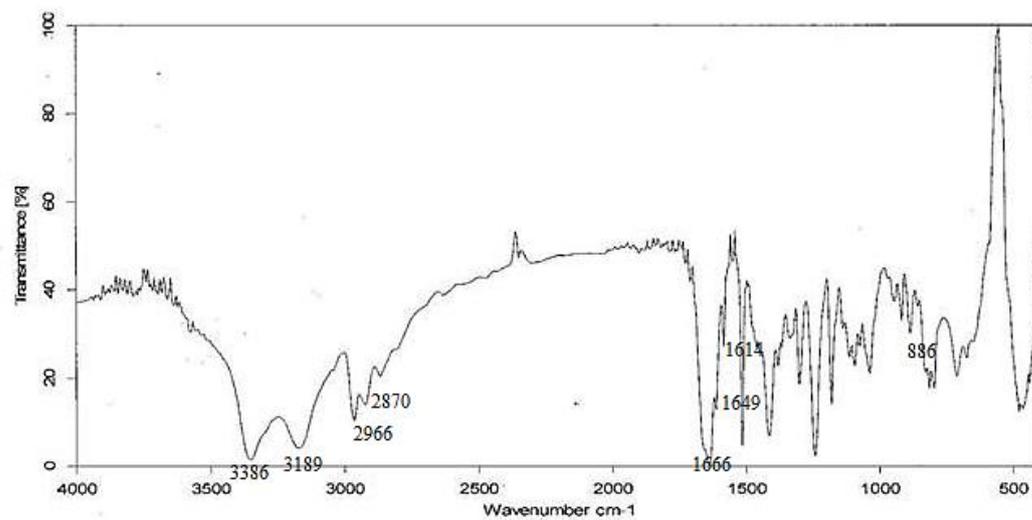


Figure 4.2: FT-IR spectrum of atenolol.

Table 4.2: FT-IR spectrum analysis of atenolol.

IR frequency band (cm ⁻¹)	Group responsible
3368	-OH
3198-3071	H-N
2966	C-CH ₃
2924	CH ₂
2870	C-H
1666	C=O
1649	O=C-NH ₂
1614	Conjugated C=C (aromatic)
886	C=CH ₂

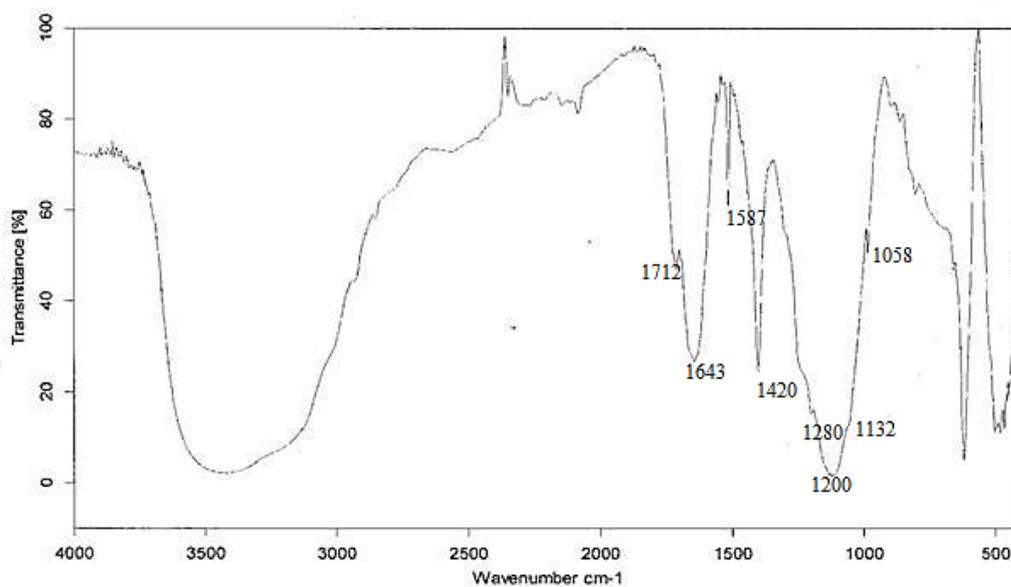


Figure 4.3: FT-IR spectrum of atenolol **ProD 1**.

The IR spectrum (Figure 4.3) shows an additional signals with absorbance 1712 cm^{-1} , 1643 cm^{-1} corresponds to C=O of the maleate moiety (Table 4.2) .

Table 4.3 : FT-IR spectrum analysis of atenolol prodrug .

IR frequency band (cm^{-1})	Group responsible
1712	C=O
1643	second (C=O)
1587	(C=C)

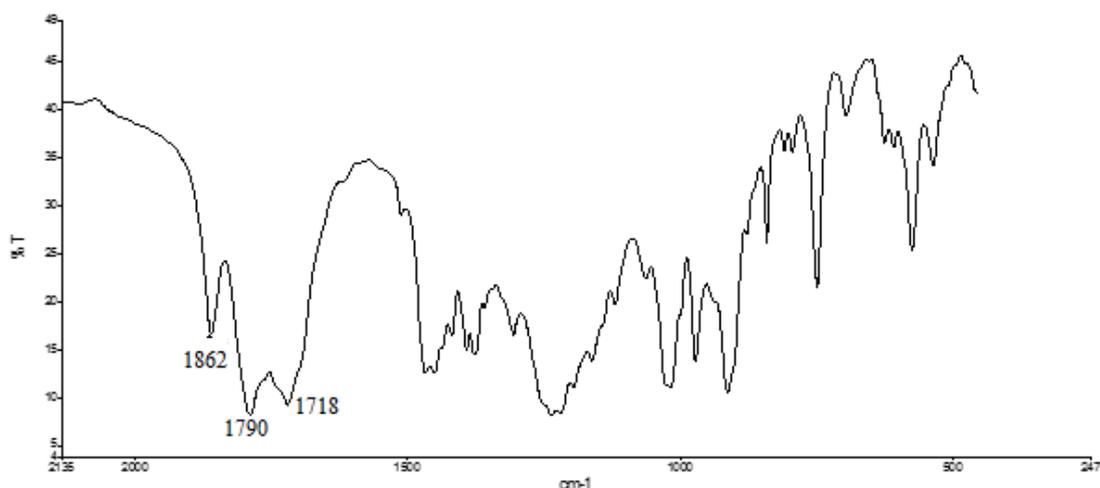


Figure 4.4: Zoomed FT-IR spectrum of atenolol **ProD 3**.

The zoomed IR spectrum (Figure 4.4) shows an additional signals with absorbance 1790 cm^{-1} , corresponds to carbonyl group ($\text{C}=\text{O}$) of the dimethyl succinate moiety .

4.2.2.5 ^1H -NMR of atenolol and atenolol proD 1 and proD 3

The following figures show the H-NMR results of atenolol and atenolol **ProD 1** and **ProD 3**, respectively.

- a. Atenolol **ProD 1**, ^1H NMR δ (ppm) (500 MHz, MeOD) 1.16–1.23 (d, 6H, CH_3CHCH_3), 3.45–3.47 (d, 2H, $J = 1.4\text{ Hz}$, $\text{NH}_2\text{-CO-CH}_2\text{-}$), 3.49–3.50 (m, 2H, $\text{N-CH}_2\text{-}$) 3.52–3.55 (m, 1H, $\text{-NCH(CH}_3)_2$), 4.00–4.01 (m, 1H, $\text{CH}_2\text{CH(OH)CH}_2$), 4.24–4.27 (m, 1H, $\text{-O-CH}_2\text{-}$), 4.41–4.43 (m, 1H, $\text{-OCH}_2\text{-}$), 5.96 (s, 2H, -NH_2), 6.04–6.07 (d, 1H, $J = 13.3\text{ Hz}$, -CH=CH-), 6.28–6.32 (d, 1H, $J = 13.3\text{ Hz}$, -CH=CH-), 6.88–6.93 (m, 2H, aromatic), 7.18–7.22 (m, 2H, aromatic).

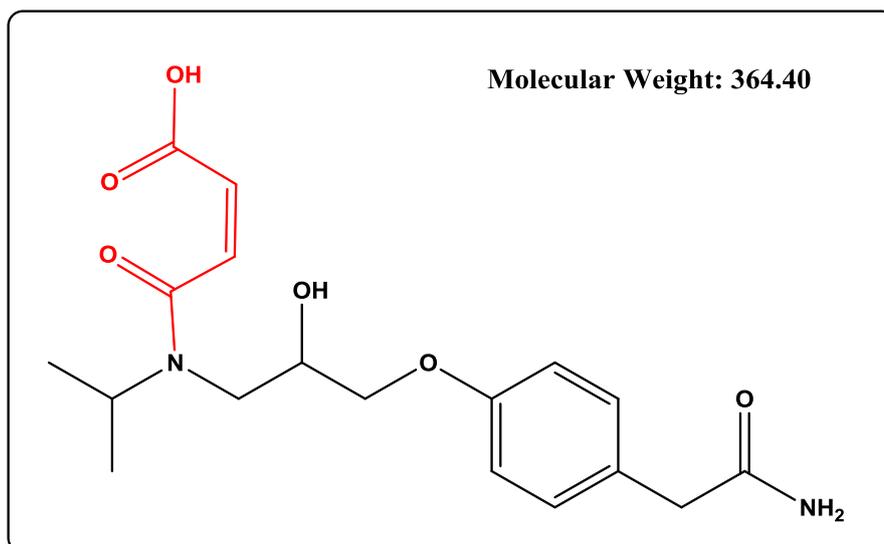


Figure 4.5: Chemical structure of atenolol **ProD 1**.

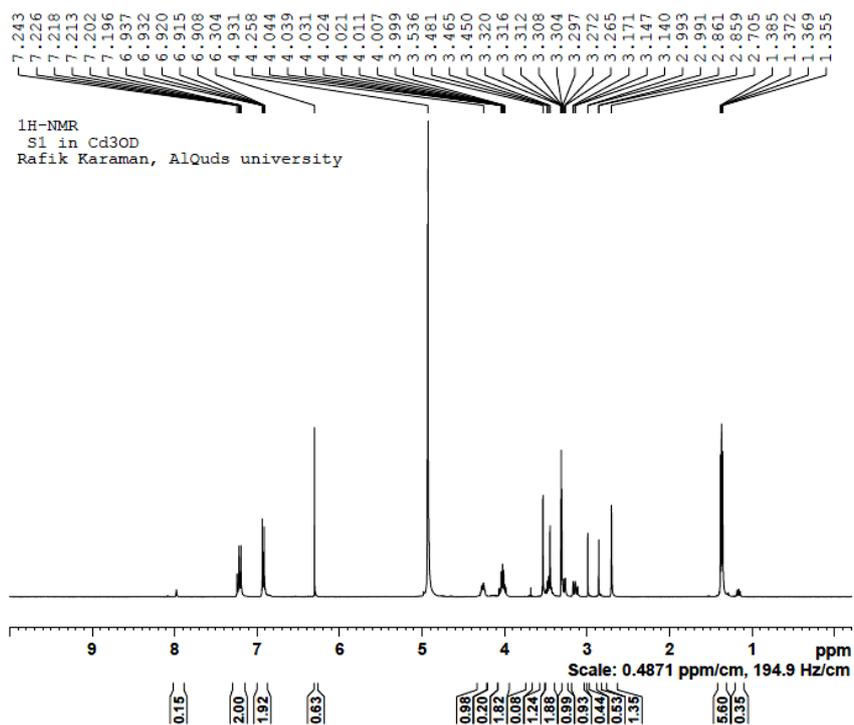


Figure 4.6: Atenolol ¹H NMR spectrum.

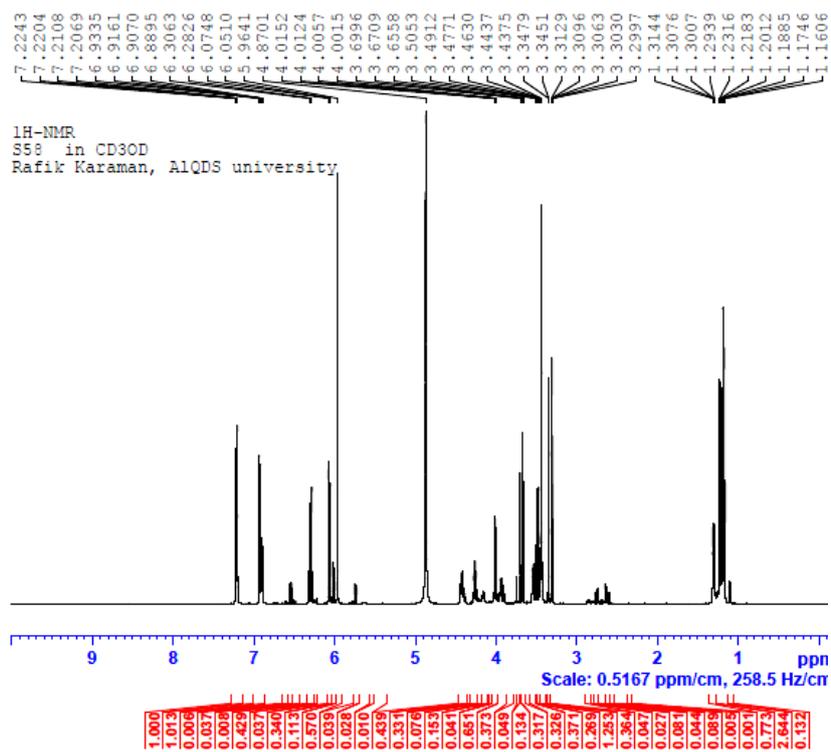


Figure 4.7 a : $^1\text{H-NMR}$ of atenolol **ProD 1**.

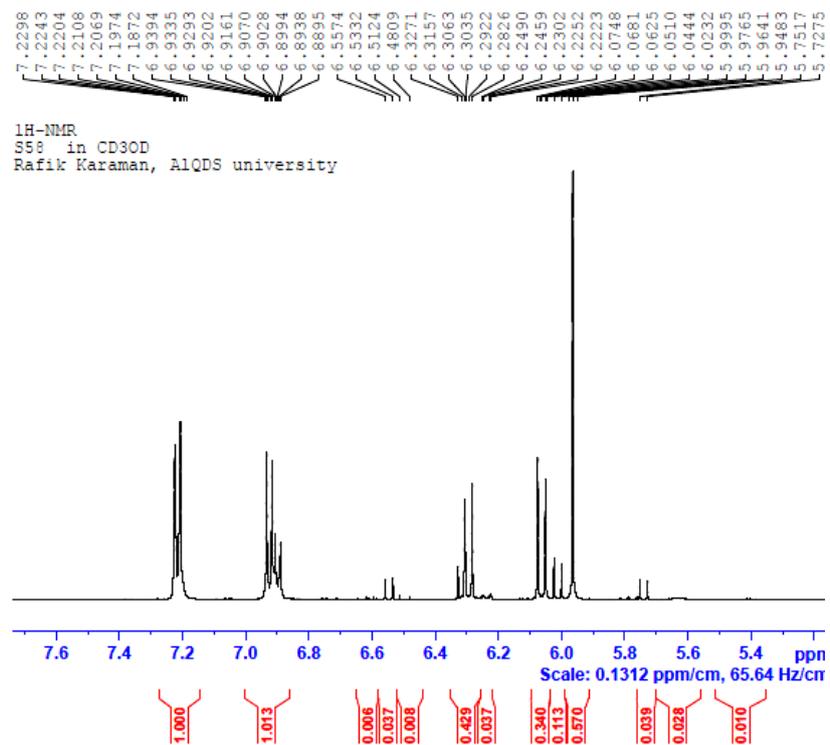


Figure 4.7 b : $^1\text{H-NMR}$ of atenolol **ProD 1**; with zooming.

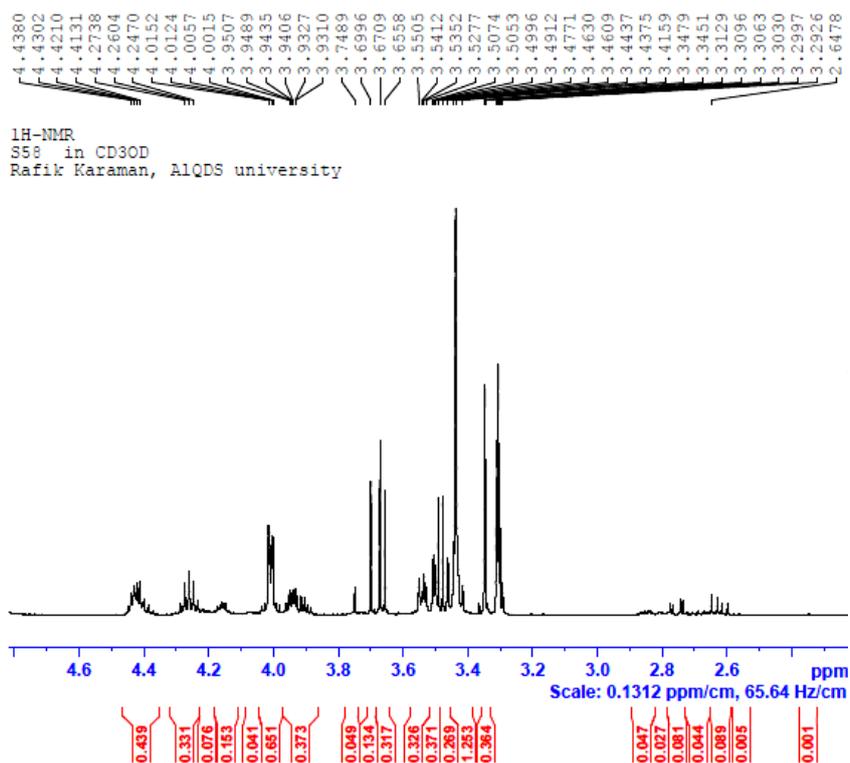


Figure 4.7 c: $^1\text{H-NMR}$ of atenolol **ProD 1**; with zooming.

- b. Atenolol **ProD 3** , $^1\text{H-NMR}$ δ (ppm) CD_3OD 1.26(d,6H) , 2.99(s,6H) , 3.28(s,2H), 3.45(s,2H) , 3.96 (m , 2H), 4.10 (m, 1H), 4.23(m,1H), 4.3(m, 2H), 6.92 (m , 2H) , 7.22 (m , 2H).

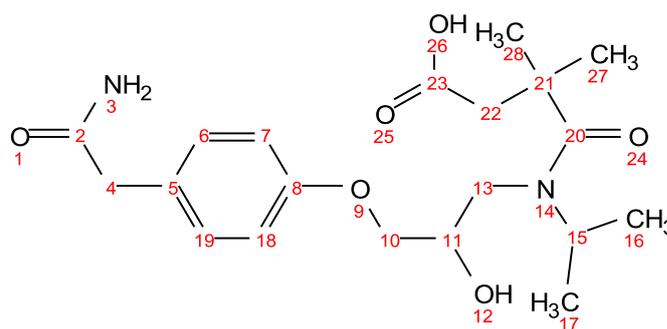
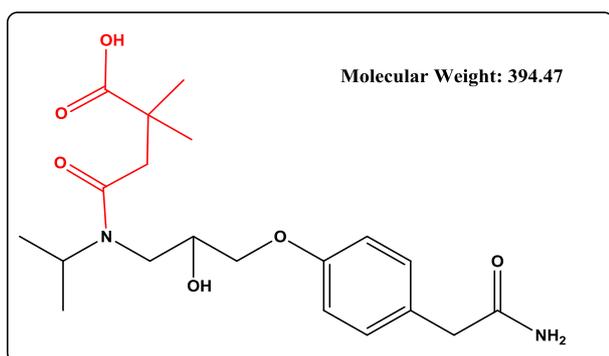


Figure 4.8: Chemical structure of atenolol **ProD 3**.

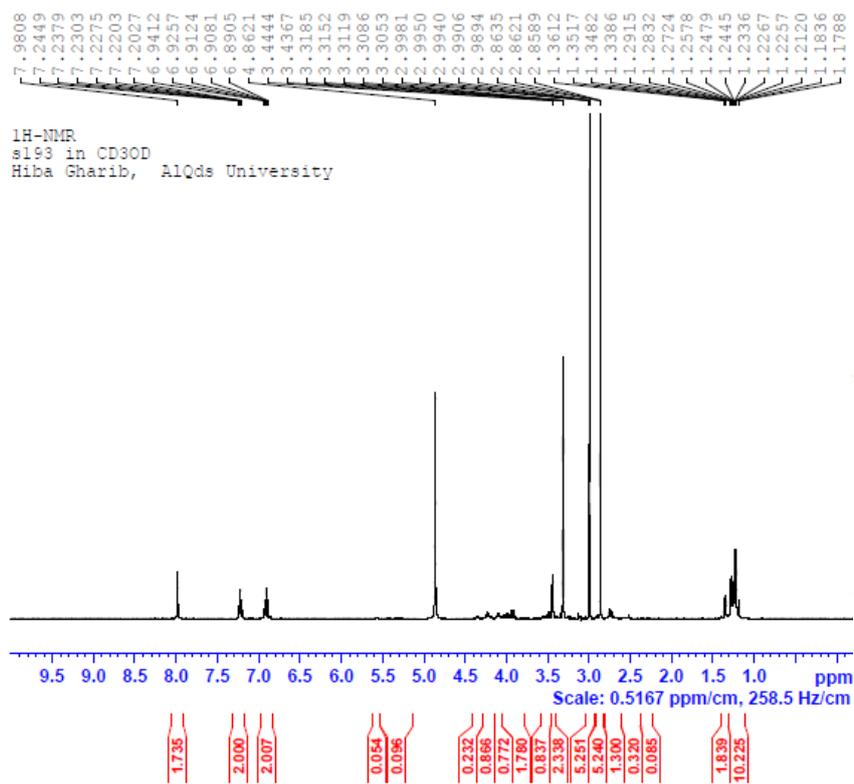


Figure 4.9 a: ¹H-NMR of atenolol **ProD 3**.

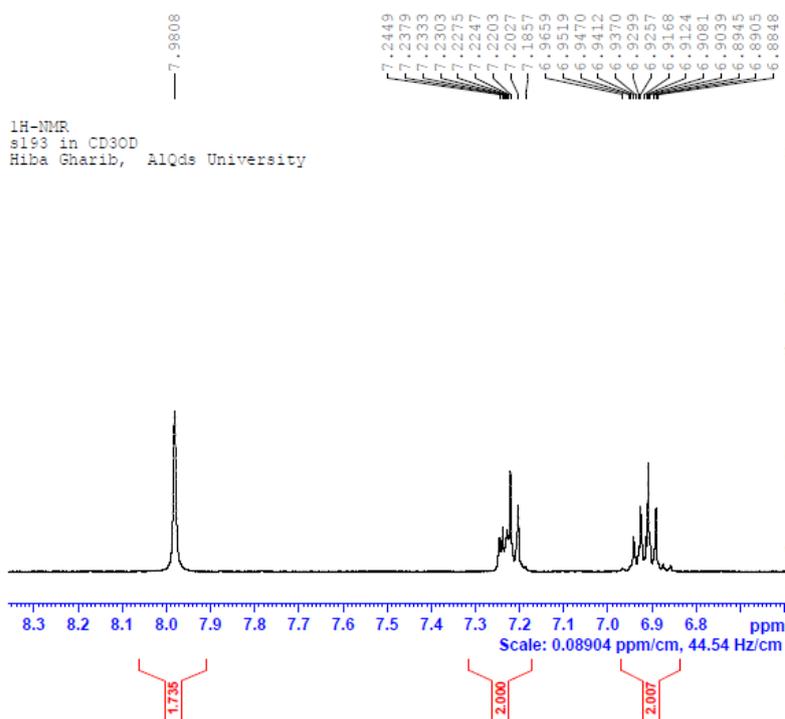


Figure 4.9 b: ¹H-NMR of atenolol **ProD 3**; with zooming.

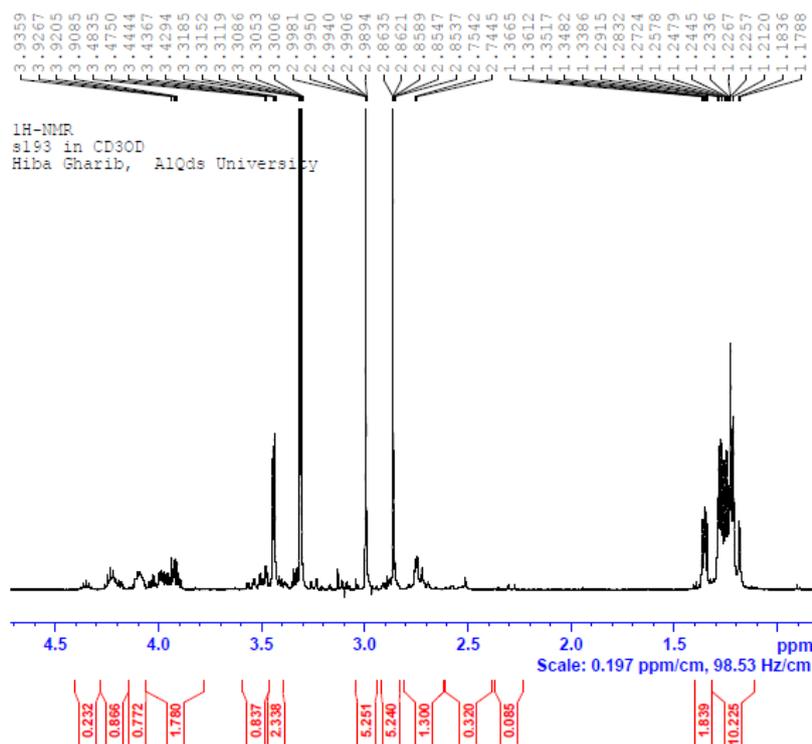


Figure 4.9 c : $^1\text{H-NMR}$ of atenolol **ProD 3**; with zooming.

4.2.2.6 LC/MS spectrum of atenolol Prodrugs

- a. Atenolol **ProD 1** : A high resolution LC-MS (Figure 4.10) shows a protonated peak at m/z 365.1705($m+1$)⁺ which is the molecular weight of the purposed atenolol ProD 1

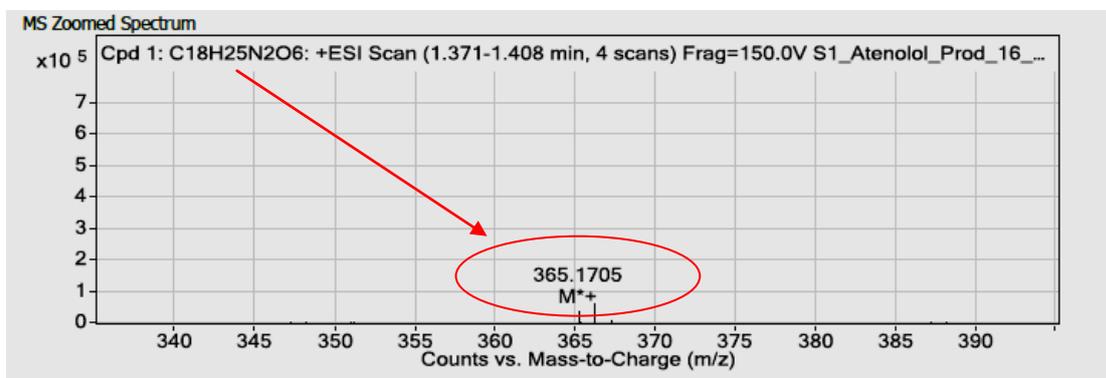


Figure 4.10 : LC- MS spectrum of atenolol **ProD 1**.

- b. Atenolol **ProD 3** : A high resolution LC-MS (Figure 4.11) shows a protonated peak at m/z 365.1705($m+1$)⁺ which is the molecular weight of the purported atenolol ProD 3.

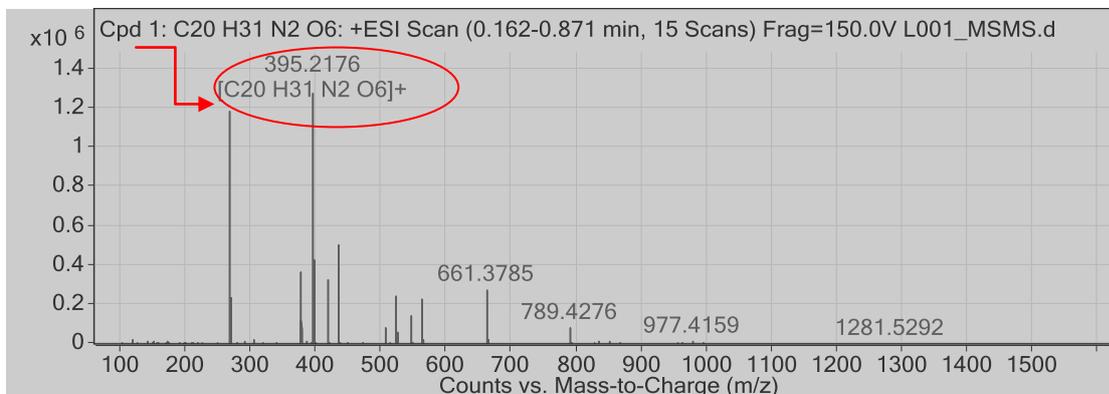


Figure 4.11 : LC- MS spectrum of atenolol **ProD 3**.

4.3.7 Atenolol **ProD 2** :

Atenolol **ProD 2** was unstable and readily cleaved after few hours of synthesis, Figure 4.12 shows a rapid hydrolysis of atenolol **ProD 2** after 3 hours of synthesis. Therefore, no H-NMR spectroscopy and LC/MS spectrum was obtained for this prodrug.

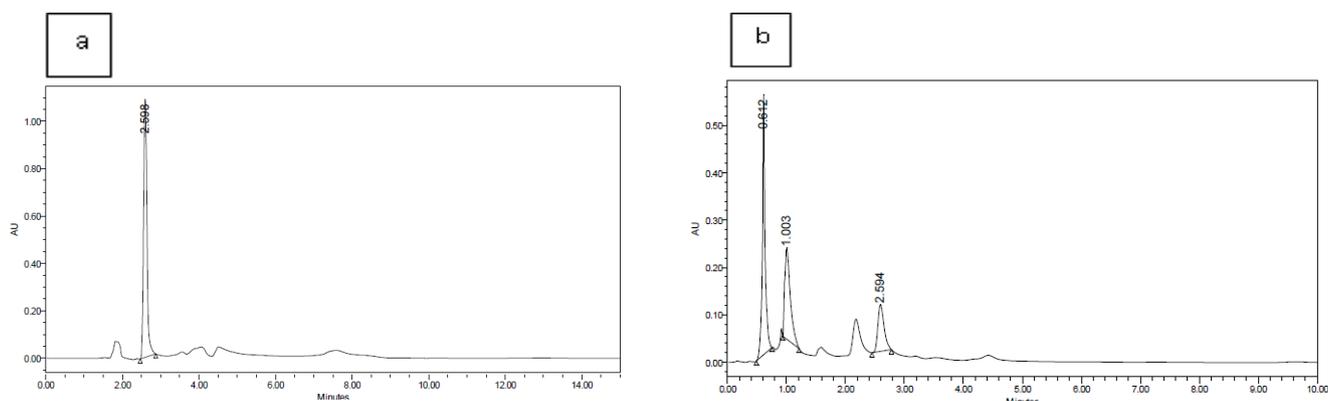


Figure 4.12: Chromatograms showing the intra-conversion of atenolol **ProD 2** (a) at zero time and (b) after 3 hours of synthesis.

4.2.3 Kinetic study:

4.2.3.1 Calibration curve

In order to follow the hydrolysis rate of the synthesized atenolol prodrugs, a calibration curve was obtained by plotting the HPLC peak area of atenolol Prodrugs versus different concentrations, as displayed in Figures 4.13 and 4.14. Excellent linearity with correlation coefficient (R^2) of 0.9996 was obtained. Quantitative analysis of the prodrug and its hydrolysis products was achieved by using a calibration curve under same conditions.

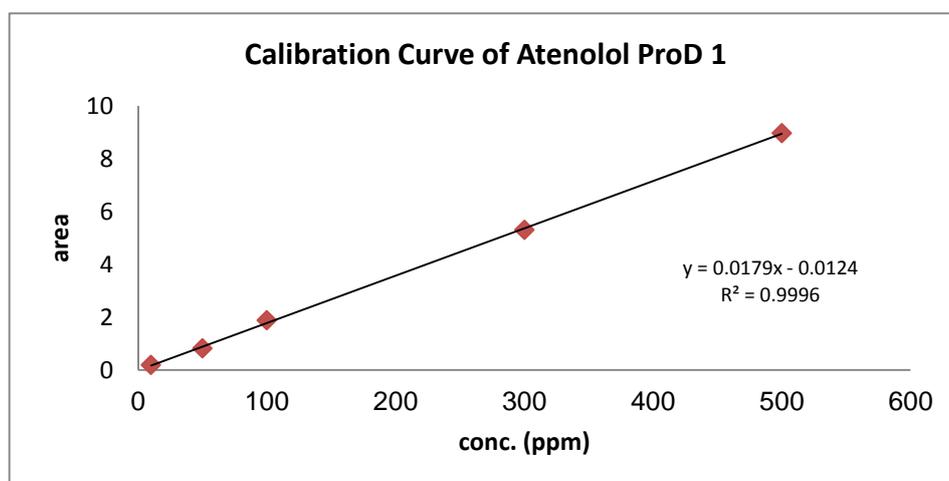


Figure 4.13: Calibration curve for atenolol **ProD 1**.

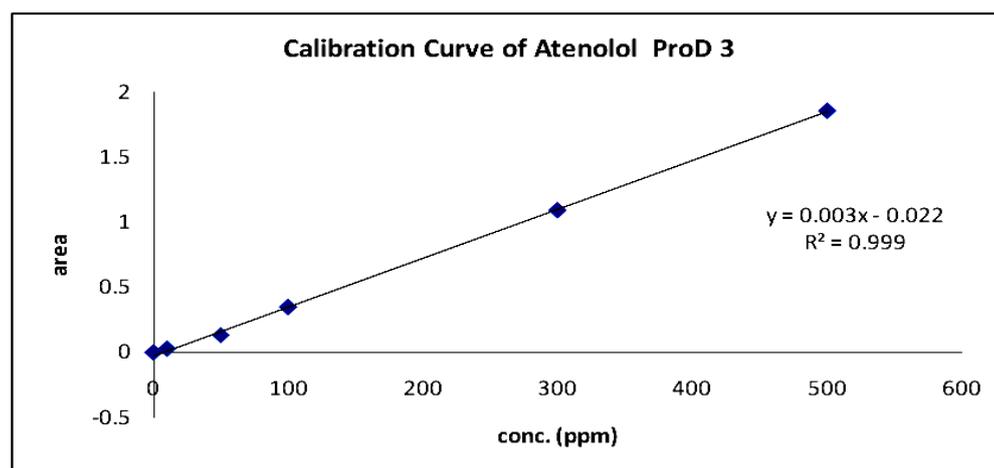


Figure 4.14: Calibration curve for atenolol **ProD 3**.

4.2.3.2 Hydrolysis studies:

The kinetics of the acid-catalyzed hydrolysis study for atenolol prodrugs were carried out in aqueous buffers in a similar manner to that done by Kirby on N-alkylmaleamic

acids [88] . This is in order to examine whether atenolol prodrugs are hydrolyzed in aqueous medium and to what extent. Acid-catalyzed hydrolysis of atenolol prodrugs were investigated in four different aqueous media: 1 N HCl and buffers pH 3, 5 and 7.4. Under the experimental conditions, atenolol **ProD 1** was hydrolyzed to release the parent drug atenolol as shown by HPLC. At constant pH and temperature, the reaction displayed strict first order kinetics as the k_{obs} was fairly constant and a straight line was obtained on plotting log concentration of residual atenolol prodrug verses time.

4.2.3.2.1 Atenolol ProD1 hydrolysis at pH 1

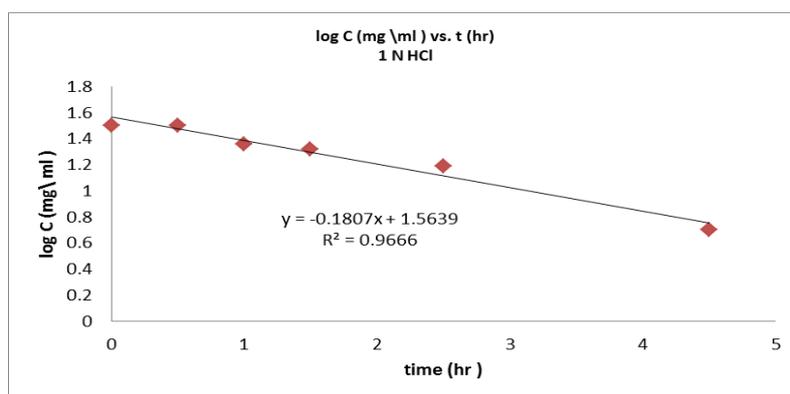


Figure 4.15: First order hydrolysis of atenolol ProD1 at pH 1.

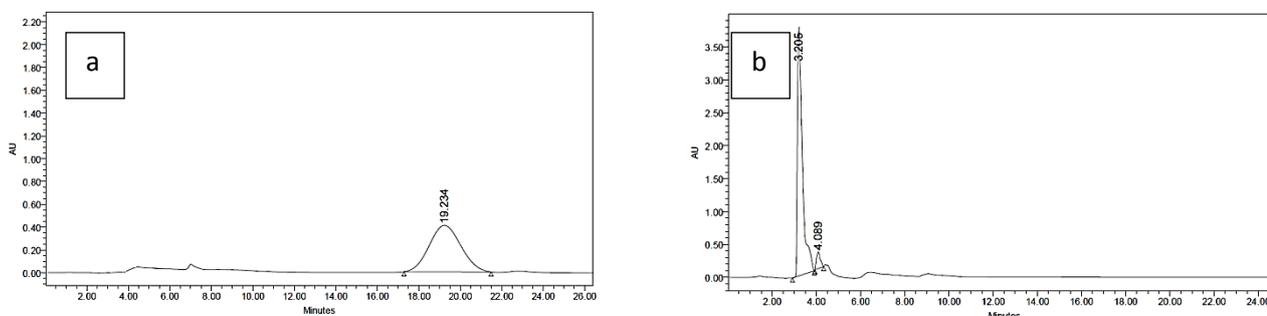


Figure 4.16: Chromatograms showing the intra-conversion of atenolol **ProD 1** in 1N HCl
(a) at zero time, (b) at the end of reaction.

4.2.3.2.2 Atenolol ProD1 hydrolysis at pH 3

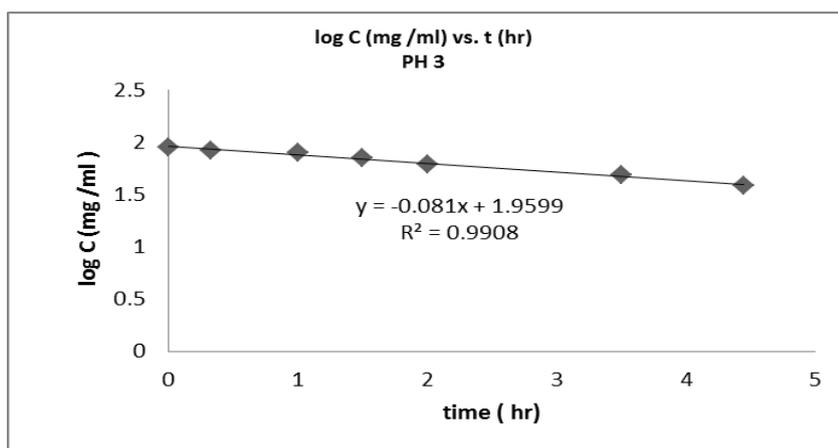


Figure 4.17: First order hydrolysis of atenolol **ProD 1** at pH 3.

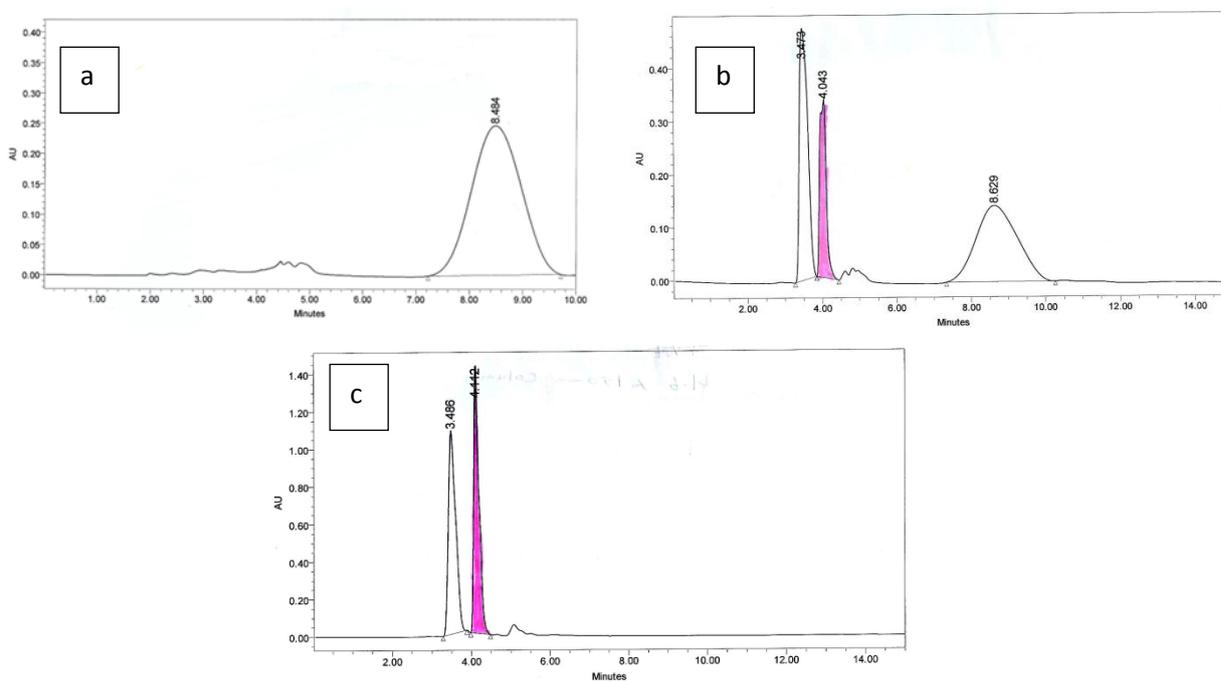


Figure 4.18: Chromatograms showing the intra-conversion of atenolol **ProD 1** at pH 3
 (a) at zero time, (b) after 2 hours and (c) at the end of reaction.

4.2.3.2.3 Atenolol ProD1 hydrolysis at pH 5

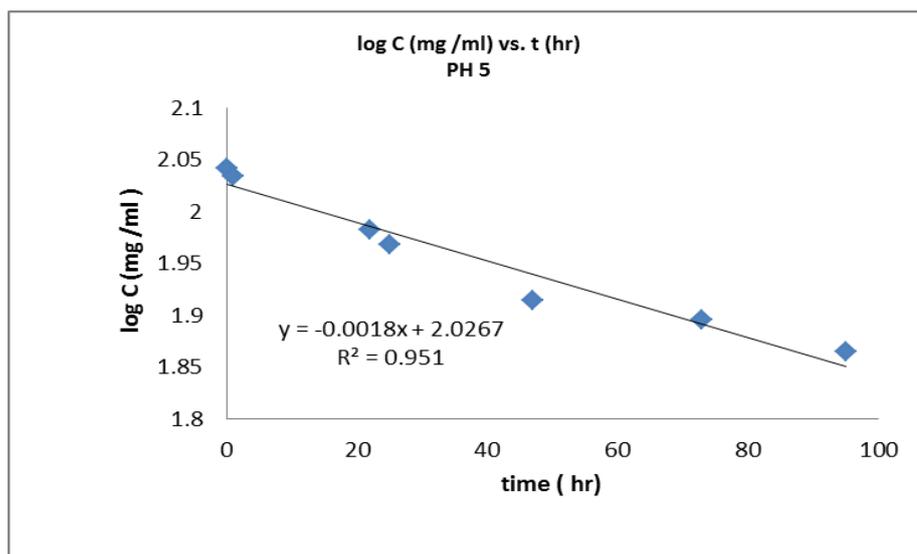


Figure 4.19 : First order hydrolysis of atenolol **ProD 1** at pH 5.

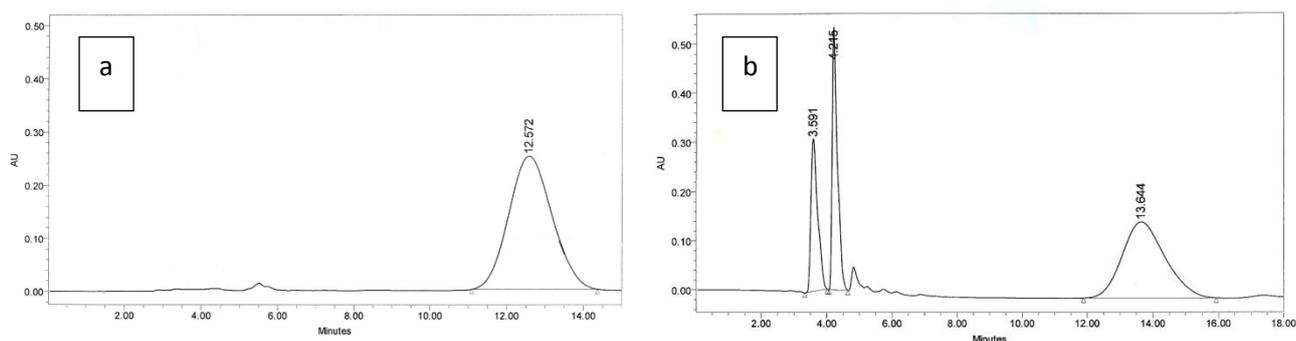


Figure 4.20 : Chromatograms showing the intra-conversion of atenolol **ProD 1** at pH 5

(a) at zero time and (b) after 250 hours.

4.2.3.2.4 Atenolol prodrug hydrolysis at pH 7.4.

No change in the atenolol prodrug was observed by the HPLC results, it was extremely stable (Figure 4.21).

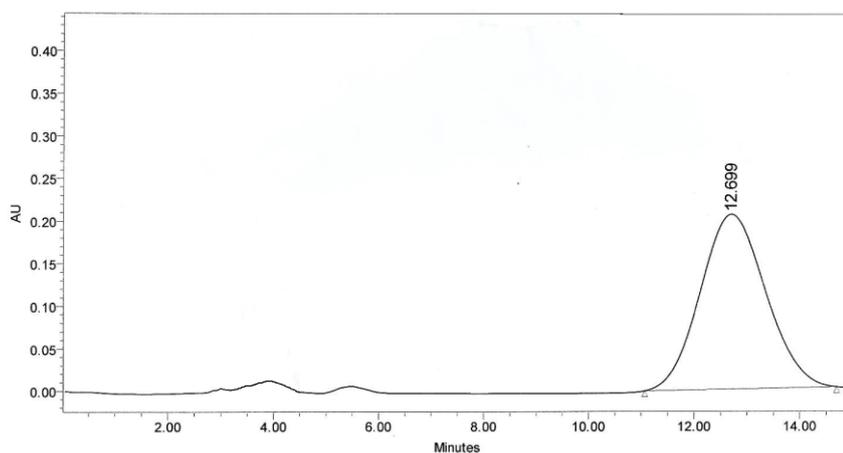


Figure 4.21: Chromatogram showing the stability of atenolol **ProD 1** at pH 7.4 after 10 days.

4.2 Discussion

4.2.1 Design of atenolol prodrugs based on DFT calculations of the acid-catalyzed hydrolysis of N-alkyl maleamic acids.

Figure 2.2 illustrates the chemical structures of two atenolol prodrugs that were designed by Karaman's group. The only difference between the proposed atenolol prodrugs and the parent drug, atenolol, is that the amine group in atenolol was replaced with an amide moiety. This chemical change is expected to increase the stability of the prodrug derivative (amide alcohol) which is formed compared to the corresponding amine alcohol, atenolol, due to general chemical stability for secondary alcohols over amine alcohols.

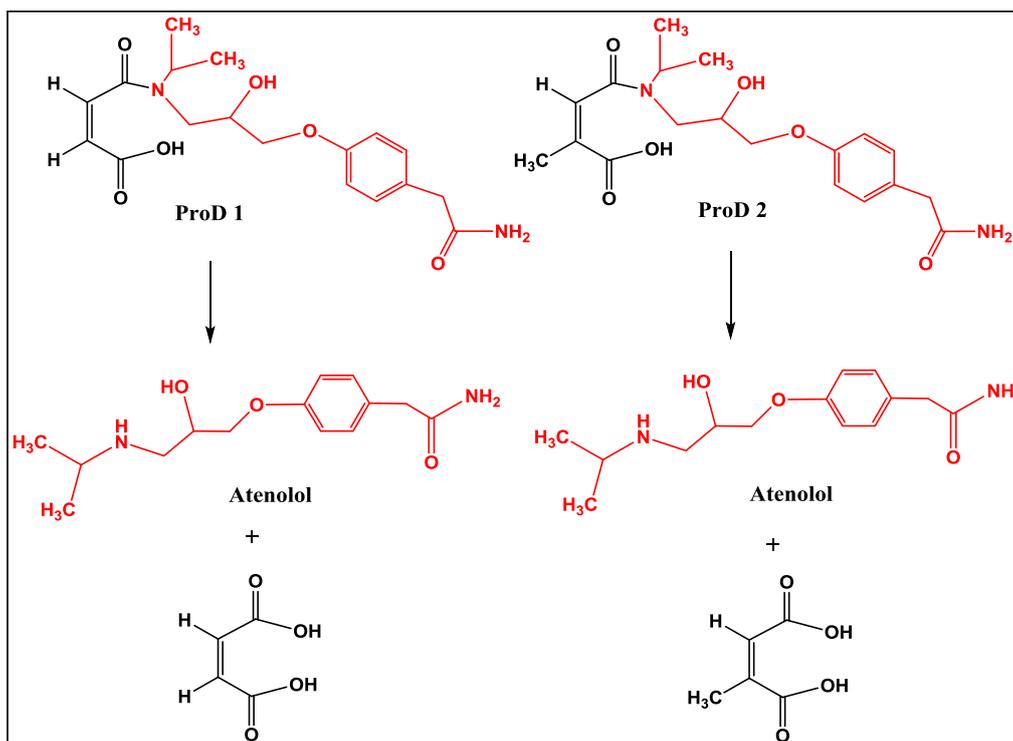


Figure 4.22 : Chemical structures for atenolol prodrugs ProD 1-2

The proposed atenolol prodrugs were designed based on the findings that emerged from the study of the acid-catalyzed hydrolysis reactions of N-alkyl maleamic acids conducted by Kirby et al. (Figure 2.3) [87]. Figure 2.4 depicts the acid-catalyzed hydrolysis reactions of N-alkyl maleamic acids studied by Kirby and Lancaster. This study findings demonstrate that the amide bond cleavage is due to intramolecular nucleophilic catalysis by the adjacent carboxylic acid group and the rate-limiting step of the process is the dissociation of the tetrahedral intermediate (Figure 2.5). Two decades later, the reaction was theoretically investigated by Katagi using AM1 semiempirical calculations. In contrast to what was proposed by Kirby, the AM1 study revealed that the rate-limiting step is the tetrahedral intermediate formation and not its collapse [89, 90]. Later on, Kluger and Chin have studied the intramolecular hydrolysis mechanism of a number of N-alkylmaleamic acids derived from aliphatic amines having a wide range of basicity [91]. Their findings revealed that the identity of the rate-limiting step is a function of both the basicity of the leaving group and the acidity of the solution.

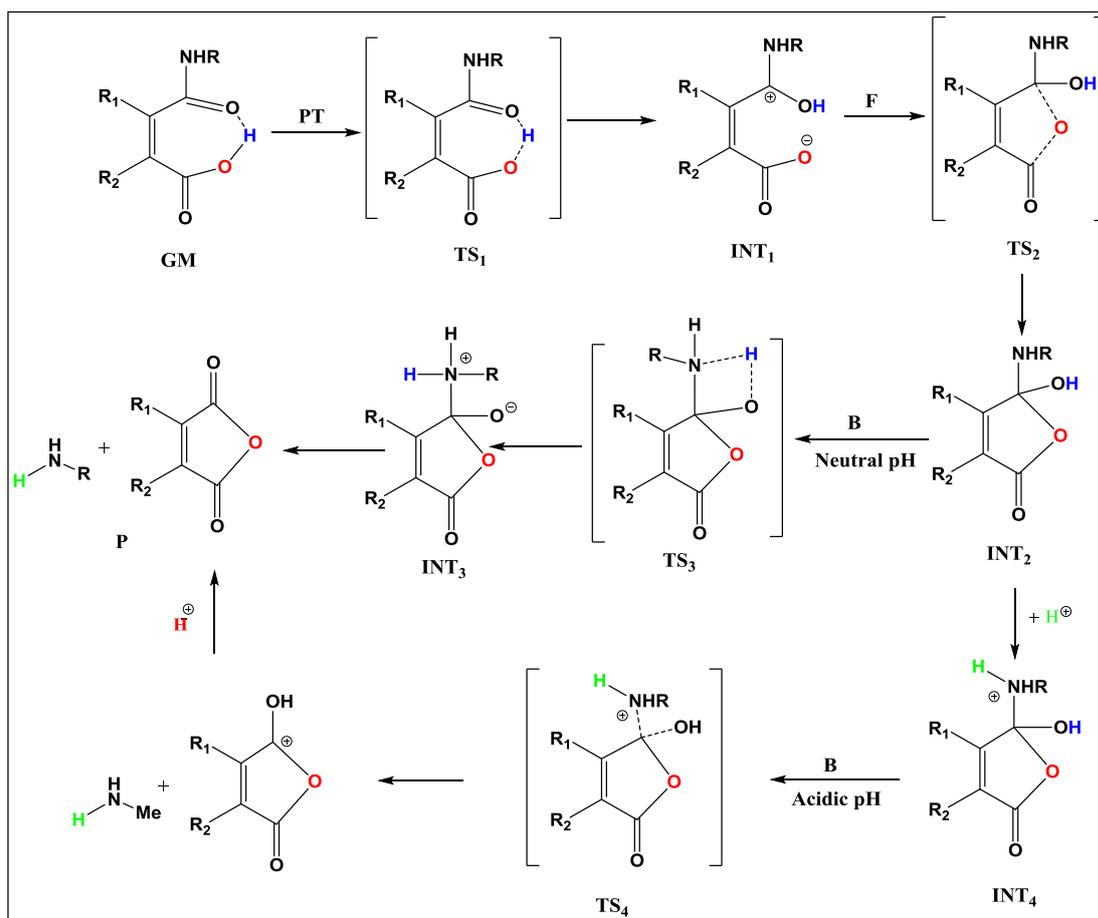


Figure 4.25: Mechanistic pathway for the acid-catalyzed hydrolysis of **1–9** and atenolol **ProD 1–ProD 2**. INT and TS are tetrahedral intermediate and transition state, respectively.

Based on the above-mentioned experimental findings, Karaman's group believes that replacing the N-methylamide group in **1–9** with atenolol molecule, as shown for atenolol **ProD 1** and **ProD 2** in (Figure 2.6) will not have any significant effect on the relative rates of these processes, if they proceed in the same mechanism. Therefore, DFT calculations of the kinetic and thermodynamic properties for processes **1–9** might fairly predict the rates for the chemical intraconversion of atenolol **ProD 1** and **ProD 2** to their parent drug, atenolol.

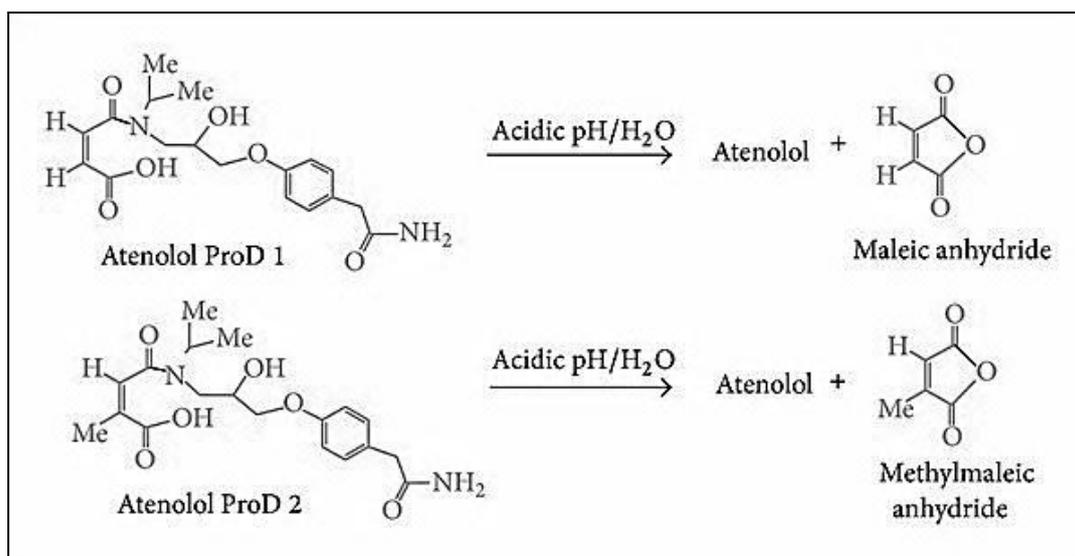


Figure 4.26: Acid-catalyzed hydrolysis for atenolol **ProD 1-ProD 2**

The DFT calculations for **1-9** and **ProD 1-2** that were conducted by Karaman's group confirmed the following findings revealed by Kirby et al. [69, 88]:

- Two types of conformations in particular were considered: one in which the amide carbonyl is *syn* to the carboxyl group and another in which it is *anti*. It was found that the global minimum structures for **1-9** and **ProD 1-2** all reside in the *syn* conformation (Figure 2.7).
- Both the substituent on the double bond and the reaction medium has a profound effect on the reaction rate.

The substituent effect

- The closest distance of approach of the carboxylic oxygen and the amide carbonyl oxygen in the ground state is less for the more reactive compounds. For example, the distance for the unsubstituted maleamic acid amide **1** is 3.16 Å whereas for the dimethyl substituted derivative **2** is 3.05 Å.
- The second structural factor that might have a significant effect on the reactivity of an acid amide is the angle by which the carboxyl group approaches the amide carbonyl carbon. The calculated θ values for the reactive derivatives were found to be larger than those for the less reactive acid amides

- The rates of the reaction for systems having less strained intermediates or products are higher than that having more strained intermediates or products.

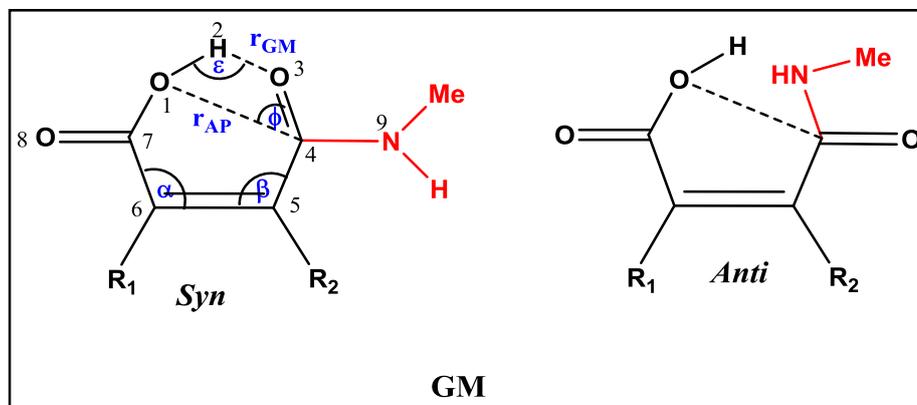


Figure 4.27: representation of acid-catalyzed hydrolysis in Kirby's acid amides 1-9. GM is the global minimum structure

The solvent effect:

The gas phase DFT calculated barriers revealed that the rate-limiting step for the hydrolysis of all N-alkyl maleamic acid amides studied regardless of the nature of the amine leaving group is a tetrahedral formation. Whereas, when the calculations were run in a dielectric constant of 78.39 (water), the rate-limiting step for the hydrolysis of the acid amides was the dissociation of the tetrahedral intermediate.

- calculations of the mechanism for the acid-catalyzed hydrolysis demonstrate that the process involves (Figure 2.5):
 - A proton transfer from the carboxylic group into the adjacent amide carbonyl carbon
 - A nucleophilic attack of the carboxylate anion thus formed onto the protonated carbonyl carbon to form a tetrahedral intermediate
 - A dissociation of tetrahedral intermediate to give products

4.2.2 Summary

As shown from the previous results, the hydrolysis studies of the atenolol **ProD 1** and **ProD 3** were carried out in aqueous buffer solutions at different pH values (1, 3, 5 and 7.4, respectively) to evaluate their fate during passage through the GIT and after absorption and reaching the blood circulation, because the mean fasting stomach pH of adult is approximately 1-2 and increases up to 5 following ingestion of food. In addition, buffer pH 5 mimics the beginning of the small intestine pathway. Finally, pH 7.4 was selected to examine the interconversion of the tested prodrug in blood circulation system.

At 1N HCl atenolol **ProD 1** was intraconverted to release the parent drug in 1.66 hour. On the other hand, at pH 7.4, the prodrug was entirely stable and no release of the parent drug was observed. Since the pKa of the carboxylic group of atenolol **ProD 1** is in the range of 3-4, it is expected that at pH 5 the anionic form of the prodrug will be dominant and the percentage of the free acid form that expected to undergo hydrolysis will be relatively low. At 1N HCl and pH 3 most of the prodrug will exist as the free acid form, whereas at pH 7.4 most of the prodrug will be in the anionic form. Thus, the difference in rates at the different pH buffers.

It is important to mention that maleic anhydride is particularly considered suitable linker, because it is produced in the body in the Krebs' cycle, so it is nontoxic when released *in vivo* after the prodrug hydrolysis, and it becomes ionized at physiological pH, so it contributes to the increased solubility of the prodrug.

Table 4.4: The observed k value and $t_{1/2}$ for the intraconversion of atenolol **ProD 1** in 1N HCl and at pH 3, pH 5 and pH 7.4.

Medium	k_{obs} (hours ⁻¹)	$t_{1/2}$ (hours)
1N HCl	4.161×10^{-1}	1.66
Buffer pH 3	1.86×10^{-1}	3.73
Buffer pH 5	4.14×10^{-3}	168
Buffer pH 7.4	No reaction	No reaction

Acid catalyzed hydrolysis of atenolol **ProD 2** was found to be readily intraconverted, according to structural feature of 2,3-dimethyl maleic moiety it contains two methyl groups on the C-C double bond (strained system) which results in a decrease of the distance between the two reactive centers (hydroxyl oxygen of the carboxylic group and the amide carbonyl carbon). Hence, the hydrolysis of atenolol **ProD 2** is very fast.

In the case of atenolol **ProD 3** the interatomic distance between the nucleophile (OH) and electrophile (C=O) is too high to make the nucleophile attack accessible. Hence, no reaction was observed with this prodrug (Table 4.4).

Table 4.5 :The observed k value and $t_{1/2}$ for the intraconversion of atenolol **ProD 3** in 1N HCl and at pH 3, pH 5 and pH 7.4.

Medium	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1N HCl	No reaction	No reaction
Buffer pH3	No reaction	No reaction
Buffer pH 5	No reaction	No reaction
Buffer pH 7.4	No reaction	No reaction

Conclusions and future directions

5.1 Conclusions

Based on Kirby's enzyme model (proton transfer in N-alkylmaleamic acids) atenolol prodrugs were synthesized and their acid-catalyzed hydrolysis reactions demonstrated that the rate-limiting step was the breakdown of the tetrahedral intermediate. The experimental value at pH 5 was 168 hours and at pH 7.4 no interconversion was observed. The lack of the reaction at the latter pH might be due to the fact that at this pH atenolol ProD 1 exists solely in the ionized form (pK_a about 3-4). As mentioned before, the free acid form is a mandatory requirement for the reaction to proceed. On the other hand atenolol ProD 2 was found to be readily intraconverted, but In the case of atenolol ProD 3 no reaction was observed.

5.2 Future directions

Future strategy to achieve more efficient atenolol prodrugs capable of increasing the liquid formulation stability, eliminating atenolol bitterness, and releasing the parent drug in a programmable manner is (i) synthesis of atenolol prodrugs having pK_a around 6 (intestine pH) such as atenolol **ProD 4**; (ii) *in vitro* kinetic studies of atenolol **ProD 4** will be performed at pH 6.5 (intestine) and pH 7.4 (blood circulation system); and (iii) *in vivo* pharmacokinetic studies will be done in order to determine the bioavailability and the duration of action of the tested prodrug. Furthermore, based on the *in vivo* pharmacokinetics characteristics of atenolol **ProD 4**, new prodrugs may be designed and synthesized.

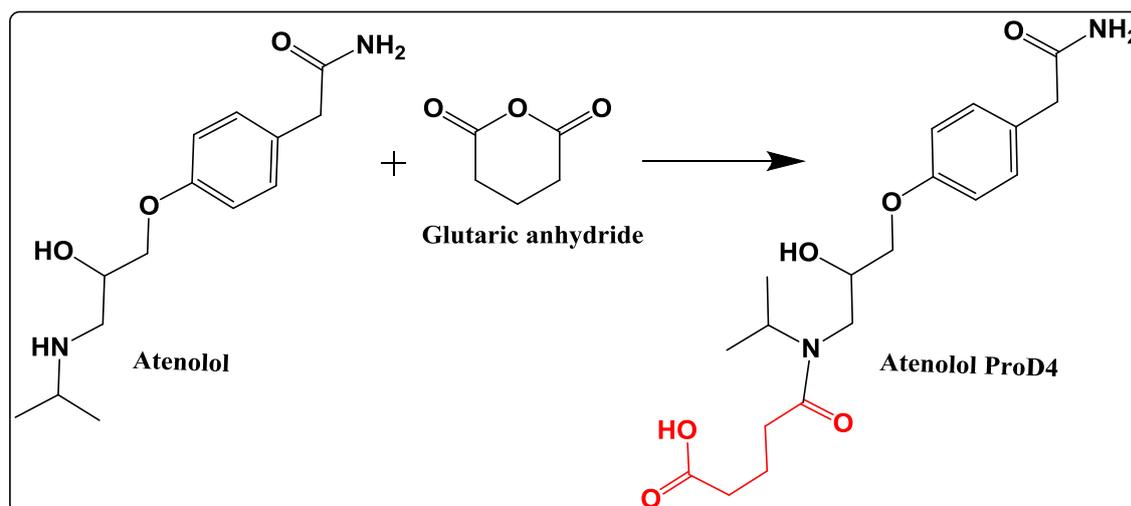


Figure 4.28: The purposed structure of atenolol ProD 4

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تصنيع ودراسة المواصفات والحركية لدواء مساعد مصمم للأنتولول

إعداد: الآء احمد محمد قطيط

المشرف الرئيسي: بروفيسور رفيق قرمان

الملخص:

Prodrugs هي عبارة عن مواد تعطى في شكل غير نشط، ثم تتحول في الجسم إلى المادة الدوائية الفعالة المقابلة. الهدف من إعطاء Prodrugs هو تحسين إمتصاص الدواء والتوزيع والتمثيل الغذائي والإخراج في الجسم. منذ أن وصفت ال Prodrugs لأول مرة سنة 1950م أصبحت منطقة خصبة للبحوث. حيث أصبحت العديد من شركات الأدوية و التكنولوجيا الحيوية الصغيرة متخصصة لإستخدام Prodrugs في تطوير الأدوية القديمة أو التي تعاني من مشاكل و لتطوير تكنولوجيات الدواء المساعد على نطاق واسع وتطبيقها على الأدوية الجديدة والمستقبلية.

مؤخرا أصبح تصميم الأدوية يعتمد على نحو متزايد على تقنيات النماذج الحاسوبية. في العقود القليلة الماضية أستخدمت أساليب الكيمياء الحاسوبية في حساب الخصائص الفيزيائية والكيميائية والجزئية للمركبات. هذه الأساليب يمكن إستخدامها أيضا لتصميم Prodrugs التي تتحول كيميائيا إلى الدواء الفعال بدون تدخل من الأنزيم المحفز. وهذا التحول إلى الدواء الفعال يعتمد بشكل أساسي على الخطوة المحددة في intramolecular process. بناء على حسابات DFT صممت ثلاثة Prodrugs لدواء الأنتولول حيث تم تصنيعها ودراسة خصائصها بإستخدام أجهزة FT-IR، LC-MS، 1H-NMR ومن ثم دراسة تحولها إلى الدواء الفعال، بناء على هذه الدراسات وجد أن $t_{1/2}$ لها تتأثر إلى حد كبير بدرجة الحموضة للوسط.

فكانت قيم $t_{1/2}$ المحددة في (pH 5, pH 3, 1N HCl) هي 168 , 3.73 , 1.66 ساعة على التوالي أما في PH 7.4 فلم يحدث أي تحول، من جهة أخرى بالنسبة ل ProD 2 فقد كان تحوله سريع جدا وفي حالة ProD 3 لم يحدث أي تحول يذكر على الاطلاق .