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Synthesis, Characterization and Evaluation of Potentially Useful Diclofenac and Mefenamic acid Prodrugs and Co-drugs

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Synthesis, Characterization and Evaluation of Potentially Useful Diclofenac and Mefenamic acid Prodrugs and Co-drugs

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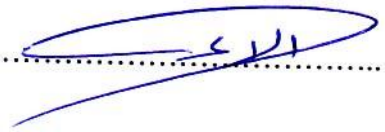
Declaration

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: 

Ala' Nadi Mustafa Abu-Jaish

Date:

Dedication

I dedicate my thesis work to:

- My loving parents, There is no doubt in my mind that without their continued support I could not have completed this process.
- My husband, I give my deepest expression of love and appreciation for the encouragement that he gave during my graduate program.
- My daughter, with a hope that she would one day realize that education is a “weapon” to fight ignorance and poverty and a key to open doors for success.

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Abstract

Background:

Non-steroidal anti-inflammatory drugs are among the most widely used medications for the treatment of various inflammatory disorders. However, the GI toxicity of NSAIDs limits their usefulness. Conventional NSAIDs prodrugs activated via enzymes distributed throughout the body. These metabolic enzymes can be affected by various factors such as age, health conditions and gender. Therefore, it is difficult to predict the bioconversion rates. A novel strategy to convert the prodrug to its active parent drug has been developed, whereby intramolecular mechanism utilized to release the drug from its corresponding prodrug.

Objectives:

The main goal of our work is to synthesize diclofenac and mefenamic acid prodrugs and codrugs lacking the bitter taste, gastric adverse effects. In addition, the proposed diclofenac and mefenamic prodrugs and codrugs should have the potential to undergo a chemical and not enzymatic driven cleavage, and release the active parent drug in a controlled manner.

Methods:

The structures of the synthesized prodrugs were confirmed and characterized by spectral analysis techniques. The release pattern of parent drug from prodrug was also studied by HPLC method. The kinetics of the prodrugs and codrugs hydrolysis was studied in four different buffer solutions at 1NHCl, pH 2.5, pH 5.5, and pH 7.4.

Results:

Novel bitterless prodrugs of mefenamic acid and diclofenac were synthesized. Codrugs of each of mefenamic acid and diclofenac with tranexamic acid were synthesized. The kinetic results of synthesized NSAIDs prodrugs revealed that hydrolysis rate is highly affected by the pH of the medium. The $t_{1/2}$ of mefenamic dimethylamine at the pH of the stomach was 10 hours while it was stable at pH 2.5, pH 5.5 and pH 7.5. The experimental $t_{1/2}$ values of diclofenac benzyl were 4 hours and 1 hour at 1NHCl and pH7.4 respectively, while it was entirely stable at pH 2.5 and pH5.5. Mefenamic tranexamic released both drugs in 1 hour at 1 NHCl. However, the release of the drugs from mefenamic tranexamic at pH 2.5, pH5.5, and pH7.4 was negligible. Diclofenac tranexamic at 1 NHCl was found to be cleaved within 30 hours.

Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviations	Definition
A^o	Angstrom
COX	Cyclooxygenase
C^o	Celsius
DFT	Density Functional Theory
DMF	Dimethylformamide
GIT	Gastrointestinal tract
HPLC	High-performance liquid chromatography
hr	Hour
IUD	Intrauterine devices
IR	Infrared
k_{obs}	The observed rate constant of hydrolysis
KBr	Potassium Bromide
LC-MS	Liquid chromatography-Mass spectrometry
M.P	Melting point
m/z	Mass-to-Charge ratio
NSAIDs	Non-steroidal anti-inflammatory drugs
NMR	Nuclear magnetic resonance
PPH	Post partumhemorrhage
ppm	Part per million
RT	Retention time
SIF	Simulated intestinal fluid
$t_{1/2}$	Half life
TLC	Thin layer chromatography
THF	Tetrahydrofuran
λ_{max}	The wavelength at which the maximum absorbance is observed
1N NaOH	Normalized sodium hydroxide
1N HCl	Normalized hydrochloric acid

Introduction

Chapter One

1. Introduction

1.1 Prodrug Background

Every drug is characterized by its biological and physicochemical properties. Some of the marketed drugs have many drawbacks in their performance that result in an inefficient delivery and unwanted side effects. The physicochemical, biopharmaceutical and pharmacokinetic properties of these drugs should be improved in order to increase their usefulness and to increase their utilization in clinical practice [1, 2].

In the past few decades, there has been a steady improvement in the pharmaceutical industry to facilitate the drug discovery phases. Many methods were developed to find new chemical entities that provide the desirable therapeutic effect with minimal unwanted side effects. However, this strategy is time consuming, costly and requires screening of thousands of molecules for biological activity of which only one might enter the drug market. One of the most powerful, attractive and promising method is the prodrug design in which the active drug molecule is linked to a promoiety to alter its undesired properties. Unfortunately, prodrugs are often considered when problems encountered with the parent drug. The design of an appropriate prodrug should be considered in the early stages of preclinical development and should not be viewed as a last resort [3, 4].

The prodrug term introduced for the first time by Adrien Albert in 1958 [5]. Prodrugs are chemically modified versions of the original drugs designed to exert the desired pharmacological effect after an enzymatic and or chemical transformation once they have been administered into the body [1, 6]. The rationale behind the use of prodrug is to alter the physicochemical, pharmacokinetic and biopharmaceutical properties of the compound. By chemical modifications on the active agent, the prodrug can overcome various barriers such as low oral drug absorption, lack of site specificity, chemical instability, toxicity and poor patient acceptance (bad taste, odor, pain at injection site, etc) [7].

The prodrug concept was used for the first time in the mid twentieth century when Parke-Davis Company conducted a modification of chloramphenicol structure to improve its unpleasant taste and poor solubility in water. As a result of this study two chloramphenicol prodrugs were synthesized: chloramphenicol sodium succinate having

a good water solubility for use in IV, IM and ophthalmic administration and chloramphenicol palmitate to be administered in the form of suspension for children [8, 9].

Prodrugs can be classified based on the type of carrier attached to the drug. There are two main classes: (1) carrier linked prodrugs; this term of prodrugs implies a bioreversible covalent linkage between an active drug and a carrier moiety. In most cases, prodrug linkers are removed by an enzymatic or chemical reaction (esters or labile amide). Ideally the linker should be nontoxic, easy to synthesize at low cost, undergo biodegradation to non-active metabolite. Carrier-linked prodrugs can be further subdivided into: (a) bipartite which is composed of one carrier (promoiety) attached directly to the drug, (b) tripartite which utilizing a spacer between the drug and a promoiety. In some cases bipartite prodrugs may be unstable due to inherent nature of the drug-promoiety bond. This can be overcome by designing a tripartite prodrug and(c) mutual prodrugs, which are consisting of two drugs linked together [10].
2) Bioprecursors prodrugs: inactive compounds that contain no promoiety but is rather based on the action of metabolism by processes such as oxidation, reduction, sulfation and phosphorylation activations to create the desired active agent [11].
Many intrinsic and extrinsic factors can influence the bioconversion of prodrugs via enzymes. The rate of bioconversion is not always predictable and can be affected by different factors such as age, gender, and health condition [12, 13].

There are two major challenges facing the prodrug approach: prodrugs designed to be activated via hydrolysis such as peptidases, phosphatases, and carboxylesterases, might be tackled by a premature hydrolysis during the absorption phase in enterocytes of gastrointestinal tract, which could produce more polar and less permeable prodrugs and are more likely to be refluxed by passive and carrier mediated processes into the lumen which results in a reduced bioavailability (50%). On other hand, cytochrome P450 enzymes are responsible for 75% of the enzymatic metabolism of prodrugs. There is accumulating evidence that genetic polymorphisms of P450 enzymes contribute to variability in prodrug activation which has an impact on the efficacy and safety of designed prodrugs [14].

Nowadays, a novel chemical prodrug approach utilized the mechanisms for intramolecular processes to design prodrugs which can chemically and not

enzymatically be cleaved to release the active parent drug in a controlled manner. In this approach the prodrugs design is based on intramolecular processes (enzyme models) using molecular orbital and molecular mechanics methods and correlations of experimental and calculated reactions rates and the rate of the drug release from its prodrug is controlled by the nature of the linker bound to the parent active drug[15, 16].

1.2 Applications of prodrugs

1.2.1 Improvement of taste

Taste is an important factor in the development of dosage forms. Unacceptable taste of certain drugs may often affect patient compliance especially in pediatric and geriatric populations. Medicines dissolve in saliva and interact with taste buds (G-protein coupled receptor-type T2R) on the tongue to give bitter taste [17]. Conventional taste masking methods such as the use of sweeteners, and flavoring agents are often inadequate in masking the taste of highly bitter drugs. To overcome this problem there is a need for orally administered bitter drugs formulated as prodrugs to mask their bitter taste. It was reported that a bitter tastant molecule requires a polar group having hydrogen bonding capability and a hydrophobic moiety. For example, paracetamol, an antipyretic and pain killer drug, has a bitter taste, it is believed that the phenolic hydroxyl group of paracetamol interacts by hydrogen bonding with bitter taste receptors. Therefore, blocking the hydroxyl group with a suitable linker could inhibit the interaction and mask the bitter taste of paracetamol [18, 19].

1.2.2 Improvement of bioavailability

Low bioavailability and low water solubility are frequent problems in drug development. Oral drug bioavailability is critical for the development of new drugs, because low oral absorption leads to inter- and intra-patient variability [20]. Oral bioavailability of lipophilic drugs depends on the dissolution in the gastrointestinal fluids, and polar drug's bioavailability depends on the transport across gastrointestinal mucosa. Therefore prodrugs are designed to increase or decrease lipophilicity. Approximately more than 30% of discovered drugs have poor aqueous solubility. Some

times formulation techniques such as salt formation andsolublizing excepients can't provide adequate solubility. Prodrugs are an alternative to increase the aqueous solubility of parent drugs by improving dissolution rate via ionizable or polar neutral functions attached to the parent drug such as phosphates, amino acids or sugar moieties [21].

Absorption of drugs through several lipid membranes has a significant influence on drug efficacy. Increasing lipophilicity of polar drugs promotes membrane permeation and oral absorption. Oral route of administration is the most common and preferred route of administration for the majority of drugs. Prodrugs are used to increase lipophilicity by masking the polar moiety of the drug so that the drugs are available for oral administration, ocular or topical drug delivery [22].

1.2.3 Overcoming toxicity problems

Many therapeutically active agents have adverse reactions that would limit their clinical use. Adverse drug reactions can change the structure and function of cells, tissues, and organs. Reduced toxicity can be accomplished by targeting drugs to desired cells via site selective drug delivery. Based on prodrug strategy, successful prodrug must be precisely transported to the site of action, where it should be transformed into the active drug to produce the therapeutic effects. Reduced toxicity can sometimes be accomplished by altering the structure of the parent drug. For example, esterification of non-steroidal anti-inflammatory drugs suppress their ulcerogenic activity [23].

1.2.4 Enhancement of chemical stability

Prodrug provides chemical stability for drugs that may destabilized at long-term storage. 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 strategy can be used by changing the functional group responsible for the instability [24].

1.2.5 Protecting from rapid metabolism and excretion

Extensive metabolic and excretion pathways cause low oral bioavailability of drugs; high first pass effect in gastrointestinal tract and liver has been bypassed by prodrug strategy. This is usually done by masking metabolically labile but pharmacologically essential functional groups. The addition of lipophilic promoieties can decrease the solubility of many drugs and prolong the duration of action of very water-soluble drugs [25].

1.3 Codrug approach

In some cases, two pharmacologically active drugs can be coupled together in a single molecule, called a codrug. In such a way that each drug acts as a promoiety for the other and vice versa. The codrug approach offers an efficient tool for improving the clinical and therapeutic effectiveness of a drug. Linking the two drugs moieties may have some additional biological action lacking in the parent drug, thus ensuring some additional benefits or providing "synergistic" effects. Mutual prodrug has given a successful results in case of well accepted and useful drugs with undesirable properties like absorption, poor bioavailability no specificity, and GIT toxicity. In the last few decades, mutual prodrug approach contributed in different therapeutic areas and a list of patents was developed in this field. The main objective of mutual prodrug designing is to bring both active drugs to their active sites with the desired pharmacological action while minimizing toxicological events [26].

In combination therapy for the management of many diseases, the therapeutic agents can be co-administered in separate dosage forms, however, the co-drug offers a potential advantages in delivering co-administered agents as a single chemical entity to increase the patient compliance [27]. The same as conventional prodrug, a mutual prodrug is converted into the component active drugs within the body through enzymatic and/or chemical reactions. The preferred linkage between the first and the second components of the codrug is the one that could be cleaved under physiological conditions.

1.4 NSAIDs

Non steroidal anti-inflammatory drugs are among the most widely used therapeutic agents in modern medicine. NSAIDs are very effective in the alleviation of pain, signs of inflammation: fever, swelling and redness [28]. Due to their anti-inflammatory effect, NSAIDs are commonly used to treat chronic health problems such as rheumatoid arthritis and lupus. However, the clinical usefulness of NSAIDs is still restricted by their GI side effects like gastric irritation, ulceration, bleeding, and perforation and in some cases may develop into life threatening conditions. NSAIDs exert their pharmacological action by inhibiting the production of prostaglandins by non-selectively blocking the COX enzymes (COX-1 and COX-2), causing analgesic, antipyretic, and anti-inflammatory benefits.COX-1 act as a housekeeping enzyme by regulating normal physiological processes such as the maintenance of gastric mucosal integrity, platelet aggregation and kidney function, whereas COX-2 is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells [29].

It is a well-accepted fact that the gastrointestinal toxicity of NSAIDs is mainly related to the mechanism of action of these agents. They are attributed to direct and/or indirect mechanisms. The free carboxylic group present in NSAIDs is thought to be responsible for direct local irritation of gastric mucosa. While the systemic inhibition of COX-1 enzyme induces indirect NSAIDs side effect, generalized inhibition of COX-1 after NSAIDs absorption mediates the gastro-intestinal toxicity. Despite the fact that this effect is decreased with selective COX-2 inhibitors, some patients undergoing chronic NSAIDs treatment demonstrated serious cardiovascular side effects [30, 31].

NSAIDs related gastrointestinal side effects categorized into three groups [32-34]:

(I) Subjective symptoms: like heartburn, abdominal pain, nausea and dyspepsia developed in 15 to 40% of NSAIDs users.

(II) Superficial gastrointestinal mucosal lesions such as erosions and asymptomatic ulcers, occurring in 5 to 20% of NSAIDs users.

(III) Serious gastrointestinal ulcers leading to life-threatening complications like perforation, symptomatic ulcers, and bleeding (perforation, ulcer, bleeding) occurring in 1% to 2% of chronic NSAIDs users, with an associated mortality rate of 10% to 15%.

Nearly all NSAIDs promote gastric ulceration and gastrointestinal bleeding. Among patients who chronically use NSAIDs 65% will develop intestinal inflammation and up to 30% will develop gastro duodenal ulceration [35, 36].

1.4.1 Diclofenac

Diclofenac is 2-(2,6-dichloranilino) phenylacetic acid. It is used to relieve acute or chronic pain states in which there is an inflammatory component like rheumatoid arthritis, osteoarthritis and in the treatment of pain resulting from minor surgery, trauma and dysmenorrheal [29]. It is one of the most frequently used NSAIDs since its introduction in 1974.Currently,diclofenac is the eighth largest-selling drug in the world [37]. Diclofenac is rapidly absorbed following oral administration, and undergoes extensive first-pass metabolism resulting in a systemic bioavailability of approximately 50% with a half-life of approximately 2 hours**.**

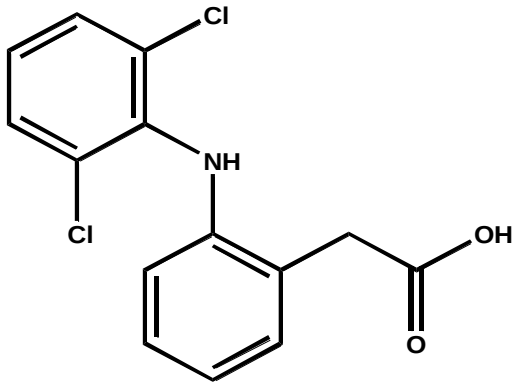


Figure 1.1: Chemical structure of diclofenac.

1.4.2 Mefenamic acid

Mefenamic acid (MA) 2-[(2,3-dimethylanilino]-benzoic acid is a member of anthranilic acid derivatives. It possesses anti-inflammatory effect due to its ability to inhibit COX enzymes and phospholipase A_2. It is typically prescribed for oral administration to relieve mild to moderate pain including headaches, dental pain, and muscular aches and most commonly used as analgesic to relive the pain associated with dysmenorrhea. The drug has a relatively short half-life of 2 hours. Mefenamic acid is available as 250mg capsules [38].

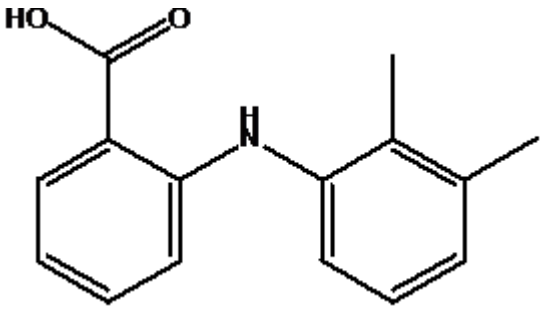


Figure 1.2: Chemical structure of mefenamic acid.

1.5 Tranexamic acid

(trans-4 (aminomethyl) cyclohexane carboxylic acid) is a synthetic derivative of the amino acid lysine that exerts its antifibrinolytic activity through competitive inhibition of the lysine binding site on plasminogen molecule. Thus, inhibits the activation of plasminogen to plasmin; plasmin is an enzyme used to degrade fibrin clot [39]. Tranexamic acid is an effective agent to treat excessive blood loss in different health condition and in surgeries such as coronary artery bypass, hip and knee replacement and liver transplantation [40]. It is an important agent to reduce mortality and morbidity caused by postpartum haemorrhage (PPH). It was reported in a Cochrane review on treatment of PPH that tranexamic acid could potentially have prevented some PPH cases if it was given to women with the risk factors for PPH[41].

Bleeding in trauma patients has also been treated with tranexamic acid. CRASH-2 study concluded that all caused mortality, relative risk and relative death due to bleeding were reduced with a tranexamic acid group more than a placebo group [42].

Tranexamic acid considered safe non-hormonal therapy to treat dysmenorrhea. It is usually prescribed with NSAIDs to treat heavy painful bleeding in women during menstrual cycle to improve women`s life quality [43]. A randomized controlled trial concluded that oral tranexamic acid is effective in decreasing blood loss during menstrual cycle by 40% [44]. Tranexamic acid acts within two to three hours after oral administration and immediately after intravenous administration. It has a Low bioavailability of (34%).

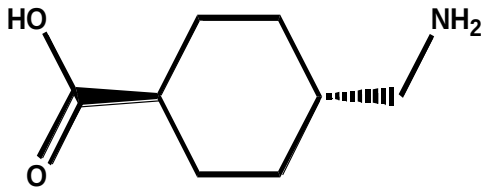


Figure 1.3: Chemical structure of tranexamic acid.

1.6 Research Problem

Although NSAIDs are potent anti-inflammatory drugs, their prolonged administration has been limited by the high incidence of gastrointestinal erosions. GI mucosal injury is generally believed to be caused by two different mechanisms:(i) local action exerted by direct effect of the drug on gastric mucosa caused by free carboxylic acid group attributed to the local inhibition of prostaglandin synthesis. While indirect effect is attributed to ion trapping in mucosal cells or back diffusion of H^+ ions from the lumen into the mucosa and (ii) generalized systemic action following absorption, that is believed to be as a result of an inhibition of COX 1 enzymes [45].

Intolerance of GI side effects leads to withdrawal rates of about 10%. Also, nonselective NSAID users are four to eight times more likely to develop gastro duodenal ulcers during therapy**.** Elderly patients are considered most at risk to develop ulcerogenic events from NSAIDs [46]. *Lanas et al.* have concluded that more than 90% of osteoarthritis patients treated with NSAIDs are at increased GI risk, among which 60% of them at high risk [47].

Much of preventive strategies have been developed in order to decrease NSAIDs GI toxicity. These are either directed at maintaining the integrity of the stomach wall and mucous layer, such as the use of COX-2 selective NSAIDs and the concomitant administration of prostaglandin analogues, or alternatively inhibiting the secretion of gastric acid, such as concomitant use of proton pump inhibitors (PPI) or histamine H2 receptor antagonists [48]. The use of certain gastro protective agents with NSAIDs has been proven to reduce serious GI adverse events, but adds to the cost of NSAID therapy [49]. Selective COX-2 inhibitors, such as celecoxib, have been clinically introduced as (GI)-sparing NSAIDs. However, these compounds are not devoid of side effects, as they can cause cardiovascular complications.

Significant amount of attention have been received from clinicians and healthcare providers since these GI side effects are costly, require hospitalization, and may be fatal [50]. Few studies have estimated mortality resulting from GI complications of NSAIDs and found the deaths to be widely varied from 3,200 to higher than 16,500 deaths per year in the United States [51].

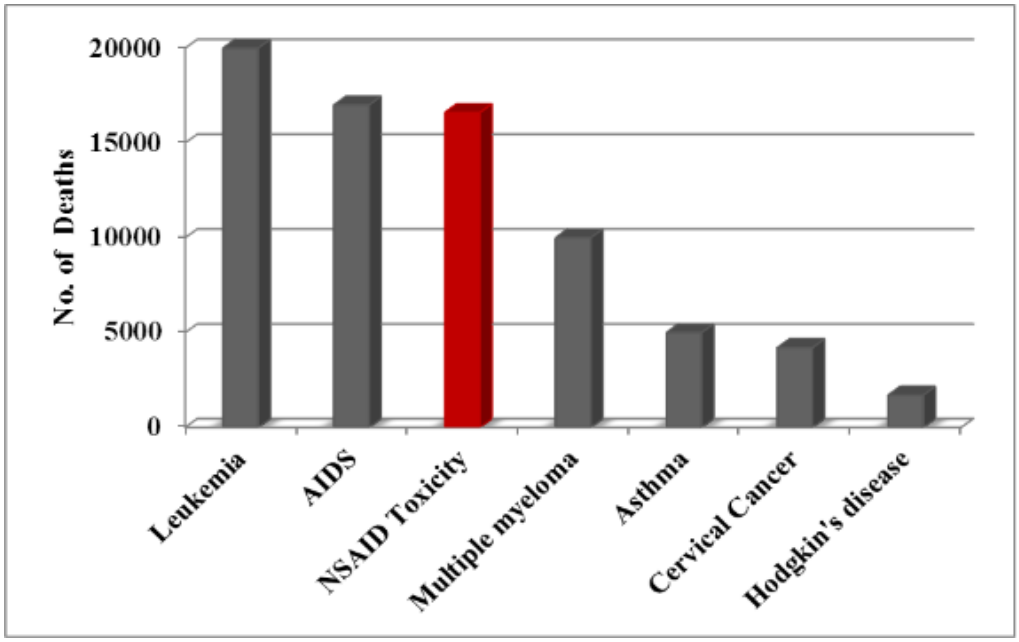


Figure 1.4. Mortality statistics for different drugs and diseases in the united states 1997[52].

On the other hand, NSAIDs have unpleasant bitter taste associated with numbness of the tongue, which leads to lack of patient compliance especially in pediatrics and geriatrics patients, creating a serious challenge to pharmacists. Different techniques such as the use of sweeteners, amino acids and flavoring agents have been developed to overcome the bitter taste. These approaches were found to be inadequate and could not overcome the problem [53].

In modern medicine, NSAIDs with tranexamic acid are the first line therapy to treat pain associated with bleeding. It has been shown to be effective in reducing heavy menstrual bleeding and pain especially in women using IUDs. Giving such co-administered drugs having complementary pharmacological activities in the form of a single chemical will increase patient compliance. Abnormal uterine bleeding and pain are the most common medical reasons for premature discontinuation of the intrauterine device (IUD). The IUD is the most common method of reversible contraception worldwide (147million current users), so premature discontinuation affects large numbers of women. Each year, almost 40 million women have an IUD inserted. Among

these, 5% to 15% discontinue IUD use within one year because of bleeding and pain [54].

Using codrug approach could increase tranexamic acid bioavailability while eliminating NSAIDs GI adverse effects. This can be achieved by making a covalent linkage between tranexamic acid and diclofenac or mefenamic acid which is expected to increase the lipophilicity of tranexamic acid.

The major problem with conventional NSAIDs prodrugs is the difficulty in predicting their bioconversion rates, and thus their pharmacological or toxicological effects. These prodrugs are metabolized via enzymes distributed through the body. Many intrinsic and extrinsic factors such as genetic polymorphisms, age-related physiological changes, and drug- drug interactions can affect the process.

Until now there is no NSAIDs prodrugs formulated that can alleviate the potential side effects completely. Therefore, there is a need to develop bitterless, safe NSAIDs devoid of gastrointestinal side effects, and have the potential to undergo conversion to their parent drugs via intramolecular reaction in a controlled manner.

1.7 General Objective

The main goal of this thesis was to synthesize, characterize and evaluate the kinetics for the following novel prodrugs: diclofenac benzyl and mefenamic dimethylamine, and the codrugs: diclofenac tranexamic and mefenamic tranexamic.

Specific objectives:

1. To synthesize mefenamic and diclofenac prodrugs, as a gastro sparing NSAIDs devoid of ulcerogenic side effects, and to mask their bitter taste by esterification or amidation of their free carboxyl group using different linkers.
2. To synthesize NSAID codrugs by conjugating mefenamic acid or diclofenac with tranexamic acid in order to ameliorate the gastric irritation by temporary blockage of the free carboxylic group present in the NSAID till its systemic absorption, increase tranexamic acid bioavailability and to produce a chemical combination therapy to treat disorders which involve pain and bleeding like menorrhagia.
3. To characterize the proposed prodrugs and codrugs using several characterization techniques.
4. To examine the prodrugs kinetics in different buffer conditions (1 NHCl, pH 2.5, pH5.5, pH 7.4).
5. To investigate the structural factors associated with high reactivity in the hydrolysis, and how the structural features of the prodrug linker can affect the interconversion process of the synthesized prodrugs and co drugs.

Literature Review

Chapter Two

2. Literature Review

2.1 NSAIDs prodrugs

In the past few years, extensive research has been oriented towards improvement of therapeutic efficacy of drugs through removal of their undesirable effects. Design and synthesis of NSAIDs prodrugs is becoming more popular, successful and have been given much attention by medicinal chemists. Many efforts have been made to synthesize NSAIDs prodrugs via masking the carboxylic acid group by forming ester and amide prodrugs [55, 56].

The literature shows clearly that most of the effort to design NSAIDs prodrugs was devoted to protect the gastrointestinal tract from ulcerogenic effects. During the past years numerous NSAIDs prodrugs have been synthesized to overcome the gastrointestinal side effects. Further studies to develop promising new NSAIDs prodrugs are in progress. In the following sections a number of examples of NSAIDs prodrugs will be discussed.

2.1.1 Mefenamic acid prodrugs and codrugs

During the past seventeen years attempts have been made to overcome the gastrointestinal side effects associated with the use of mefenamic acid. In 1997 *Jilani et al*. have synthesized several hydroxyl ethyl esters of diclofenac and mefenamic acid and studied their stability in 1N HCl, buffer pH 7.4 and human plasma. Their study revealed that mefenamic acid prodrugs were much more stable than their corresponding diclofenac prodrugs. The $t_{1/2}$ values for mefenamic acid prodrugs were 38 hours in buffer pH 10 and 7.8 hours in plasma and that of diclofenc prodrugs were 22 hours and 1.12 hours, respectively. Based on this result they concluded that mefenamic ester prodrugs are not suitable for use as prodrugs due to their high stability in plasma [57].

In 2002, *Tantishaiyakul et al*. have synthesized a mefenamic-guaiacol ester prodrug by reacting mefenamic acid, guaiacol, N,N′-dimethylaminopyridine, and N,N′-dicyclohexyl carbodiimide. The physicochemical properties, stability and transport across Caco-2 monolayers for the synthesized prodrug were researched. The prodrug

has shown to be completely stable in aqueous buffer solutions of pH 1-10. However, it underwent hydrolysis in the presence of porcine liver esterase and Caco- 2 homogenate. The transported amount of the ester was 14.63% after 3 hours with a lag time of 23 minutes. The Papp for the ester was 4.72×10^{-6} cm s^{-1}. This value suggests that the prodrug absorption was moderate [58].

In 2005, *Almasirad et al*. have made a number of mefenamic acid prodrugs by which the non-steroidal anti-inflammatory drug was attached to N-arylhydrazone derivative. The aim of their study was to obtain new compounds having analgesic and anti-inflammatory activity without GI side effects. The synthesized prodrugs were tested for analgesic and anti-inflammatory activities by abdominal constriction (writhing test) and carrageenan-induced rat paw edema tests, respectively. Their study revealed that most of the synthesized prodrugs induced significant reduction in the writhing response compared to the control samples [59].

In 2005, *Khan et al*. reacted mefenamic acid with 1, 2, 3-trihydroxy propane 1, 3-dipalmitate/stearate to provide new mefenamic acid ester prodrugs. The aim of their study was to make novel mefenamic acid prodrugs lacking the gastrointestinal side effects associated with their parent drug, mefenamic acid. The synthesized prodrugs were tested for gastric toxicity, anti-inflammatory activity by the carageenan induced paw oedema test and analgesic activity by the acetic acid induced writhing method. The cleavage rate of the ester prodrugs to their parent active drug was studied at pH 3, 4, 5 and 7.4 and monitored by HPLC method. The kinetic results revealed very low hydrolysis rate at pH 5 when compared to pH 7.4. This result indicates that the drug release from the prodrugs in the pH of stomach was negligible; however, the release of mefenamic acid at pH 7.4 was in adequate amounts [60].

In 2007, *Dev.et al*. have synthesized mefenamic acid-β-cyclodextrin prodrug. The primary hydroxy group of β- cyclodextrins was used to block the free acid group of mefenamic acid. The synthesis consisted of several protection and deprotection steps. The study demonstrated that mefenamic acid-β-cyclodextrin prodrug has retained its pharmacological activity as was evident by the percentage inhibition of oedema and in acetic acid induced writhing method and comparison with the activity of its active parent drug. In addition, the study showed that the maximum activity

of the ester prodrug was obtained after 6 hours indicating that there is no drug absorption in the stomach. Further, in vitro studies showed the ester was completely stable in simulated gastric and intestinal fluid whereas it underwent complete hydrolysis in rat fecal contents representing the colon. Ulcerogenicity studies showed that the ester prodrug is not ulcerogenic indicating that masking the carboxyl group in mefenamic acid is a good approach to reduce the ulcerogenicity, a major side effect of the active parent drug, mefenamic acid[61].

In 2009, *Rasheed et al*. have synthesized mefenamic amide prodrugs by an amidation reaction of methyl esters of amino acids such as histidine and tryptophan with mefenamic acid. The goal of their study was to mask the free hydroxyl group of mefenamic acid which is responsible for the adverse effects of gastrointestinal origin associated with the use of the NSAIDs drugs. The hydrolysis rates, anti-inflammatory and analgesic activities as well as ulcer index of the synthesized amide prodrugs were investigated. The results indicated marked reduction of ulcer index and comparable anti-inflammatory activity of the prodrugs as compared to mefenamic acid. In addition, the amide prodrugs showed excellent pharmacological response and encouraging hydrolysis rate both in SIF and SIF+ 80% human plasma. Based on these results the authors concluded that both amide prodrugs are more efficient than their active parent drug and are advantageous due to the fact that they possess lesser gastrointestinal side effects than mefenamic acid [62].

In 2010, *Rasheed et al*. have synthesized two mefenamic acid-amide prodrugs, mefenamic acid-tyrosine and mefenamic acid-glycine via multi-step synthesis which involved protection and deprotection reactions. Pharmacological activity test and kinetic studies on both prodrugs were carried out. The two prodrugs kinetic studies were accomplished in simulated gastric fluid, simulated intestinal fluid, and 80% plasma. In addition, the analgesic, anti-inflammatory, and ulcerogenic activities for both prodrugs were evaluated. Mefenamic acid glycine prodrug showed analgesic activity of 86%, and both mefenamic acid-tyrosine and mefenamic acid-glycine prodrugs showed more efficient anti-inflammatory activity (74% and 81%, respectively) than that of their parent drug, mefenamic acid (40%). Moreover, the study indicated that the average ulcer index of the two newly synthesized prodrugs was lower (9.1 and 4.5) than that of mefenamic acid (24.2). Based on the study results the authors concluded that both

prodrugs are more efficient than mefenamic acid and are advantageous since their gastrointestinal side effects are lesser than of their parent drug [63].

In 2011, *Uludag et al*. have synthesized ibuprofen, ketoprofen, and mefenamic acid ester and amide prodrugs and they investigated their pharmacological activities and stability in physiological media. Their findings revealed that the synthesized prodrugs were completely stable in simulated gastric (SGF, pH 1.2) and intestinal fluids (SIF, pH 6.8). Furthermore, they found that these prodrugs were more lipophilic than their parent active drugs, thus resulting in higher absorption than their parent drugs. Based on the lack of the hydrolysis of these prodrugs by esterases and amidases they concluded that these NSAID derivatives have potent analgesic and anti-inflammatory activity themselves and lack any gastrointestinal side effects (non-ulcerogenic). These results were supported by docking experiments of the synthesized prodrugs with the active sites of esterases and amidases which revealed a strong binding between the prodrugs and enzymes [64].

In 2011, *Velingkar et al*. synthesized a number of mefenamic acid codrugs with and without spacer. The synthesized codrugs were tested for anti-inflammatory activity by carrageenan induced rat paw edema method; for analgesic activity by Eddy's hot plate and tail-flick method; and for ulcerogenicity and acute oral toxicity. The tests results for thecodrugs revealed efficient analgesic and anti-inflammatory activity and a lack of ulcerogenicity. Hydrolysis studies demonstrated that the codrugs were stable at pH 1.2, indicating a lack of cleavage of the codrugs in the stomach. However, in human plasma (pH7.4) the codrugs released 80% of the parent drug upon hydrolysis, whereas much lower percentage of the drug was released in aqueous buffer of 7.4, suggesting that the rate of hydrolysis in human plasma was markedly accelerated when compared to that in aqueous buffers [65].

In 2012, *Mahdi et al*. have synthesized a number of NSAIDs- gabapentin codrugs by which the two active parent drugs were connected by glycol spacers to reduce the gastrointestinal adverse effects associated with the use of NSAIDs. The hydrolysis of the ester bond connecting the two drugs via glycol in two different non enzymatic buffers at pH 1.2 and 7.4, as well as in 80% human plasma was monitored by HPLC. The codrugs connected via ethylene glycol spacers showed complete stability at buffer solutions with half-lives ranging from about 8–25 hours, whereas they underwent 49%–

88% hydrolysis (within 2 hours) in 80% human plasma. The kinetic study results of some of the codrugs indicate that these compounds may be stable during their passage through the GIT until reaching the blood circulation[66].

In 2013, *Shah et al*. synthesized a novel codrug consisting of paracetamol and mefenamic acid with the aim to reduce the ulcererogenic adverse effects associated with the use of NSAIDs. The codrug was completely characterized by standard methods, its stability at different pH values was investigated and its pharmacological properties were evaluated. The kinetic study of the codrug was followed by HPLC at pH 2, pH 7.4 as well as in human plasma. The kinetics results showed the codrug to be stable at pH 2 and pH 7.4; however, it underwent cleavage to the parent drugs in human plasma with hydrolysis rate of 1.8908 x $10^{4}s^{-1}$and half-life ($t_{1/2}$) of 61.07 minutes, indicating rapid hydrolysis in plasma to release the two parent drugs. The pharmacological activities (anti-inflammatory, analgesic and ulcerogenic) of the codrug were evaluated. The ulcerogenic reduction in terms of gastric wall mucosa, hexosamine and total proteins were also determined in glandular stomach of rats. The results revealed that the codrug has an ulcer index lower than the parent drug, indicating low ulcerogenic side effects [67].

In 2014, *Dhokchawle* et al. have synthesized a number of mefenamic acid prodrugs by which the free carboxyl group in mefenamic acid was connected via a covalent bond with natural compounds, eugenol and vanillin. The synthesized ester prodrugs were fully characterized by standard methods and by solubility studies, partition coefficient and hydrolytic studies. The synthesized prodrugs were tested for their anti-inflammatory analgesic and ulcerogenic activity. The tests results revealed that the synthesized prodrugs have shown retention of the anti-inflammatory activity with a reduced ulcerogenicity when compared to their active parent drug, mefenamic acid [68].

In 2014, *Kemisetti et al*. have synthesized mefenamic acid prodrugs by which the NSAID drug was covalently attached to either polyethylene glycol 1500 or polyethylene glycol 6000 via a glycine spacer. The synthesized prodrugs were fully characterized and their hydrolysis at buffers of pH 1.2 and 7.4 were investigated. In addition, their anti-inflammatory activity using Carrageenan induced rat paw edema method and ulcerogenicity using Pylorus ligation method were tested. The study results demonstrated that the hydrolysis rates of the prodrugs at pH 7.4 were higher than that at

pH 1.2 and the anti-inflammatory activity of the prodrugs was comparable to that of their active parent drug, mefenamic acid. Based on these results the authors concluded that the synthesized prodrugs possess anti-inflammatory activity as well as good ulcer protecting activity and can be used as a better replacement to their parent NSAID drug [69].

2.1.2 Diclofenac prodrugs and codrugs

In 1993, morpholinoalkyl esters of diclofenac were synthesized and their hydrolysis in phosphate buffer pH 7.4 and plasma of rats was evaluated. The prodrugs were found to have better absorption than their parent drug and they underwent cleavage in fast rates. In addition, the study showed that in vivo irritation of gastrointestinal mucosa of rat was significantly lower using these prodrugs compared to that after a single and chronic oral administration of diclofenac (the active parent drug) [70].

Tabrizi and coworkers have made several diclofenac prodrugs by attaching diclofenac to polychloromethylstyrene, polyvinyl chloroacetate and polyethylene glycol through a labile ester bond. The group found that these polymers are useful as polymeric prodrugs while the polyvinyl chloroacetate was evaluated as a good carrier for in vivo release of the drug. The hydrolysis of polymer-drug conjugates in cellophane membrane dialysis bags containing buffer solutions pH 8 at 37 C^{o} was studied and the rate of the hydrolysis was determined However, no sharp results were obtained and further study should be undertaken to draw conclusions on the feasibility of using these prodrugs in human [71].

In 1996, several NSAIDs such as ibuprofen, naproxen, diclofenac and ketorolac were reacted with R-(-)-2-amino-1-butanolwith the aim of providing the corresponding amide prodrugs with better bioavailability and less gastrointestinal side effects. The analgesic activity and toxicity of the synthesized prodrugs were investigated and compared to that of the corresponding active parent drugs [72].

In 1997, *Jilani et al*. have synthesized hydroxyl ethyl esters of diclofenac and mefenamic acid aiming to provide NSAIDs prodrugs with efficient analgesic activity and lesser gastrointestinal side effects than their active parent drugs. Stability study on those prodrugs was conducted in 1N HCl, buffer solutions of pH 7.4 and human plasma.

The hydrolytic degradation rate of the diclofenac ester in aqueous buffer solutions was very slow with a half-life of more than 22 hours, whereas the degradation rate in human plasma was very fast with a half-life value of less than 1.2 hours. This indicates that the diclofenac prodrug is quite stable in the stomach conditions and hence It is expected that its gastrointestinal side effects will be lesser than that of diclofenac due to masking the free carboxylic group in diclofenac, which is believed to be responsible in part for these adverse effects [57].

In 1999, *Mahfouz et al*. have conducted ulcerogenicity study, using electron microscopy on rat's stomach; the rats were treated for 4 days with the synthesized NSAIDs ester prodrugs before the ulcerogenicity test. The study revealed that the synthesized prodrugs have shown lesser irritation to the stomach's mucosa than their active parent drugs [73].

In 2000, Bandarage and coworkers synthesized diclofenac ester prodrugs containing a nitrosothiol (S-NO) group aiming to provide NSAIDs with the capability to donate N-O group. These prodrugs were orally administered to mice for bioavailability and toxicity evaluation. The study demonstrated that those prodrugs released the active parent drug, diclofenac, in a significant amount within 15 minutes and showed an efficient inflammatory effect. The S-NO diclofenac prodrugs were shown to be much saferthan their active parent drug, diclofenac. In addition, the study demonstrated that rat stomach lesions caused by S-NO-diclofenac derivatives were less than lesions caused by the parent drug, diclofenac [74].

In 2002, Hirabayashi and coworkers have carried out a study on in vivo disposition at whole body, organ and cellular levels of bisphosphonic prodrug of diclofenac (DIC-BP) upon administration of a dose in the range of 0.32-10 mg/kg. Their study indicated that both total body clearance and volume of distribution at steady state were reduced while the plasma half-life was prolonged. In addition, the study revealed that more than 50% of DIC-BP was transported into osseous tissues when was given in a dose of up to 1mg/kg, however when the dose was increased the skeletal distribution was decreased and both hepatic and splenic accumulations were increased. This is because bisphosphonates cannot be distributed in tissues but they can form a large complex with endogenous metalsin plasma and are recognized as foreign substances from macrophages and thus being taken by the reticuloendothelial system. In order to

optimize the DIC-BP prodrug's delivery, the dosage regimen should be such that the plasma concentration of DIC- BP is maintained at a level lower than that required for precipitate complexes, similar to that of other bisphosphonates [75].

In 2004, Dalpiaz and coworkers have studied the in vitro intracellular uptake of diclofenac and its conjugate ascorbic acid (AA-Diclo) and their affinity for the SVCT2 transporter. In addition, the AA-Diclo prodrug stability was investigated. The hydrolysis study followed a first-order kinetics with a half-life of about 10 hoursin plasma and about 3 hours in the whole blood, suggesting that AA-Diclo prodrug is a potential candidate to enhance the short half-life of diclofenac in vivo [76].

In 2004, Khan and coworkers have synthesized a number of glyceride derivatives of diclofenac and have studied their gastrointestinal side effects, anti-inflammatory and analgesic activity. In addition, the group has investigated the release of the active parent drug from these prodrugs in a wide range of pHs. Their results revealed that the synthesized glyceride prodrugs were found to lack any gastrointestinal side effects and their analgesic and anti-inflammatory effects were significantly greater than that of the active parent drug, diclofenac [77].

In 2009, *Manon et al*. have synthesized a number of diclofenac - antioxidant mutual prodrugs by conjugating diclofenac with different antioxidants having anti-ulcerogenic activity. The study screening revealed that the synthesized mutual prodrugs retained the anti-inflammatory activity as diclofenac, however with lesser ulcerogenic side effects [78].

In 2010, *Nemmani et al*. have designed, synthesized and evaluated new NO-releasing NSAID prodrugs such as NO-Aspirin and NO-diclofenac. NO-diclofenac showed excellent pharmacokinetic, anti-inflammatory properties. In addition, this prodrug showed significant NO-releasing properties and protected rats from NSAID-induced gastric damage which could be attributable to the beneficial effects of NO released from this prodrug [79].

In 2011, diclofenac ester prodrugs were synthesized and their in vitro and in vivo pharmacokinetic and pharmacodynamics properties were evaluated. A study which conducted after oral administration of ester prodrugs, revealed that these compounds have a very good analgesic and anti-inflammatory activity with lesser gastrointestinal

irritation than their active parent drug, diclofenac, and they underwent a rapid enzymatic hydrolysis to their parent drug[80].

In 2012, *Santos et al.* have synthesized 1-(2,6-dichlorophenyl)indolin-2-one,a diclofenac prodrug, and studied its therapeutic activity. The study demonstrated that the new prodrug has shown relevant anti-inflammatory properties without gastrointestinal side effects. Furthermore, the study showed that the prodrug decreased PGE2 levels, COX-2 expression and cellular influx into peritoneal cavity induced by carrageenan treatment. The pharmacokinetic studies on the prodrug have shown *in vivo* enzyme catalyzed interconversion of the prodrug to its parent active drug, diclofenac. Santos et al. have concluded based on this study that the synthesized new nonulcerogenic NSAID prodrug is useful to treat inflammatory conditions by long-term therapy [81].

In 2012, *Ghosh et al*. have synthesized four codrugs of naltrexone and diclofenac linked together via phenolic or alcoholic linker. Transdermal flux, permeability and skin concentration of both parent drugs and codrugs were quantified to form a structure permeability relationship. The results revealed that all codrugs underwent bioconversionin the skin. The extent of the bioconversion was found to be dependent on the structure; phenol linked codrugs were less stable compared to the secondary alcohol linked ones. The flux of naltrexone across microneedle treated skin and the skin concentration of diclofenac were higher for the phenol linked codrugs. The polyethylene glycol link enhanced solubility of the codrugs, which translated into flux enhancement. Based on the study results, *Gosh et al*.concluded that polyethylene glycol linked naltrexone diclofenac codrug is better suited for a 7 day drug delivery system both in terms of stability and drug delivery [82].

In 2014, *Suryawanshi et al*. have synthesized five diclofenac ester prodrugs by reacting the corresponding alcohol with the NSAID drug aiming at reducing the undesired side effects, the most important being (GI) irritation and ulceration, associated with the use of NSAIDs. It is widely believed that using the prodrug approach by temporary blocking the free carboxylic group present in the NSAIDs till their systemic absorption, is the best way to retain the anti-inflammatory effect of the NSAID and eliminate all gastrointestinal adverse effects associated with its use. All five diclofenac ester prodrugs were evaluated for anti-inflammatory activity by Carrageenan Induced Rat hand Paw method and all of the prodrugs without exception showed quite

appreciable anti-inflammatory activity, so the mutual prodrug will specifically and efficiently target the cancerous cells [83].

In 2014, *Hasan et al*. have synthesized eight diclofenac- chalcone mutual prodrugs aiming to provide anti-inflammatory agents with enhanced anti-inflammatory activity and less ulcerogenic adverse effects than their parent drugs. The mutual prodrugs were synthesized by conjugation of diclofenac with chalcone derivatives by the Claisen–Schmidt condensation of acetophenone or p-hydroxy acetophenone with benzaldehyde or appropriately substituted benzaldehyde in the presence of a catalyst. The anti-inflammatory activity of the synthesized fluorinated chalcone derivative was performed using the cotton pellet-induced granuloma in rats as a model, and found to be comparable to that of dexamethasone. Based on this study, Hasan et al.have concluded that chalcones with their pronounced anti-inflammatory activity can synergize the activity of diclofenac when both are in the same compound (codrug) [84].

2.2 Prodrugs design based on intramolecular processes

The striking efficiency of enzyme catalysis has inspired many organic chemists and biochemists to explore enzyme mechanisms by investigating particular intramolecular processes such as enzyme models which proceed faster than their intermolecular counterparts. A novel prodrug approach of intramolecular processes (enzyme models) was utilized to design prodrugs which can chemically release the active parent drug in a controlled manner. In this approach, the design of the prodrugs is based on computational calculations using quantum mechanics and molecular mechanics methods and correlations of experimental and calculated reactions rates. The rate of the drug release is solely dependent on the rate limiting step for the intraconversion reaction.

Currently, computational methods including quantum mechanics such as ab initio, a semi-empirical, density functional theory (DFT), and molecular mechanics are increasingly and widely used as reliable tool that provide structure-energy calculations for prediction the potential drugs and prodrugs [85].

The ab initio method is based on rigorous utilization of the Schrodinger equation and is restricted to small systems that do not have more than thirty atoms due to the extreme cost of computation time. Calculations of molecules exceeding 50 atoms can be done using semi-empirical methods. Density functional theory (DFT) is a semi-empirical

method used to calculate geometric and energies for medium-sized systems(up to 60 atoms) of biological and pharmaceutical interest and is not restricted to the second row of the periodic table [3].

Various intramolecular processes were studied to understand enzyme catalysis in order to design novel prodrug linkers. These processes include (i) proton transfer between two oxygen in Kirby's acetals, and proton transfer between nitrogen and oxygen in Kirby's enzyme models. (ii) intramolecular acid-catalyzed hydrolysis in Kirby's maleamic acid amide derivatives[16] and (iii) proton transfer between two oxygen in rigid systems as investigated by Menger.

Based on the computational calculations conducted by Karman's group on the above mentioned intramolecular processes (enzyme models), it was concluded that: (i) rates acceleration in intramolecular processes is a result of both entropy and enthalpy effects. In intramolecular ring-closing reactions where enthalpic effects were predominant, steric effects were the driving force for the acceleration, whereas proximity orientation was the determining factor in proton-transfer reactions. (ii) The distance between the two reacting centers is the main factor in determining whether the reaction type is intermolecular or intramolecular. When the distance exceeded 3 Å, an intermolecular engagement was preferred because of the engagement with a water molecule (solvent). When the distance between the electrophile and nucleophile was <3 Å, an intramolecular reaction was dominant. (iii)The efficiency of proton transfer between two oxygen and between nitrogen and oxygen in Kirby's enzyme models is attributed to relatively strong hydrogen bonding in the products and the transition states leading to them [3].

Modern computational approach was utilized for the design of innovative prodrugs. During the past seven years, mechanisms of intramolecular processes for a number of enzyme models have been studied by Karaman's group and were used to design novel prodrug linkers. Among the enzyme models have been investigated: proton transfer between two oxygens and proton transfer between oxygen and nitrogen in Kirby's acetals [86]

Karaman's group successfully designed and synthesized several novel prodrugs. Examples of these prodrugs include: the anti-Parkinson's agent dopamine [87], anti-viral agent acyclovir [88], anti-malarial agent atovaquone [89], antihypertensive atenolol [90], antibacterial cefuroxime [91]and the anti-psoriasis monomethyl maleate [92].

2.2.1 Design of mefenamic and diclofenac prodrugs using Kirby`s enzyme model (Proton transfer in N-alkylmaleamic acids)

The design of mefenamic and diclofenac prodrugs was accomplished using Kirby's enzyme model that describes proton transfer reactions in N-alkylmaleamic acids [92] (Figure2.1). The DFT calculations run on these systems revealed that the hydrolysis reaction occurs *via* an intramolecular general acid catalysis mechanism and the reaction rate is dependent on the following factors: (1)The difference between the strain energies of intermediate and product and intermediate and reactant. (2)The distance between the two reacting centers. (3)The attack angle. Further, a linear correlation between the calculated proton transfer reaction and the experimental rates established the credibility of using DFT methods in predicting energies and rates for proton transfer reactions [86, 93].

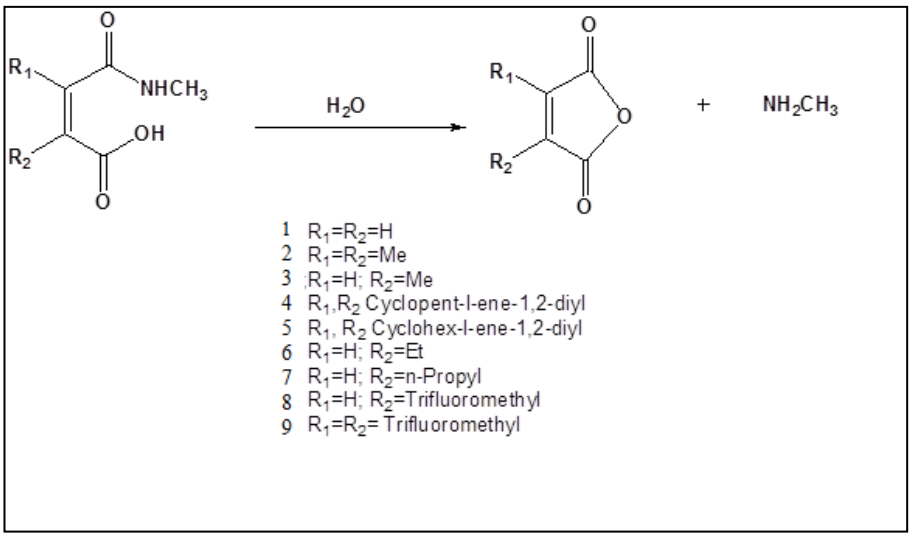


Figure 2.1: Acid-catalyzed hydrolysis of N-alkylmaleamic acids

The calculations also demonstrated that the acid catalyzed 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.

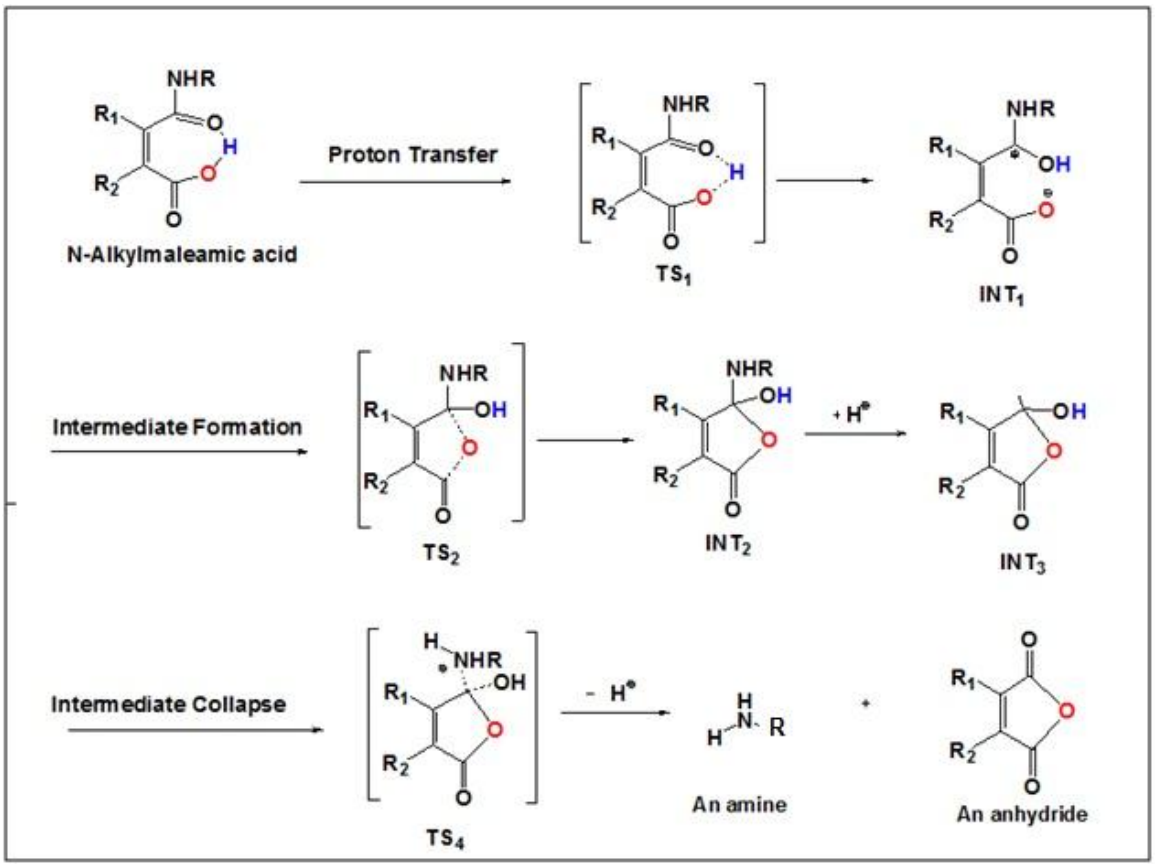


Figure 2.2: Proposed mechanism for the acid-catalyzed hydrolysis of N-alkylmaleamic acids.

Experimental

Chapter Three

3. Experimental Part

3.1 Materials

All organic salts were of analytical grade and were used without further purification. Organic buffer components were distilled or recrystallized. Distilled water was redistilled twice before use. Dimethylamine, triethylamine, anhydrous sodium dihydrogen phosphate, oxalyl chloride, diclofenac sodium and diclofenac potassium, tranexamic acid,and mefenamic acid were commercially obtained from Sigma Aldrich. Methanol, acetonitrile and water for analysis were for HPLC grade and were purchased from Sigma Aldrich. High purity chloroform, tetrahydrfuran (THF), dichloromethane, hexane, ethylacetate, dimethylformamide, and diethyl ether (> 99%) were purchased from Biolab (Israel).

3.2 Instrumentation

HPLC measurements were carried out using Shimadzu prominence high performance liquid chromatography system HPLC-PDA, (Shimadzu corp. Japan). LC-Esi-MS measurements were performed employing an agilent 1200 series liquid chromatography coupled with a 6520 accurate mass quadruple time of flight mass spectrometer (Q-TOF LC/MS)The high pressure liquid chromatography system consisted of a model 2695 HPLC from Waters (Israel) equipped with a Waters 2996 Photodiode array. Data acquisition and control were carried out using Empower ™ software (Waters: Israel). Analytes were separated on a 4.6 mm x150 mm C18 XBridge® column (5 µm particle size) used in conjunction with a 4.6 mm, 20 µm, XBridge® C18 guard column. Microfilters 0.45µm porosity were normally used (Acrodisc® GHP, Waters). pH meter model HM-30G: TOA electronics™ was used in this study to measure the pH value for the buffers. 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. ^{1}H-NMR: Data were collected using Varian Unity Inova400 MHz spectrometer equipped with a 5-mm switchable and data were processed using the VNMR software. For ^{1}H-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). All infrared spectra (FTIR) were obtained from a KBr matrix (4000–400 cm^{-1}) using a PerkinElmer Precisely, Spectrum 100, FT-IR spectrometer.

3.3 Synthesis of the Prodrugs

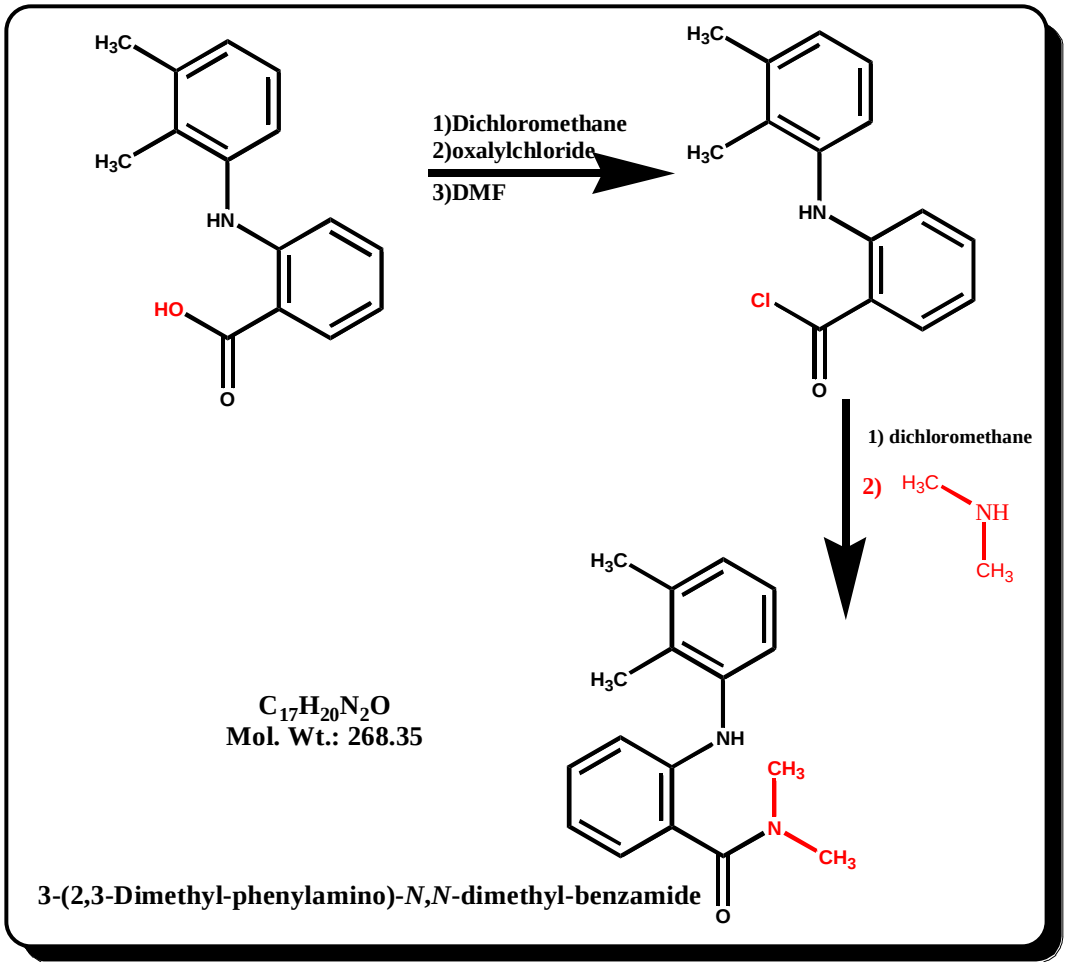
3.3.1 Mefenamic dimethylamine (Scheme 1)

First step: synthesis of mefenamic acyl chloride

In a 250 ml round-bottom flask, 1 equivalent of of mefenamic acid (4.28gm, 20 mmol) was dissolved in anhydrous dichloromethane (50 ml), then 2 equivalents of of oxalyl chloride(40 mmol, 3.4 ml) was added and 0.5 ml of DMF was slowly added as a catalyst, the resulting solution was stirred at room temperature overnight, then the solvent and excess oxalyl chloride were removed under reduced pressure.

Second step: mefenamic acyl chloride–dimethylamine reaction

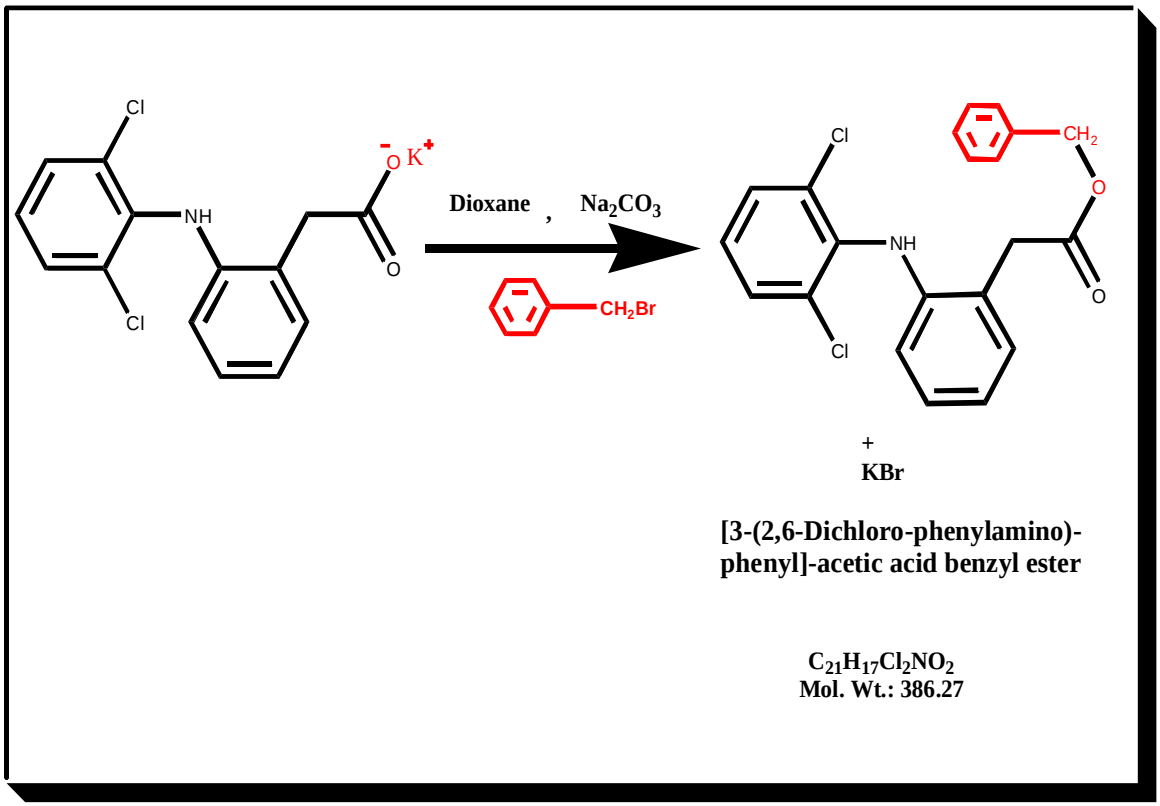
Product from step 1) was dissolved in 40ml dried dichloromethane. 1 equivalent of dimethylamine (5 ml) was added to the reaction. The mixture was allowed to stir overnight at room temperature until the reaction was completed. The progress of the reaction was monitored by TLC. After completion of the reaction 50 ml dichloromethane was added to the reaction mixture. The reaction mixture was transferred to a separatory funnel and the organic layer was extracted with 100ml 1N NaOH then with 100ml 1N HCl. The organic layer was collected and washed with 100 ml water. Sodium sulfate was added to dry excess water followed by filtration. The solution was evaporated under vacuum to furnish the crude product which was purified by column chromatography. The yellow precipitate formed was collected and dried (3.8 gm) with yield of (88.7%) and m.p (300C^{o}).



Scheme 1: Synthesis of mefenamic dimethylamine

3.3.2 Diclofenac benzyl (Scheme 2)

In a 250 ml round-bottom flask 1 equivalent of diclofenac potassium (3.43gm,20mmol) was dissolved in 100ml dioxane, 2 gm of sodium carbonate was added, the resulting solution was stirred for 30 minutes. Then, 4 equivalents of benzyl bromide (5ml, 80mmol) was added to reaction mixture. The reaction was allowed to heat until reflux for 3 days until the reaction was completed. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was filtered and washed with dioxane. The filtrate was evaporated under vacuum to furnish a product which was purified by column chromatography. The white precipitate formed was collected and dried (3 gm) with yield (87.4%), m.p (300C^{o})



Scheme 2: Synthesis of diclofenac benzyl

3.3.3 Mefenamic-tranexamic (Scheme 3)

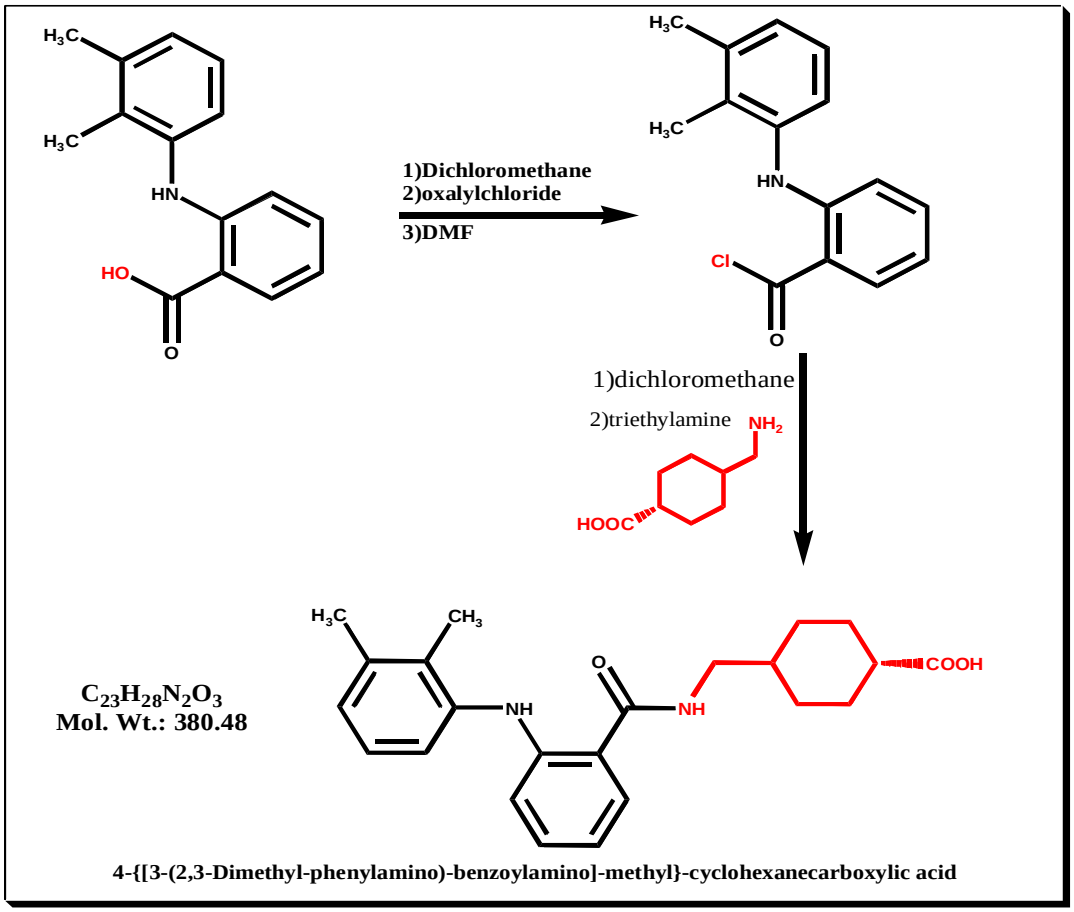
First step: synthesis of mefenamic acyl chloride

In a 250 ml round-bottom flask, 1 equivalent of of mefenamic acid 4.28 gm (20 mmol) was dissolved in anhydrous dichloromethane (50 ml), then 2 equivalents of of oxalyl chloride (3.4 ml,40mmol) and 0.5 ml of DMF were slowly added, the resulting solution was stirred at room temperature overnight, then the solvent and excess oxalyl chloride were removed under reduced pressure.

Second step: reaction of mefenamic acyl chloride with tranexamic acid

In a 250 ml round-bottom flask mefenamic chloride (product from step 1) was dissolved in dichloromethane (30 ml), a solution of 1 equivalents tranexamic acid(3.14 g,20mmol) in dry DMF (20ml) and 5ml triethylamine were added, the resulting solution was stirred for 3 days at room temperature until the reaction was completed. The progress of the reaction was monitored by TLC. The organic layer was extracted with 100 ml 1N HCl,

the organic layer was dried over $MgSO_4$ anhydrous, filtered and evaporated to dryness. The product was a yellow precipitate (4gm) with a yield of 93% , and m.p over 300C^o.



Scheme 3: Synthesis of mefenamic tranexamic

3.3.4 Diclofenac tranexamic (Scheme 4)

First step : diclofenac protonation

In a 250 ml round-bottom flask, 1 equivalent of diclofenac potassium (6.68 gm ,20 mmol) was dissolved in 30 ml methanol, then 3-4 equivalents of concentrated HCl (4ml**)** was added. The mixture was allowed to stir at room temperature overnight. The reaction solvent was removed under reduced pressure and the residue was dissolved in THF (50 ml), filtered and evaporated.

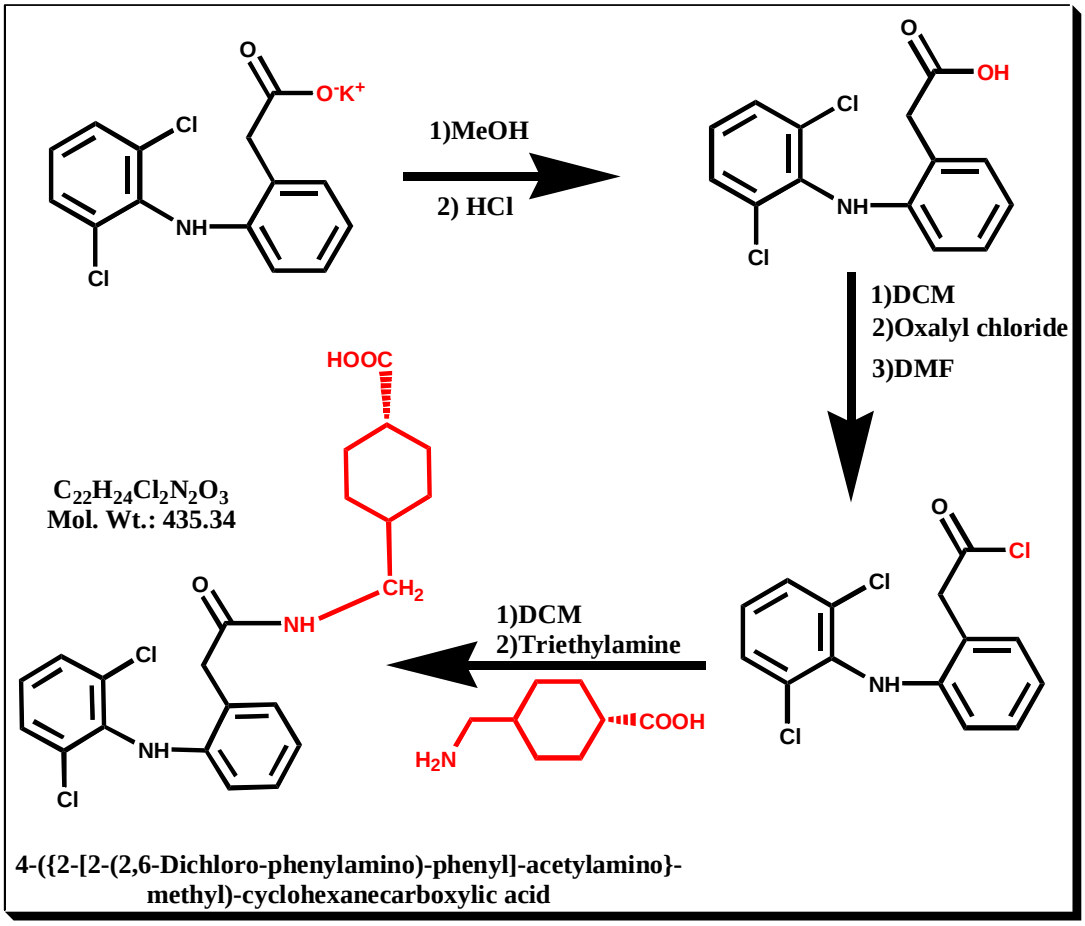
Second step: synthesis of diclofenac acyl chloride

In a 250 ml round-bottom flask, 1 equivalent of the protonated diclofenac (product from step 1) (20 mmol) was dissolved in anhydrous dichloromethane (50 ml), 2 equivalents

of oxalyl chloride(3.4 ml) and 0.5 ml of DMF were slowly added, the resulting solution was stirred at room temperature overnight, then the solvent and excess oxalyl chloride were removed under reduced pressure.

Third step: reaction of diclofenac acyl chloride with tranexamic acid

In a 250 ml round-bottom flask diclofenac chloride (product from step 2) was dissolved in dichloromethane (30 ml), a solution of 1 equivalent of tranexamic acid 3.14 g in dry DMF (20ml) and 5ml triethylamine was added, the resulting solution was stirred for 3 days at room temperature until the reaction was completed. The progress of the reaction was monitored by TLC. After completion of the reaction, the organic layer was extracted with 100 ml 1N HCl. The combined organic layer was dried over $MgSO_4$ anhydrous, filtered and evaporated to dryness. The product was a white precipitate(5.5 gm) with a yield of 83% and m.p (100C^o)



Scheme 4: Synthesis of diclofenac tranexamic**.**

3.4 Chemical hydrolysis

Acid catalyzed hydrolysis of diclofenac and mefenamic prodrugs and codrugs were carried out in aqueous buffers in the same manner to that executed by Kirby *et al.* on maleamic acids [94]. This is to investigate whether the prodrugs or codrug undergoes hydrolysis in aqueous medium and to what extent or not, suggesting the fate of the prodrugs or codrug in the system. The synthesized prodrugs and codrugs were studied using high performance liquid chromatography (HPLC) at constant temperature (37 ^{o}C) and at ambient pressure in different buffers 1N HCl, pH 2.5 (stomach), pH 5.5 (intestine) and pH 7.4 (blood) which correspond to the physiological environments in the human body.

3.4.1 Buffer Preparation

Potassium dihydrogen phosphate(6.8 g) were dissolve in 900 ml water for HPLC, the pH of buffers pH 2.5 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 5.5 and pH7.4, however, the required pH was adjusted using 1 N NaOH.

Intraconversion of 500 ppm mefenamic acid dimethylamine and diclofenac solutions, in 1N HCl, buffer pH 2.5, buffer pH 5.5 or buffer pH 7.4, to its parent drug was monitored by HPLC at a wavelength of 254 nm. Conversion reactions were run mostly at 37.0 ˚C. Intra-conversion of 500 ppm mefenamic tranexamic, diclofenac tranexamic solutions, in 1N HCl, buffer pH 2.5, buffer pH 5.5 and buffer pH 7.4, to its parent drug were monitored by HPLC at a wavelength of 254 nm. Conversion reactions were run mostly at 37.0 ˚C.

3.4.2 Calibration curve

A 100 ml stock solution of mefenamic dimethylamine and diclofenac benzyl with a final concentration of 500 ppm were prepared by dissolving 50 mg from each prodrug in 100 ml methanol. The following diluted solutions were prepared from the stock solution: 100, 200, 300 and 400 ppm. Each solution was then injected to the HPLC apparatus using 4.6 mm x 250 mm, 5 µm C18 XBridge ® column, mobile phase

contains water: acetonitrile (25:75) a flow rate of 1 ml min^{-1} and UV detection at a wavelength of 254 nm.

Peak area vs. concentration of the pharmaceutical (ppm) was then plotted, and R^2 of the plot was recorded.

A 100 ml stock solution of mefenamic tranexamic and diclofenac tranexamic with a final concentration of 500 ppm were prepared by dissolving 50 mg from each codrug in 100 ml methanol. The following diluted solutions were prepared from the stock solution: 100, 200, 300 and 400 ppm. Each solution was then injected to the HPLC apparatus using 4.6 mm x 250 mm, 5 µm C18 XBridge ® column, mobile phase for mefenamic tranexamic water: acetonitrile (10:90) and (75:25) for diclofenac tranexamic, a flow rate of 1 ml min^{-1} and UV detection at a wavelength of 254 nm.

Peak area vs. concentration of the pharmaceutical (ppm) was then plotted, and R^2 of the plot was recorded.

3.4.3 Preparation of standard and sample solution

A 500 ppm of standard (mefenamic acid, diclofenac potassium) were prepared by dissolving 50 mg of each drug (mefenamic acid, diclofenac) in 100 ml of 1N HCl, buffer pH 2.5, buffer pH 5.5 or buffer pH 7.4, then each sample was injected into HPLC to detect the retention time of mefenamic.

A 500 ppm of standard linker (dimethylamine and benzyl bromide) was prepared by dissolving 50 mg of each linker in 100 ml of 1N HCl, buffer pH 2.5, buffer pH 5.5 or buffer pH 7.4, then each sample was injected into HPLC to determine the retention time of linker.

A 500 ppm of each prodrug was prepared by dissolving 50 mg of each prodrugin 100 ml of 1N HCl, buffer pH 2.5, buffer pH 5.5 or buffer pH 7.4 then each sample was injected into the HPLC to determine the retention time.

The reaction progress was followed by monitoring the disappearance of the prodrug and appearance of the drug and linker versus time**.**

Results and discussion

Chapter Four

4. Results and Discussion

4.1 Characterization

Mefenamic acid and mefenamic dimethylamine were characterized by FT-IR, LC-MS and ^{1}H-NMR spectroscopy.

Mefenamic acid , IR spectrum (Figure 4.1) shows an absorbance at 1653 cm^{-1} corresponds to C=O of carboxylic acid and 3312 cm^{-1}corresponds to N-H.

^{1}H-NMR (Figure 4.2) δ (ppm) CD_3OD:2.16 (s, 3H, Ar-(CH3)) , 2.35 (s , 3H, Ar-(CH3) , 6.63(t, 1H, Ar-H), 7.01(d, 2H, J=6.7 ,Ar-H), 7.11(t, 1H, Ar-H), 7.23(t, 1H, Ar-H), 7.94 (d, 2H, J=1.65, Ar-H).

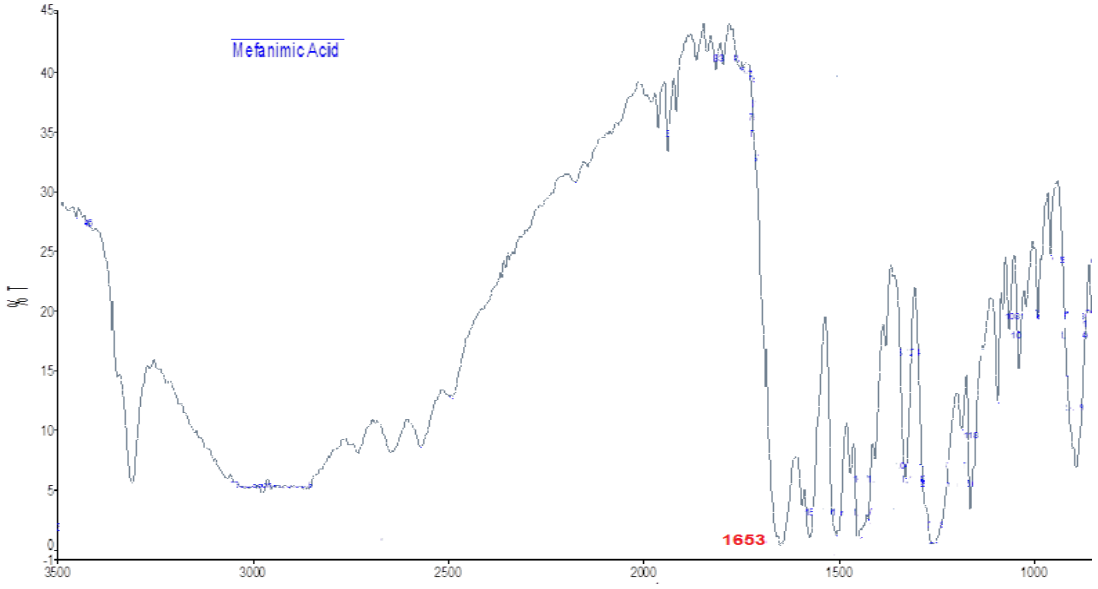


Figure 4.1: FT-IR spectrum of mefenamic acid.

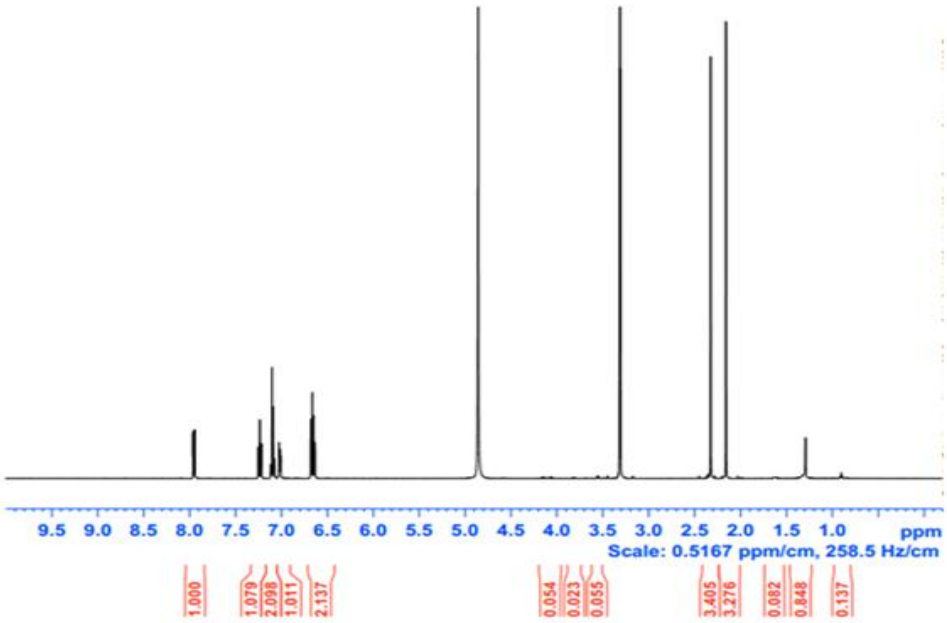


Figure 4.2: ^{1}H-NMR spectrum of mefenamic acid.

Mefenamic dimethylamine.

M.P: 300 C^{o}

H^{1}NMR: in (Acetone-d6, ppm): 2.45(s, 3H, Ar-(CH3)), 2.49(s, 3H, Ar-(CH3)), 2.76(S, 6H, N-(CH3)), 2.80 (s, 6H, N-(CH3)), 7.10(d, J=7.12, 2H, Ar-H), 7.23 (t, 1H, Ar-H), 7.66 (t, 1H, Ar-H), 7.74 (t, 1H, Ar-H), 8.12(d, 1H,Ar-H), 8.29(d, J=1.08, 2H, Ar-H)

IR (KBr/ν_{max} cm^{-1}) 1614 (C=O), 2963 (N-H), *m*/*z* (M+1)$^{+}$269.1648

The IR spectrum (Figure 4.3) shows an additional signals at frequencies of1712 cm^{-1}, 1643 cm^{-1}correspond to C=O of the amide. A high resolution LC-MS (Figure4.4) shows a protonated peak at *m*/*z* 269.1648(M+1)$^{+}$ The ^{1}H-NMR (Figure4.5) dimethylamine proton show singlet peaks at 2.76and 2.80 ppm.

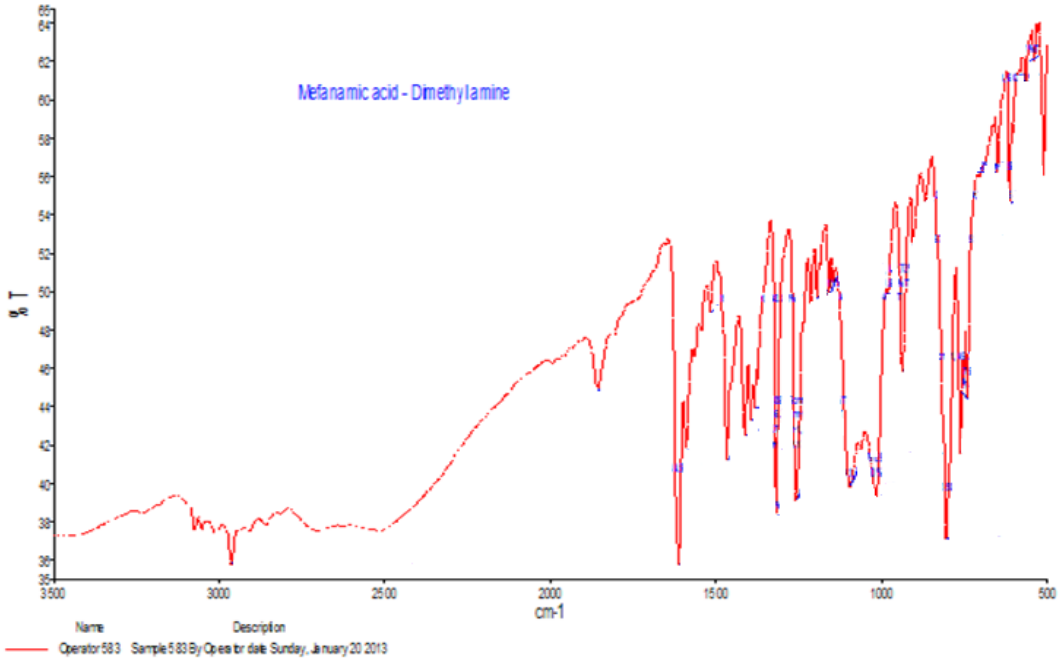


Figure 4.3 : FT-IR spectrum of mefenamic dimethylamine**.**

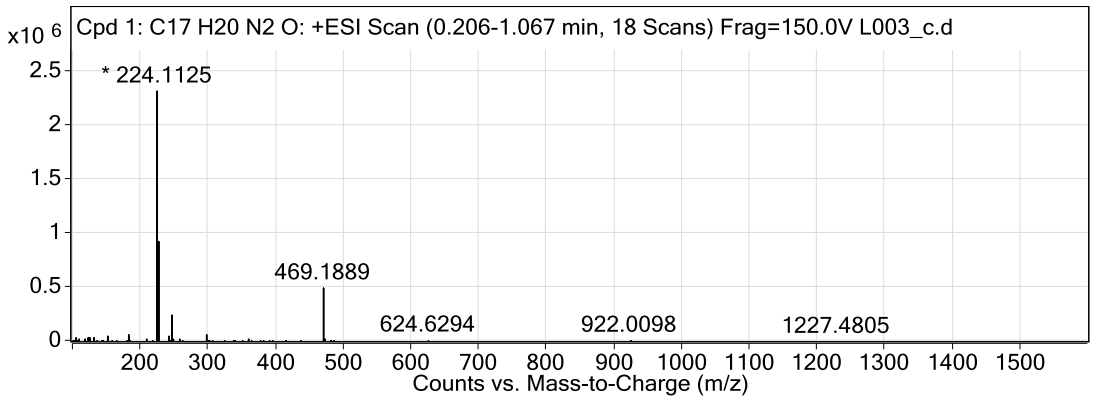


Figure 4.4: LC-MS spectrum of mefenamic dimethylamine**.**

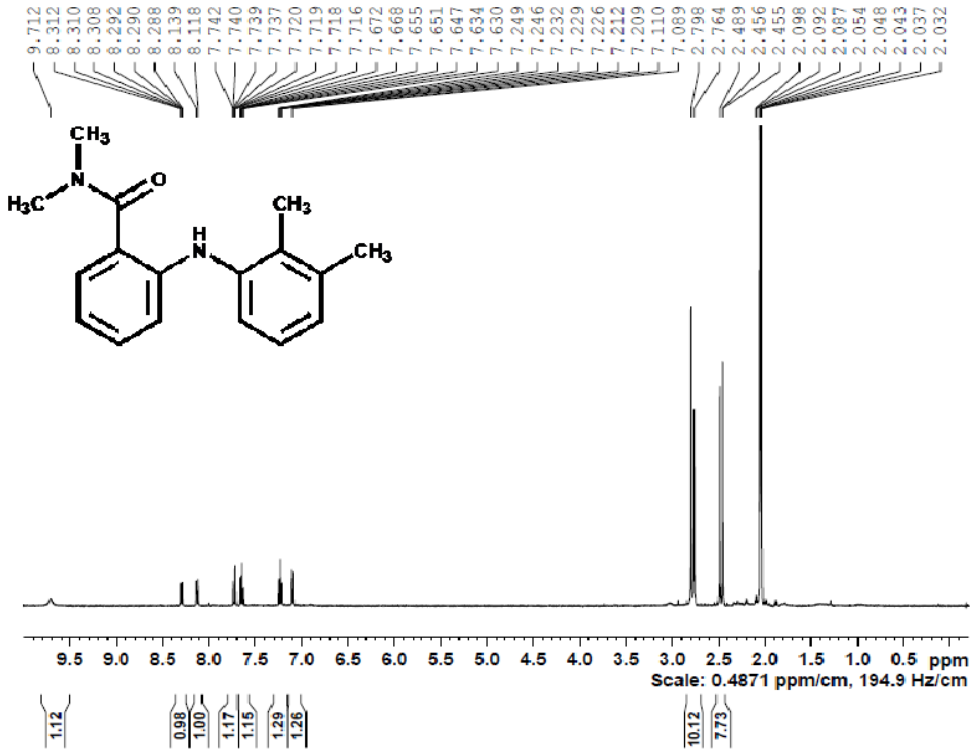


Figure 4.5: ^{1}H-NMR spectrum of mefenamic dimethylamine**.**

Diclofenac: The IR spectrum (Figure 4.6) shows an absorbance at 1578 cm^{-1}corresponds to C=O. The H^1NMR (Figure 4.7): 400MHz ($CDCl_3$, ppm): 3.63 (s, 2H, COC$\underline{H_2}$) , 6.35 (d, 1H, J= 0.8, Ar-H),6.80 (t , 2H, Ar-H), 6.98 (t, 1H, Ar-H), 7.20(d, 1H , J=1.2 , Ar-H), 7.35(d,2H, J=3.6 , Ar-H).

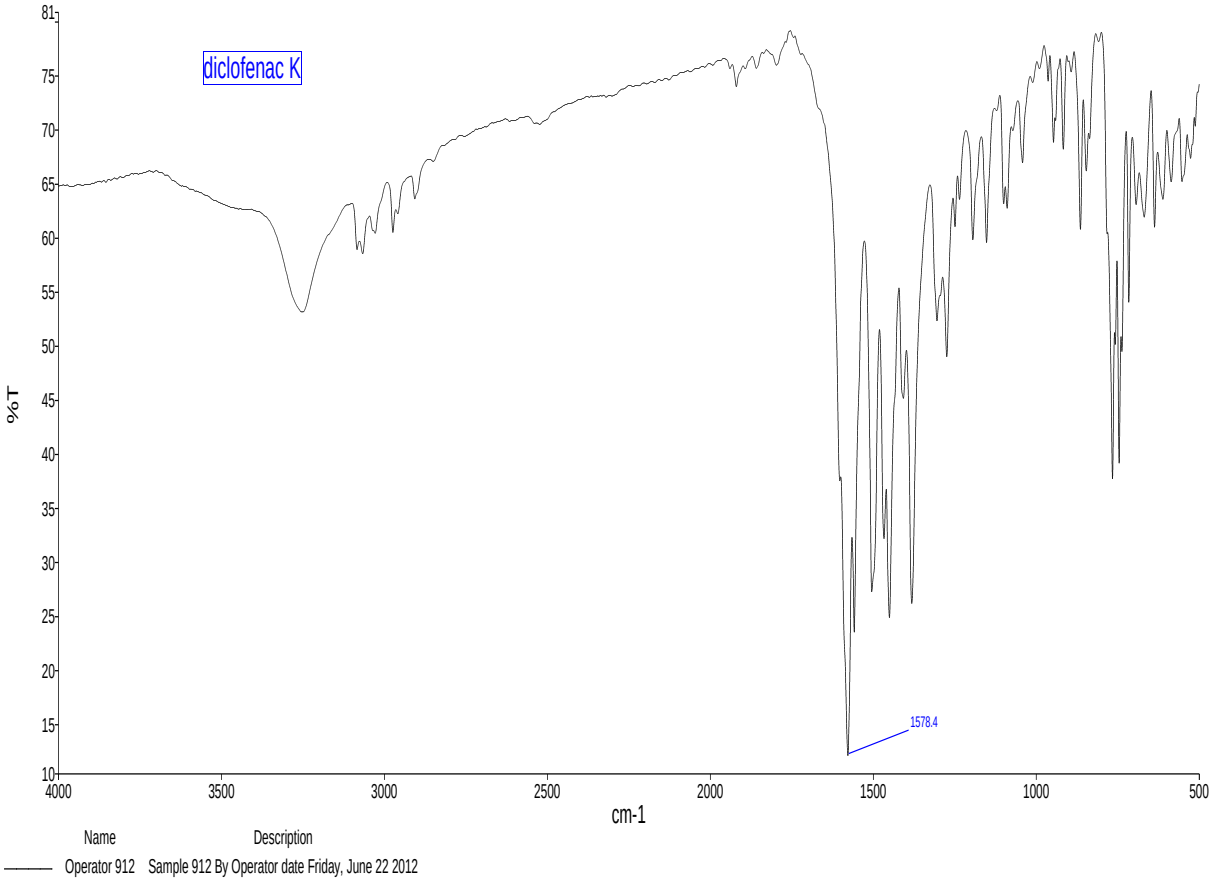


Figure 4.6 FT-IR spectrum of diclofenac potassium.

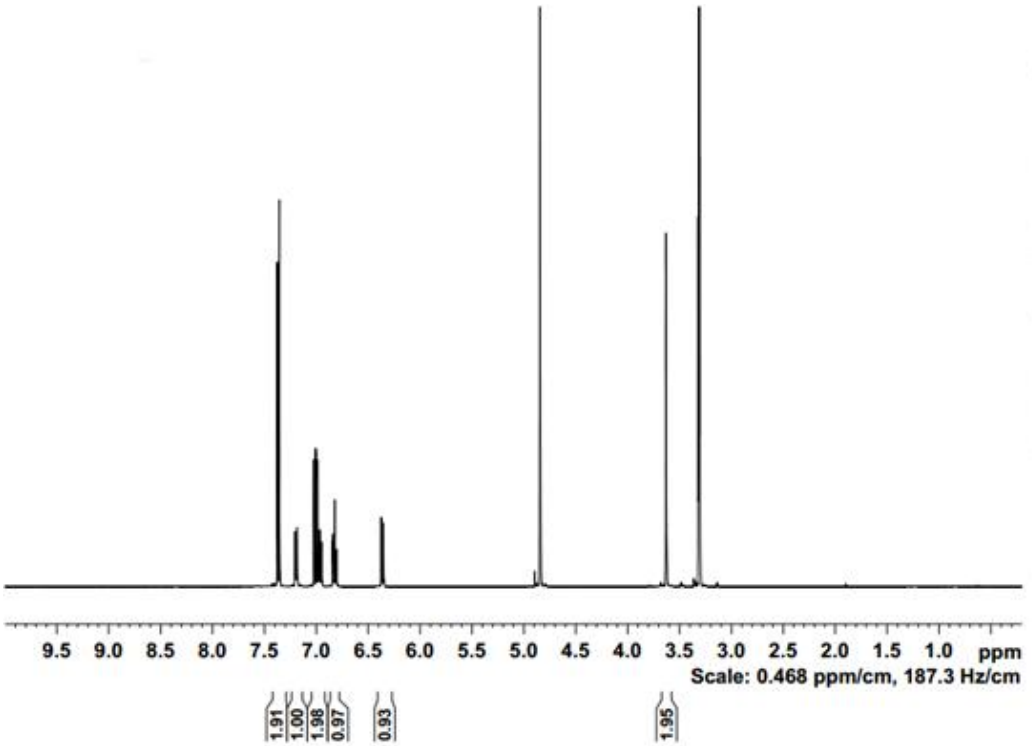


Figure 4.7: ^{1}H-NMR spectrum of diclofenac.

Diclofenac benzyl.

M.P: 300 C^{o}.

^{1}H-NMR ($CDCl_3$, ppm): 3.86 (s,2H,COCH2) , 5.18 (s, 2H, CH2-Ar) , 6.54(d, 1H, J= 8, Ar-H),6.88 (d, 2H, J= 21.6, CH_2-Ar-H),6.97 (t, 1H, Ar-H), 7.14(t, H, Ar-H), 7.24(d, 1H, J=1.6 ,Ar-H),7.31 (d, 1H, J= 3.2, Ar-H), 7.37(t, 5H, Ar-H).

IR (KBr/ν_{max} cm^{-1}) 1743(C=O), 3361(N-H).*m*/*z* 386.07(M+1)$^{+}$.

IR spectrum(Figure 4.8) shows an additional signals with frequencies of 1743 cm^{-1},3361 cm^{-1} correspond to carbonyl group (C=O) and (N-H), respectively. A high resolution LC-MS (Figure 4.9) shows a protonated peak at *m*/*z* 386.07(M+1)$^{+}$.The ^{1}H-NMR (Figure 4.10)shows additional singlet peak at 5.18ppm and doublet and triplet peaks at 6.30- 7.32 corresponding to the protons of the benzyl moiety.

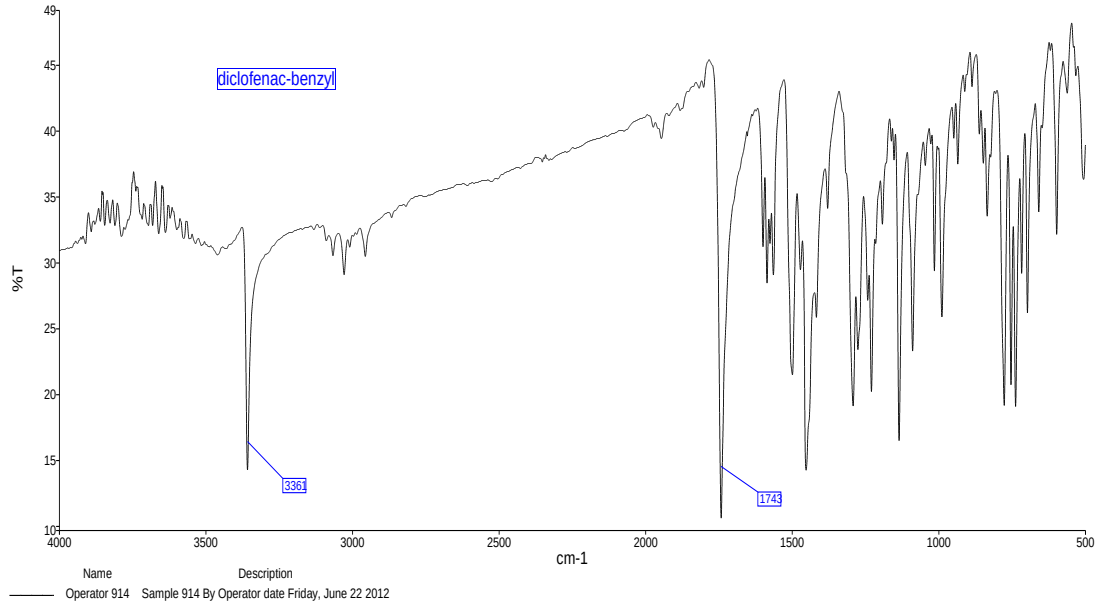
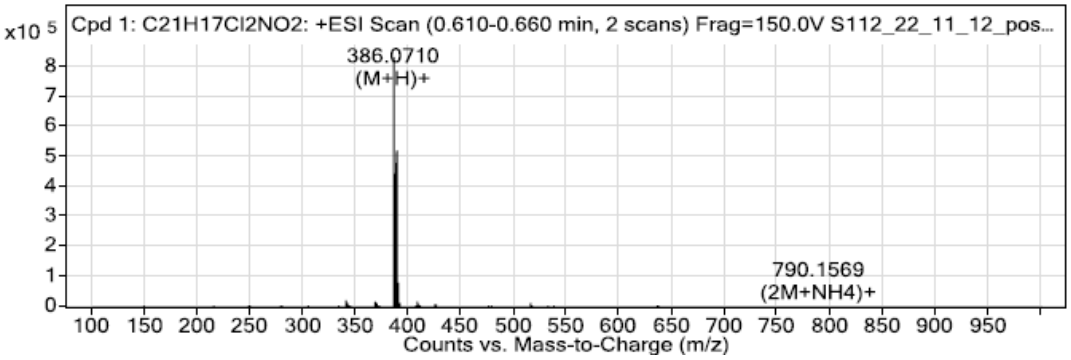


Figure 4.8: FT-IR spectrum of diclofenac benzyl.



Peak List

m/z	z	Abund	Formula	Ion
368.0599	1	20027	C21 H16 Cl2 N O	(M+H)+[-H2O]
385.0617		548	C21 H17 Cl2 N O2	M*+
386.071		834888	C21 H18 Cl2 N O2	(M+H)+
387.0737		169303	C21 H18 Cl2 N O2	(M+H)+
388.0684		528144	C21 H18 Cl2 N O2	(M+H)+
389.0711		103733	C21 H18 Cl2 N O2	(M+H)+
390.066		80043	C21 H18 Cl2 N O2	(M+H)+
408.0519	1	17222	C21 H17 Cl2 N Na O2	(M+Na)+
424.0271	1	8362	C21 H17 Cl2 K N O2	(M+K)+
790.1569	1	80	C42 H38 Cl4 N3 O4	(2M+NH4)+

Figure 4.9. LC- MS spectrum of diclofenac benzyl.

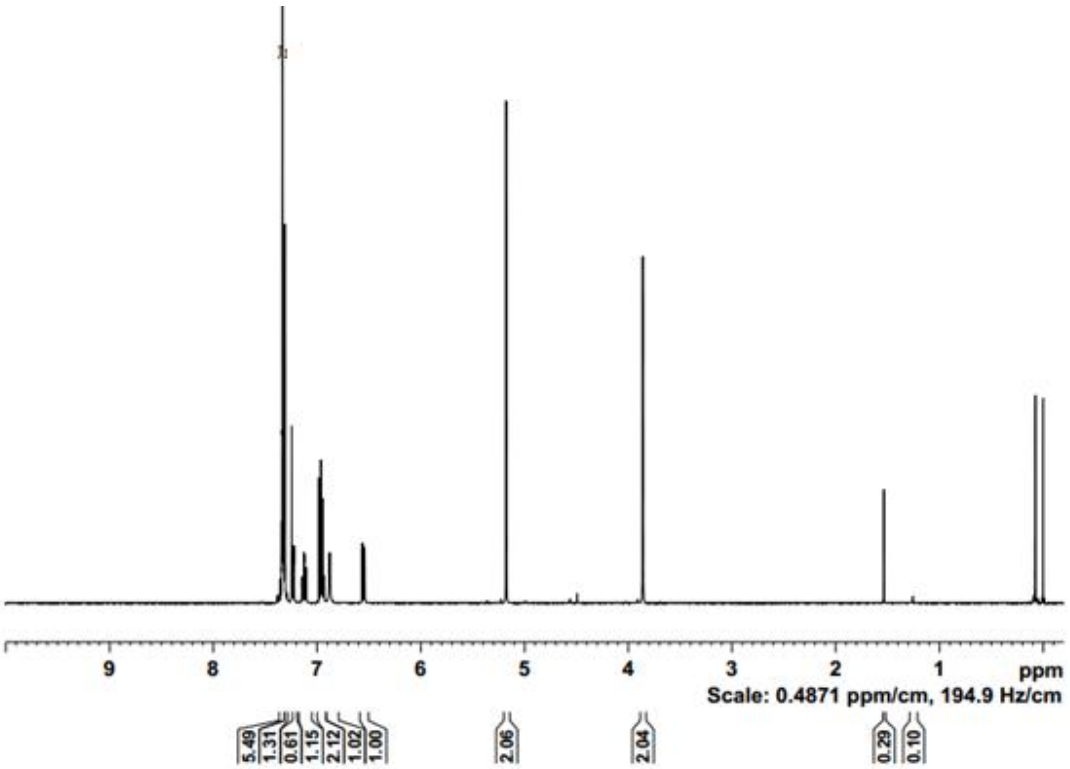


Figure 4.10: ^{1}H-NMR spectrum of diclofenac benzyl.

Tranexamic acid. The IR spectrum (Figure 4.11) shows an absorbance at 1642 cm^{-1} corresponds to C=O. A high resolution LC-MS (Figure 4.12) at the ESI (positive mode) shows a protonated peak at *m*/*z* 158.1176 $(M+1)^+$, an adduct of $[M^+Na]^+$ appeared at *m*/*z* of 180.0993. The ^{1}H-NMR (Figure 4.13) peaks occur at 1.06 ppm (q, 2H, CH-$\underline{CH_2}$-CH_2), 1.40 ppm (q, 2H, CH-$\underline{CH_2}$-CH_2), 1.54 ppm (m, 1H, CH_2-$\underline{CH}$-CH_2-CH_2), 1.84 ppm (m, 2H, CH_2-$\underline{CH_2}$-CH), 2.02 ppm (m, 2H, CH_2-$\underline{CH_2}$-CH), 2.22 ppm (m, 1H, CH_2-$\underline{CH}$-CH_2-CH2), 3.17 ppm (d, 2H, $\underline{CH_2}$-N).

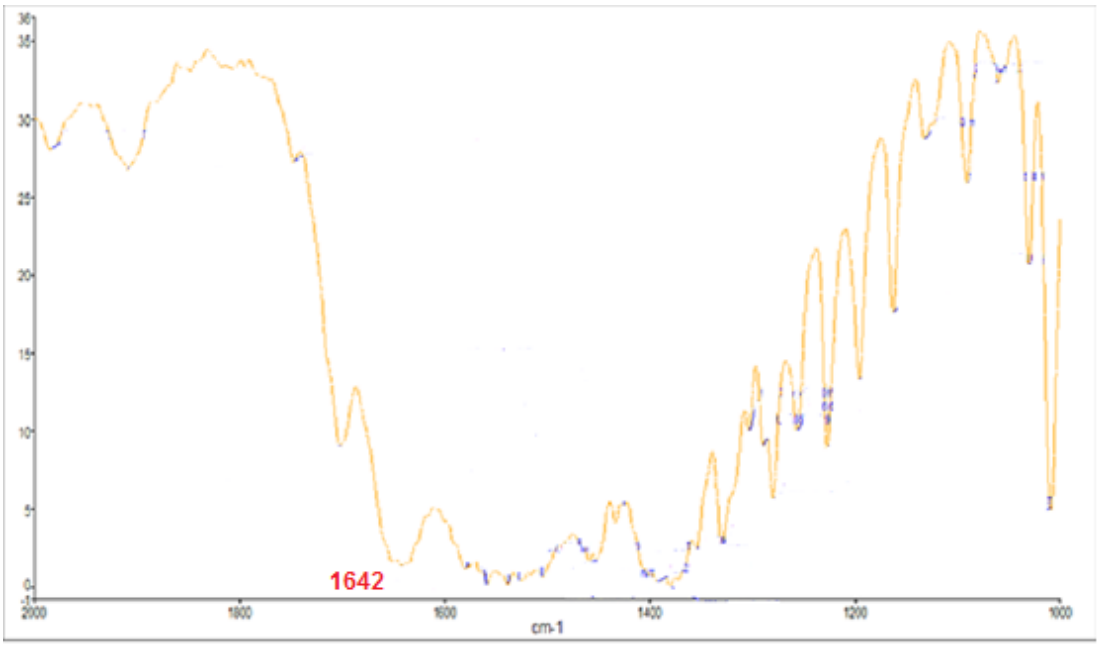


Figure 4.11: FT-IR spectrum of tranexamic acid.

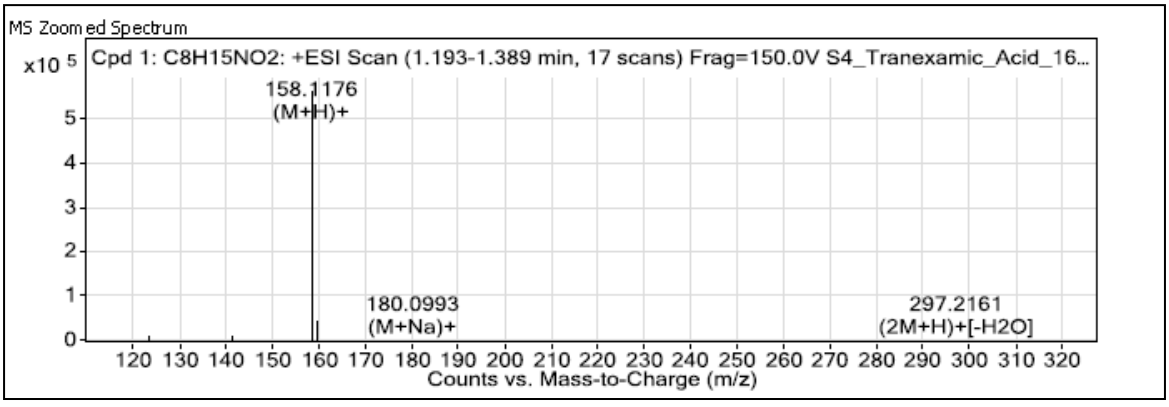


Figure 4.12: LC-MS spectrum of tranexamic acid.

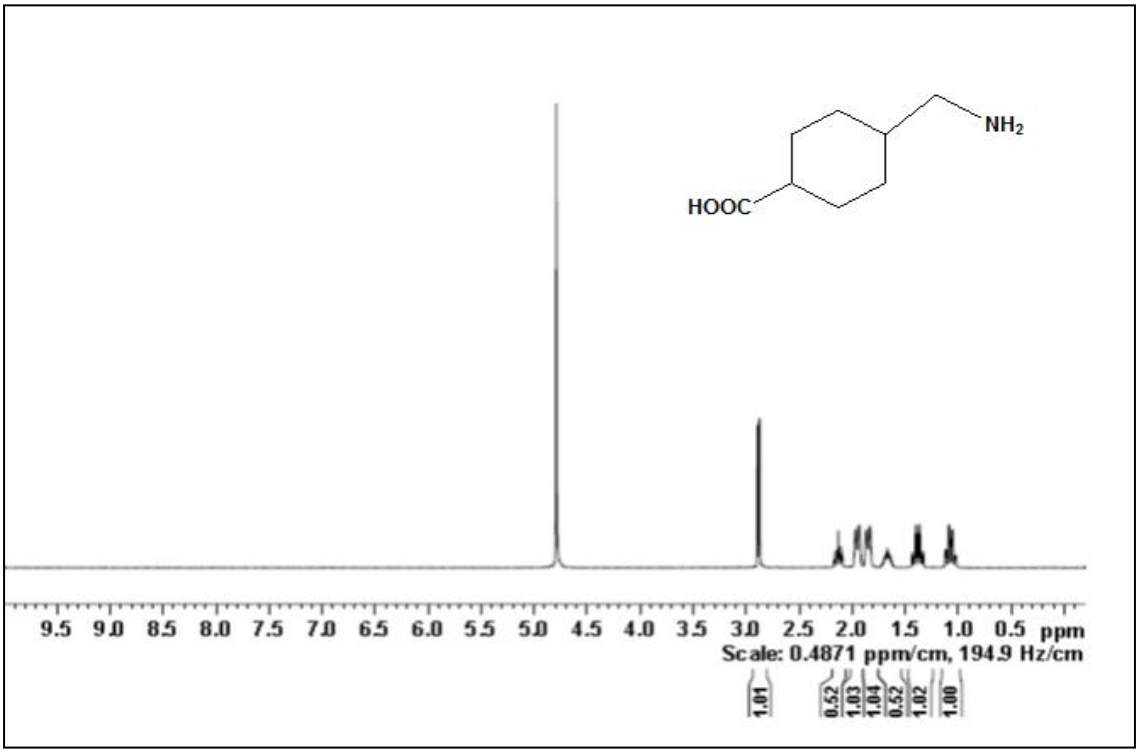


Figure 4.13: ^{1}H-NMR spectrum of tranexamic acid.

Mefenamic tranexamic.

M.P over 300 °C

^{1}H-NMR ($CDCl_3$) 0.88 (m, 1H, cyclohexane-H), 1.12(q, 8H, cyclohexane-H), 1.14 (m, 1H, cyclohexane-H), 1.54(s, 6H, Ar-($CH3)_2$), 2.46 (d, 2H, J=10.8, NH(CH_2), 7.11(d,1H, J=8.4 ,Ar-H), 7.63(t, 2H, Ar-H), 7.67 (d, 2H, J=1.6, Ar-H), 8.2 (d, 2H, J=8.4, Ar-H).

IR (KBr/ν_{max} cm^{-1})1653 (C=O), 3312(N-H)

.*m*/*z*386.07$(M+1)^{+}$.

IR spectrum (Figure4.14) shows an additional peaks with frequencies of1623 cm^{-1}(C=O) and 3266 cm^{-1}(N-H). A high resolution LC-MS (Figure4.15) shows a protonated peak at *m*/*z* 381.3227$(M+1)^{+}$. The ^{1}H-NMR (Figure4.16) shows additional doublet peakat 2.46 ppm with coupling constant of 10.8 Hz and multiplet at 0.88-1.14 ppm correspond to the cyclohexyl ring of the tranexamic acid moiety.

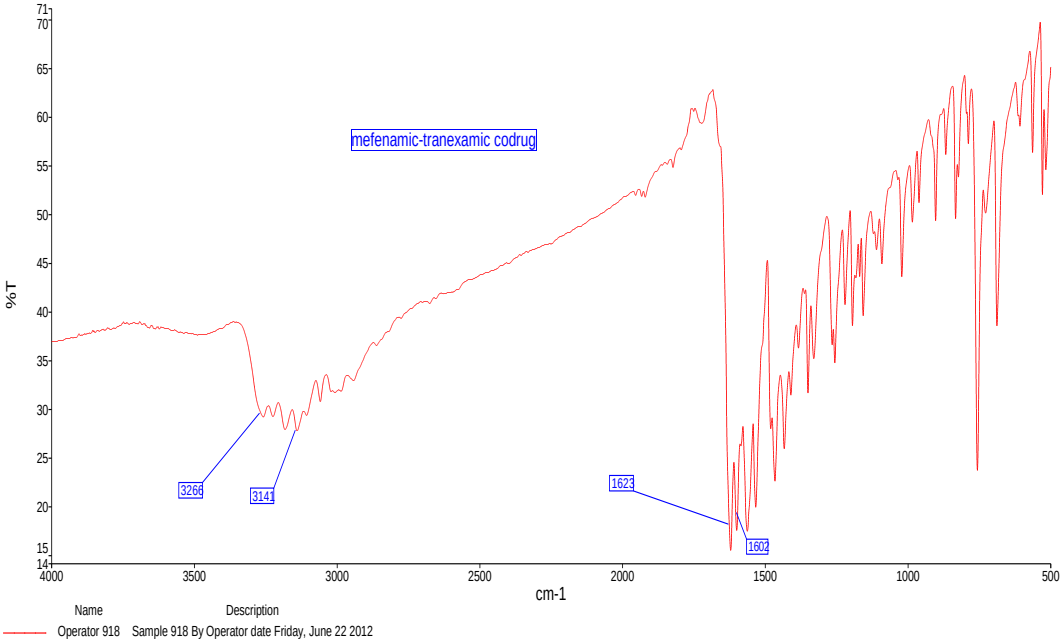


Figure 4.14: FT-IR spectrum of mefenamic tranexamic

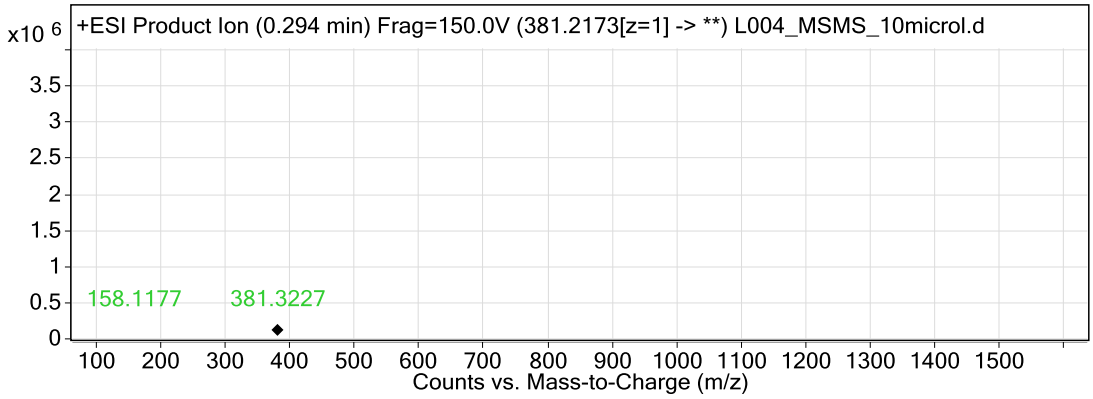


Figure 4.15 LC-MS spectrum of mefenamic tranexamic**.**

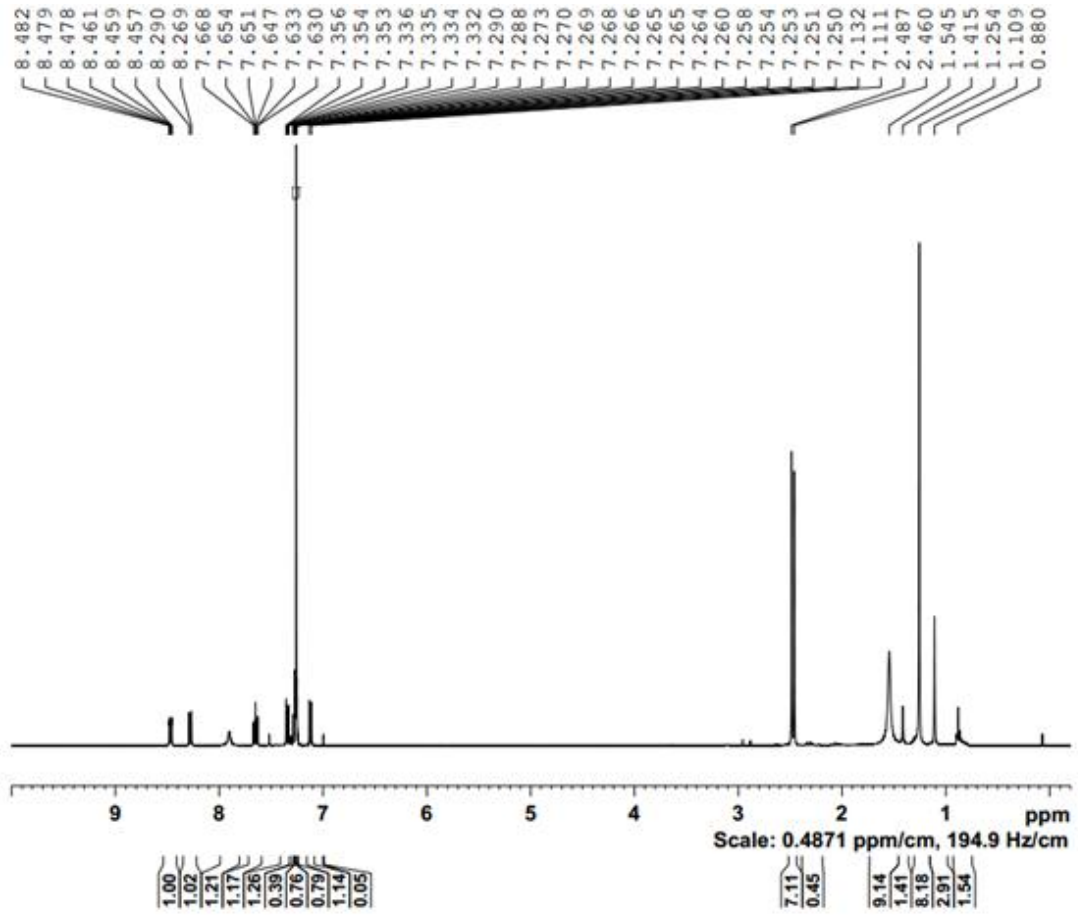


Figure 4.16: ^{1}H-NMR spectrum of mefenamic tranexamic.

Diclofenac tranexamic.

M.P: 100C^{o}

H^{1}NMR (CDCl$_3$, ppm): 3.75 (s, 2H, COC$\underline{H_2}$), 3.82(d , 2H, CH2-cyclohexane), 6.54(d, 1H, J=0.8, Ar-H), 6.97 (t, 2H , Ar-H), 7.14 (t, 2H, Ar-H), 7.22 (d, H, Ar-H), 7.24(d, 2H, J=1.6 , Ar-H).

FTIR: (KBr,cm^{-1}) in diclofenac tranexamic 1738(C=O), 3353(NH)

m/*z* 435.1160 [M+1]$^{+}$

IR spectrum (Figure4.17) shows additional two peaks at1738(C=O) and 3353(NH). A high resolution LC-MS (Figure4.18) shows a protonated peak at *m*/z 435.1160(M+1)$^{+}$. The ^{1}H-NMR (Figure 4.19) shows an additional doublet peak at 3.82 ppm corresponds to the protons of the methyl groups of the tranexamic acid moiety.

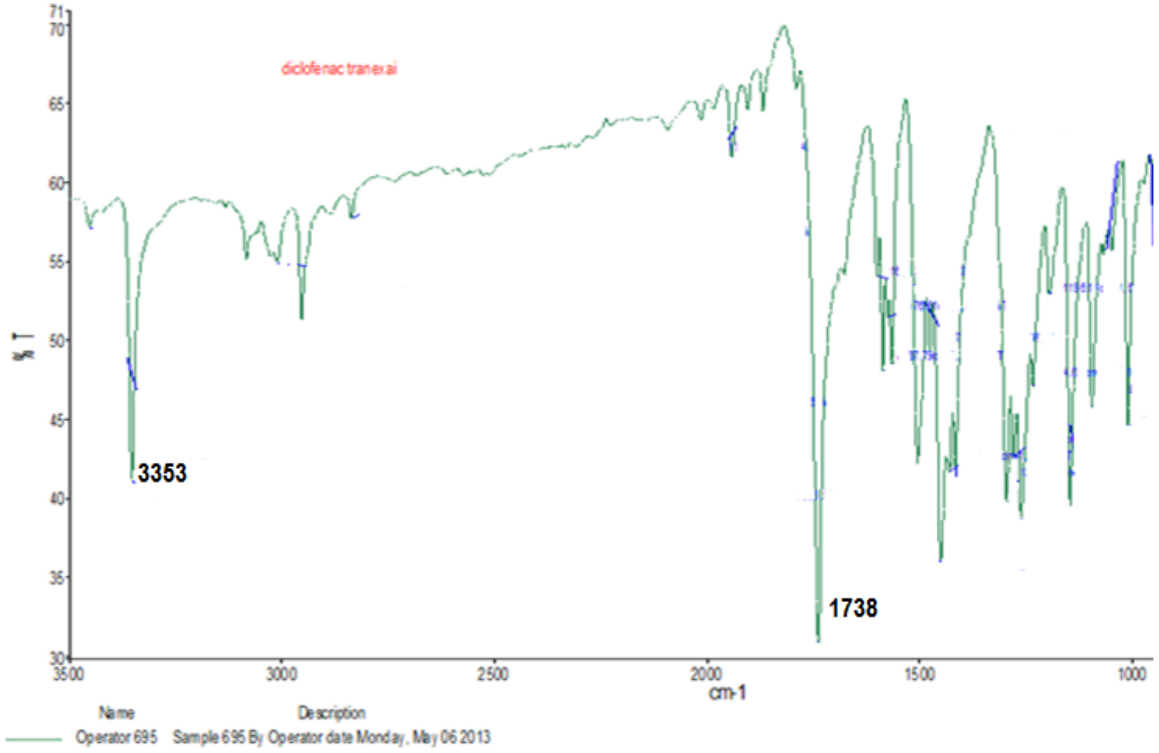


Figure 4.17: FT-IR spectrum of diclofenac tranexamic

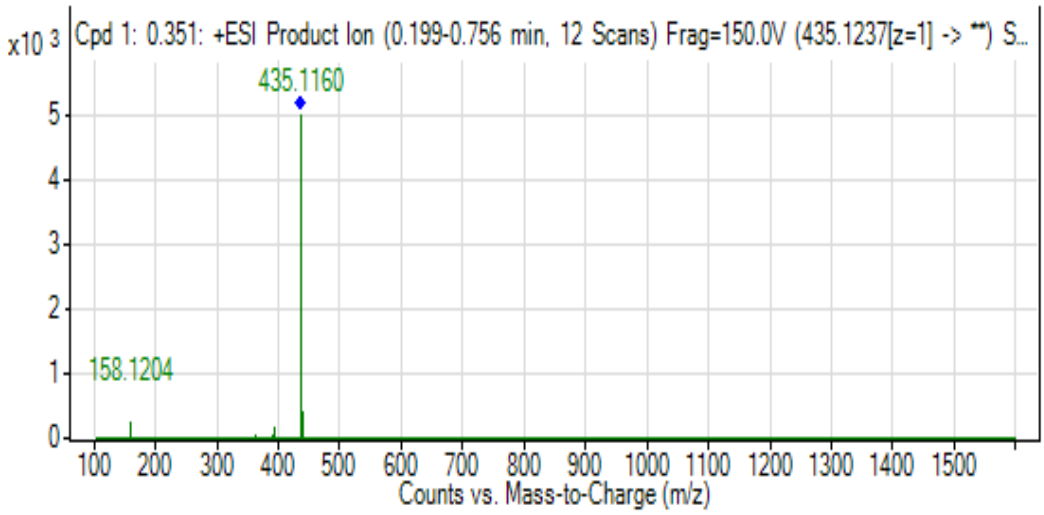


Figure 4.18 LC-MS spectrum of diclofenac tranexamic**.**

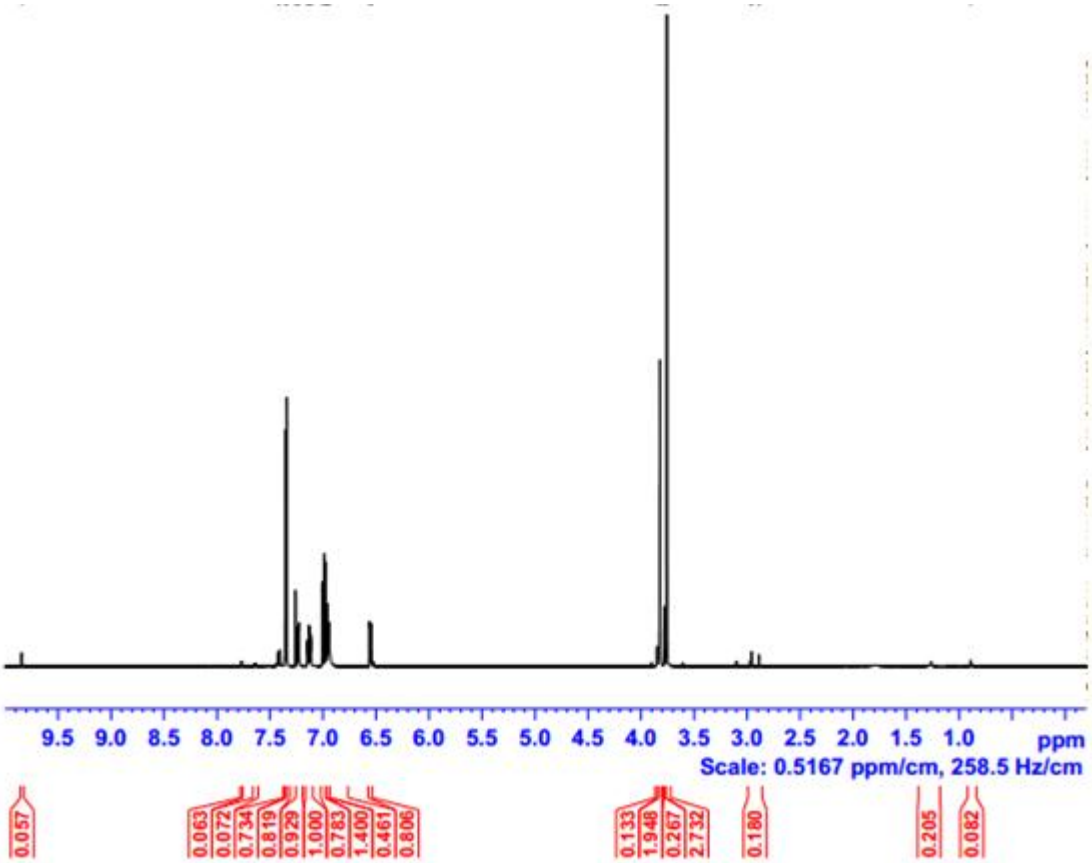


Figure 4.19: ^{1}H-NMR spectrum of diclofenac tranexamic**.**

4.2 Calibration curves

Calibration curves were obtained by plotting the peak area versus concentration as displayed in Figure (4.20) for mefenamic dimethylamine, diclofenac benzyl ,mefenamic tranexamic and and diclofenac benzyl. As shown in the figure, excellent linearity with correlation coefficient (R^2) above 0.95was obtained.

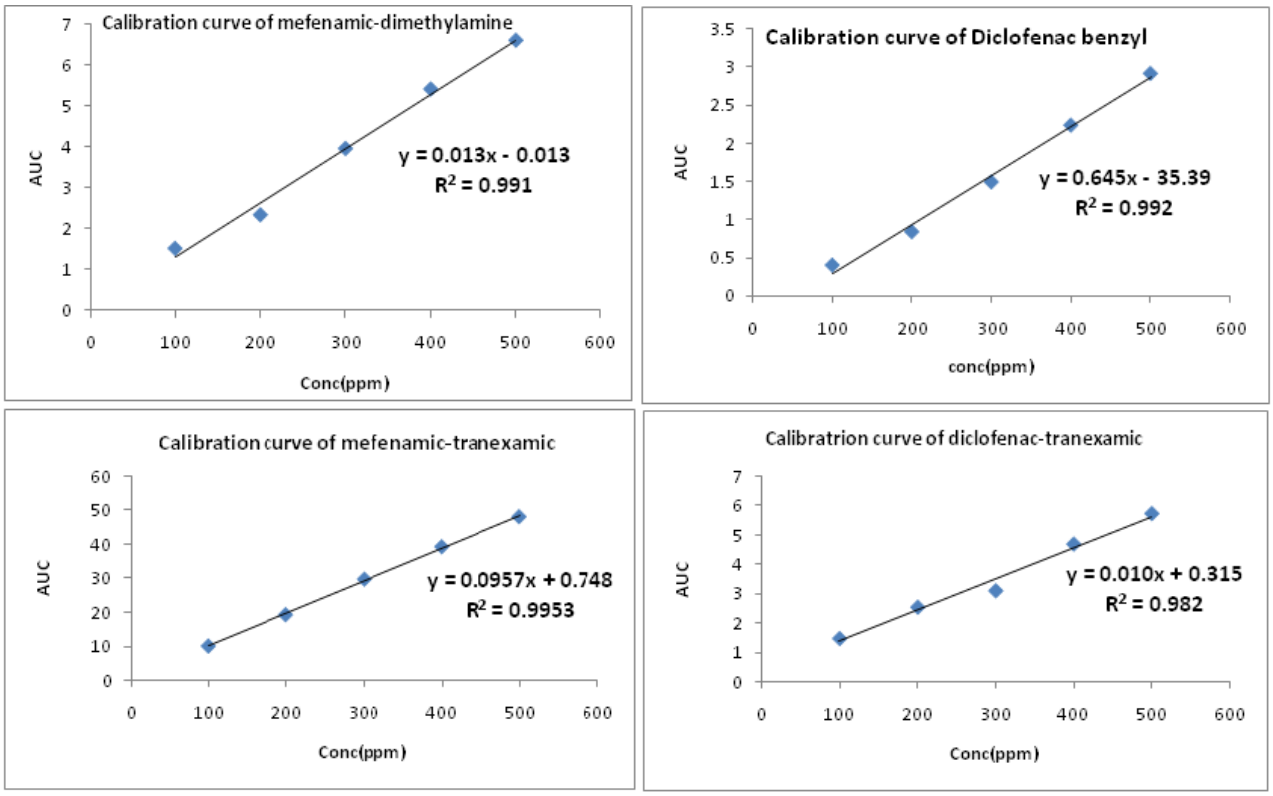


Figure 4.20: Calibration curves for mefenamic dimethylamine, diclofenac benzyl ,mefenamic tranexamic and and diclofenac tranexamic.

4.3 Kinetic studies

4.3.1 Mefenamic dimethylamine and diclofenac benzyl

4.3.1.1 Mefenamic dimethyl amine

Mefenamic dimethyl amine was hydrolyzed in 1N HCl to release the corresponding parent drug within 10 hrs, the reaction displayed strict first order kinetics as the k_{obs} was fairly constant and a straight plot was obtained on plotting log concentration of residual prodrug vs. time. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$) for these prodrugs were calculated from the linear regression equation correlating the log concentration of the residual prodrug vs. time figure (4.21). On the other hand, at pH 2.5, pH 5.5 and pH 7.4, mefenamic dimethylamine was entirely stable and no release of the parent drug was observed. This is due to the fact that mefenamic dimethylamine undergoes acid-catalyzed hydrolysis (intramolecular process), where the proton is transferred from the nitrogen of mefenamic moiety to the carbonyl of the amide followed by cyclization and dimethylamine departure. This reaction occurs at low pH where the nitrogen of mefenamic acid can accept a proton. In higher pHs the mefenamic acid moiety cannot be protonated and therefore, no intramolecular proton transfer is occurred.Kinetic data is listed in Table 1.

Table1 : The observed *k* value and $t_{1/2}$ for the intraconverion of Mefenamic dimethylamine in 1N HCl and at pH 2.5 , pH 5.5 and pH 7.4.

Medium	k_{obs} (h^{-1})	$t_{½}$ (h)
1N HCl	**$6.909*10^{-2}$**	**10**
Buffer pH 2.5	No reaction	No reaction
Buffer pH 5.5	No reaction	No reaction
Buffer pH 7.4	No reaction	No reaction

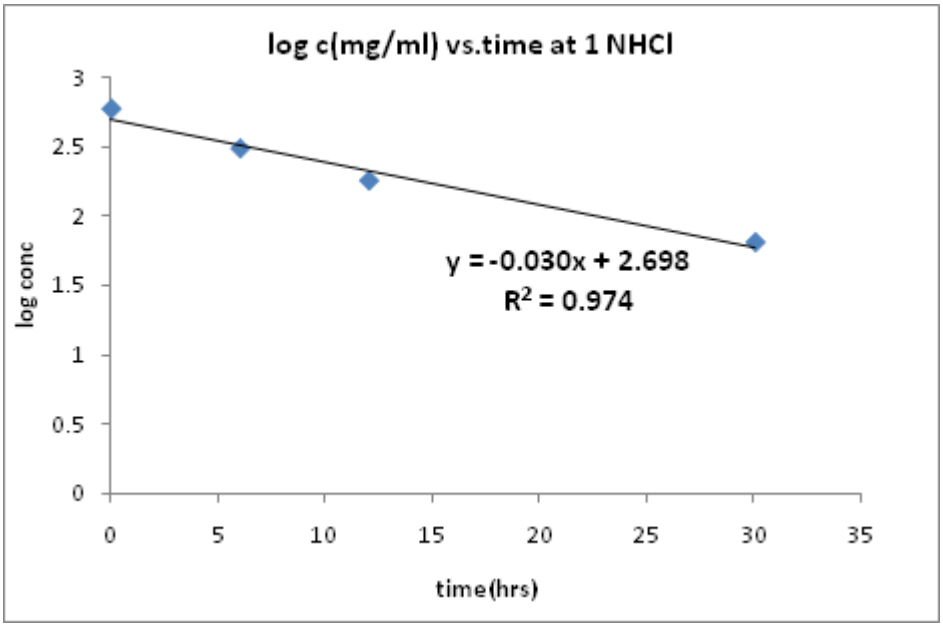


Figure 4.21: First order hydrolysis plot for the intraconverion of Mefenamic dimethylamine in 1N HCl.

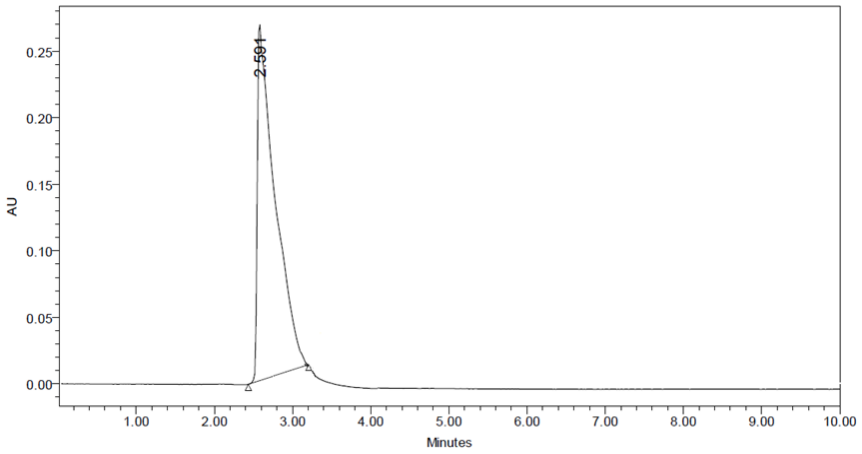


Figure 4.22:Chromatogram of mefenamic acid , Rt=2.5min

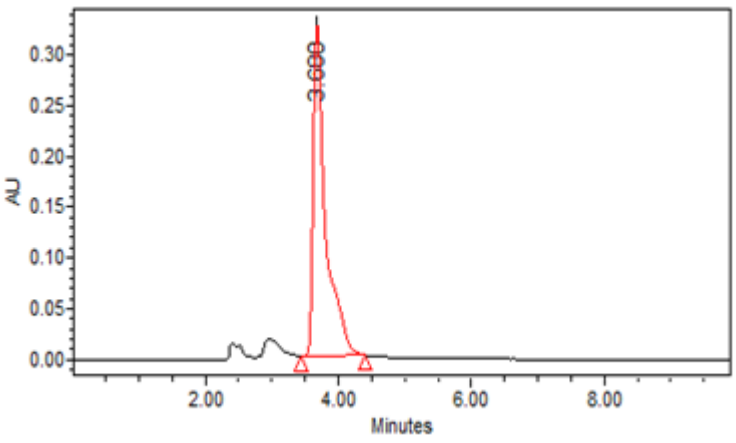


Figure 4.23: Chromatogram mefenamic-dimethylamine Rt=3.6min

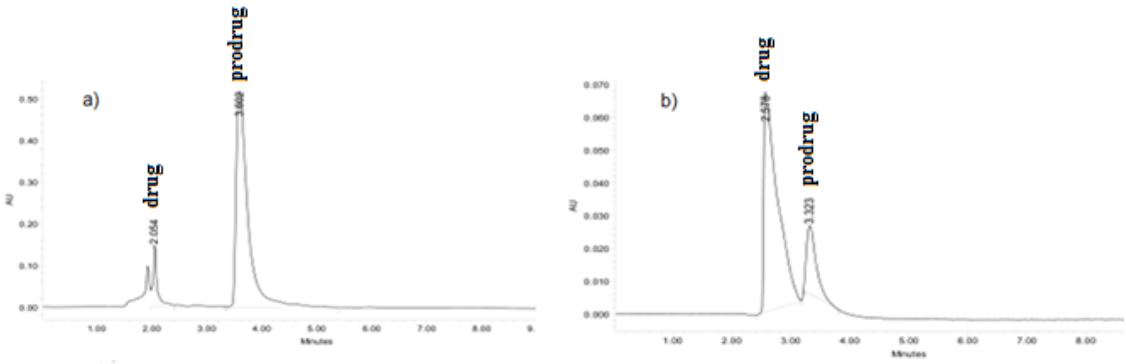


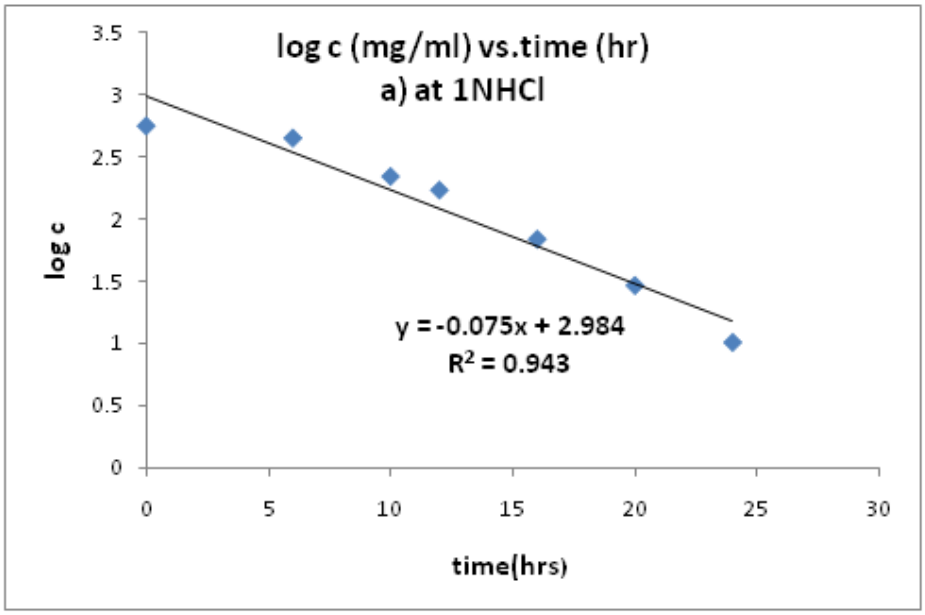
Figure 4.24 : Chromatograms showing the intra-conversion of mefenamic dimethylamine at 1 NHCl (a) at zero time, (b) after 30 hours.

4.3.1.2 Diclofenac benzyl

Diclofenac benzyl was hydrolyzed in 1N HCl and pH 7.4 to release the corresponding parent drug within 4 hrs and 1 hrs respectively (Table 2). The reaction displayed strict first order kinetics as the k_{obs} was fairly constant and a straight plot was obtained on plotting log concentration of residual prodrug vs. time. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$) for these prodrugs were calculated from the linear regression equation correlating the log concentration of the residual prodrug vs. time figure(4.25). At pH 2.5, pH 5.5 diclofenac benzyl was entirely stable and no release of the parent drug was observed. This is due to the fact that diclofenac-benzyl is an ester. Generally esters are hydrolyzed in strong acidic or basic media. Therefore, it is expected that at pH 1 or less (very acidic condition) or pH 7.4 (a relatively basic condition, where the nitrogen of diclofenac is not protonated) the ester will undergo hydrolysis to give diclofenac and benzyl alcohol. pHs of 2.5 and 5.5 are not sufficient basic medium for providing OH- as a nucleophile needed to attack the ester carbonyl for the hydrolysis to occur. At low pH (1N HCl or pH less than 1) the hydrolysis reaction is acid-catalyzed hydrolysis, whereas, at pH 7.4 the hydrolysis reaction is base-catalyzed hydrolysis.

Table 2 : The observed *k* value and $t_{1/2}$ for the intraconverion of diclofenac benzyl in 1N HCl and at pH 2.5 , pH 5.5 and pH 7.4.

Medium	**k_{obs} (h^{-1})**	**$t_{½}$ (h)**
1N HCl	**$17.27*10^{-2}$**	**4**
Buffer pH 2.5	No reaction	No reaction
Buffer pH 5.5	No reaction	No reaction
Buffer pH 7.4	**$66.78*10^{-2}$**	**1**



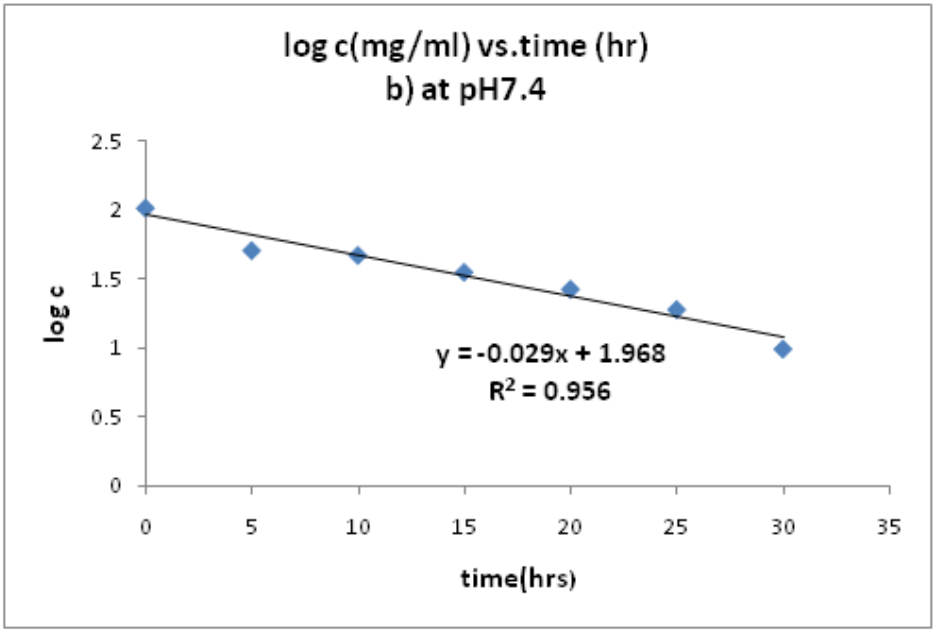


Figure 4.25: First order hydrolysis plot for the intraconverion of diclofenac benzyl in a) 1NHCl , b) pH 7.4.

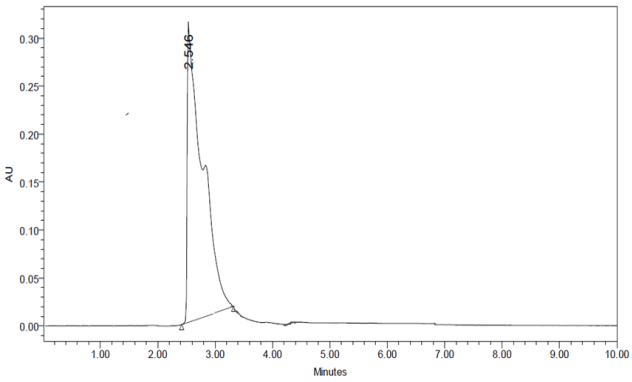
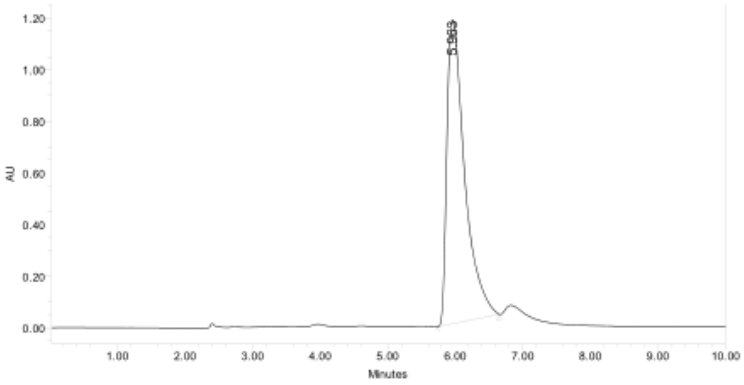


Figure 4.26: Chromatogram of diclofenac Rt=2.5min



	RT	Area	% Area	Height
1	5.963	21631255	100.00	1179232

Figure 4.27: Chromatogram of diclofenac benzyl ,Rt=5.9

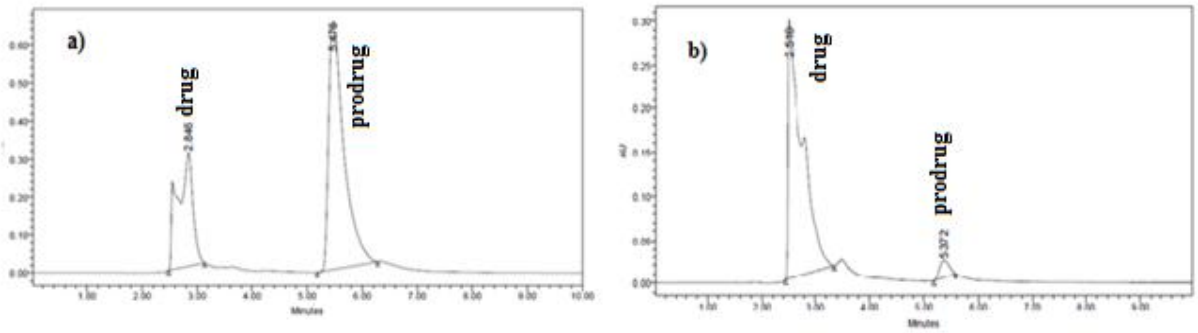


Figure 4.28: Chromatograms showing the intra-conversion of diclofenac benzyl at 1 NHCl (a) at zero time, (b) after 24 hours.

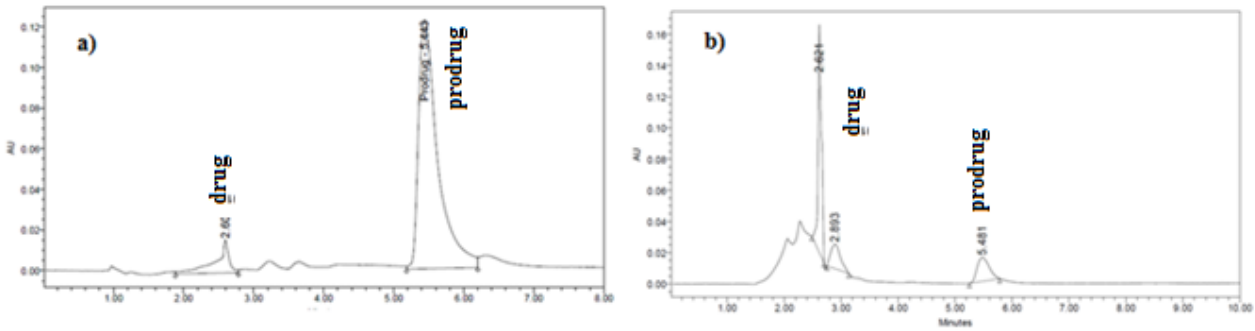


Figure 4.29: Chromatograms showing the intra-conversion of diclofenac benzyl at pH=7.4 (a) at zero time, (b) after 24 hours.

4.2.1 Mefenamic tranexamic and diclofenac tranexamic

Under the experimental conditions, mefenamic tranexamic and diclofenac tranexamic hydrolyzed to release the parent drugs as evident by HPLC analysis. At constant pH and temperature, the reaction displayed strict first order kinetics as the k_{obs} was fairly constant and a straight plot was obtained on plotting log concentration of residual codrug vs. time. The rate constant (*k*obs) and the corresponding half-lives ($t_{1/2}$) mefenamic tranexamic and diclofenac tranexamic in the different media were calculated from the linear regression equation correlating the log concentration of the residual codrug vs. time. It is worth noting that 1N HCl and pH 2.5 were selected to examine the intraconversion of mefenamic tranexamic and diclofenac tranexamic in the pH as of stomach, since the mean fasting stomach pH of adult is approximately 1-2.5. Furthermore, environment of buffer pH 5.5 mimics that of beginning small intestine route, whereas pH 7.4 was selected to determine the intraconversion of the tested prodrugs in blood circulation system. Acid-catalyzed hydrolysis of mefenamic tranexamic and diclofenac tranexamic was found to be much higher in 1N HCl than at pH 2.5, pH 5.5and pH 7.4. Mefenamic tranexamic in 1NHCl was hydrolyzed to release the parent drugs nearly in one hour while the $t_{1/2}$ value of diclofenac tranexamic in 1 NHCl was about 30 hrs. On the other hand, at pH 2.5, pH 5.5 and pH 7.4, both codrugs were entirely stable and no release of the parent drugs was observed. Since the pK_avalues of tranexamic acid is in the range of 3-4, it is expected that at pH 5.5 and 7.4 the anionic form of the codrugs will be dominant and the percentage of the free acid form that

undergoes the acid-catalyzed hydrolysis will be negligible. The discrepancy in hydrolysis rate between mefenamic tranexamic and diclofenac tranexamic at 1NHCl is attributed to the effect of the distance between the two reacting centers. It is worth noting that previous DFT calculations and experimental data on the acid catalyzed hydrolysis revealed that the efficiency of the intramolecular acid-catalyzed hydrolysis by the carboxyl group is remarkably sensitive to the distance between the electrophile and nucleophile. Systems having short distance between the two reacting centers experience low rates and vice versa. The kinetic data for mefenamic tranexamic and diclofenac tranexamic are listed in **Tables 3** and **4**.

Table 1: The observed *k* value and $t_{1/2}$ for the intraconverion of mefenamic tranexamic acid in 1N HCl, pH 2, pH 5.5 and pH 7.4.

Medium	k_{obs} (h^{-1})	$t_{½}$ (h)
1N HCl	**$63.6*10^{-2}$**	**1**
Buffer pH 2.5	No reaction	No reaction
Buffer pH 5.5	No reaction	No reaction
Buffer pH 7.4	No reaction	No reaction

Table 4: The observed *k* value and $t_{1/2}$ for the intraconverion of diclofenac tranexamic acid in 1N HCl, pH 2, pH 5.5 and pH 7.4.

Medium	k_{obs} (h^{-1})	$t_{½}$ (h)
1N HCl	**$2.303*10^{-2}$**	**30**
Buffer pH 2.5	No reaction	No reaction
Buffer pH 5.5	No reaction	No reaction
Buffer pH 7.4	No reaction	No reaction

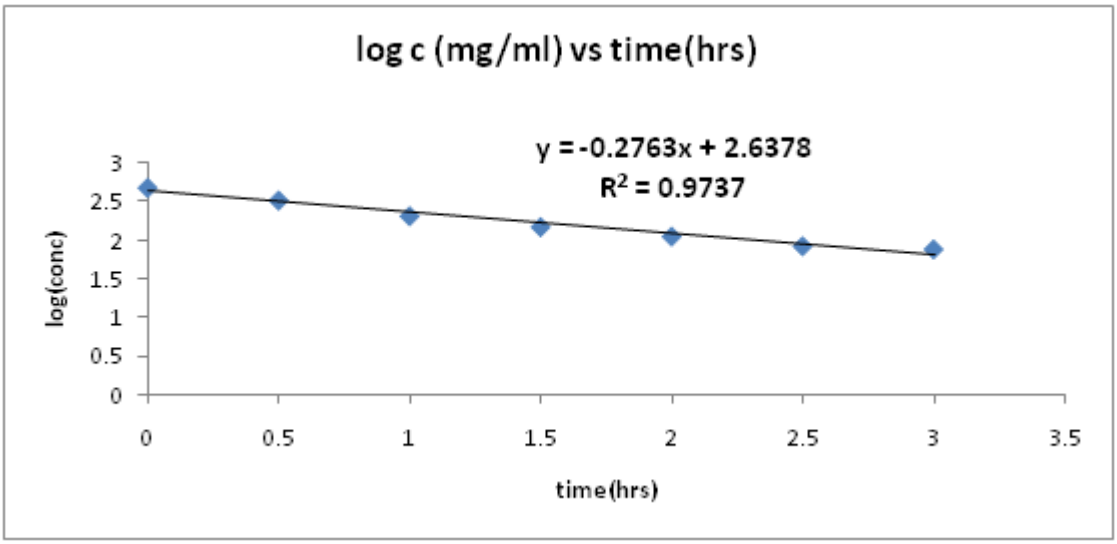


Figure 4.30: First order hydrolysis plot for the intraconverion of mefenamic tranexamic in 1N HCl

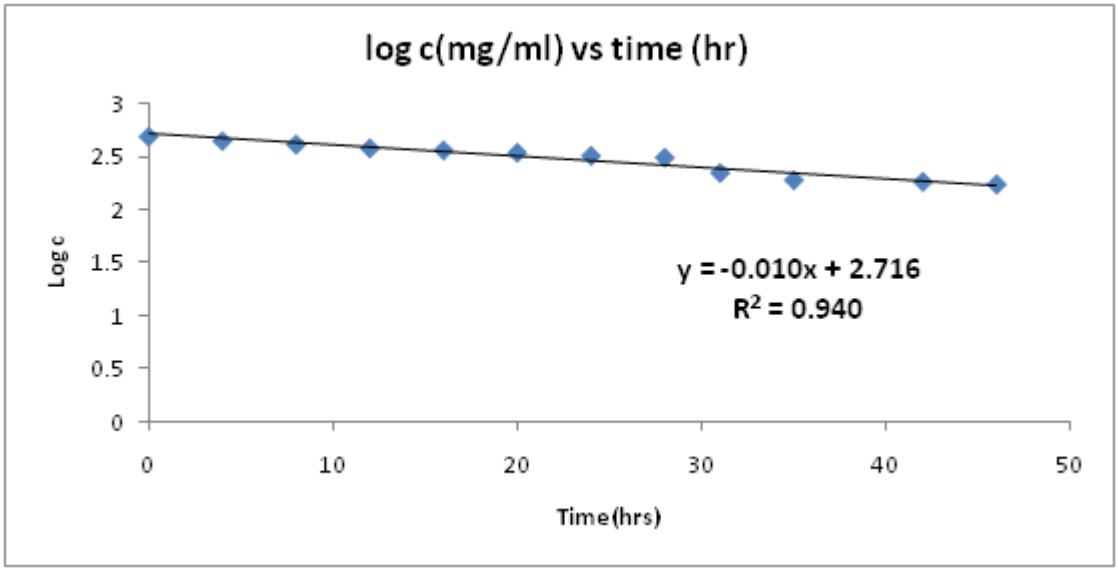


Figure 4.31: First order hydrolysis plot for the intraconverion of diclofenac tranexamic in 1N HCl

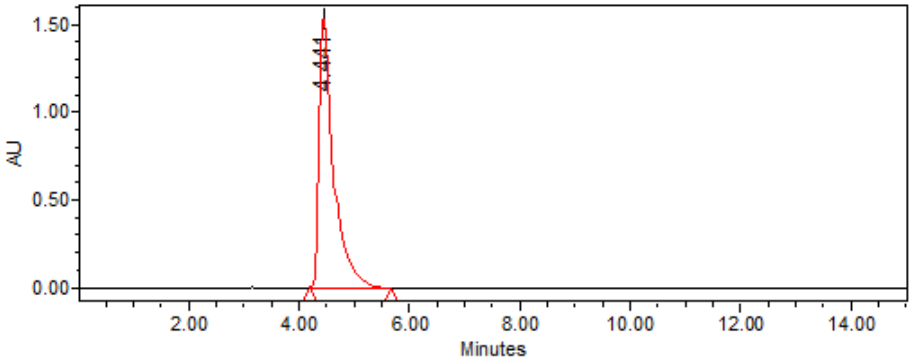


Figure 4.32: Chromatogram of mefenamic Rt=4.4min

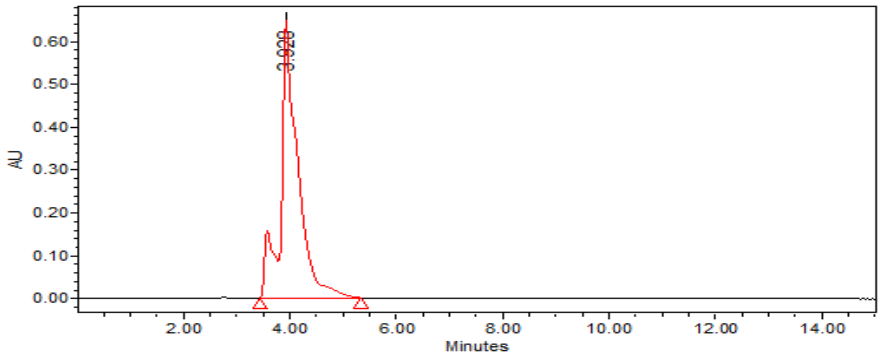


Figure 4.33: Chromatogram of mefenamic tranexamic Rt = 3.9min

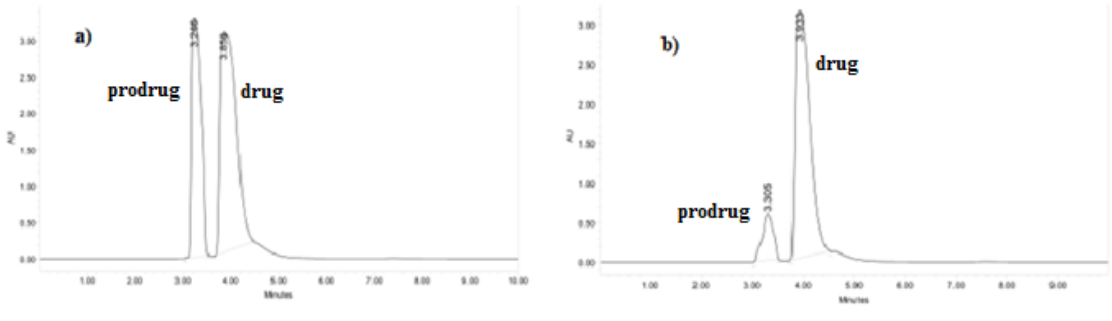
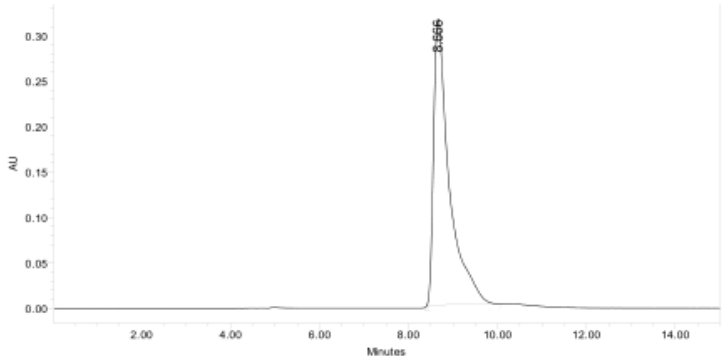
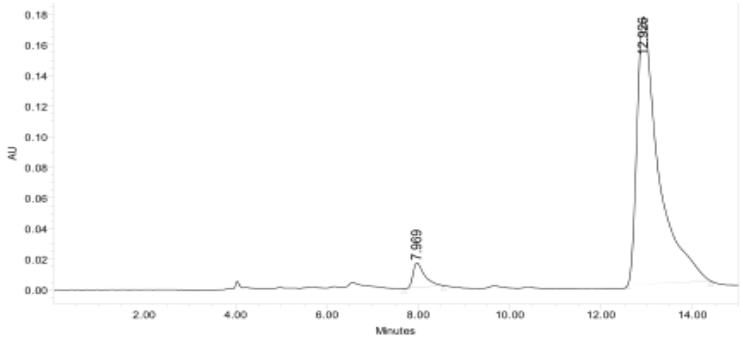


Figure 4.34: Chromatograms showing the intra-conversion of mefenamic tranexamic in 1NHCl at a) zero time b) after 3 hrs



	RT	Area	% Area	Height
1	8.666	7699715	100.00	314892

Figure 4.35: Chromatogram of diclofenac Rt=8.6min



	RT	Area	% Area	Height
1	7.969	282058	4.68	16173
2	12.926	5742856	95.32	174807

Figure 4.36: Chromatogram of diclofenac tranexamic Rt=12.6 min

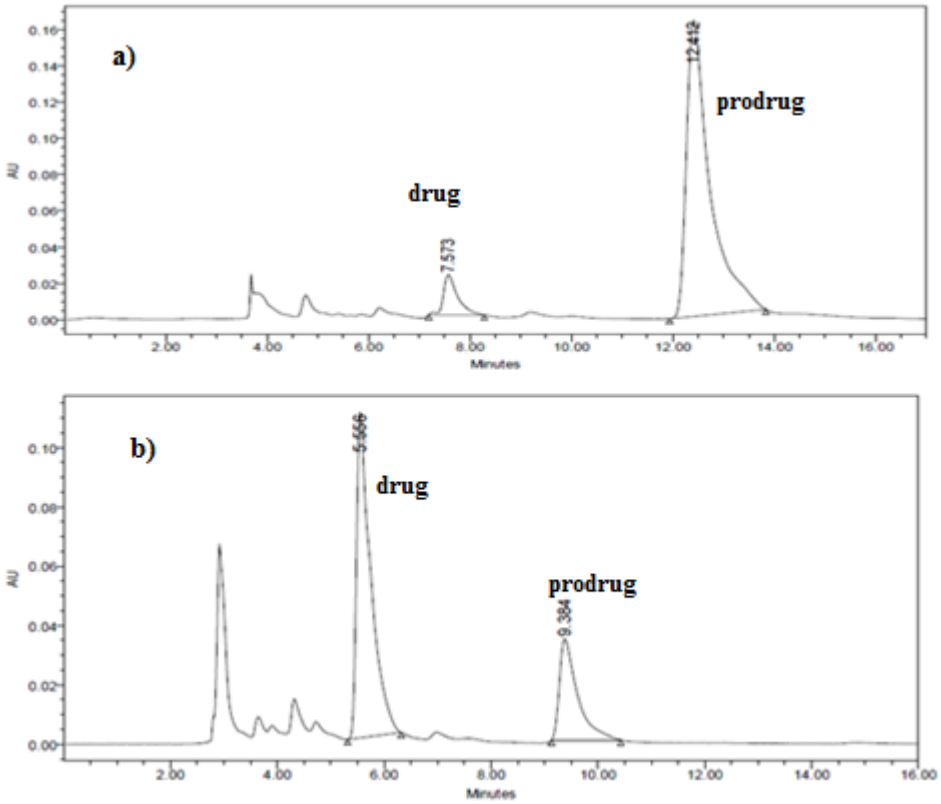


Figure 4.37: Chromatograms showing the intraconversion of diclofenac tranexamic in 1NHCl at a) zero time b) after 60 hrs.

5. Conclusion

Novel prodrugs and codrugs of mefenamic acid and diclofenac were synthesized and their *in vitro* pharmacokinetic properties were evaluated. The designed linkers by Karaman's group were used to mask the bitter taste and GI toxic effects of NSAIDs. Based on Kirby's enzyme model the synthesized prodrugs were hydrolyzed to release the parent drugs via intramolecular acid catalyzed hydrolysis.

The predicted $t_{1/2}$ and k_{obs} of mefenamic dimethylamine, diclofenac benzyl, mefenamic tranexamic and diclofenac tranexamic were computationally calculated. It should be emphasized that the $t_{1/2}$ of prodrugs will be determined on two major factors: (1) the pH of the medium and (2) the chemical structure of the linker (promoiety). Kinetic studies revealed that the synthesized prodrugs and codrugs exist as a free carboxylic acid form in the low pHs such as the stomach, whereas in the blood circulation system (pH = 7.4), the carboxylate anion is the predominant form. Therefore, the interconversion rates of NSAIDs prodrugs to NSAIDs can be programmed according to the nature of the prodrug linker.

Based on Kirby`s enzyme model novel prodrugs and codrugs of mefenamic acid and diclofenac were synthesized and their *in vitro* pharmacokinetic properties were evaluated. The designed linkers by Karaman's group were used to mask the bitter taste, GI toxic effects and have the potential to release the parent drugs *via* intramolecular reaction without the need for enzyme catalysis. The rate of intramolecular acid catalyzed hydrolysis is dependent on the following factors:(1) the difference between the strain energies of intermediate and product and intermediate and reactant, (2) the distance between the two reacting centers and (3) the attack angle. Thus, the rate by which the pro-drug releases the anti-inflammatory drug can be determined according to the nature of the linker (Kirby's enzyme model)

The experimental $t_{1/2}$ value for mefenamic dimethylamine in 1NHCl was found to be 10 hrs, while at pH 2.5, pH 5.5 and pH 7.4 the prodrug showed complete stability. Mefenamic dimethylamine undergoes acid-catalyzed hydrolysis (intramolecular process) at low pH where the nitrogen of mefenamic acid can accept a proton. In higher pHs the mefenamic acid moiety cannot be protonated and therefore, no intramolecular proton transfer can occur. Diclofenac benzyl was hydrolyzed to release the parent drug

within 4 and 1 hrs at 1 NHCl and pH 7.4, respectively. On the other hand, no hydrolysis was observed at pH 2.5 and pH 5.5. The lack of the hydrolysis at pH these two pHs might be due to the fact that ester hydrolysis is catalyzed by an acid or a base and the media of the mentioned two pHs are not sufficiently acidic nor basic to catalyze the ester (diclofenac benzyl) hydrolysis.

Mefenamic tranexamic in 1NHCl was hydrolyzed to release the parent drugs within one hour while the $t_{1/2}$ value of diclofenac tranexamic in 1 N HCl was about 30 hrs. On the other hand, at pH 2.5, pH 5.5 and pH 7.4, both codrugs were entirely stable and no release of the parent drugs was observed.

The discrepancy in hydrolysis rate between mefenamic tranexamic and diclofenac tranexamic at 1 N HCl is attributed to the fact that in mefenamic tranexamic there is a possibility of intramolecular acid-catalyzed hydrolysis which stems from the short distance between the two reacting centers (the proton and the nitrogen of the mefenamic acid moiety), whereas in diclofenac tranexamic the hydrolysis is occur via intermolecular acid-catalyzed hydrolysis due to the long distance between the proton and the nitrogen of the diclofenac moiety.

In vitro binding test to bitter taste receptors for mefenamic dimethylamine, diclofenac benzyl , mefenamic tranexamic and diclofenac tranexamic were found to be bitterless. This suggests that NSAIDs prodrugs and codrugs can replace their parent drugs for the use as safe and bitterless anti-inflammatory drugs for geriatrics and pediatrics.

The ability of NSAIDs prodrugs and codrugs to reduce ulcerogenic side effects while retaining the anti-inflammatory events when administered makes this class of compounds promising new anti-inflammatory agents that should be further investigated and developed for future therapeutic use.

In the next decades, it is expected that the prodrug approach will become an integral part of the drug discovery processes and not as a hindsight approach to the solution of problems associated with older drugs.

6. Future directions:

1- *In vivo* pharmacokinetic studies will be conducted in order to determine the bioavailability and the duration of action of the tested prodrugs.

2- *In vitro* and in vivo studies will be done to determine the anti-inflammatory and anti-bleeding effects of the synthesized prodrugs and codrugs.

References

1. Stella, V. J. (2010). Prodrugs: Some thoughts and current issues. *Journal of pharmaceutical sciences*, *99*(12), 4755-4765.
2. Dahan, A., Khamis, M., Agbaria, R., & Karaman, R. (2012). Targeted prodrugs in oral drug delivery: the modern molecular biopharmaceutical approach. *Expert opinion on drug delivery*, *9*(8), 1001-1013.
3. Karaman, R. (2013). Prodrug Design by Computation Methods: A New Era. *Drug Designing: Open Access*, *2013*.
4. Jana, S., Mandlekar, S., & Marathe, P. (2010). Prodrug design to improve pharmacokinetic and drug delivery properties: challenges to the discovery scientists. *Current medicinal chemistry*, *17*(32), 3874-3908.
5. Albert, A. (1958). Chemical aspects of selective toxicity. *Nature*, *182*(4633), 421.
6. Rautio, J. (2010). Prodrug strategies in drug design. *Prodrugs and targeted delivery: towards better ADME properties*, 1-30.
7. Huttunen, K. M., Raunio, H., & Rautio, J. (2011). Prodrugs—from serendipity to rational design. *Pharmacological reviews*, *63*(3), 750-771.
8. Stella, V., Borchardt, R., Hageman, M., Oliyai, R., Maag, H., & Tilley, J. (Eds.). (2007). *Prodrugs: challenges and rewards*. Springer Science & Business Media.
9. Glazko, A. J., Carnes, H. E., Kazenko, A., Wolf, L. M., & Reutner, T. F. (1956). Succinic acid esters of chloramphenicol. *Antibiotics annual*, *5*, 792-802.
10. Higuchi, T., & Stella, V. J. (1975). *Pro-drugs as novel drug delivery systems*. The Society.
11. Stella, V. J., & Nti-Addae, K. W. (2007). Prodrug strategies to overcome poor water solubility. *Advanced drug delivery reviews*, *59*(7), 677-694.
12. Draganov, D. I., & La Du, B. N. (2004). Pharmacogenetics of paraoxonases: a brief review. *Naunyn-Schmiedeberg's archives of pharmacology*, *369*(1), 78-88.
13. Ngawhirunpat, T., Kawakami, N., Hatanaka, T., Kawakami, J., & Adachi, I. (2003). Age dependency of esterase activity in rat and human keratinocytes. *Biological and Pharmaceutical Bulletin*, *26*(9), 1311-1314.
14. Karaman, R. (2013). Computationally designed enzyme models to replace natural enzymes in prodrug approaches. *Drug Designing: Open Access*, *2013*.
15. Karaman, R. (2013). Prodrugs Design Based on Inter-and Intramolecular Chemical Processes. *Chemical biology & drug design*, *82*(6), 643-668.

16. Kirby, A. J. (1997). Efficiency of proton transfer catalysis in models and enzymes. *Accounts of chemical research*, *30*(7), 290-296.
17. Chandrashekar, J., Mueller, K. L., Hoon, M. A., Adler, E., Feng, L., Guo, W., ... & Ryba, N. J. (2000). T2Rs function as bitter taste receptors. *Cell*, *100*(6), 703-711.
18. Hejaz, H., Karaman, R., & Khamis, M. (2012). Computer-assisted design for paracetamol masking bitter taste prodrugs. *Journal of molecular modeling*, *18*(1), 103-114.
19. Karaman, R. (2013). Computationally designed prodrugs for masking the bitter taste of drugs. *Drug Designing: Open Access*, *2013*.
20. Gomez-Orellana, I. (2005). Strategies to improve oral drug bioavailability. *Expert opinion on drug delivery*, *2*(3), 419-433.
21. Müller, C. E. (2009). Prodrug approaches for enhancing the bioavailability of drugs with low solubility. *Chemistry & Biodiversity*, *6*(11), 2071-2083.
22. Dahan, A., Khamis, M., Agbaria, R., & Karaman, R. (2012). Targeted prodrugs in oral drug delivery: the modern molecular biopharmaceutical approach. *Expert opinion on drug delivery*, *9*(8), 1001-1013.
23. Stella, V. J., Charman, W. N. A., & Naringrekar, V. H. (1985). Prodrugs. *Drugs*, *29*(5), 455-473.
24. Huttunen, K. M., Raunio, H., & Rautio, J. (2011). Prodrugs—from serendipity to rational design. *Pharmacological reviews*, *63*(3), 750-771.
25. Svensson, L. Å., & Tunek, A. (1988). The design and bioactivation of presystemically stable prodrugs. *Drug metabolism reviews*, *19*(2), 165-194.
26. Aljuffali, I. A., Lin, C. F., Chen, C. H., & Fang, J. Y. (2016). The codrug approach for facilitating drug delivery and bioactivity. *Expert opinion on drug delivery*, (just-accepted).
27. Bhosle, D., Bharambe, S., Gairola, N., & Dhaneshwar, S. S. (2006). Mutual Prodrug Concept: Fundamentals and Applications. *Indian journal of pharmaceutical sciences*, *68*(3).
28. Nuki, G. (1990). Pain control and the use of non-steroidal analgesic anti-inflammatory drugs. *British medical bulletin*, *46*(1), 262-278.
29. Vane, J. R., & Botting, R. M. (1998). Mechanism of action of nonsteroidal anti-inflammatory drugs. *The American journal of medicine*, *104*(3), 2S-8S.

30. Cioli, V., Putzolu, S., Rossi, V., Barcellona, P. S., & Corradino, C. (1979). The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats. *Toxicology and applied pharmacology*, *50*(2), 283-289.

31. Tammara, V. K., Narurkar, M. M., Crider, A. M., & Khan, M. A. (1993). Synthesis and evaluation of morpholinoalkyl ester prodrugs of indomethacin and naproxen. *Pharmaceutical research*, *10*(8), 1191-1199.

32. Vonkeman, H. E., & van de Laar, M. A. (2010, February). Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. In *Seminars in arthritis and rheumatism* (Vol. 39, No. 4, pp. 294-312). WB Saunders.

33. Brun, J., & Jones, R. (2001). Nonsteroidal anti-inflammatory drug–associated dyspepsia: the scale of the problem. *The American journal of medicine*, *110*(1), S12-S13.

34. Langman, M. J. S., Weil, J., Wainwright, P., Lawson, D. H., Rawlins, M. D., Logan, R. F. A., & Colin-Jones, D. G. (1994). Risks of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs. *The Lancet*, *343*(8905), 1075-1078.

35. Blower, A. L. (1996). Considerations for nonsteroidal anti-inflammatory drug therapy: safety. *Scandinavian Journal of Rheumatology*, *25*(sup105), 13-26.

36. Bjarnason, I., & Macpherson, A. J. (1994). Intestinal toxicity of non-steroidal anti-inflammatory drugs. *Pharmacology & therapeutics*, *62*(1), 145-157.

37. Karmakar, U., & Faysal, M. M. (2009). Diclofenac as microspheres. *The Internet Journal of Third World Medicine*, *8*(1).

38. Mozayani, A., & Raymon, L. (Eds.). (2003). *Handbook of drug interactions: a clinical and forensic guide*. Springer Science & Business Media.

39. Thorsen, S. (1975). Differences in the binding to fibrin of native plasminogen and plasminogen modified by proteolytic degradation influence of ω-aminocarboxylic acids. *Biochimica et Biophysica Acta (BBA)-Protein Structure*, *393*(1), 55-65.

40. Liumbruno, G. M., Bennardello, F., Lattanzio, A., Piccoli, P., & Rossetti, G. (2011). Recommendations for the transfusion management of patients in the peri-operative period. II. The intra-operative period. *Blood Transfus*, *9*(2), 189-217.

41. Mousa, H. A., & Alfirevic, Z. (2007). Treatment for primary postpartum haemorrhage (Review). *Cochrane Database Syst Rev*, *1*, CD003249.
42. Williams-Johnson, J. A., McDonald, A. H., Strachan, G. G., & Williams, E. W. (2010). Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. *West Indian Medical Journal*, *59*(6), 612-624.
43. Coulter, A., Kelland, J., Peto, V., & Rees, M. C. (1995). Treating menorrhagia in primary care: an overview of drug trials and a survey of prescribing practice. *International journal of technology assessment in health care*, *11*(03), 456-471.
44. Lukes, A. S., Kouides, P. A., & Moore, K. A. (2011). Tranexamic acid: a novel oral formulation for the treatment of heavy menstrual bleeding. *Women's Health*, *7*(2), 151-158.
45. Musumba, C., Pritchard, D. M., & Pirmohamed, M. (2009). Review article: cellular and molecular mechanisms of NSAID-induced peptic ulcers. *Alimentary pharmacology & therapeutics*, *30*(6), 517-531.
46. Emery, P. (1996). Considerations for nonsteroidal anti-inflammatory drug therapy: benefits. *Scandinavian Journal of Rheumatology*, *25*(sup105), 5-12.
47. Lanas, A., Garcia-Tell, G., Armada, B., & Oteo-Alvaro, A. (2011). Prescription patterns and appropriateness of NSAID therapy according to gastrointestinal risk and cardiovascular history in patients with diagnoses of osteoarthritis. *BMC medicine*, *9*(1), 1.
48. Roth, S. H. (1996). NSAID gastropathy: a new understanding. *Archives of internal medicine*, *156*(15), 1623-1628.
49. Johnson, R. E., Hornbrook, M. C., Hooker, R. S., Woodson, G. T., & Shneidman, R. (1997). Analysis of the costs of NSAID-associated gastropathy. *Pharmacoeconomics*, *12*(1), 76-88.
50. Rahme, E., Joseph, L., Kong, S. X., Watson, D. J., & LeLorier, J. (2001). Cost of prescribed NSAID-related gastrointestinal adverse events in elderly patients. *British journal of clinical pharmacology*, *52*(2), 185-192.
51. Singh, G., & Triadafilopoulos, G. (1999). Epidemiology of NSAID induced gastrointestinal complications. *The Journal of rheumatology. Supplement*, *56*, 18-24.

52. Singh, G., & Triadafilopoulos, G. (1999). Epidemiology of NSAID induced gastrointestinal complications. *The Journal of rheumatology. Supplement*, *56*, 18-24.

53. Sohi, H., Sultana, Y., & Khar, R. K. (2004). Taste masking technologies in oral pharmaceuticals: recent developments and approaches. *Drug development and industrial pharmacy*, *30*(5), 429-448.

54. Grimes, D. A., Hubacher, D., Lopez, L. M., & Schulz, K. F. (2006). Non-steroidal anti-inflammatory drugs for heavy bleeding or pain associated with intrauterine-device use. *The Cochrane Library*.

55. Mishra, A., Veerasamy, R., Jain, P. K., Dixit, V. K., & Agrawal, R. K. (2008). Synthesis, characterization and pharmacological evaluation of amide prodrugs of ketorolac. *European journal of medicinal chemistry*, *43*(11), 2464-2472.

56. Mishra, A., Veerasamy, R., Jain, P. K., Dixit, V. K., & Agrawal, R. K. (2008). Synthesis, characterization and pharmacological evaluation of amide prodrugs of flurbiprofen. *European journal of medicinal chemistry*, 19(1):89-100.

57. Jilani, J. A., Pillai, G. K., Salem, M. S., & Najib, N. M. (1997). Evaluation of hydroxyethyl esters of mefenamic acid and diclofenac as prodrugs. *Drug development and industrial pharmacy*, *23*(3), 319-323.

58. Tantishaiyakul, V., Wiwattanawongsa, K., Pinsuwan, S., Kasiwong, S., Phadoongsombut, N., Kaewnopparat, S., ... & Rojanasakul, Y. (2002). Characterization of mefenamic acid-guaiacol ester: stability and transport across Caco-2 cell monolayers. *Pharmaceutical research*, *19*(7), 1013-1018.

59. Almasirad, A., Tajik, M., Bakhtiari, D., Shafiee, A., Abdollahi, M., Zamani, M. J., ... & Esmaily, H. (2005). Synthesis and analgesic activity of N-arylhydrazone derivatives of mefenamic acid. *J Pharm Pharm Sci*, *8*(3), 419-425.

60. Khan, M. S. Y., & Akhter, M. (2005). Glyceride derivatives as potential prodrugs: synthesis, biological activity and kinetic studies of glyceride derivatives of mefenamic acid. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *60*(2), 110-114.

61. Dev, S., Mhaske, D. V., Kadam, S. S., & Dhaneshwar, S. R. (2007). Synthesis and Pharmacological Evaluation of Cyclodextrin Conjugate Prodrug of Mefenamic acid. *Indian journal of pharmaceutical sciences*, *69*(1).

62. Rasheed, A. (2012). Synthesis, hydrolysis and pharmacodynamic profiles of novel prodrugs of some non steroidal anti inflammatory drugs.

63. Rasheed, A., & Ashok Kumar, C. K. (2010). Tyrosine and glycine derivatives as potential prodrugs: design, synthesis, and pharmacological evaluation of amide derivatives of mefenamic acid. *Journal of enzyme inhibition and medicinal chemistry*, *25*(6), 804-811.

64. ULUDAĞ, M. O., ERGÜN, B. Ç., Alkan, D. A., ERCAN, N., ÖZKAN, G. Y., & BANOĞLU, E. (2011). Stable ester and amide conjugates of some NSAIDs as analgesic and antiinflammatory compounds with improved biological activity. *Turkish Journal of Chemistry*, *35*(3), 427-439.

65. Velingkar, V. S., Desai, D. M., & Panda, V. S. (2011). Synthesis, characterization, computational ADME studies, pharmacological screening and in vitro hydrolysis studies of potential novel mutual prodrugs of N-(2, 3-xylyl anthranilic acid). *Int J Drug Des Discov*, *2*(3), 548-558.

66. Mahdi, M. F., & Alsaad, H. N. (2012). Design, Synthesis and hydrolytic behavior of mutual prodrugs of NSAIDs with Gabapentin using glycol spacers. *Pharmaceuticals*, *5*(10), 1080-1091.

67. Shah, K., Shrivastava, S. K., & Mishra, P. (2012). Synthesis, kinetics and pharmacological evaluation of mefenamic acid mutual prodrug. *Acta poloniae pharmaceutica*, *70*(5), 905-911.

68. Dhokchawle, B. V., Kamble, M. D., Tauro, S. J., & Bhandari, A. B. (2014). Synthesis, spectral studies, hydrolysis kinetics and pharmacodynamic profile of mefenamic acid prodrugs. *Der Pharma Chemica*, *6*(3).

69. Kemisetti, D. P., Manda, S., Aukunuru, J., Chinnala, K. M., & Rapaka, N. K. (2014). Synthesis of prodrugs of mefenamic acid and their in vivo evaluation. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(7), 437-442.

70. Tammara, V. K., Narurkar, M. M., Crider, A. M., & Khan, M. A. (1994). Morpholinoalkyl ester prodrugs of diclofenac: synthesis, in vitro and in vivo evaluation. *Journal of pharmaceutical sciences*, *83*(5), 644-648.

71. Tabrizi, M. N., Davaran, S., & Entezami, A. (1996). Synthesis of diclofenac polymeric prodrugs and their hydrolysis reactivity. *Iran Polym J*, *5*(4), 243-251.

72. Akgün, H., Tozkoparan, B., Ertan, M., Aksu, F., & Inan, S. Y. (1996). Synthesis of some 2-arylpropionic acid amides as prodrugs. *Arzneimittel-Forschung*, *46*(9), 891-894.

73. Mahfouz, N. M., Omar, F. A., & Aboul-Fadl, T. (1999). Cyclic amide derivatives as potential prodrugs II: N-hydroxymethylsuccinimide-/isatin esters of some NSAIDs as prodrugs with an improved therapeutic index. *European journal of medicinal chemistry*, *34*(7), 551-562.

74. Bandarage, U. K., Chen, L., Fang, X., Garvey, D. S., Glavin, A., Janero, D. R., ... & Shumway, M. J. (2000). Nitrosothiol esters of diclofenac: Synthesis and pharmacological characterization as gastrointestinal-sparing prodrugs. *Journal of medicinal chemistry*, *43*(21), 4005-4016.

75. Hirabayashi, H., Sawamoto, T., Fujisaki, J., Tokunaga, Y., Kimura, S., & Hata, T. (2002). Dose-dependent pharmacokinetics and disposition of bisphosphonic prodrug of diclofenac based on osteotropic drug delivery system (ODDS). *Biopharmaceutics & drug disposition*, *23*(8), 307-315.

76. Dalpiaz, A., Pavan, B., Scaglianti, M., Vitali, F., Bortolotti, F., Biondi, C., ... & Manfredini, S. (2004). Transporter-mediated effects of diclofenamic acid and its ascorbyl pro-drug in the in vivo neurotropic activity of ascorbyl nipecotic acid conjugate. *Journal of pharmaceutical sciences*, *93*(1), 78-85.

77. Khan, M. S. Y., Akhter, M., & Husain, A. (2006). Synthesis, biological evaluation and kinetic studies of glyceride prodrugs of biphenyl acetic acid. *INDIAN JOURNAL OF CHEMISTRY SECTION B*, *45*(4), 1014.

78. Manon, B., & Sharma, P. D. (2009). Design, synthesis and evaluation of diclofenacantioxidant mutual prodrugs as safer NSAIDs. *Indian journal of chemistry. Section B, Organic including medicinal*, *48*(9), 1279.

79. Pathan, A. R., Karwa, M., Pamidiboina, V., Deshattiwar, J. J., Deshmukh, N. J., Gaikwad, P. P., ... & Sharma, S. D. (2010). Oral bioavailability, efficacy and gastric tolerability of P2026, a novel nitric oxide-releasing diclofenac in rat. *Inflammopharmacology*, *18*(4), 157-168.

80. Bandgar, B. P., Sarangdhar, R. J., Ahamed, F. A., & Viswakarma, S. (2011). Synthesis, characterization, and biological evaluation of novel diclofenac prodrugs. *Journal of medicinal chemistry*, *54*(5), 1202-1210.

81. Santos, J. L. D., Moreira, V., Campos, M. L., Chelucci, R. C., Barbieri, K. P., P. C. M. D. C., ... & Chin, C. M. (2012). Pharmacological evaluation and preli pharmacokinetics studies of a new diclofenac prodrug without gastric ulc effect. *International journal of molecular sciences*, *13*(11), 15305-15320.

82. Ghosh, P., Lee, D., Kim, K. B., & Stinchcomb, A. L. (2014). Optimization of naltrexone diclofenac codrugs for sustained drug delivery across microneedle-treated skin. *Pharmaceutical research*, *31*(1), 148-159.

83. Suryawanshi, S. B., OSMAN, H. A., Shaikh, Y. I., & Nazeruddin, G. M. (2014). Synthesis of various esters of diclofenac (NSAIDs) as pro-drugs and their biological evaluation. *Chemical Science Transactions*, *3*(2), 562-565.

84. Hasan, S. A., & Elias, A. N. (2014). Synthesis of new Diclofenac derivatives by coupling with chalcone derivatives as possible mutual prodrugs. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(1), 239-245.

85. Reddy, M. R., & Erion, M. D. (2001). *Free energy calculations in rational drug design*. Springer Science & Business Media.

86. Karaman, R. (2010). The efficiency of proton transfer in Kirby's enzyme model, a computational approach. *Tetrahedron Letters*, *51*(16), 2130-2135.

87. Karaman, R. (2011). Computational-Aided Design for Dopamine Prodrugs Based on Novel Chemical Approach. *Chemical biology & drug design*, *78*(5), 853-863.

88. Karaman, R., Dajani, K. K., Qtait, A., & Khamis, M. (2012). Prodrugs of Acyclovir–A Computational Approach. *Chemical biology & drug design*, *79*(5), 819-834.

89. Karaman, R., & Hallak, H. (2010). Computer-Assisted Design of Pro-drugs for Antimalarial Atovaquone. *Chemical biology & drug design*, *76*(4), 350-360.

90. Karaman, R., Dajani, K., & Hallak, H. (2012). Computer-assisted design for atenolol prodrugs for the use in aqueous formulations. *Journal of molecular modeling*, *18*(4), 1523-1540.

91. Karaman, R. (2013). Prodrugs for masking bitter taste of antibacterial drugs—a computational approach. *Journal of molecular modeling*, *19*(6), 2399-2412.

92. Karaman, R., Dokmak, G., Bader, M., Hallak, H., Khamis, M., Scrano, L., & Bufo, S. A. (2013). Prodrugs of fumarate esters for the treatment of psoriasis and multiple sclerosis—a computational approach. *Journal of molecular modeling*, *19*(1), 439-452.

93. Karaman, R., & Pascal, R. (2010). A computational analysis of intramolecularity in proton transfer reactions. *Organic & biomolecular chemistry*, *8*(22), 5174-5178.

94. Karaman, R. (2011). Analyzing the efficiency in intramolecular amide hydrolysis of Kirby's N-alkylmaleamic acids–A computational approach. *Computational and Theoretical Chemistry*, *974*(1), 133-142.

تصنيع ودراسة مواصفات وتقييم الادوية المساعدة الاولية والمشتركة المفيدة للديكلوفيناك و حمض الميفيناميك

الملخص:

الخلفية:

مضادات الالتهاب اللاستيرويدية هي من بين الادوية الأكثر استخداما على نطاق واسع لعلاج انواع مختلفة من الالتهابات. ومع ذلك، فإن سمية هذه الادوية للجهاز الهضمي يحد من فائدتها. الادوية المساعدة التقليدية لمضادات الالتهاب اللاستيرويدية يتم تفعيلها عن طريق الانزيمات الموزعة في جميع أنحاء الجسم بحيث يمكن أن تتأثر هذه الإنزيمات بعوامل مختلفة مثل العمر والظروف الصحية والجنس .بحيث من الصعب أن نتوقع معدلات التحويل البيولوجي.تم تطوير استراتيجية جديدة لتحويل الدواء المساعد للدواء النشط . استخدمت الية التفاعل داخل الجزئ لتحرير الدواء من الدواء المساعد. بهذه الطريقة لاحاجة لانزيم التحفيز لتفعيل الدواء المساعد ,العملية تعتمد فقط على الخطوة المحددة للتفاعل داخل الجزئ.

الاهداف:

الهدف الرئيسي لعملنا هو تصنيع ادوية مساعدة ومشتركة لكل من حمض الميفيناميك والديكلوفيناك. بحيث تكون خالية من الطعم المر, الاثار السلبية للمعدة. بالاضافة لذلك ,الادوية المساعدة المقترحة حمض الميفيناميك والديكلوفيناك يجب ان يكون لديها القدرة للتحلل الكيميائي وليس الانزيمي وقادرة على تحرير الدواء بشكل مسيطر عليه.

الطريقة:

تم التأكد ودراسة مواصفات الادوية المساعدة الاولية والمشتركة التي تم تصنيعها باستخدام تفنيات التحليل الطيفي. ومعدل تحلل الادوية تمت دراسته ايضا باستخدام جهاز ال HPLC في اربعة محاليل مختلفة 1NHCl, pH2.5, pH5.5 وpH7.4.

النتائج:

تم تصنيع ادوية مساعدة اولية مميزة لحمض الميفيناميك والديكلوفيناك.وكذلك تم تصنيع ادوية مشتركة من حمض الميفيناميك والديكلوفيناك مع الترانيكساميك تفتقر للطعم المر.وبينت نتائج حركية الادوية للمركبات التي تم تصنيعها ان معدل تحلل الادوية المساعدة يتأثر بشدة من درجة حموضة الوسط. كان عمر النصف (t1/2) ل حمض ميفيناميك دايميثيل امين في درجة حموضة المعدة 10ساعة بينما كان مستقرا عند درجات الحموضة 2.5 ، 5.5 و 7.5. اما بالنسبة ل ديكلوفيناك بينزل كان (t1/2) 4 ، 1 ساعة في درجة الحموضة 1, 7.4 على التوالي. في حين أنه كان مستقر تماما في درجة الحموضة 5.5 و 7.4.ميفيناميك ترانيكساميك تحلل بسهولة لينتج كل من الدوائين في حمض الهيدروكلوريك(درجة الحموضة 1). ومع ذلك، فان تحلله في درجة الحموضة 2.5 , 5.5, 7.4 لا يكاد يذكر. اما ديكلوفيناك ترانيكساميك فان عمر النصف له في 1NHClهو 30 ساعة.

عمادة الدراسات العليا

جامعة القدس

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رسالة ماجستير

فلسطين ـ القدس

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المشرف الرئيسي: بروفيسور رفيق قرمان

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

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