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Prodrugs for Masking the Bitter Taste of Drugs

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1. Introduction

The palatability of the active ingredient of a drug is a significant obstacle in developing a patient friendly dosage form. Organoleptic properties, such as taste, are an important factor when selecting a certain drug from the generic products available in the market that have the same active ingredient. It is a key issue for doctors and pharmacists administering the drugs and particularly for the pediatric and geriatric populations. Nowadays, pharmaceutical companies are recognizing the importance of taste masking and a significant number of techniques have been developed for concealing the objectionable taste [1].

The word “medicine” for a child is considered a bad thing to administer because of its aversive taste. Medicines dissolve in saliva and bind to taste receptors on the tongue giving a bitter, sweet, salty, sour, or umami sensation. Sweet and sour taste receptors are concentrated on the tip and lateral borders of the tongue respectively. Bitter taste is sensed by the receptors on the posterior part of the tongue and umami taste receptors are located all over the tongue. A short period after birth, infants reject bitter tastes and prefer sweet and umami tastes[1]. Children have larger number of taste buds than adults which are responsible for sensitivity toward taste. These taste buds regenerate every two weeks. Taste becomes altered as a function of the aging process, which explains why most children find certain flavors to be too strong when adults do not. The American Academy of Pediatrics estimates that compliance in children is as low as 53%, indicating that children frequently fail to take medications properly. Noncompliance can lead to: (1) persistent symptoms, (2) need for additional doctor visits or even hospitalizations, (3) worsening of condition, (4) need for additional medications, (5) increased healthcare costs and (6) development of drug-resistant organisms in cases of infectious diseases [2].

In mammals, taste buds are groups of 30-100 individual elongated "neuroepithelial" cells which are often embedded in special structure in the surrounding epithelium known as papillae. Just below the taste bud apex, taste cells are joined by tight junctional complexes that prevent gaps

between cells. Food molecules cannot therefore squeeze between taste cells and get into the taste bud.

Taste papillae located on the tongue appear as little red dots, or raised bumps, particularly at the front of the tongue called "fungi form" papillae. There are three other kinds of papillae, foliate, circumvallates and the non-gustatory filiform. In mammals taste buds are located throughout the oral cavity, in the pharynx, the laryngeal epiglottis and at the entrance of the esophagus. Taste perception fades with age; on average, people lose half their taste receptors by time they turn 20 [3]. The sensation of taste can be categorized into five basic tastes: sweetness, sourness, saltiness, bitterness, and umami. Taste buds are able to differentiate among different tastes through detecting interaction with different molecules or ions. Sweet, umami, and bitter tastes are triggered by the binding of molecules to G protein-coupled receptors on the cell membranes of taste buds. Saltiness and sourness are perceived when alkali metal or hydrogen ions enter taste buds, respectively [4]. As taste senses both harmful and beneficial things, all basic tastes are classified as either aversive or appetitive, depending upon the effect the things they sense have on our bodies [5]. Sweetness helps to identify energy-rich foods, while bitterness serves as a warning sign of poisons [6].

For a long period, it was commonly accepted that there is a finite and small number of "basic tastes" of which all seemingly complex tastes are ultimately composed. As of the early twentieth century, physiologists and psychologists believed there were four basic tastes: sweetness, sourness, saltiness, bitterness. At that time umami was not proposed as a fifth taste but now a large number of authorities recognize it as the fifth taste [7]. In Asian countries within the sphere of mainly Chinese and Indian cultural influence, pungency (piquancy or hotness) had traditionally been considered a sixth basic taste. Today, the consensus is that sweet, amino acid (umami), and bitter taste converge one common transduction channel, the transient receptor potential channel TRPM5, *via* phospholipase C (PLC). TRPM5 is a newly discovered TRP related to other channels in sensory signaling systems. It has been shown that PLC, a major signaling effect of G-protein coupled receptors (GPCRs), and TRPM5 are co-expressed with T1Rs and T2Rs and are vital for sweet, amino acid, and bitter taste transduction. Activation of T1R or T2R receptors by their respective taste molecules would stimulate G proteins, and in turn PLC (PLC- β 2). The activation of PLC generates two intracellular messengers-IP3 and diacylglycerol (DAG)-from the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) and opens the TRPM5 channel, resulting in the generation of a depolarizing receptor potential. Other additional pathways may modulate sweet, amino acid, or bitter taste reception but would not, themselves, trigger a taste response. It is not at present known how PLC activates TRPM5 or whether DAG is involved [8-18].

2. Taste masking

There are numerous pharmaceutical and over the counter (OTC) preparations that contain active ingredients, which are bitter in taste. With respect to OTC preparations, such as cough and cold syrups, the bitterness of the preparation leads to lack of patient compliance. Among

examples that are commonly used drugs with bitter taste: (1) pseudoephedrine (1) (Figure 1), a sympathomimetic drug of the phenethylamine (2) (Figure 1) and amphetamine (3) (Figure 1) chemical classes. It may be used as a nasal/sinus decongestant, as a stimulant, or as a wakefulness-promoting agent [19], (2) dextromethorphan (4) (Figure 1), an antitussive (cough suppressant) drug. It is one of the active ingredients in many over-the-counter cold and cough medicines. Dextromethorphan has also found other uses in medicine, ranging from pain relief to psychological applications. It is sold in syrup, tablet, spray, and lozenge forms. In its pure form, dextromethorphan occurs as a white powder [20], (3) dyphylline (5) (figure 1) also known as dipprophyllinea xanthine derivative with bronchodilator and vasodilator effects. It is used in the treatment of respiratory disorders like asthma, cardiac, and bronchitis. It acts as an adenosine receptor antagonist and phosphodiesterase inhibitor [21]. (4) phenylephrine (6) (Figure 1), is a selective α 1-adrenergic receptor agonist used primarily as a decongestant, as an agent to dilate the pupil, and to increase blood pressure [22]. Phenylephrine is marketed as a substitute for the decongestant pseudoephedrine, (5) chlorhexidine (7) (Figure 1), a chemical antiseptic. It is effective on both Gram-positive and Gram-negative bacteria, although it is less effective with some Gram-negative bacteria. It has both bactericidal and bacteriostatic mechanisms of action, the mechanism of action being membrane disruption, not ATPase inactivation as previously thought [23]. It is also useful against fungi and enveloped viruses, though this has not been extensively investigated, (6) atorvastatin (8) (Figure 1), a member of the drug class known as statins, used for lowering blood cholesterol. It also stabilizes plaque and prevents strokes through anti-inflammatory and other mechanisms. Like all statins, atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body [22], (7) loperamide (9) (Figure 1), a piperidine derivative, is an opioid drug used against diarrhea resulting from gastroenteritis or inflammatory bowel disease. In most countries it is available generically [24]. (8) terfenadine (10) (Figure 2), was an antihistamine formerly used for the treatment of allergic conditions. It was brought to market by Hoechst Marion Roussel (now Sanofi-Aventis) and marketed under various brand names. According to its manufacturer, terfenadine had been used by over 100 million patients worldwide as of 1990 [25]. It was superseded by fexofenadine (11) (Figure 2) in the 1990s due to the risk of a particular type of disruption of the electrical rhythms of the heart (specifically cardiac arrhythmia caused by QT interval prolongation) [22], (9) prednisolone (12) (Figure 2), is a synthetic glucocorticoid, a derivative of cortisol, which is used to treat a variety of inflammatory and auto-immune conditions. It is the active metabolite of the drug prednisone and is used especially in patients with hepatic failure, as these individuals are unable to metabolize prednisone into prednisolone [22], (10) salbutamol (13) (Figure 2), or albuterol (USAN) is a short-acting β 2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease. It is marketed as Ventolin among other brand names. Salbutamol was the first selective β 2-receptor agonist to be marketed – in 1968. It was first sold by Allen & Hanburys under the brand name Ventolin. The drug was an instant success, and has been used for the treatment of asthma ever since [26]. (11) guaifenesin (14) (Figure 2), or guaiphenesin (former BAN), also glyceryl guaiacolate, is an expectorant drug sold over the counter and usually taken orally to assist the bringing up (expectoration) of phlegm from the airways in acute respiratory tract infections

[22] and (12) amoxicillin (15) (Figure 2), a moderate-spectrum, bacteriolytic, β -lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β -lactam antibiotics. Amoxicillin is one of the most common antibiotics prescribed for children. The drug became available in 1972 [22].

Figure 1. Chemical structures for 1-9.

3. Challenges and criteria for pursuing masking bitter taste approaches

The most significant challenges that facing developers when pursuing masking bitter taste drugs approaches are: (i) Safety, tolerability and efficacy of the compound which are based on non-clinical testing, and physicochemical properties such as solubility, permeability and stability, (ii) lack of robust and reliable techniques for early taste screening of compounds with limited toxicity data, (iii) structure–taste relationships of pharmaceutically active molecules is limited, (iv) The perception of taste of pharmaceuticals has been shown to be different between adults and children and it might differ between healthy and patient children [4] and (v) ethical concerns to perform taste studies in healthy children unless the study is a ‘swill and spit’ one with drugs known to have a good safety profile [27-29].

4. Bitter taste masking approaches (techniques)

A variety of taste masking approaches has been used to address the patient compliance problem. With strongly bad tasting medications even a little exposure is sufficient to perceive the bad taste. Conventional taste masking methods such as the use of sweeteners, amino acids and flavoring agents alone are often inadequate in masking the taste of highly bitter drugs. Drugs such as macrolide antibiotics, non-steroidal anti-inflammatory such as ibuprofen (16) (Figure 2), quinine (17) (Figure 2), celecoxib (18) (Figure 3), etoricoxib (19) (Figure 3), levofloxacin (20) (Figure 3) and penicillins have a pronounced bitter taste [30]. Masking the taste of water soluble bitter drugs, especially those given in high doses, is difficult to achieve by using sweeteners alone. As a consequence, several approaches have been investigated and have resulted in the development of more efficient techniques for masking the bitter taste of active ingredients. All of the developed techniques are based on the physical modification of the formulation containing the bitter tastant. Among the approaches used to mask bitter taste of pharmaceuticals are: (1) taste masking using flavors, sweeteners, and amino acids. This technique is the foremost and the simplest approach for taste masking, especially in the case of pediatric formulations, chewable tablets, and liquid formulations. However, it is not an ideal to be used for highly bitter and highly water soluble drugs. An example for such approach is the use of monosodium glycyrrhizinate together with flavors to mask the bitter taste of guaiphenesin (14) (an expectorant drug) Taste masked lamivudine (antiretroviral drug) was prepared by using lemon, orange and coffee flavors [3,31]; (2) taste masking with lipophilic vehicles such as: i) Lipids; acetaminophen granules are sprayed with molten stearyl stearate, mixed with suitable tablet excipients, and incorporated into a taste masked, chewable tablet formulation and (ii) lecithin and Lecithin-like substances; formulations with lecithin or lecithin-like substances in large quantities are believed to efficiently mask bitter taste of pharmaceuticals [3]. An example of a drug formulation containing lecithin-like substance is the one composed of magnesium aluminum silicate with soybean lecithin and talampicillin HCl (21) (antibiotic drug) (Figure 3); (3) coating is one of the most efficient and commonly used taste mask-

ing techniques. It is more efficient technology for aggressively bitter drugs even though coating imperfections, if present, reduce the efficiency of the technique. Coating of tablets, pellets or any other kind of particles with a film-forming polymer is a successful approach to provide a physical barrier, concealing unpleasant odors and bitter taste. Additionally, it can prevent penetration of moisture into the formulation. Coating materials can be selected from a wide range of hydrophobic and hydrophilic polymers such as polyvinylpyrrolidone, polyvinyl alcohol and cellulose derivatives. The ideal polymer for taste-masking, odor suppression and moisture protection should prevent dissolution of the dosage form in the mouth, but should be readily soluble in the stomach. Coating is classified based on the type of coating material, coating solvent system, and the number of coating layers. Taste masked famotidine (a drug for ulcer treatment) formulated by using a combination of water soluble polymers like polyvinylpyrrolidone and insoluble polymers like cellulose acetate is an example of such technique. Other various inert coating agents can be used to coat bitter drugs. These coating agents simply provide a physical barrier over the drug particles. Examples for such coating agents are starch, povidone, gelatin, methylcellulose, ethyl cellulose and etc. One of the most efficient Method of drug particle coating is the fluidized bed processor [4]. In this approach, powders as fine as 50 μm are fluidized in an expansion chamber by means of heated, high-velocity air, and the drug particles are coated with a coating solution introduced usually from the top as a spray through a nozzle. Increasing the length of the coating cycle can increase coating thickness. Taste masking of Ibuprofen (16)(Figure 2) has been successfully achieved by this technique [4]; (4) microencapsulation is a technique applicable to protect materials from oxidation, volatilizing as well as to mask their bitter tastes [6]. Microencapsulation processes are commonly based on the principle of solvent extraction or evaporation. Microencapsulation as a process has been defined by Bakan [6] as a means of applying relatively thin coating to small particles of solid, droplets of liquid and dispersion. This process can be used for masking the bitter taste of drugs by microencapsulating drug particles with various coating agents. Coating agents employed includes gelatin, povidone, HPMC, ethyl cellulose, Bees wax, carnauba wax, acrylics and shellac. Bitter-tasting drugs can be first encapsulated to produce free flowing microcapsules, which are then blended with other excipients and compressed into tablets. Microencapsulation also increases the stability of the drug. It can be accomplished by a variety of methods, including air suspension, coacervation-phase separation, spray drying and congealing, pan coating, solvent evaporation and multi-orifice centrifugation techniques; (5) taste suppressants and potentiators such as the Linguagen's bitter blockers (e.g. adenosine monophosphate) are used for masking bitter taste of various compounding by competing with the latter on binding to the G-protein coupled receptor sites (GPCR) [32]; (6) ion exchange resins are water insoluble, cross-linked polymers containing salt forming groups in repeating position on the polymer chain. Drug can be bound to the ion exchange resin by either repeated exposure of the resin to the drug in a chromatographic column or by prolonged contact of resin with the drug solution. The resins forms insoluble adsorbates or resinates through weak ionic bonding with oppositely charged drugs. The exchange of counter ions from resin is competitive.

Most of the bitter drugs have amine as a functional group, which is the cause of their obnoxious taste. If the functional groups are blocked by complex formation the bitterness of the drug reduces drastically. A drug-resin complex is made from the bitter drugs and ion-exchange resins. The nature of the drug-resin complex is such that the average pH of 6.7 and cation concentration of about 40 meq/ lit in saliva are not able to break the drug-resin complex but it is weak enough to be broken down by the hydrochloric acid present in the stomach. Thus the drug: resin complex is absolutely tasteless and stable, with no after taste, but at the same time its bioavailability is not affected. Ion exchange resin like Amberlite was used to formulate taste masked fast dissolving orally consumable films of dextromethorphan (cough suppressant drug) [33,34]; (7) inclusion complexes in which the drug molecule fits into the cavity of a complexing agent forming a stable complex. The obtained complexing agent has the potential to mask the bitter taste of a drug by either decreasing its oral solubility on ingestion, or decreasing the amount of drug particles exposed to taste buds, thus reducing the perception of bitter taste. The inclusion complexes with cyclodextrin owe their existence to van der Waals forces between the host and guest. Cyclodextrin is the most widely used complexing agent for inclusion type complexes. It is a sweet, nontoxic, cyclic oligosaccharide derived from starch. Cyclodextrin forms inclusion complexes with organic molecules both in solid state and in solution [35]; (8) pH modifiers are capable of generating a specific pH microenvironment in aqueous media that has the ability to facilitate *in situ* precipitation of the bitter drug compound in saliva thus reducing the overall taste sensation for liquid dosage forms like suspension [36]; (9) adsorbates which are commonly used with other taste masking technologies to mask pharmaceuticals bitterness. The pharmaceutical may be adsorbed or/and entrapped in the matrix of the adsorbate porous, which may result in a delayed release of the bitter tastant during the passage through the oral cavity and hence achieving taste masking [37]; (10) chemicals; the solubility and absorption of drugs can be modified by the formation of molecular complexes. Lowering drug solubility through molecular complexation can decrease the intensity of bitterness. Higuchi and Pitman [38] reported that caffeine (22) (Figure 3) forms complexes with organic acids that are less soluble than xanthenes and as such can be used to decrease the bitter taste of caffeine; (11) solid dispersions; solid dispersion have been defined as dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by melting (fusion) solvent or melting solvent method. Solid dispersion is also called as co precipitates for those preparation obtained by solvent method such as co precipitates of sulphathiazole (23) (Figure 3) and povidone. Solid dispersions using insoluble matrices or bland matrices may be used to mask the bitter taste of drugs. Also using them as adsorbates on various carriers may increase the stability of certain drugs [39]; (12) multiple emulsions; a novel technique for taste masking of drugs employing multiple emulsions has been prepared by dissolving drug in the inner aqueous phase of w/o/w emulsion under conditions of good shelf stability. The formulation is designed to release the drug through the oil phase in the presence of gastrointestinal fluid [40]; (13) using liposomes is another way of masking the unpleasant taste of therapeutic agent is to entrap them into liposome. For example, incorporating it into a liposomal

formulation prepared with egg phosphatidyl choline masked the bitter taste of an antimalarial, chloroquine phosphate(24) (Figure 4)in HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid) buffer at pH 7.2 [41];and (14) prodrugs; chloramphenicol palmitate ester (25) (Figure 4), clindamycin palmitate ester (26) (Figure 4)and triamcinolone diacetate ester (27)(Figure 4) [42].

Figure 2. Chemical structures for 10-17.

Figure 3. Chemical Structures for 18-23.

Although the mentioned approaches have helped to improve the taste of some drugs formulations, the problem of the bitter taste of drugs in pediatric and geriatric formulations still creates a serious challenge to pharmacists. Thus, different strategies should be developed in order to overcome this serious problem. The novel chemical approach discussed in this chapter involves the design of prodrugs for masking bitter taste of pharmaceuticals based on intramolecular processes using density functional theory (DFT) and ab initio methods [43] and correlations of experimental and calculated reactions rates. No enzyme is needed to catalyze the interconversion of a prodrug to its corresponding

drug. The rate of drug release is controlled by the nature of the linker bound to the drug. Bitter tastant molecules interact with taste receptors on the tongue to give bitter sensation. Altering the ability of the drug to interact with bitter taste receptors could reduce or eliminate its bitterness. This could be achieved by an appropriate modification of the structure and the size of a bitter compound. Bitter molecules bind to the G-protein coupled receptor-type T2R on the apical membrane of the taste receptor cells located in the taste buds [44,45].

Due to the large variation of structural features of bitter tasting molecules, it is difficult to generalize the molecular requirements for bitterness. Nevertheless, it was reported that a bitter tastant molecule requires a polar group and a hydrophobic moiety. A quantitative structure activity relationship (QSAR) model was developed and has been established for the prediction of bitterness of several tastant analogues. For example, it was reported that the addition of a pyridinium moiety to an amino acid chain of a variety of bitter amino acid compounds decreases bitterness, such as in the case of glycine. Other structural modifications, such as an increase in the number of amino groups/residues to more than 3 and a reduction in the poly-hydroxyl group/ COOH, have been proven to decrease bitterness significantly. Moreover, changing the configuration of a bitter tastant molecule by making isomer analogues was found to be important for binding affinity to enhance bitterness agonist activity (e.g. L-tryptophan is bitter while D-tryptophan is sweet) [46].

Our recent studies on intramolecularity have demonstrated that there is a necessity to further explore the mechanisms for the intramolecular processes to be utilized in the design for determining the factors playing dominant role in determining the reaction rate. Unraveling the reaction mechanism would allow for an accurate design of an efficient chemical device to be used as a prodrug linker that can be covalently linked to a drug which can chemically, and not enzymatically be cleaved to release the active parent drug in a controlled manner. For instance, exploring the mechanism for a proton transfer in Kirby's acetals [47] has led to a design and synthesis of novel prodrugs of aza-nucleosides to treat myelodysplastic syndromes [48] and statins to treat high cholesterol levels in the blood [49]. In the above mentioned examples, the prodrug moiety was attached to the hydroxyl group of the active drug such that the drug promoiety (prodrug) has the potential to degrade upon exposure to physiological environment such as stomach, intestine, and/or blood circulation, with rates that are solely dependent on the structural features of the pharmacologically inactive promoiety (Kirby's enzyme model). Other different linkers such as Kirby's maleamic acid enzyme model [50] was also investigated for the design of some prodrugs such as tranexamic acid prodrugs to treat bleeding conditions [51] and acyclovir as anti-viral drug to treat Herpes Simplex [52]. Menger's Kemp acid enzyme model [53] was also utilized for the design of dopamine prodrugs for the treatment of Parkinson's disease [54]. Prodrugs for dimethyl fumarate to treat psoriasis were also designed, synthesized and currently under *in vitro* and *in vivo* kinetic studies [55].

The same approach was utilized for masking the bitter taste of antibacterial drugs such as cefuroxime (28) (Figure 4), atenolol (29) (Figure 4), paracetamol (30) (Figure 4), amoxicil-

lin (15) (Figure 2) and cephalexin (31) (Figure 4) [56]. The role of the promoiety in the antibacterial (cefuroxime) prodrugs is to block the free amine or the hydroxyl group which is responsible for the drug bitter taste, and to enable the release of a drug in a programmable manner. The only difference between the proposed prodrugs and their parent drugs is that the amine group in the parent drug is replaced with an amide moiety. Replacing the amine group with an amide eliminates the capability of the molecule to hydrogen bond with the bitter taste receptor, thus masking the bitter taste of the parent drug. For example, paracetamol, a widely used pain killer and fever-reducer found in the urine of patients who had taken phenacetin (32) (Figure 4) has a very unpleasant bitter taste. Phenacetin, on the other hand, lacks or has very slight bitterness. The difference in the structural features of both drugs is only in the nature of the group in the *para* position of the benzene ring. While in the case of paracetamol the group is hydroxyl, in phenacetin it is ethoxy. Acetanilide (33) (Figure 4) has a chemical structure similar to that of paracetamol and phenacetin but lacks the group in the *para* position of the benzene ring, making it lack the bitter taste characteristic of paracetamol. These combined facts suggest that the presence of the hydroxy group on the *para* position of paracetamol is the major contributor for its bitter taste. It is believed that paracetamol interacts with the bitter taste receptors *via* hydrogen bonding which involves its phenolic group. Blocking the phenolic hydroxyl of paracetamol is expected to inhibit its binding to the bitter taste receptor and hence to eliminate its bitterness. Similarly, it is expected that blocking the free amine group in atenolol, amoxicillin or cephalexin with a suitable linker might inhibit the interaction between the amine group of the parent drug and its bitter taste receptors and hence masks its bitterness. The nature of the bitter taste receptors with paracetamol (via the phenolic group) or atenolol, amoxicillin or cephalexin (via the amine group) is likely to be as a result of hydrogen bonding between the substrate and the receptor.

In this chapter, the novel prodrug approach to be presented is based on enzyme models that have been made to understand the mechanism by which enzymes catalyze biochemical reactions. The tool exploited in the design is computational calculations using molecular orbital and molecular mechanics methods and correlations between experimental and calculated rate values for some intramolecular processes. In this approach, no enzyme is needed to catalyze the conversion of a prodrug to its active parent drug. The conversion rate is solely determined by the factors affecting the rate limiting step in the intramolecular (conversion) process. Knowledge gained from the mechanisms of the previously studied enzyme models was exploited in the design.

It is believed that the use of this approach might eliminate all disadvantages related to prodrug conversion by the metabolic (enzyme catalyzed process) approach. The bioconversion of prodrugs is perhaps the most vulnerable link in the chain, because there are many intrinsic and extrinsic factors that can affect the process. For example, the activity of many prodrug activating enzymes may be varied due to genetic polymorphisms, age-related physiological changes, or drug interactions, leading to adverse pharmacokinetic, pharmacodynamic, and clinical effects. In addition, there are wide interspecies variations in both the expression and

Figure 4. Chemical structures for 24-33.

function of the major enzymes activating prodrugs, and these can pose some obstacles in the preclinical optimization phase.

5. Enzyme models utilized for the design of potential bitterless prodrugs for bitter drugs such as atenolol, amoxicillin, cephalixin, paracetamol and guaiphenesin

Scholar studies of enzyme mechanisms by several chemists and biochemists, over the past five decades, have had a significant contribution for understanding the mode and scope of enzymes catalysis.

Nowadays, the scientific community has reached to the conclusion that enzyme catalysis is based on the combined effects of the catalysis by functional groups and the ability to reroute intermolecular reactions through alternative pathways by which substrates can bind to preorganized active sites. It is believed that rate accelerations by enzymes can be proceed by (i) covalently enforced proximity, as seen in the case of chymotrypsin, [57] (ii) non-covalently enforced proximity, as represented in the catalysis of metallo-enzymes, [58] (iii) covalently enforced strain, [59], and (iv) non-covalently enforced strain, which has been extensively studied on models mimicking the lysozyme enzyme which is most closely associated with rate acceleration due to this kind of strain [60].

Rates for the majority of enzymatic reactions ranges between 10^{10} and 10^{18} fold their non-enzymatic bimolecular counterparts. For instance, biochemical reactions involving the catalysis of the enzyme cyclophilin are enhanced by 10^5 and those by the enzyme orotidine monophosphate decarboxylase are accelerated by 10^{17} [61]. The significant enhancement in rate manifested by enzymes is a result of the substrate binding within the confines of the enzyme active site. The substrate-enzyme binding energy is the dominant driving force and the major contributor to catalysis. A consensus has been reached that in all enzymatic processes binding energy is used to overcome physical and thermodynamic factors that make barriers to the reaction (free energy). These factors are: (1) the change in entropy (ΔS°), in the form of the freedom of motions of the reactants in solution; (2) the hydrogen bonding net around bio-molecules in aqueous solution; (3) a proper alignment of catalytic functional groups on the enzyme; and (4) the distortion of a substrate that must occur before the reaction takes place [62,63].

Scholarly studies have been done by Bruice, Cohen, Menger, Kirby and others to design enzyme models having the potential to reach rates comparable to rates of biochemical reactions catalyzed by enzymes. Examples for such models are those based on rate enhancements driven by covalently enforced proximity. The most cited example is the intramolecular cyclization of dicarboxylic semi esters to anhydrides advocated by Bruice *et al.* [64,65]. Bruice *et al.* has demonstrated that a relative rate of anhydride formation can reach 5×10^7 upon cyclization of a dicarboxylic semi ester when compared to a similar counterpart's bimolecular process.

Other examples of rate acceleration based on proximity orientation include: (a) acid-catalyzed lactonization of hydroxy-acids as studied by Cohen *et al.* [66-68] and Menger [63, 69-75], (b) intramolecular S_N2 -based cyclization reactions as researched by Brown *et al.* [76] and Mandonini's group [77], (c) proton transfer between two oxygens in Kirby's acetals [78-84], and proton transfer between nitrogen and oxygen in Kirby's enzyme models [78-84], (d) proton transfer

between two oxygens in rigid systems as investigated by Menger [63, 69-75], and (e) proton transfer from oxygen to carbon in some of Kirby's enol ethers [78-84]. The conclusions emerged from these studies are (1) the driving force for enhancements in rate for intramolecular processes are both entropy and enthalpy effects. In the cases by which enthalpy effects were predominant such as ring-closing and proton transfer reactions proximity or/and steric effects were the driving force for rate accelerations. (2) The nature of the reaction being intermolecular or intramolecular is determined on the distance between the two reacting centers. (3) In S_N2 -based ring-closing reactions leading to three-, four- and five-member rings the *gem*-dialkyl effect is more dominant in processes involving the formation of an unstrained five-member ring, and the need for directional flexibility decreases as the size of the ring being formed increases. (4) Accelerations in the rate for intramolecular reactions are a result of both entropy and enthalpy factors. (5) An efficient proton transfer between two oxygens and between nitrogen and oxygen in Kirby's acetal systems were affordable when a strong hydrogen bonding was developed in the products and the transition states leading to them [85-103].

In the past few years some prodrugs based on the trimethyl lock system have been reported. Borchardt et al. has shown that the pro-prodrug 3-(2'-acetoxy-4', 6'-dimethyl dimethyl)-phenyl-3, 3-dimethylpropionamide is capable of releasing the biologically active amine drug upon acetate hydrolysis by enzyme triggering. Another successful example exploiting a stereopopulation control model is the prodrug Taxol which enhances the drug water solubility and hence affords it to be administered to the human body *via* intravenous injection. Taxol is the brand name for paclitaxel, a natural diterpene, approved in the USA for use to treat cancer [104-108].

6. Computational methods used in the design of bitterless prodrugs for bitter tastant drugs

Nearly sixty five years ago, organic, bioorganic and medicinal chemists alike have started using computational methods for calculating molecular properties of ground and transition states. These computational methods use principles of computer science to aid in solving chemical problems. Theoretical results emerged from these methods, incorporated into efficient computer programs, for calculating the structures and physical and chemical properties of molecules.

Equilibriums energy-based and reactions rates calculations for systems having medicinal interests are of a vast importance to the health community. Today, quantum mechanics (QM) such as *ab initio*, semi-empirical and density functional theory (DFT), and molecular mechanics (MM) are commonly and increasingly being used and broadly accepted as precise tools for predicting structure-energy calculations for drugs and prodrugs alike [109-112].

Ab initio methods typically are adequate only for small systems. *Ab initio* methods are based entirely on theory from first principles. The *ab initio* molecular orbital methods (QM) such as HF, G1, G2, G2MP2, MP2 and MP3 are based on rigorous use of the Schrodinger equation with

a number of approximations. Ab initio electronic structure methods have the advantage that they can be made to converge to the exact solution, when all approximations are sufficiently small in magnitude and when the finite set of basis functions tends toward the limit of a complete set. The convergence is usually not monotonic, and sometimes the smallest calculation gives the best result for some properties. The disadvantage of ab initio methods is their enormous computational cost. They take a significant amount of computer time, memory, and disk space [109-112]. On the other hand, empirical or semi-empirical methods are less accurate because they employ experimental results, often from acceptable models of atoms or related molecules, to approximate some elements of the underlying theory. Example for such methods is the semi-empirical quantum chemistry methods based on the Hartree–Fock formalism, but make many approximations and obtain some parameters from empirical data. These methods are especially important for calculating large molecules where the full Hartree–Fock method without the approximations is too expensive. Semi-empirical calculations are much faster than their ab initio counterparts. Their results, however, can be imprecise if the molecule being computed is not similar enough to the molecules in the database used to parameterize the method. Among the commonly used semiempirical methods are MINDO, MNDO, MINDO/3, AM1, PM3 and SAM1. Calculations of molecules exceeding 60 atoms can be handled using semiempirical methods [113-116].

Another widely used quantum mechanical method is the density functional theory (DFT). With this theory, the properties of many-electron systems can be determined by using functionals, i.e. functions of another function, which in this case is the spatially dependent electron density. Therefore, the name density functional theory comes from the use of functionals of the electron density. DFT is among the most popular and versatile methods available in condensed-matter physics, computational physics, and computational chemistry. The DFT method is adequate for calculating structures and energies for medium-sized systems (30-60 atoms) of biological, pharmaceutical and medicinal interest and is not restricted to the second row of the periodic table [43].

Although the use of DFT method is significantly increasing some difficulties still encountered when describing intermolecular interactions, especially van der Waals forces (dispersion); charge transfer excitations; transition states, global potential energy surfaces and some other strongly correlated systems. Incomplete treatment of dispersion can adversely affect the DFT degree of accuracy in the treatment of systems which are dominated by dispersion.

On the other hand, molecular mechanics is a mathematical approach used for the computation of structures, energy, dipole moment, and other physical properties. It is widely used in calculating many diverse biological and chemical systems such as proteins, large crystal structures, and relatively large solvated systems. However, this method is limited by the determination of parameters such as the large number of unique torsion angles present in structurally diverse molecules [117].

Molecular mechanics simulations, for example, use a single classical expression for the energy of a compound, for instance the harmonic oscillator. The database of compounds used for parameterization, i.e., the resulting set of parameters and functions is called the force field, is crucial to the success of molecular mechanics calculations. A force field parameterized against

a specific class of molecules, for instance proteins, would be expected to only have any relevance when describing other molecules of the same class. These methods can be applied to proteins and other large biological molecules, and allow studies of the approach and docking of potential drug molecules. Since the size of the system which *ab initio* calculations can handle is relatively small despite the large sizes of biomacromolecules surrounding solvent water molecules such as in the cases of enzymes and receptors, isolated models of areas of proteins such as active sites have been investigated using *ab initio* calculations. However, the disregarded proteins and solvent surrounding the catalytic centers have also been shown to contribute to the regulation of electronic structures and geometries of the regions of interest. To overcome these discrepancies, quantum mechanics/molecular mechanics (QM/MM) calculations are used, in which the system is divided into QM and MM regions where QM regions correspond to active sites to be studied and are described quantum mechanically. MM regions correspond to the remainder of the system and are treated molecular mechanically. The pioneer work of the QM/MM method was accomplished by Warshel and Levitt [118], and since then, there has been a significant progress on the development of a QM/MM algorithm and applications to biological systems [119,120].

7. Mechanistic study of the acid-catalyzed hydrolysis of maleamic acids 34-42 used for the design of atenolol, amoxicillin and cephalexin prodrugs

The acid-catalyzed hydrolysis of **34-42** (Figure 5) was kinetically investigated by Kirby et al. [84]. The study demonstrated that the amide bond cleavage is due to intramolecular nucleophilic catalysis by the adjacent carboxylic acid group and the rate-limiting step is the tetrahedral intermediate breakdown (Figure 6) [84]. In 1996, the reaction was computationally investigated by Katagi using AM1 semiempirical calculations. In contrast to what was suggested by Kirby, Katagi's study demonstrated that the rate-limiting step is the formation of the tetrahedral intermediate and not its dissociation [121]. Later on Kluger and Chin have experimentally researched the mechanism of the intramolecular hydrolysis process utilizing several N-alkylmaleamic acids derived from aliphatic amines with a wide range of basicity [122]. The study findings demonstrated that the identity of the rate-limiting step is a function of both the basicity of the leaving group and the solution acidity.

In order to utilize Kirby's enzyme model [84] for the design of prodrugs of the following drugs: atenolol, amoxicillin and cephalexin, a mechanistic study using DFT calculation methods at B3LYP/6-31G (d,p), B3LYP/311+G (d,p) levels and hybrid GGA (MPW1k) on an intramolecular acid catalyzed hydrolysis of maleamic (4-amino-4-oxo-2-butenic) acids (Kirby's N-alkylmaleamic acids) **34-42** was conducted. The calculations confirmed that the reaction involves three steps: (1) proton transfer from the carboxylic group to the adjacent amide carbonyl oxygen, (2) nucleophilic attack of the carboxylate anion onto the protonated carbonyl carbon; and (3) dissociation of the tetrahedral intermediate to provide products (Figure 6). Moreover, the calculations demonstrate that the rate-limiting step is dependent on the reaction medium. When the calculations were run in the gas phase the rate-limiting step was the tetrahedral intermediate formation, whereas when the calculations were conducted in the presence of a

cluster of water the dissociation of the tetrahedral intermediate was the rate-limiting step. When the leaving group (methylamine) in **34-42** was replaced with a group having a low pKa value the rate-limiting step of the hydrolysis in water was the formation of the tetrahedral intermediate. In addition, the calculations revealed that the efficiency of the intramolecular acid-catalyzed hydrolysis by the carboxyl group is remarkably sensitive to the pattern of substitution on the carbon-carbon double bond. The rate of hydrolysis was found to be linearly correlated with the strain energy of the tetrahedral intermediate or the product. Systems having strained tetrahedral intermediates or products experience low rates and vice versa [51,52,54,91].

Figure 5. Acid-catalyzed hydrolysis of maleamic acids **34-42**.

8. Bitterless atenolol prodrugs based on Kirby's maleamic acids enzyme model

Atenolol is a relatively polar hydrophilic compound with water solubility of 26.5 mg/mL at 37 °C and a log partition coefficient (octanol/ water) of 0.23. Atenolol is a selective β₁-adrenoceptor antagonist, applied in the treatment hypertension, angina, acute myocardial infarction, supraventricular tachycardia, ventricular tachycardia, and the symptoms of alcohol withdrawal. The net effect of atenolol on controlling both the heart rate and blood pressure is the reduction in myocardial work and oxygen requirement which reduces cardiovascular stress, thereby preventing arrhythmia and angina attacks.

Atenolol has a pKa of 9.6; it undergoes ionization in the stomach and intestine thus its oral bioavailability is low due to inefficient absorption through membranes.

NHR = atenolol, acyclovir, cefuroxime, tranexamic acid or methyl
R1 and R2; H, methyl or trifluoromethyl

Figure 6. Proposed mechanism for the acid-catalyzed hydrolysis of maleamic acids.

The bioavailability of atenolol is 45%-55% of the given dose and is not increased by administration of the drug in a solution form [123-125]. About 50% of administered atenolol is absorbed; however, most of the absorbed quantity reaches the systemic circulation. Atenolol peak blood levels are reached within two to four hours after ingestion. Differently from propranolol or metoprolol, atenolol is resistant to metabolism by the liver and the absorbed dose is eliminated by renal excretion. More than 85% of I.V. dose is excreted in urine within 24 hours compared with 50% for an oral dose. Only 6-16% is protein-bound resulting in relatively consistent plasma drug levels with about a four-fold inter-patient variation. The elimination half-life of atenolol is between 6 to 7 hours and there is no alteration of kinetic profile of a drug by chronic administration.

Atenolol is one of the most important medicines used for prevention of several types of arrhythmias in childhood, but unfortunately it is still unlicensed [126]. On the other hand, atenolol is indicated as a first-step therapy for hypertension in elderly patients, who have difficulty in swallowing and, thus, tablets and capsules are frequently avoided.

Atenolol is available as 25, 50 and 100 mg tablets for oral administration. However, most of these medicines are not formulated for easy or accurate administration to children for the migraine indication or in elderly patients who may have a difficulty swallowing tablets. Attempts to prepare a liquid formulation was challenging because atenolol is unstable in solutions. Studies showed that the degradation rate of atenolol is dependent on the temperature, indicating higher stability at 4 °C. Atenolol syrup is stable only for 9 days. Furthermore, oral doses of atenolol are incompletely absorbed (range 46-62%), even when formulated as a solution. Furthermore, atenolol bitterness is considered as a great challenge to health sector when used among children and geriatrics [125]. The main problem in oral administration of bitter drugs such as atenolol is incompliance by the patients [1] and this can be overcome by masking the bitterness of a drug either by decreasing its oral solubility on ingestion or eliminating the interaction of drug particles to taste buds [2]. Thus the development of bitterless and more lipophilic prodrug that is stable in aqueous medium is a significant challenge. Improvement of atenolol pharmacokinetic absorption properties and hence its effectiveness may increase the absorption of the drug *via* a variety of administration routes. The aims of the study described in this section were: (1) design of atenolol prodrugs that can be (i) formulated in aqueous solutions and be stable over a long period of time, (ii) bitterless compounds having the capability to convert in physiological environment to the parent active drug, atenolol, in a controlled manner and (2) synthesis, characterization and *in vitro* kinetic study of the conversion of the designed prodrugs to their parent drug in different pHs (physiological media).

The proposed atenolol prodrugs that were designed based on the acid-catalyzed hydrolysis reactions of N-alkyl maleamic acids **34-42** (Figure 5) are depicted in Figure 7.

As shown in Figure 7, the only difference exists between the proposed atenolol prodrugs and their parent drug is that the amine group of atenolol was replaced with an amide moiety. Replacing the free amine in atenolol with an amide is expected to increase the stability of the prodrug thus formed due to general chemical stability for tertiary alcohols over amine alcohols. In addition, recent stability studies on atenolol esters have demonstrated that the esters were more stable than their corresponding alcohol, atenolol, when formulating in aqueous solutions. Furthermore, kinetic study on atenolol and propranolol demonstrated that increasing the lipophilicity of the drug leads to an increase in the stability of its aqueous solutions. Based on that, it is expected that atenolol prodrugs shown in Figure 7 will have the potential to be more resistant to heat or/oxidation when formulated in aqueous solutions [128-131]. Atenolol's bitter-taste can be masked by using the prodrug chemical approach. For example, paracetamol (**30**), a widely used pain killer found in the urine of patients who had taken phenacetin has a very unpleasant bitter taste. Phenacetin (**31**), on the other hand, lacks or has very slight bitter taste. The difference in the structural features of both drugs is only the group in the *para* position of the benzene ring. While in the case of paracetamol the group is hydroxyl, in phenacetin it is ethoxy. On the other hand, acetanilide (**32**) is a bitterless compound with a chemical structure similar to that of paracetamol and phenacetin but lacks the group in the *para* position of the benzene ring. These facts suggest that the presence of the hydroxyl group on the *para* position of the benzene ring is the major contributor for the bitterness of paracetamol. It is likely that paracetamol bitterness is a result of interactions *via* hydrogen bonding

of the phenolic group in paracetamol with the bitter taste receptors. Similarly, it is expected that blocking the amine group in atenolol with a suitable linker might inhibit the hydrogen bonding between the amine group in atenolol and its bitter taste receptors and hence masking the drug's bitterness [132].

The proposed atenolol prodrugs, atenolol **ProD 1** and atenolol **ProD 2**, have a hydroxyl and carboxylic acid groups (hydrophilic moiety) and the rest of the prodrug molecule is a lipophilic moiety (Figure 7), where the combination of both groups ensures a moderate hydrophilic lipophilic balance (HLB).

It is worth noting that the HLB value of atenolol prodrug will be largely determined on the pH of the physiological environment by which the prodrug is exposed to. For example, in the stomach pH, the atenolol prodrugs, **ProD 1** and **ProD 2**, will exist in the free carboxylic acid form whereas in the blood circulation the carboxylate form will be dominant. It was planned that atenolol **ProD 1-2** (Figure 7) will be formulated as sodium salts since the carboxylate form is expected to be quite stable in neutral aqueous medium. However, upon dissolution in the stomach (pH less than 3) the proposed prodrugs will exist mainly as a carboxylic acid form thus enabling the acid-catalyzed hydrolysis to commence.

Figure 7. Acid-catalyzed hydrolysis for atenolol ProD 1 and atenolol ProD 2.

9. Calculation of the $t_{1/2}$ values for the cleavage reactions of atenolol prodrugs ProD 1-2

The effective molarity (EM) parameter is a commonly tool used to predict the efficiency of intramolecular reactions when bringing two functional groups such as an electrophile and a nucleophile in a close proximity. Intramolecularity is usually measured by the effective

molarity parameter. The effective molarity is defined as the rate ratio ($k_{\text{intra}}/k_{\text{inter}}$) for corresponding intramolecular and intermolecular processes driven by identical mechanisms. Ring size, solvent and reaction type are the major factors affecting the EM value. Ring-closing reactions *via* intramolecular nucleophilic addition are much more efficient than intramolecular proton transfer reactions. EM values in the order of 10^9 - 10^{13} M were determined for intramolecular processes occurring through nucleophilic addition. Whereas for proton transfer processes values of less than 10 M were measured for proton transfer processes until recently where values of 10^{10} was documented by Kirby on the hydrolysis of some enzyme models [60,78-84].

For obtaining the EM values for processes **34-42** and atenolol **ProD1-2** the kinetic and thermodynamic parameters for their corresponding intermolecular process, **Inter** (Figure 8) were calculated.

Using equations 1-4, equation 5 was derived, and describes the EM term as a function of the difference in the activation energies of the intra-and the corresponding inter-molecular processes. The calculated EM values for processes **34-42** and **ProD 1-2** were calculated using equation 5.

$$EM = k_{\text{intra}}/k_{\text{inter}} \quad (1)$$

$$\Delta G_{\text{inter}}^{\ddagger} = -RT \ln k_{\text{inter}} \quad (2)$$

$$\Delta G_{\text{intra}}^{\ddagger} = -RT \ln k_{\text{intra}} \quad (3)$$

$$\Delta G_{\text{intra}}^{\ddagger} - \Delta G_{\text{inter}}^{\ddagger} = -RT \ln k_{\text{intra}}/k_{\text{inter}} \quad (4)$$

$$\ln EM = (\Delta G_{\text{intra}}^{\ddagger} - \Delta G_{\text{inter}}^{\ddagger})/RT \quad (5)$$

Where T is the temperature in Kelvin and R is the gas constant.

Figure 8. Acid catalyzed hydrolysis for process **Inter**.

The calculated EM values from eq. 5 for processes **34-38** were correlated with the corresponding EM values [101] (Figure 9a). Good correlation with a correlation coefficient of $r=$ was obtained. The correlation results demonstrate that processes **35** and **37** were the most efficient among **34-38**, whereas process **4** was the least. The discrepancy in the rates of processes **35** and **38** on one hand and process **37** on the other hand might be attributed to strain effects.

In addition, for further support to the credibility of our DFT calculations the calculated free activation energies ($\Delta G_{BW}^\ddagger$) were correlated with the corresponding experimental free activation energies (Exp $\Delta G^\ddagger$). Good correlation was obtained with R value of 0.96 (Figure 9b).

Utilizing eq. 6 obtained from the correlation of $\log k_{rel}$ vs. $\Delta G^\ddagger$ and the experimental $t_{1/2}$ value measured for process **2** ($t_{1/2}=1$ second) [103], the $t_{1/2}$ values for atenolol **ProD 1** and atenolol **ProD 2** at pH 2 were calculated and their values were 65.3 hours and 11.8 minutes, respectively.

$$\log k_{rel} = - 0.44 \Delta G^\ddagger + 13.53 \quad (6)$$

10. In vitro intraconversion of atenolol ProD 1 to the parent drug atenolol

Kinetics of the acid-catalyzed hydrolysis for atenolol **ProD 1** was carried out in an aqueous buffer in a similar manner to that done by Kirby on N-alkylmaleamic acids **34-38**. This is in order to examine whether atenolol prodrug is hydrolyzed in aqueous medium and to what extent, suggesting its fate in the system. Acid-catalyzed hydrolysis of atenolol **ProD 1** was investigated in four different aqueous media: 1 N HCl and buffers pH 2, pH 5 and pH 7.4. Under the experimental conditions, atenolol **ProD 1** was hydrolyzed to release the parent drug, atenolol, (Figure 10) as was evident by HPLC measurements. At constant pH and temperature, the reaction displayed strict first order kinetics as the k_{obs} was fairly constant and a straight line was obtained on plotting log concentration of residual atenolol prodrug versus time. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$) for atenolol prodrug **ProD 1** in the different media were calculated from the linear regression equation correlating the log concentration of the residual prodrug versus time. The kinetic data, k_{obs} and $t_{1/2}$ values, are listed in Table 1. 1N HCl, pH 2 and pH 5 were selected to examine the intraconversion of atenolol **ProD 1** in pH as of stomach, because the mean fasting stomach pH of adult is approximately 1-2 and increases up to 5 following ingestion of food. In addition, buffer pH 5 mimics the beginning of the small intestine environment. The medium at pH 7.4 was selected to examine the intraconversion of the tested prodrug in the blood circulation system. Acid-catalyzed hydrolysis of atenolol **ProD 1** was found to be higher in 1N HCl than at pH 2 and 5 (Figure 10). At 1N HCl atenolol **ProD 1** was intraconverted to release the parent drug in 2.53 hour. On the other hand, at pH 7.4, the prodrug was entirely stable and no release of the parent drug was observed. Since the pK_a of the carboxylic group of atenolol **ProD1** is in the range of 3-4, it is expected at pH 5 the anionic form of the prodrug will be dominant and the percentage of the free acid form that expected to undergo hydrolysis will be relatively low. At 1N HCl and pH 2 most of the prodrug will exist as the free acid form, whereas at pH 7.4 most of the prodrug will be in the anionic form. Thus, the difference in rates at the different pH buffers.

Figure 9. (a) log calculated effective molarity vs. experimental effective molarity for processes **34-38**. (b) DFT calculated activation energy (kcal/mol) vs. experimental activation energy (kcal/mol) for processes **34-38**.

Figure 10. First order hydrolysis plot of atenolol **ProD 1** in (a) 1N HCl, (b) buffer pH 2 and (c) buffer pH 5.

$t_{1/2}$ (h)	k_{obs} (h ⁻¹)	Medium
2.53	4.95×10^{-4}	1 N HCl
3.82	2.22×10^{-4}	Buffer pH 2
133	2.75×10^{-6}	Buffer pH 5
-----	No Reaction	Buffer pH 7.4

In 1N HCl and at pH 2, 5 and 7.4

Table 1. The observed k value and $t_{1/2}$ of atenolol **ProD 1**

11. Bitterless amoxicillin and cephalexin prodrugs based on Kirby’s maleamic acids enzyme model

Most of the antibacterial agents that are commonly used suffer unpleasant taste and a respected number of them are characterized with bitter taste. For example, amoxicillin, cephalexin and cefuroxime axetil have an extremely unpleasant and bitter taste which is difficult to mask. This is a particular problem in geriatric patients who cannot swallow whole tablets or when small doses are required. Even the antibacterial suspension is difficult for pediatrics to administer due to its better and unpleasant taste [133-139].

It is widely assumed that the extremely bitter and unpleasant taste of these antibacterial drugs is due to a formation of intermolecular force/s between the drug and the active site of the bitter taste receptor/s. The intermolecular bond/s is/are most likely due to formation either *via* hydrogen bond or ionic bond of the amido (in cefuroxime)or amine (in amoxicillin and cephalexin) group to the active site of the bitter taste receptors.

Antimicrobial agents are classified according to their specific mode of action against bacterial cell. By which these agents may interfere with cell wall synthesis, inhibit protein synthesis, interfere with nucleic acid synthesis or inhibit a metabolic pathway. They have a broad spectrum of activity against both gram-positive and gram-negative bacteria. Among these agents, β -lactams – penicillins, cephalosporins, carbapenems and monobactams, by which represent 60% of all antimicrobial use by weight. They are preferred because of their efficacy, safety, and because their activity can be extended or restored by chemical manipulation. Inevitably, however, their usage has been restricted because of their bacterial resistance.

11.1. Amoxicillin

Amoxicillin is an oral semi-synthetic penicillin, moderate-spectrum, bacteriolytic, β -lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms by which it is susceptible to the action of the β -lactamases. Amoxicillin has a bactericidal action and acts against both Gram positive and Gram-negative microorganisms by inhibiting the biosynthesis and repair of the bacterial mucopeptide wall. It is usually the drug of choice within its class because it is well absorbed following oral administration. Amoxicillin presents some outstanding advantages in comparison with other amino-penicillins, such as: a better absorption

from the intestinal tract, better capacity for reaching effective concentrations at the sites of action and a more rapid capacity for penetrating the cellular wall of Gram-negative microorganisms. Amino-penicillins are frequently prescribed agents for the oral treatment of lower respiratory tract infections and are generally highly effective against *S. pneumonia* and non- β -lactamase-producing *H. influenza*. Amoxicillin is mostly common antibiotics prescribed for children. It has high absorption after oral administration which is not altered and affected by the presence of food. Amoxicillin dose reaches C_{max} about 2 hours after administration and is quickly distributed and eliminated by excretion in urine (about 60%-75%). The antibacterial effect of amoxicillin is extended by the presence of a benzyl ring in the side chain. Because amoxicillin is susceptible to degradation by β -lactamase-producing bacteria, which are resistant to a broad spectrum of β -lactam antibiotics, such as penicillin, for this reason, it is often combined with clavulanic acid, a β -lactamase inhibitor. This increases effectiveness by reducing its susceptibility to β -lactamase resistance. Amoxicillin has two ionizable groups in the physiological range (the amino group in α -position to the amide carbonyl group and the carboxyl group). Amoxicillin has a good pharmacokinetics profile with bioavailability of 95% if taken orally, its half-life is 61.3 minutes and it is excreted by the renal and less than 30 % bio-transformed in the liver [140-142].

11.2. Cephalexin

Cephalexin is a first-generation cephalosporin antibiotic, which was chosen as the model drug candidate to obtain dosage with improved stability, palatability and attractive pediatric elegance, cost effective with ease of administration. Cephalosporins are the most widely used for treatment of skin infections because of their safety profile, and their wide range of activity against both gram positive and gram negative microorganism. Cephalexin is also used for the treatment of articular infections as a rational first-line treatment for cellulitis, it is a useful alternative to penicillins hypersensitivity, and thought to be safe in a patient with penicillin allergy but caution should always be taken, that's because cephalexin and other first-generation cephalosporins are known to have a modest cross-allergy in patients with penicillin hypersensitivity. In addition, cephalexin is also effective and used in the treatment of group A β -hemolytic streptococcal throat infections. Cephalexin works by interfering with the bacteria's cell wall formation, causing it to rupture, and thus killing the bacteria. The compound is zwitterion by which it contains both a basic and an acidic group, the isoelectric point of cephalexin in water is approximately 4.5 to 5. Cephalexin has a good pharmacokinetic profile by which it is well absorbed, 80% excreted unchanged in urine within 6 hours of administration. Cephalexin's half-life is 0.5-1.2 hours and it is excreted *via* the renal. It is used for the treatment of infections including otitis media, streptococcal pharyngitis, bone and joint infections, pneumonia, cellulitis and UTI, and so it may be used to prevent bacterial endocarditis [142-145].

11.3. Cefuroxime axetil

Cefuroxime axetil is a semi-synthetic, broad-spectrum cephalosporin antibiotic for oral administration. Cefuroxime axetil is an orally active antibacterial agent though its absorption is incomplete. The range of its bioavailability is 25-52%. The axetil moiety is metabolized to acetaldehyde and acetic acid. Peak plasma concentration is reached 2-3 hours after an oral

administration. Up to 50% of cefuroxime in the circulation is bound to plasma proteins. The plasma half-life is about 70 minutes and is prolonged in patients with renal impairments and in neonates. Cefuroxime axetil is widely distributed in the body including plural fluid, sputum bone synovial fluid, and aqueous humor, but only achieves therapeutic concentration in the CSF when the meninges are inflamed. It crosses the placenta and has been detected in breast milk. Cefuroxime is excreted unchanged, by glomerular filtration and renal tubular secretion, and high concentration is achieved in urine [146].

Amoxicillin, cephalixin and cefuroxime axetil as mentioned before suffer low stability and bitter taste sensation. Several attempts were made in order to enhance their aqueous solubility and bioavailability. Among several research approaches, the prodrug approach has been widely used for an improvement of drugs delivery to their site of action by physicochemical modulation properties that affect absorption or by targeting to specific enzymes or membrane transporters [147,148]. Generally, enzymatic catalysis is required for most of prodrugs that are in clinical use in order to be converted into the parent drug. This is mostly particular for those prodrugs designed to liberate the parent drug in the blood stream following gastro-intestinal absorption. These prodrugs are typically ester derivatives of drugs containing carboxyl or hydroxyl groups which are converted into the parent drug by esterase catalyzed hydrolysis. However, a high chemical reactivity that precludes either liquid or solid formulation of the prodrug (e.g. some phenol esters) or low chemical reactivity, resulting in reduced regeneration of the parent drug due to enzymatic activation for other functional groups. Thus, non-enzymatic pathways for some prodrugs that can regenerate the parent drug, have emerged as an alternative approach by which prodrug activation is not influenced by inter-and intra-individual variability that affects the enzymatic activity. In particular, since the middle-1980s, cyclization-activated prodrugs have been capturing the attention of medicinal chemists, and reached maturity in prodrug design in the late 1990s. Activation of prodrugs *via* a cyclization pathway allows a fine tuning of the rate of drug release through the appropriate choice of the functional groups involved in ring closure and stereoelectronic constraints in the course of the cyclization step. As noticed from the history of prodrugs mostly in preclinical and clinical consideration of prodrug bioconversion, the most common that several hydrolyses-activated prodrugs of penicillins, cephalosporins, and angiotensin-converting enzyme inhibitors have less than complete absorption which was observed and highlights yet another challenge with prodrugs susceptible to esterase hydrolysis. The oral bioavailability of these mentioned types of prodrugs is typically around 50% since these prodrugs undergo premature hydrolysis during the absorption process in the enterocytes of the gastrointestinal tract [149]. Another approach which has been utilized to enhance bioavailability of antibacterial drugs is by making the corresponding prodrugs with optimum lipophilicity. Some drugs remain poorly absorbed from most of the administration routes due to their poor lipophilicity. Two approaches were utilized to enhance the bioavailability of antibacterial drugs by increasing their lipophilicity: (a) membrane/water partition coefficient of the lipophilic form of a drug has been enhanced as compared to the hydrophilic form, thus favoring passive diffusion such as in the cases of pivampicillin, bacampicillin and talamipicillin (prodrugs of ampicillin) which are more lipophilic and better absorbed than amoxicillin and are rapidly interconverted and (b) the

lipophilic prodrugs have poor solubility in gastric fluids and thus greater stability and absorption example for such approach is erythromycin esters [150].

Some ampicillin esters were prepared for improving the bioavailability of ampicillin. For example, the pivaloyloxyethyl (pivampicillin), phthalidyl (talampicillin), and ethoxycarbonyloxyethyl (bacampicillin) were found to have two fold the oral bioavailability of their parent drug, ampicillin. Complete hydrolysis of these esters was occurred in the gastrointestinal mucosa, whereas methoxymethylester of ampicillin was partially hydrolyzed by gut and hepatic first-pass metabolism and appears in the systemic circulation and tissues as intact ester [151-154].

12. In vitro intraconversion of amoxicillin and cephalexin prodrugs to their parent drugs

Based on our previously reported DFT calculations and on experimental data for the acid-catalyzed hydrolysis of amide acids **34-42** (Figure 5) [84,91], two amoxicillin and cephalexin prodrugs were proposed (Figures 11 and 12, respectively). As shown in Figures 11 and 12, the antibacterial prodrugs, amoxicillin **ProD 1** and cephalexin **ProD 1** molecules are composed of an amide acid promoiety, containing a carboxylic acid group (hydrophilic moiety) and the rest of the antibacterial prodrug molecule (a lipophilic moiety).

Figure 11. Acid-catalyzed hydrolysis of amoxicillin ProD 1.

The combination of both, the hydrophilic and lipophilic groups provides a prodrug entity with a potential to be with a high permeability (a moderate HLB). It should be emphasized, that the HLB value of the prodrug entity will be determined upon the pH of the target physiological environment. In the stomach where the pH is in the range 1-2, it is expected that prodrugs, amoxicillin **ProD1** and cephalexin **ProD1** will be in a free carboxylic acid form (a relatively high hydrophobicity) whereas in the blood stream circulation where the is pH 7.4 a carboxylate anion (a relatively low hydrophobicity) is expected to be predominant form. Our strategy was to prepare amoxicillin **ProD 1** and cephalexin **ProD 1** as sodium or potassium carboxylates due to their high stability in neutral aqueous medium. It should be indicated that compounds

34-42 undergo a relatively fast hydrolysis in acidic aqueous medium whereas they are quite stable at neutral pH.

Figure 12. Acid-catalyzed hydrolysis of cephalixin ProD 1.

The hydrolysis kinetic studies for amoxicillin **ProD 1** and cephalixin **ProD 1** were carried out in aqueous buffers in the same manner to that executed by Kirby *et al.* on maleamic acids **34-40**. This is to investigate whether the antibacterial prodrugs undergo hydrolysis in aqueous medium and to what extent or not, suggesting the fate of the prodrugs in the system. The kinetics for the acid-catalyzed hydrolysis of the synthesized amoxicillin **ProD 1** and cephalixin **ProD 1** were carried out in four different aqueous media: 1 N HCl, buffer pH 2.5, buffer pH 5 and buffer pH 7.4. Under the experimental conditions the two antibacterial prodrugs intraconverted to release the parent drugs (Figures 13 and 14) as was determined by HPLC analysis. For both amoxicillin and cephalixin prodrugs, at constant temperature and pH the hydrolysis reaction displayed strict first order kinetics as the k_{obs} was quite constant and a straight line was obtained on plotting log concentration of residual prodrug versus time. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$) for amoxicillin **ProD 1** and cephalixin **ProD 1** in the different media were calculated from the linear regression equation obtained from the correlation of log concentration of the residual prodrug versus time. The kinetic data for amoxicillin **ProD 1** and cephalixin **ProD 1** are listed in Tables 2 and 3, respectively. It is worth noting that 1N HCl, pH 2.5 and pH 5 were selected to examine the intraconversion of amoxicillin **ProD 1** and cephalixin **ProD 1** 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 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 both, amoxicillin **ProD 1** and cephalixin **ProD 1** was found to be much higher in 1N HCl than at pH 2.5 and 5 (Figures 13 and 14). At 1N HCl the $t_{1/2}$ values for the intraconversion of amoxicillin **ProD 1** and cephalixin **ProD 1** were about 2.5 hours. On the other hand, at pH 7.4, both prodrugs amoxicillin **ProD 1** and cephalixin **ProD 1** were quite stable and no release of the parent drugs was observed. At pH 5 the hydrolysis of both prodrugs amoxicillin **ProD 1** and cephalixin **ProD 1** was too slow. This is because the pK_a of amoxicillin **ProD 1** and

cephalexin **ProD 1** is in the range of 3-4, it is expected that at pH 5 the anionic form of the prodrug will be dominant and the percentage of the free acidic form that undergoes an acid-catalyzed hydrolysis will be relatively low. At 1N HCl and pH 2.5 most of the prodrug will exist as the free acid form and at pH 7.4 most of the prodrug will be in the anionic form. Thus, the discrepancy in rates at the different pH buffers.

Figure 13. First order hydrolysis plot of amoxicillin **ProD 1** in (a) 1N HCl, (b) buffer pH 2.5 and (c) buffer pH 5.

$t_{1/2}$ (h)	k_{obs} (h^{-1})	Medium
2.5	2.33×10^{-4}	1 N HCl
7	9.60×10^{-5}	Buffer pH 2.5
81	7.55×10^{-6}	Buffer pH 5
----	No reaction	Buffer pH 7.4

Table 2. The observed k value and $t_{1/2}$ of amoxicillin **ProD 1** in 1N HCl and at pH 2, 5 and 7.4

$t_{1/2}$ (h)	k_{obs} (h ⁻¹)	Medium
2.4	2.41×10^{-4}	1 N HCl
14	4.17×10^{-5}	Buffer pH 2.5
---	No reaction	Buffer pH 5.5
---	No reaction	Buffer pH 7.4

in 1N HCl and at pH 2, 5 and 7.4

Table 3. The observed k value and $t_{1/2}$ of cephalixin ProD 1

Figure 14. First order hydrolysis plot of cephalixin ProD 1 in (a) 1N HCl, (b) buffer pH 2.5 and (c) buffer pH 5.

13. Mechanistic study of Bruce’s hydrolysis of di-carboxylic semi-esters 43-47 used for the design of bitterless paracetamol prodrugs

Five decades ago, Bruce and Pandit have investigated the kinetics of for the hydrolysis reaction of di-carboxylic semi-esters **43-47** depicted in Figure 15 [64,65]. Their findings revealed the relative rate (k_{rel}) for **47>46>45>44>43**. They attributed the discrepancy in rates to differences in the proximity orientation of the nucleophile to electrophile. Using the observation that alkyl substituent on succinic acid influences rotamer distributions, the ratio between the reactive gauche and the unreactive anti-conformers, they proposed that *gem*-dialkyl substitution increased the probability of the resultant rotamer adopting the more reactive conformation. Hence, for ring-closing reaction to precede, the two reacting centers, the nucleophile and electrophile, must be in the gauche conformation. In the unsubstituted reactant, the nucleophile and electrophile are almost entirely in the anti-conformation in order to minimize steric interactions [81-82]. In order to design paracetamol prodrugs, *via* linking the active drug with a di-carboxylic semi-ester linker (Bruce’s enzyme model), lacking the bitterness of their parent drug, paracetamol, and have the capability to chemically and not enzymatically undergo

hydrolysis in physiological environment we have unraveled the mechanism for the ring-closing reaction of **43-47** using DFT and molecular mechanics calculation methods [93].

Quantum molecular mechanics using DFT methods at B3LYP 6-31G (d,p) and B3LYP/311+G (d,p) levels were exploited to calculate the thermodynamic and kinetic parameters for all reactants, transition states, intermediates and products involved in the proposed mechanism for process **43-47** (Figure 16). As shown in Figure 16 the mechanism for these processes consists of two steps; (1) formation of a tetrahedral intermediate and (2) collapse of a tetrahedral intermediate to furnish a cyclic anhydride and p-bromophenolate anion.

Figure 15. Hydrolysis of di-carboxylic semi-esters **43-47**.

The phenomenon of rate enhancements in several intramolecular processes was ascribed by Bruice and Menger to the importance of the proximity of the nucleophile to the electrophile of the ground state molecules [64,65,155]. Menger in his “spatiotemporal” hypothesis advocated a mathematical equation correlating activation energy to distance and based on this that, he came to the conclusion that enormous rate accelerations in reactions catalyzed by enzymes are feasible when imposing short distances between the reactive centers of the substrate and enzyme [155]. Differently from Menger, Bruice attributed the catalysis by enzymes to favorable ‘near attack conformations’; systems that have a high quota of near attack conformations will have a higher intramolecular reaction rate and *vice versa*. Bruice’s idea invokes a combination of distance between the two reacting centers and the angle of attack by which the nucleophile approaches the electrophile [64,65].

In contrast to the proximity orientation proposal, others proposed the high rate enhancements in intramolecular processes to steric effects (relief of the strain energy of the reactant) [156].

To test whether the acceleration in rates for processes **43-47** (Figure 15) is a result of proximity orientation or due to steric effects (difference in strain energies of the reactants), the strain energy values for the reactants and the intermediates in systems **43-47** were calculated using Allinger’s MM2 method. The calculated strain energy values for **43-47** were correlated with

Figure 16. Proposed mechanism for the hydrolysis of di-carboxylic semi-esters **43-47**.

the corresponding experimental relative rates ($\log k_{\text{rel}}$) [64,65]. The results demonstrated good correlation between the two parameters. On the other hand, attempts to correlate the distance between the two reactive centers and $\log k_{\text{rel}}$ failed to provide any correlation between the two parameters. This reveals that the driving force for acceleration in rates of **43-47** is driven by strain effects and not proximity orientation stemming from Bruice's near attack conformation [64,65]. In addition, in accordance with Bruice and Pandit's findings [64,65] we have found that the ring-closing reactions proceed by one mechanism, by which the rate-limiting step is the tetrahedral intermediate dissociation and not its formation.

14. Paracetamol Prodrugs Based on Bruice's Enzyme Model

Paracetamol is an odorless, bitter crystalline compound used as an over the counter analgesic and anti-pyretic drug. Paracetamol is used to relief minor aches. it is used as pain killer by decreasing the synthesis of prostaglandin due to inhibiting cyclooxygenases (COX-1 and COX-2). Paracetamol is favored over aspirin as pain killer in patients have excessive gastric secretion or prolonged bleeding. It was approved to be used as fever reducer in all ages. Pharmacokinetic studies have shown that urine of patients who had taken phenacetin contained paracetamol. Later was demonstrated that paracetamol was a urinary metabolite of acetanilide. Phenacetin known historically to be one of the first non-opioid analgesics without anti-inflammatory properties lacks or has a very slight bitter taste [157,158]. Comparison of the structures of paracetamol and phenacetin shows that shows close similarity between both analgesics except of the nature of the group on the *para* position of the benzene ring. While in

paracetamol the group is hydroxyl, in phenacetin it is ethoxy. On the other hand, acetanilide has a chemical structure similar to that of paracetamol and phenacetin but it lacks any group at the *para* position of the benzene ring. Acetanilide lacks the bitter taste characteristic for paracetamol. The comparisons of the three compounds might suggest that the presence of hydroxy group on the *para* position of the benzene ring plays a major role for paracetamol bitterness. Therefore, it is expected that masking the hydroxyl group in paracetamol with a suitable linker could inhibit the binding of paracetamol to its bitter taste receptor/s and hence masking its bitterness. It is likely that paracetamol binds to the active site of its bitter taste receptor via hydrogen bonding interactions by which its phenolic hydroxyl group is engaged. It is worth noting that linking paracetamol with Bruice's enzyme model linker *via* its phenolic hydroxyl group might hinder paracetamol bitter taste.

Based on the DFT calculations on the cyclization of Bruice's **43-47** (Figure 15), two paracetamol prodrugs were proposed (Figure 17). As shown in Figure 17, the paracetamol prodrugs, **ProD 1-2**, have a carboxylic acid group as a hydrophilic moiety and the rest of the prodrug, acetanilide, as a lipophilic moiety, where the combination of both groups provides a moderate HLB. It should be noted that the HLB value will be determined upon the physiologic environment by which the prodrug is dissolved. For example, in the stomach, the paracetamol prodrugs will primarily exist in the carboxylic acid form whereas in the blood circulation the carboxylate anion form will be predominant. Since Bruice's cyclization reaction occurs in basic medium paracetamol **ProD 1-2** were obtained as carboxylic free acid form, since this form is expected to be stable in acidic medium such as the stomach.

Figure 17. Hydrolysis of paracetamol ProD 1 and paracetamol ProD 2.

15. In vitro intraconversion of Paracetamol ProD 1 to the parent drug paracetamol

The hydrolysis of paracetamol **ProD 1** was studied in four different media; 1N HCl, buffer pH 3, buffer pH 6.6 and buffer pH 7.4. The prodrug hydrolysis was monitored using HPLC analysis. At constant pH and temperature the release of paracetamol from its prodrug was followed and showed a first order kinetics. k_{obs} (h^{-1}) and $t_{1/2}$ values for the intraconversion of paracetamol **ProD 1** was calculated from regression equation obtained from plotting log concentration of residual of paracetamol **ProD 1** vs. time. The kinetics results in the different media are summarized in Table 4 and Figure 18.

Medium	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1N HCl	No reaction	No reaction
Buffer pH 3	6.3×10^{-5}	3
Buffer pH 7.4	6.1×10^{-4}	0.3

Table 4. The observed k value and $t_{1/2}$ of paracetamol **ProD 1** In 1N HCl and buffers pH 3 and 7.4.

As shown in Table 4 the hydrolysis rate of paracetamol **ProD 1** at pH 7.4 was the fastest among all media, followed by pH 6.6 medium. In 1N HCl no conversion of the prodrug to the parent dug was observed.

Figure 18. First order hydrolysis plot of paracetamol **ProD 1** in (a) buffer pH 3 and (b) buffer pH 7.4.

At pH 7.4 and 6.6 paracetamol **ProD 1** is mainly exists as the carboxylate anion form which is expected to undergo fast hydrolysis according to Bruice’s mechanism shown in Figure 16. At pH 3, the prodrug exists in both form, the carboxylate anion and the carboxylic free acid forms since the pKa of the prodrug is about 3. In 1N HCl, the prodrug is entirely exists as the

carboxylic free acid form and since only the carboxylate anion form undergoes Bruice's cyclization the hydrolysis rate in 1N HCl is almost negligible or zero.

16. Conclusions and future directions

The quantum mechanics (QM) calculations in different methods revealed that the acid-catalyzed hydrolysis efficiency of processes **34-42**, atenolol **ProD 1-ProD 2**, amoxicillin **ProD1** and cephalexin **ProD 1** is significantly sensitive to the pattern of substitution on the carbon-carbon double bond and nature of the amine leaving group. The linear correlation found between the reaction rate and strain energy difference between the intermediate and the reactant (E_s INT-GM) supports the notion that the reaction is governed by strain effects. Furthermore, the linear correlation of the calculated DFT and experimental EM values reinforce the credibility of using DFT methods for energy and rate predictions for the kind of processes reported in this section.

Comparisons of the calculated DFT properties for processes **34-40** and atenolol prodrugs **ProD1-ProD2** with the calculated DFT properties for the acid-catalyzed hydrolysis of acyclovir prodrugs and cefuroxime (Figure 19) demonstrate that while for processes **34-40** and atenolol prodrugs **ProD 1-ProD 2**, the rate-limiting step was the collapse of the tetrahedral intermediate in the processes of cefuroxime prodrugs and acyclovir prodrugs the rate-limiting step was the tetrahedral intermediate formation. This is might attributed to the nature of the amine leaving group involved in the tetrahedral intermediate collapse step.

Figure 19. Chemical structures for acyclovir and cefuroxime prodrugs.

Comparison of the calculated $t_{1/2}$ value (63.2 hours) for atenolol **ProD 1** to the experimental value (3.82 hours) indicates that while the B3LYP/6-31G (d,p) value is overestimated (about 17

times larger than the experimental) the one obtained by mpwpw91/6-31+G(d,p) was much more closer (6.3 hours). The discrepancy between the calculated B3LYP/6-31G (d,p) and experimental values might be attributed to (i) B3LYP/6-31 G(d,p) is a DFT method without dispersion corrections and (ii) PCM solvation model (calculations in presence of solvent) is not capable of handling calculations in acidic aqueous solvent.

The experimental $t_{1/2}$ value for atenolol **ProD 1** at pH 5 was 133 hours and at pH 7.4 no hydrolysis was observed. The lack of the hydrolysis at the latter pH might be due to the fact that at this pH atenolol **ProD 1** exists mainly in the ionized form (pK_a about 3-4). As mentioned before the free acid form is a mandatory requirement for the acid-catalyzed hydrolysis to proceed.

In a similar manner to that observed in the intraconversion of atenolol **ProD 1**, the acid-catalyzed hydrolysis of both, amoxicillin **ProD 1** and cephalixin **ProD 1** was much faster in 1N HCl than in pH 2.5 and 5 (Figures 13 and 14). At 1N HCl the $t_{1/2}$ values for the intraconversion of amoxicillin **ProD 1** and cephalixin **ProD 1** was in both cases about 2.5 hours. On the other hand, in pH 7.4, both amoxicillin **ProD 1** and cephalixin **ProD 1** were entirely stable and no intraconversion to the parent drugs was detected. The salient points emerged from our study on Bruice's system are as follows: (i) the cyclization rate of Bruice's system was found to be dependent on the difference in the strain energies of the intermediate and reactant, and no relationship was found between the reaction rate and the distance between the nucleophile and the electrophile. (ii) The reactions of strained di-carboxylic semi-esters are more efficient than the less strained ones, and the reactivity extent was linearly correlated with the strain energy difference between the intermediate and reactant. (iii) The activation energy required to give a stable transition state for a strained di-carboxylic semi-ester is less than that for the unstrained semi-ester, since the conformational change from the reactant to the transition state in the former is smaller, and (iv) based on the linearity found between the relative rate, the activation energy and the difference in strain energies of the intermediate and reactant for Bruice's di-carboxylic semi-esters we have proposed two paracetamol prodrugs, which were synthesized and their in vitro kinetics was studied. Future strategy to achieve more efficient atenolol prodrugs capable of increasing the liquid formulation stability, eliminating atenolol bitterness and releasing the parent drug in a programmable manner is synthesis of atenolol prodrugs having pK_a around 6 (intestine pH). At the pH of the intestine the planned prodrugs will exist mainly in the acidic form which has the capability to undergo an acid-catalyzed hydrolysis to provide the active drug, atenolol.

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References

- [1] Sohi H, Sultana Y, Khar RK. Taste Masking Technologies in oral pharmaceuticals, recent development and approaches. *Drug Develop. Ind. Pharm.* 2004;30(5): 429-448.
- [2] Reilly WJ. *Pharmaceutical necessities in Remington: The science and practice of pharmacy*, Mack Publishing Company; 2002. p 1018-1020.
- [3] Gowan Jr. WG, Richard DB. Aliphatic esters as a solventless coating for pharmaceuticals. No. CA2082137. 19 Aug. 2003.
- [4] Gowthamarajan K, Kulkarni G T, Kumar MN. Pop the pills without bitterness. *Resonance* 2004;9(12): 25-32.
- [5] Davis JD. *Drug Cosmet. India: Encyclopedia of pharmaceutical technology* 2000;2: 1-5.
- [6] Bakan JA. *Microencapsulation, Theory and practice of Industrial Pharmacy*, Third Edition 1986. p 412-429.
- [7] Ikeda K. New seasonings. *Chemical senses* 2002;27(9): 847-849.
- [8] Maehashi K, Matano M, Wang H, Vo LA, Yamamoto Y, Huang L. Bitter peptides activate hTAS2Rs, the human bitter receptors. *Biochemical and biophysical research communications* 2008;365(4): 851-855.
- [9] Lindemann B. Receptors and transduction in taste. *Nature* 2001;413(6852): 219-225.
- [10] Behrens M, Meyerhof W. Bitter taste receptors and human bitter taste perception. *Cell Mol. Life Sci.* 2006;63: 1501-1509.
- [11] Meyerhof W, Born S, Brockhoff A, Behrens M. Molecular biology of mammalian bitter taste receptors. A review. *Flavour Frag. J.* 2011;26: 260-268.
- [12] Behrens M, Meyerhof W. Mammalian bitter taste perception. *Results Probl. Cell. Differ.* 2009;47: 203-220.

- [13] Brockhoff A, Behrens M, Massarotti A, Appendino G, Meyerhof W. Broad tuning of the human bitter taste receptor hTAS2R46 to various sesquiterpene lactones, clerodane and labdane diterpenoids, strychnine, and denatonium. *J. Agric. Food Chem.* 2007;55: 6236–6243.
- [14] Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G, Behrens M. The molecular receptive ranges of human TAS2R bitter taste receptors. *Chem. Senses* 2010;35: 157–170.
- [15] Wiener A, Shudler M, Levit A, Niv MY. Bitter DB: a database of bitter compounds. *Nucleic acids research* 2012;40(D1): D413-D419.
- [16] Bufe B, Hofmann T, Krautwurst D, Raguse JD, Meyerhof W. The human TAS2R16 receptor mediates bitter taste in response to beta-glucopyranosides. *Nat. Genet.* 2002;32: 397–401.
- [17] Sainz E, Cavenagh MM, Gutierrez J, Battey JF, Northup JK, Sullivan SL. Functional characterization of human bitter taste receptors. *Biochem. J.* 2007;403: 537–543.
- [18] Sakurai T, Misaka T, Ishiguro M, Masuda K, Sugawara T, Ito K, Kobayashi T, Matsuo S, Ishimaru Y, Asakura T. et al. Characterization of the beta-D-glucopyranoside binding site of the human bitter taste receptor hTAS2R16. *J. Biol. Chem.* 2010;285: 28373–28378.
- [19] Hunter G, Wayne ED, Timothy DN, Peter S, Alicia E, Gary G. Pseudoephedrine is without ergogenic effects during prolonged exercise. *Journal of Applied Physiology* 1996;81: 2611–2617.
- [20] Malladi M, Jukanti R, Nair R, Wagh S, Padakanti H, Matet A. Design and Evaluation of Taste Masked Dextromethorphan Hydrobromide Oral Disintegrating Tablets. *Acta Pharmaceutica* 2010; 60: 267–280.
- [21] Schwabe U, Ukena D, Lohse MJ. Xanthine derivatives as antagonists at A1 and A2 adenosine receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1985;330(3): 212–21.
- [22] Mycek MJ, Harvey RA, Champe RC. *Lippincott's illustrated reviews pharmacology*. Philadelphia: Lippincott-Raven; 1997.
- [23] Grover R, Frank ME. Regional Specificity of Chlorhexidine Effects on Taste Perception; *Chem Senses* 2008;33(4): 311-318.
- [24] Stokbroekx RA, Vanenberk J, Van Heertum AHMT, Van Laar GMLW, Van der Aa MJMC, Van Bever WFM, Janssen PAJ. Synthetic Antidiarrheal Agents. 2,2-Diphenyl-4-(4'-aryl-4'-hydroxypiperidino)butyramides. *Journal of Medicinal Chemistry* 1973;16(7): 782–786.

- [25] Thompson D, Oster G. Use of Terfenadine and Contraindicated Drugs. *Journal of the American Medical Association (American Medical Association)* 1996;275 (17): 1339–1341.
- [26] Rossi S. *Australian Medicines Handbook*. AMH.ISBN 0-9578521-4-2; 2004.
- [27] Karaman R. Computationally Designed Prodrugs for Masking the Bitter Taste of Drugs. *Journal of Drug Designing* 1:e106. doi:10.4172/2169-0138.1000e106; 2012.
- [28] Karaman R. “A Solution to Aversive Tasting Drugs for Pediatric and Geriatric Patients” *Journal of Drug Designing*, 2013, *Drug Des*, DOI:10.4172/2169-0138.1000e116; 2013.
- [29] Karaman R. “The Future of Prodrugs designed by Computational Chemistry” *Journal of Drug Designing*, 2012, 1:e103. doi:10.4172/ddo.1000e103; 2012.
- [30] Ayenew Z, Puri V, Kumar L, Bansal AK. Trends in Pharmaceutical Taste Masking Technologies: A Patent Review. *Recent Patents on Drug Delivery & Formulation* 2009;3: 26-39.
- [31] Fawzy AA. Pleasant Tasting Aqueous Liquid Composition of a Bitter-Tasting Drug, *PCT Int. Appl.* WO9805312, 2; 1998.
- [32] Zelalem A, Puri V, Kumar L, Bansal A. Trends in Pharmaceutical Taste Masking Technologies: A Patent Review. *Recent Patents on Drug Delivery & Formulation* 2009;3, 26-39.
- [33] Jain NK. *Advances in controlled and Novel Drug delivery* 2001; 1stEd, 290-306.
- [34] Bress WS, Kulkarni N, Ambike S, Ramsay MP. EP1674078; 2006.
- [35] Mendes WR. *Theory and practice of Industrial pharmacy*, Third Edition; 1976.
- [36] Redondo AMJ, Abanades LB. WO047550; 2003.
- [37] Kashid N, Chouhan P, Mukherji G. WO2007108010; 2007.
- [38] Burgard A. JP026665; 2003.
- [39] Lachman L, Liberman HA. *Pharmaceutical Dosage Forms:Tablet”, Volume I*; 1989.p 11-14.
- [40] Rao MY, Bader F. Masking the taste of chloroquine by Multiple Emulsion. *The East. Pharm.* 1993;123: 11.
- [41] Kasturagi Y. Selective inhibition of bitter taste of various drugs by lipoprotein. *Pharm. Res.* 1995;12(5): 658-662.
- [42] Brahmankar DM., Jaiswal SB. *Biopharmaceutics & Pharmaceutics*. First Edition; 1995:pp 162-163.

- [43] Parr RG, Yang W. Density Functional Theory of Atoms and Molecules. Oxford University Press, Oxford; 1989.
- [44] Ley JP. Masking bitter taste by molecules. Chem. Precept. 2008;1: 58-77.
- [45] Chandreshekar J, Mueller K, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJP. T2Rs function as bitter taste receptor. Cell (Cambridge, Mass.) 2000;100: 703-711.
- [46] Scotti L, Scotti MT, Ishiki HM, Ferreira MGP, Emerenciano VP, Menezes CMS, Ferreira EI. Quantitative elucidation of the structure-bitterness relationship of cynaropicrin and grosheimin derivatives. Food Chem. 2007;105: 77-83.
- [47] Kirby AJ. Efficiency of proton transfer catalysis in models and enzymes. Acc Chem. Res 1997; 30:290– 296.
- [48] Karaman R. Prodrugs of Aza Nucleosides Based on Proton Transfer Reactions. J. Comput. Mol. Des. 2010;24: 961-970.
- [49] Karaman R, Amly W, Scrano L, Mecca G, Bufo SA. Computationally designed prodrugs of statins based on Kirby's enzyme model. Journal of molecular modeling 2013; 1-14. DOI 10.1007/s00894-013-1929-2.
- [50] Kirby AJ, Hollfelder F. From Enzyme Models to Model Enzymes, RSC Publishing, Cambridge UK; 2009. p 1-273.
- [51] Karaman R, Ghareeb H, Dajani KK, Scrano L, Hallak H, Abu-Lafi S, Bufo SA. Design, synthesis and in-vitro kinetic study of tranexamic acid prodrugs for the treatment of bleeding conditions. J. Molecular Aided Computer Design 2013; 27(7): 615-635.
- [52] Karaman R, Dajani KK, Qtait A, Khamis M. Prodrugs of Acyclovir-A Computational Approach. Chemical Biology and Drug Design 2012;79: 819-834.
- [53] Menger FM, Ladika M. Fast hydrolysis of an aliphatic amide at neutral pH and ambient temperature. A peptidase model. J Am Chem Soc 1988;110: 6794-6796
- [54] Karaman R. Computational aided design for dopamine prodrugs based on novel chemical approach. Chem. Biol. Drug Design 2011;78: 853-863.
- [55] Karaman R, Dokmak G, Bader M, Hallak H, Khamis M, Scrano L, Bufo SA. Prodrugs of fumarate esters for the treatment of psoriasis and multiple sclerosis (MS)-A computational approach. J. Molecular Modeling 2013;19: 439-452.
- [56] Karaman R. Prodrugs for Masking Bitter Taste of Antibacterial Drugs-A Computational Approach. J. Molecular Modeling. 2013;19: 2399–2412.
- [57] Blow DM, Birktoff JJ, Hartly BS. Role of a Buried Acid Group in the Mechanism of Action of Chymotrypsin. Nature (London) 1969;221: 337-340.
- [58] Page MI. (Ed.) The Chemistry of Enzyme Action, Elsevier, Amsterdam; 1984.

- [59] Gandour RD, Schowen RL. (Eds.), *Transition States of Biochemical Processes*, Plenum Press, New York; 1978.
- [60] Kirby AJ. *Enzyme Mechanisms, Models, and Mimics*. *Angewandte Chemie International Edition in English* 1996;35 (7): 706–724.
- [61] Nelson DL, Cox MM, *Lehninger Principles of Biochemistry*, Worth Publishers, New York; 2003.
- [62] Fersht A. *Structure and Mechanism in Protein Science: A guide to Enzyme Catalysis and Protein Folding*, W.H. Freeman and Company, New York; 1999.
- [63] Czarnik AW, in: Liebman JF., Greenberg A. (Eds.), *Mechanistic Principles of Enzyme Activity*, VCH Publishers., New York, NY; 1988.
- [64] Bruice TC, Pandit UK. The effect of geminal substitution ring size and rotamer distribution on the intramolecular nucleophilic catalysis of the hydrolysis of monophenyl esters of dibasic acids and the solvolysis of the intermediate anhydrides. *J Am Chem Soc* 1960; 82: 5858–5865.
- [65] Bruice TC, Pandit UK. Intramolecular models depicting the kinetic importance of “Fit” in enzymatic catalysis. *Proc Natl Acad Sci U S A* 1960;46: 402–404.
- [66] Milstein S, Cohen LA. Concurrent general-acid and general-base catalysis of esterification. *J Am Chem Soc* 1970;92: 4377–4382.
- [67] Milstein S, Cohen LA. Rate acceleration by stereopopulation control: models for enzyme action. *Proc Natl Acad Sci USA* 1970;67: 1143–1147.
- [68] Milstein S, Cohen LA. Stereopopulation control I. Rate enhancement in the lactonizations of o-hydroxyhydrocinnamic acids. *J Am Chem Soc* 1972;94: 9158–9165.
- [69] Menger FM, Ladika M. Remote enzyme-coupled amine release. *J Org Chem* 1990;35: 3006–3007.
- [70] Menger FM. On the source of intramolecular and enzymatic reactivity. *Acc Chem Res* 1985;18: 128–134.
- [71] Menger FM, Chow JF, Kaiserman H, Vasquez PC. Directionality of proton transfer in solution. Three systems of known angularity. *J Am Chem Soc* 1983;105: 4996–5002.
- [72] Menger FM. Directionality of organic reactions in solution. *Tetrahedron* 1983;39: 1013–1040.
- [73] Menger FM, Grossman J, Liotta DC. Transition-state pliability in nitrogen-to-nitrogen proton transfer. *J Org Chem* 1983;48: 905–907.
- [74] Menger FM, Galloway AL, Musaev DG. Relationship between rate and distance. *Chem Commun* 2003: 2370–2371.

- [75] Menger FM. An alternative view of enzyme catalysis. *Pure Appl Chem* 2005;77: 1873–1876.
- [76] Brown RF, van Gulick NM. The geminal alkyl effect on the rates of ring closure of bromobutylamines. *J Org Chem* 1956;21: 1046-1049.
- [77] Galli C, Mandolini L. The role of ring strain on the ease of ring closure of bifunctional chain molecules. *Eur J Org Chem* 2000: 3117-3125.
- [78] Barber SE, Dean KES, Kirby AJ. A mechanism for efficient proton-transfer catalysis. Intramolecular general acid catalysis of the hydrolysis of 1-arylethyl ethers of salicylic acid. *Can. J. Chem.* 1999;77(5-6): 792-801.
- [79] Kirby AJ, de Silva MF, Lima D, Roussev CD, Nome F. Efficient intramolecular general acid catalysis of nucleophilic attack on a phosphodiester. *J. Am. Chem. Soc.* 2006;128: 16944-16952.
- [80] Kirby AJ, Williams NH. Efficient intramolecular general acid catalysis of enol ether hydrolysis. Hydrogen-bonding stabilization of the transition state for proton transfer to carbon. *J. Chem. Soc., Perkin Trans. 2* 1994: 643-648.
- [81] Kirby AJ, Williams NH. Efficient intramolecular general acid catalysis of vinyl ether hydrolysis by the neighbouring carboxylic acid group. *J. Chem. Soc. Chem. Commun* 1991:1643-1644.
- [82] Fife TH, Przystas TJ. Intramolecular general acid catalysis in the hydrolysis of acetals with aliphatic alcohol leaving groups. *J. Am. Chem. Soc.* 1979;101: 1202-1210.
- [83] Kirby AJ. effective molarities for intramolecular reactions. *J. Phys. Org. Chem.* 2005;18: 101-278.
- [84] Kirby AJ., Lancaster PW. Structure and efficiency in intramolecular and enzymatic catalysis. Catalysis of amide hydrolysis by the carboxy-group of substituted maleamic acids. *J. Chem. Soc., Perkin Trans. 2* 1972; 1206-1214.
- [85] Karaman R. Analysis of Menger's spatiotemporal hypothesis. *Tetrahedron Lett* 2008;49: 5998-6002.
- [86] Karaman R. Cleavage of Menger's aliphatic amide: a model for peptidase enzyme solely explained by proximity orientation in intramolecular proton transfer. *J Mol Struct (THEOCHEM)* 2009; 910: 27-33.
- [87] Karaman R. The efficiency of proton transfer in Kirby's enzyme model, a computational approach. *Tetrahedron Lett* 2010;51: 2130-2135.
- [88] Karaman R, Pascal RA. Computational Analysis of Intramolecularity in Proton Transfer Reactions. *Org & Bimol Chem* 2010;8: 5174-5178.
- [89] Karaman R. A General Equation Correlating Intramolecular Rates with Attack" Parameters: Distance and Angle. *Tetrahedron Lett* 2010;51: 5185-5190.

- [90] Karaman R. Analyzing the efficiency of proton transfer to carbon in Kirby's enzyme model-a computational approach. *Tetrahedron Lett* 2011;52: 699-704.
- [91] Karaman R. Analyzing the efficiency in intramolecular amide hydrolysis of Kirby's N-alkylmaleamic acids-A computational approach. *Comput Theor Chem* 2011;974: 133-142.
- [92] Karaman R. A New Mathematical Equation Relating Activation Energy to Bond Angle and Distance: A Key for Understanding the Role of Acceleration in Lactonization of the Trimethyl Lock System. *Bioorganic Chemistry* 2009;37: 11-25.
- [93] Karaman R. Revaluation of Bruice's Proximity Orientation. *Tetrahedron Lett.* 2009;50: 452-458.
- [94] Karaman R. Accelerations in the Lactonization of Trimethyl Lock Systems are Due to Proximity Orientation and not to Strain Effects. *Research Letters in Org. Chem.* doi: 10.1155/2009/240253.
- [95] Karaman R. The gem-disubstituent effect-a computational study that exposes the relevance of existing theoretical models. *Tetrahedron Lett.* 2009;50: 6083-6087.
- [96] Karaman R. Analyzing Kirby's amine olefin – a model for amino-acid ammonia lyases. *Tetrahedron Lett.* 2009;50: 7304-7309.
- [97] Karaman R. The Effective Molarity (EM) Puzzle in Proton Transfer Reactions. *Bioorganic Chemistry* 2009;37:106-110.
- [98] Karaman R. Effects of substitution on the effective molarity (EM) for five membered ring-closure reactions-a computational approach. *Journal of Molecular Structure (THEOCHEM)* 2010;939: 69-74.
- [99] Karaman R. The Effective Molarity (EM) Puzzle in Intramolecular Ring-Closing Reactions. *Journal of Molecular Structure (THEOCHEM)* 2010;940: 70-75.
- [100] Karaman R, Fattash B, Qtait A. The Future of Prodrugs-Design By Quantum Mechanics Methods. *Expert Opinion on Drug Delivery* 2013; 10(5): 713-729.
- [101] Karaman R. The Effective Molarity (EM) – a Computational Approach" *Bioorganic Chemistry* 2010;38: 165-172.
- [102] Karaman R. Proximity vs. Strain in Ring-Closing Reactions of Bifunctional Chain Molecules-a Computational Approach. *J. Mol. Phys.* 2010;108: 1723-1730.
- [103] Karaman R. The role of proximity orientation in intramolecular proton transfer reactions. *Journal of Computational and Theoretical Chemistry* 2011;966: 311-321.
- [104] Shan D, Nicolaou MG, Borchardt RT, Wang B. Prodrug strategies based on intramolecular cyclization reactions. *Journal of pharmaceutical sciences* 1997;86(7): 765-767.
- [105] Testa B. Prodrug research: futile or fertile?. *Biochemical pharmacology* 2004;68(11): 2097-2106.

- [106] Ueda Y, Mikkilineni AB, Knipe JO, Rose WC, Casazza AM, Vyas DM. Novel water soluble phosphate prodrugs of Taxol® possessing in vivo antitumor activity. *Bioorganic & Medicinal Chemistry Letters* 1993;3(8): 1761-1766.
- [107] Pauletti GM, Gangwar S, Siahaan TJ, Aubé J, Borchardt RT. Improvement of oral peptide bioavailability: Peptidomimetics and prodrug strategies. *Advanced drug delivery reviews* 1997;27(2): 235-256.
- [108] Gangwar S, Pauletti GM, Siahaan TJ, Stella VJ, Borchardt RT. Synthesis of a novel esterase-sensitive cyclic prodrug of a hexapeptide using an (acyloxy) alkoxy promoiety. *The Journal of Organic Chemistry* 1997;62(5): 1356-1362.
- [109] Reddy MR, Erion MD. *Free Energy Calculations in Rational Drug Design*, Kluwer Academic/Plenum Publishers; 2001.
- [110] Parr RG, Craig DP, Ross IG. Molecular Orbital Calculations of the Lower Excited Electronic Levels of Benzene, Configuration Interaction included. *Journal of Chemical Physics* 1950;18(12): 1561-1563.
- [111] Parr RG. On the genesis of a theory. *Int. J. Quantum Chem.* 1990;37(4): 327-347.
- [112] Chen TC. Expansion of Electronic Wave Functions of Molecules in Terms of 'United-Atom' Wave Functions. *J. Chem. Phys.* 1955;23(11): 2200-2201.
- [113] Dewar MJS, Thiel W. Ground states of molecules. The MNDO method. Approximations and parameters. *J Am Chem Soc* 1977;99: 4899-4907.
- [114] Bingham RC, Dewar MJS, Lo DH. Ground states of molecules. XXV. MINDO/3. An improved version of the MNDO semiempirical SCF-MO method. *J Am Chem Soc* 1975;97: 1285-1293.
- [115] Dewar MJS, Zoebisch EG, Healy EF, Stewart JJP. AM1: A new general purpose quantum mechanical molecular model. *J Am Chem Soc* 1985;107: 3902-907.
- [116] Dewar MJS, Jie C, Yu J. The first of new series of general purpose quantum mechanical molecular models. *Tetrahedron* 1993;49: 5003-5038.
- [117] Burkert U, Allinger NL. *Molecular Mechanics*, American Chemical Society, Washington, DC, ACS Monograph 177; 1982.
- [118] Warshel A, Levitt M. Theoretical studies of enzymatic reactions: dielectric, electrostatic and steric stabilization of the carbonium ion in the reaction of lysozyme, *Journal of Molecular Biology* 1976;103(2): 227-249.
- [119] Field MJ. Simulating enzyme reactions: challenges and perspectives. *Journal of Computational Chemistry* 2002;23(1): 48-58.
- [120] Mulholland AJ. Modelling enzyme reaction mechanisms, specificity and catalysis. *Drug Discovery Today* 2005;10(20): 1393-1402.

- [121] Katagi T. AM1 study of acid-catalyzed hydrolysis of maleamic (4-amino-4-oxo-2-butenic) acids. *J Comp Chem* 1990;11(9): 1094-1100.
- [122] Kluger R, Chin J. Carboxylic acid participation in amide hydrolysis. Evidence that separation of a nonbonded complex can be rate determining. *J Am Chem Soc* 1982;104: 2891-2897.
- [123] Melander APHBE, Stenberg P, Liedholm H, Schersten B, Wåhlin-Boll E. Food-induced reduction in bioavailability of atenolol. *European journal of clinical pharmacology* 1979;16(5): 327-330.
- [124] McAinsh J, Holmes BH, Fitzsimons TJ, Young J. Bioavailability in man of atenolol and chlorthalidone from a combination formulation. *Biopharmaceutics & drug disposition* 1986;7(3): 223-231.
- [125] Standing JF, Tuleu C. Paediatric formulations—Getting to the heart of the problem. *International journal of pharmaceutics* 2005;300(1): 56-66.
- [126] Garner S, West D, Reynolds E. Stability of atenolol in an extemporaneously compounded oral liquid. *American Journal of Hospital Pharmacy* 1994; 51: 508-511.
- [127] Anroop B, Ghosh B, Parcha V, Khanam J. Transdermal delivery of atenolol: effect of prodrugs and iontophoresis. *Curr Drug Delivery* 2009;6: 280-290.
- [128] Foppa T, Murakami FS, Silva MA. Development, validation and stability study of pediatric atenolol syrup. *Pharmazie* 2007;62 (7): 519-521.
- [129] Vergin H, Nitsche V. Oral bioavailability of atenolol. *J Int Med Res* 1989;17 (5): 417-425.
- [130] Shin S-C, Choi J-S. Enhanced bioavailability of atenolol by transdermal administration of the ethylene-vinyl acetate matrix in rabbits. *European Journal of Pharmaceutics and Biopharmaceutics* 2003;56 (3): 439-443.
- [131] Chan K, Swenden J. Pilot study of the short-term physicochemical stability of atenolol tablets stored in a multi-compartment compliance aid. *European Journal of Hospital Pharmacy Science* 2007;13 (3): 60-66.
- [132] Hejaz H, Karaman R, Khamis M. Computer-Assisted Design for paracetamol Masking Bitter Taste Prodrugs. *J Mol Model* 2012;18: 103-114.
- [133] Finn A, Straugun A, Meyer M, Chubb J. Effect of dose and food on the bioavailability of cefuroxime axetil. *Biopharm Drug Dispos.* 1987;8: 519-26.
- [134] Kees FK, Lukassek U, Naber KG, Grobecker H. Comparative investigations on the bioavailability of cefuroxime axetil. *Arzneimittel-Forschung* 1991;41(8): 843-846.
- [135] Patel AR, Vavia PR. Preparation and evaluation of taste masked famotidine formulation using drug/beta-cyclodextrin/polymer ternary complexation approach. *AAPS PharmSci Tech.* 2008;9(2): 544-50.

- [136] Bora D, Borude P, Bhise K. Taste Masking by Spray-Drying Technique. *AAPS PharmSci Tech.* 2008;9(4): 1159-64.
- [137] Yajima T, Nogata A, Demachi M, Umeki N, Itai S, Yunoki N. et al. Particle design for taste-masking using a spray-congealing technique. *Chem Pharm Bull.* 1996;44(1): 187-91.
- [138] Al-Omran MF, Al-Suwayeh SA, El-Helw AM, Saleh SI. Taste masking of diclofenac sodium using microencapsulation. *J Microencapsul.* 2002;19(1): 45-52.
- [139] Shidhaye S, Malke S, Kadam V. Taste masked, orally disintegrating tablet containing microspheres for immediate release. *J Pharm Res.* 2008;1: 225-9.
- [140] Neu HC, Winshell EB. Pharmacological studies of 6 (D(-)-amino-p-hydroxyphenylacetamido) penicillanic acid in humans. *Antimicrob Agents Chemother* 1970;10: 423-426.
- [141] Parr Jr TR, Bryan LE. Non-enzymatic resistance to B-lactam antibiotics and resistance to other cell wall synthesis inhibitions. *Antimicrobial drug resistance.* Academic Press, Inc., New York; 1984;pp 81-111.
- [142] Waxman DJ, Strominger JL. Penicillin-binding proteins and the mechanism of action of beta-lactam antibiotics. *Annu Rev Biochem* 1983;52: 825-69.
- [143] Brogden RN, Speight TM, Avery GS. Amoxycillin: A review of its antibacterial and pharmacokinetic properties and therapeutic use. *Drugs* 1975; 9(2): 88-140.
- [144] Davis JL, Salmon JH, Papich MG. Pharmacokinetics and tissue fluid distribution of cephalexin in the horse after oral and i.v. administration. *J Vet Pharmacol Ther* 2005;28(5): 425-431.
- [145] Tack KJ, Keyserling CH, McCarty J, Hedrick JA. Study of use of cefdinir versus cephalexin for treatment of skin infections in pediatric patients. The Cefdinir Pediatric Skin Infection Study Group. *Antimicrobial agents and chemotherapy* 1997;41(4): 739-742.
- [146] Jacobs MR, Jones RN, Giordano PA. Oral β -lactams applied to uncomplicated infections of skin and skin structures. *Diagnostic microbiology and infectious disease* 2007; 57(3): S55-S65.
- [147] Disney FA, Dillon H, Blumer JL, Dudding BA, McLinn SE, Nelson DB, Selbst SM. Cephalexin and Penicillin in the Treatment of Group A β -Hemolytic Streptococcal Throat Infections. *Archives of Pediatrics & Adolescent Medicine* 1992;146(11): 1324.
- [148] Perry CM, Brogden RN. Cefuroxime axetil. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs.* 1996;52(1): 125-58.
- [149] Han HK, Amidon GL. Targeted prodrug design to optimize drug delivery. *AAPS PharmSci* 2000;2(1): 48-58.

- [150] Sinkula AA, Yalkowsky SH. Rationale for design of biologically reversible drug derivatives: prodrugs. *Journal of pharmaceutical sciences* 1975;64(2): 181-210.
- [151] Beaumont K, Webster R, Gardner I, Dack K. Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. *Current drug metabolism* 2003;4(6): 461-485.
- [152] Amidon GL, Leesman GD, Elliott RL. Improving intestinal absorption of water-insoluble compounds: A membrane metabolism strategy. *Journal of pharmaceutical sciences* 1980;69(12): 1363-1368.
- [153] Ehrnebo M, Nilsson SO, Boréus LO. Pharmacokinetics of ampicillin and its prodrugs bacampicillin and pivampicillin in man. *Journal of pharmacokinetics and Biopharmaceutics* 1979;7(5): 429-451.
- [154] Jones KH. Bioavailability of talampicillin. *Br. Med. J.* 1977;2: 232-233.
- [155] Menger FM, Karaman R. A Singularity Model for Chemical Reactivity. *Eur. J. Chem.* 2010;16: 1420-1427.
- [156] Houk KN, Tucker JA, Dorigo AE. Quantitative modeling of proximity effects on organic reactivity. *Acc. Chem. Res.* 1990;23: 107-113.
- [157] Graham GG, Scott KF. Mechanism of Action of Paracetamol. *American Journal of Therapeutics* 2005;12: 46-55.
- [158] Hoftiezer JW, O'Laughlin JC, Ivey KJ. Effects of 24 hours of aspirin, Buffer in, paracetamol and placebo on normal human gastroduodenal mucosa. *Gut* 1982;23(8): 692-697.