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ANTIBACTERIAL PREDRUGS-FROM 1899 TILL 2015

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ABSTRACT

The predrug (prodrug) term involves chemically modified inert compound which upon an administration releases the active parent drug to elicit its pharmacological response within the body. For many years, the predrug strategy has been extensively developed to solve many unwanted drug properties. This approach has several advantages over conventional drug administration and it has the potential to be quite effective method for the treatment of diseases in the future. In this mini-review we describe a number of antibacterial agents' predrugs, and the ways by which predrug strategy was exploited to overcome many pharmaceutical and pharmacokinetic problems that the parent active antibacterial drugs suffer from such as, low bioavailability by increasing or decreasing lipophilicity, site selectivity for higher absorption and less toxicity, short duration of action to increase patient compliance, rapid metabolism to increase oral bioavailability and masking bitter sensation which is crucial for geriatric and pediatric patient compliance.

KEYWORDS: Predrugs, Prodrugs, Antibacterials predrugs, Antibacterial agents, Predrug chemical approach, Intramolecular process, Bitter sensation.

INTRODUCTION

The prodrug or more precisely "predrug" term was reported for the first time by Albert as a pharmacologically inactive compound which is metabolized to an active form upon administration to the body.[1] Predrugs have been successfully used to modify the physicochemical and pharmacokinetic properties (absorption, distribution, metabolism and excretion, ADME) of drugs and to eliminate or decrease the associated toxicity upon their administration.[2]

Generally, a predrug undergoes chemical and/or enzymatic reaction prior to exert its therapeutic activity.[3] Basically, the use of the term predrug implies a chemical entity in which a non-toxic promoiety is covalently linked to an active drug.[4] This approach is designed to overcome biological and/or biochemical barriers through a chemical approach rather than a physical (formulation) approach.[5-14]

In general, predrugs contain a promoiety (linker) that is cleaved by enzymatic or chemical reactions, while other predrugs liberate their active forms after molecular modification, such as an oxidation or reduction reactions. In other cases, two therapeutically active drugs can be attached together in a single molecule named a "codrug". In these cases, each drug acts as a linker (promoiety) for the other drug. It is mandatory that the predrug should be pharmacologically inactive or weakly active, rapidly converted to its active parent drug and a non-toxic moiety by catalyzed or uncatalayzed chemical reaction.

Antibacterials Predrugs

The first examples of antibacterials predrugs, methenamine and prontosil , were discovered by an accident and their discovery illustrate how serendipity has contributed to predrugs development.[2]

Methenamine was discovered in 1899 by Schering (Germany) as inactive predrug that upon an exposure to the urinary tract releases the antibacterial formaldehyde. This predrug is useful in the treatment of urinary tract infections, when transported to an acidic medium such as the urinary bladder it undergoes protonation which results in a complete breakdown of the molecule to yield ammonia and the antibacterial agent formaldehyde (Figure 1).[15]

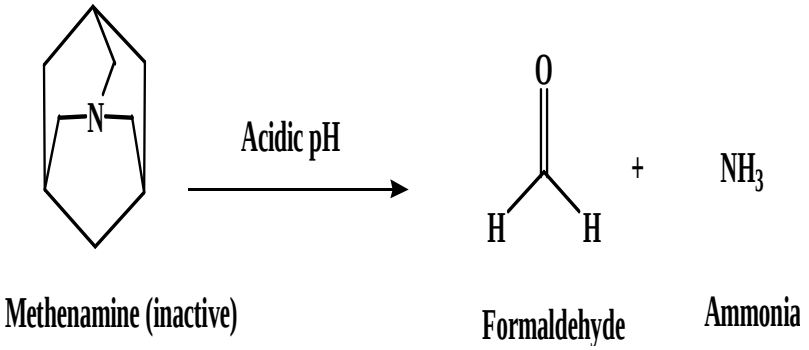

Figure 1. Formation of the antibacterial agent formaldehyde from Methenamine predrug at acidic pH.

Prontosil, the first sulfa drug and a predrug of sulfanilamide was found to be therapeutically effective against microorganisms only in vivo, and not in vitro. Upon administration it is metabolized by the enzyme azo-reductase to provide sulfanilamide (Figure 2).[16]

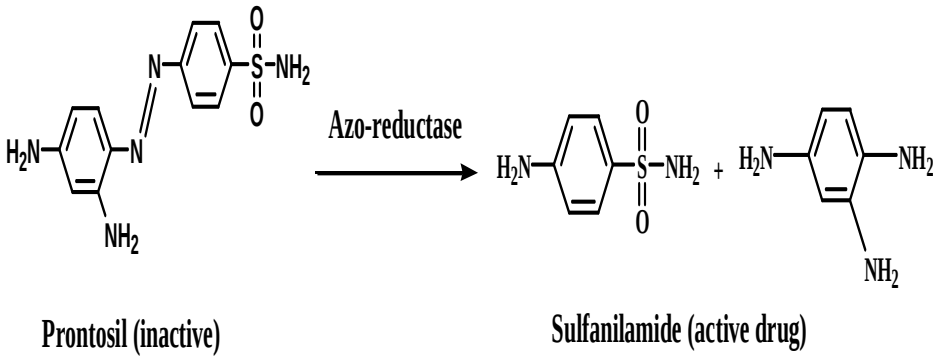

Figure 2. Metabolic activation of Prontosil by azo-reductase.

The predrug approach was not utilized till the mid twentieth century when Parke-Davis Company was intentionally used it for the first time to modify the chemical structure of the antibacterial agent chloramphenicol aiming at improving its bitter taste and poor water solubility.

Two predrugs of chloramphenicol were synthesized by Parke-Davis Company; chloramphenicol sodium succinate with improved water solubility for IV, IM, and ophthalmic administrations, and the bitterless chloramphenicol palmitate formulated into suspension for pediatrics and geriatrics administration (Figure 3).[2, 17]

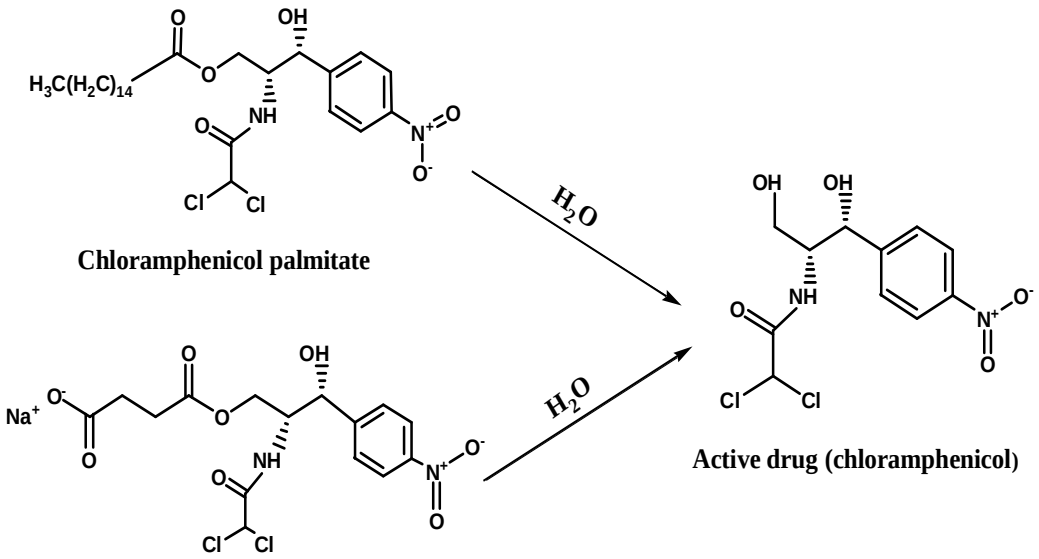

Figure 3. Conversion of chloramphenicol predrugs to their active parent drug, chloramphenicol.

The mutual predrug approach (codrug approach) was utilized in the case of sulfasalazine. Sulfasalazine is a colon selective mutual predrug, of 5-aminosalicylic acid (5-ASA) and sulfapyridine given for the treatment of ulcerative colitis.[18] This mutual predrug was the first sulfa drug to be utilized in inflammatory bowel disease after its development in the mid twentieth century. It consists of 5-ASA attached to sulfapyridine through a diazo bond (Figures 4 and 5). In vivo, the diazo bond is easily cleaved by bacterial azo-reductases found in the colon. Studies on this mutual predrug revealed that 5-ASA moiety was found to be therapeutically active compound, while sulfapyridine is believed to act solely as a carrier moiety to deliver 5-ASA to the affected area of the lower gastrointestinal tract.[19, 20] This approach has a significant advantage since the mutual predrug undergoes a metabolic cleavage to provide 5-ASA prior to its absorption, thus preventing its systemic absorption and aids in concentrating the active parent drug at the site of action. Although sulfapyridine has proved to be an excellent targeting carrier for 5-ASA to the colon, it gave rise to many adverse effects as a result of its systemic toxicity.

Due to this disadvantage, another interesting mutual predrug of 5-ASA, olsalazine, has been emerged. Olsalazine (OSZ) is a dimer of 5-ASA, where a molecule of 5-ASA is attached to another similar molecule via an azo linkage. When this dimer reaches the large intestine, it undergoes cleavage to furnish two molecules of 5-ASA for every molecule of olsalazine

administered (Figure 5). This strategy completely eliminates the side effects associated with sulfasalazine, targets 5-ASA to the colon, and improves the bioavailability of 5-ASA.

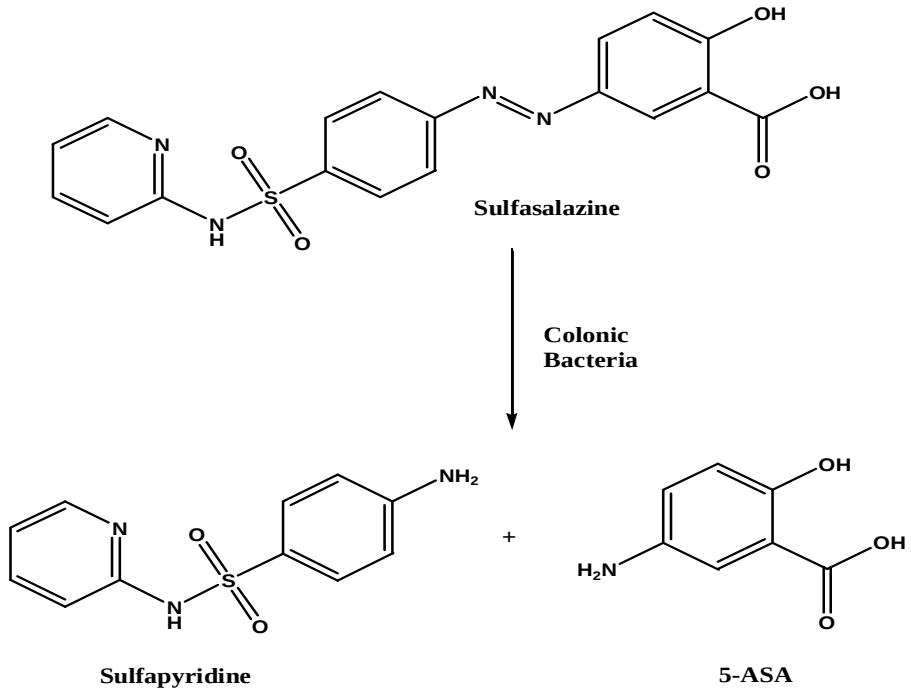

Figure 4. Metabolic conversion of the mutual predrug, sulfasalazine, to sulfapyridine and 5-ASA.

Balsalazine was also synthesized by linking 5-ASA and 4-aminobenzoyl-b-alanine (Figure 5) which has shown good efficiency with fewer side effects than SASP.[21]

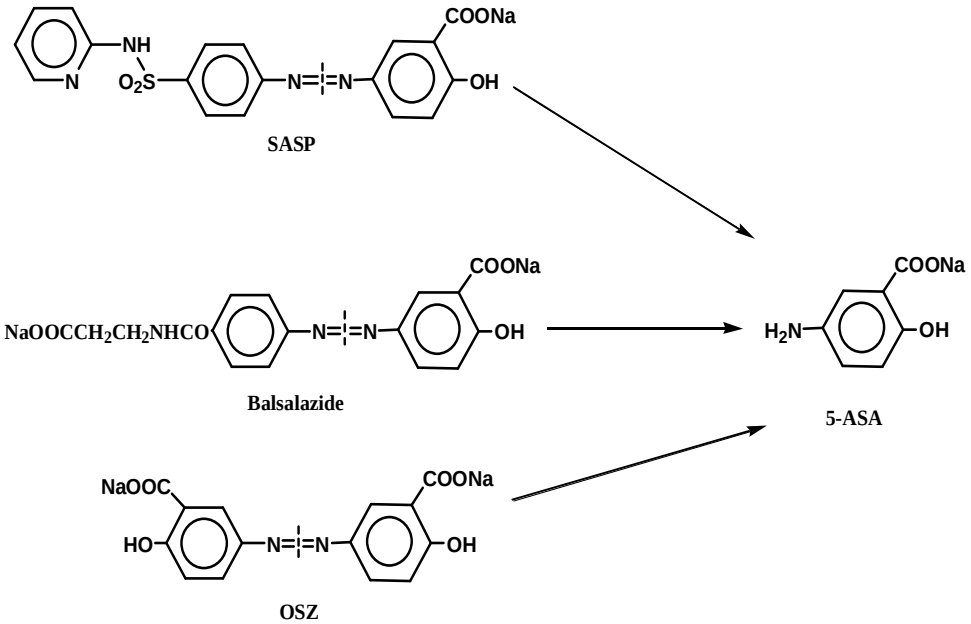

Figure 5: Another azo bond containing predrugs OSZ (2 units of 5-ASA linked together) and balsalazine (5-ASA linked to 4-aminobenzoyl-b-alanine).

A large number of carrier linkage predrugs are designed to be activated by metabolic esterases. A variety of esterases is distributed throughout the whole body and differs in their specificity.

The most important group of enzymes involved in ester bioactivation is carboxylesterases (CESs). This kind of enzymes is a multi-gene whose genes are localized in the endoplasmic reticulum (ER) of different tissues. The CESs catalyze the hydrolysis of a variety of ester- and amide-containing predrugs to the corresponding parent drugs. CESs show ubiquitous tissue expression profiles with the highest levels of CESs activity present in the liver microsomal site.[21] Therefore, the potential for their substrates to become involved in drug-drug interactions is expected to be negligible.[22] Example of antibacterial predrug that is activated by this type of esterases is pivampicillin (Figure 6).

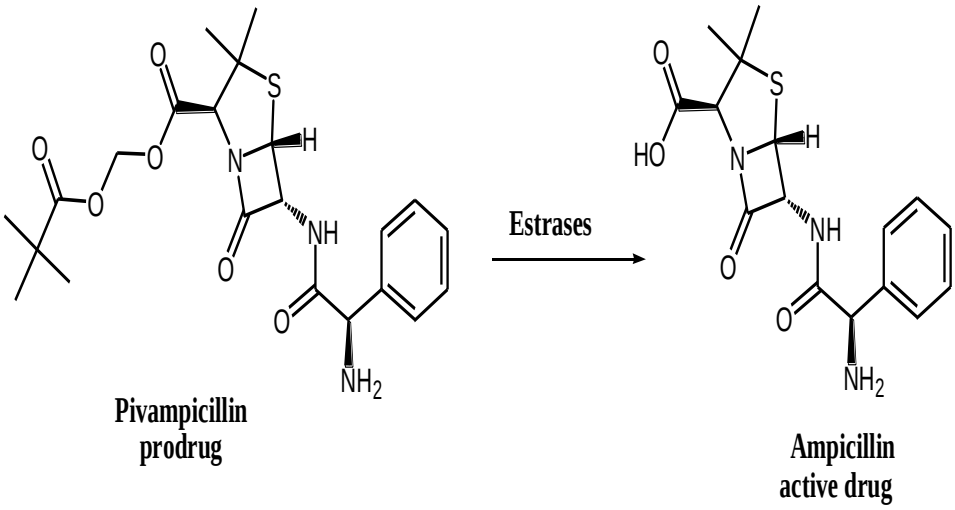

Figure 6: Activation of pivampicillin via carboxylesterase.

Other examples of antibacterials predrugs that were obtained to improve oral bioavailability and clinical profile for their parent active forms are carbenicillin, carfecillin (phenyl ester) and carindacillin (indanyl ester) (Figure 7).[23]

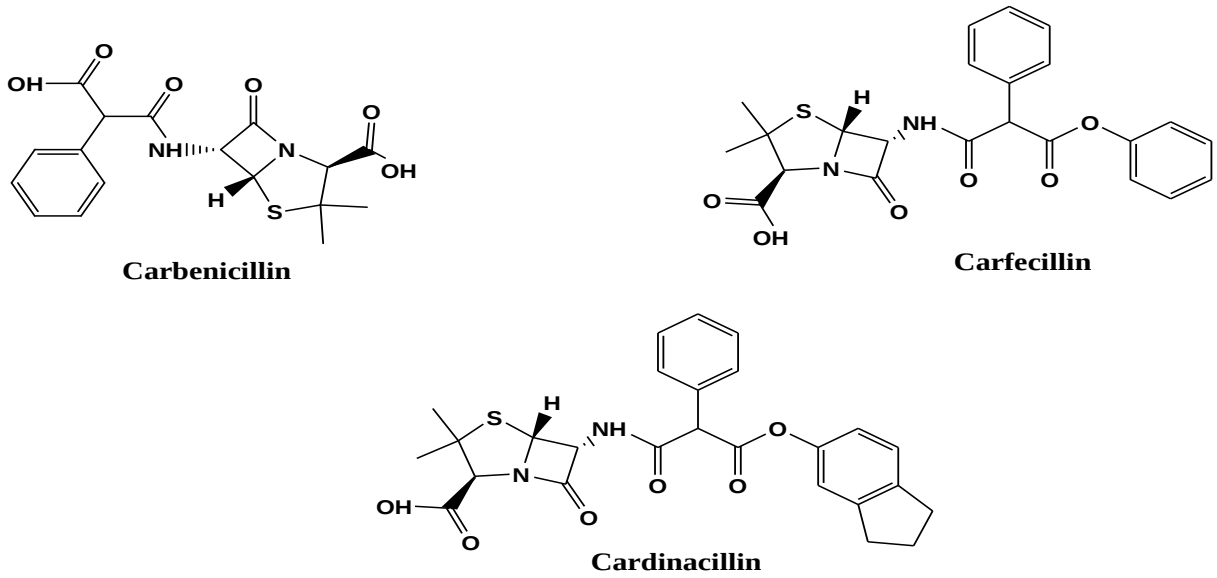

Figure 7: Chemical structures of carbenicillin, carfecillin and cardinacillin.

Acyloxy and alkyl or [(alkoxycarbonyl) oxy] methyl esters are another class of carboxylic acid esters. Practically, acyloxy and alkyl [(alkoxycarbonyl) oxy] methyl esters both are cleaved in vivo in an efficient manner.[2] Acyloxyalkyl esters of benzyl penicillin are enzymatically cleaved in a fast manner into their active parent drugs. The rapid cleavage rate is due to the spacing provided by the acyloxycarbonyl linker.[24] On the other hand, alkyl ester predrugs of other β-lactam antibacterial agents are slowly converted into their active parent drugs due to the crowding surroundings the carbonyl group contained in their structures (Figure 8).[2]

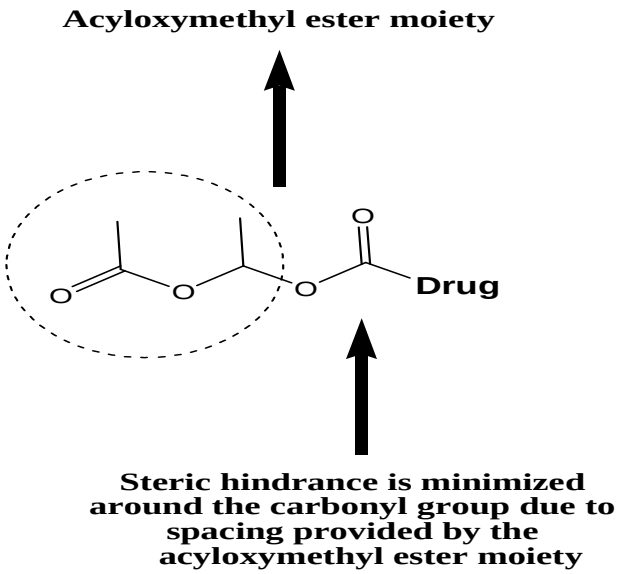

Figure 8: An illustration of the steric hindrance around the carbonyl group in the acyloxymethyl ester moiety.

Sultamicillin is a member of this class in which the irreversible β-lactamase inhibitor, sulbactam, is attached through an ester linkage to an ampicillin molecule to provide a mutual predrug. One of the advantages of this mutual predrug is the fact that it possesses a synergistic effect.[25] and upon an oral administration it is completely hydrolyzed to equimolar proportions of sulbactam and ampicillin, thereby acting as an efficient mutual predrug (Figure 9).[26]

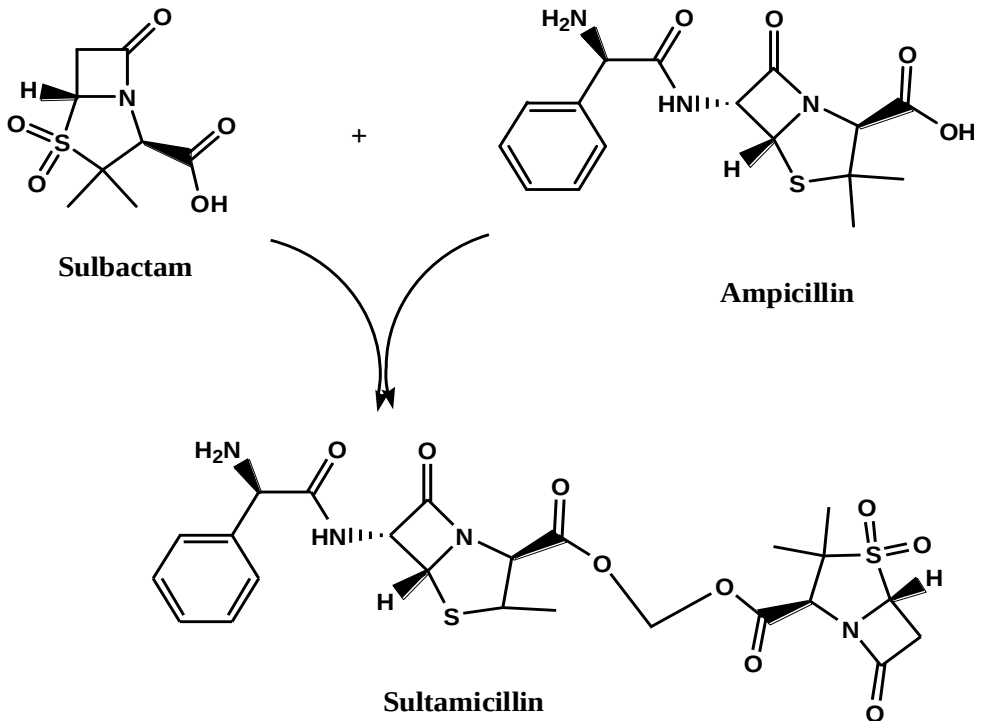

Figure 9: A conversion of sultamicillin to ampicillin and sulbactam.

Hetacillin is synthesized from a condensation of ampicillin with acetone.[27] and is considered as an example of 4- imidazolidinones (Figure 10) predrugs. It was designed to overcome the polymerization phenomenon.[28] associated with ampicillin. Polymerization of ampicillin occurs at high concentrations as a result of the intermolecular nucleophilic attack by the free amino terminal present in ampicillin molecule on the beta lactam ring of an adjacent molecule. Hetacillin predrug exhibits a six-fold increase in stability compared to ampicillin which is achieved by the equilibrium obtained between the drug and the predrug. In a later stage, the predrug converts back to ampicillin within 11 minutes. Using this approach the oral bioavailability of ampicillin was slightly increased.[29]

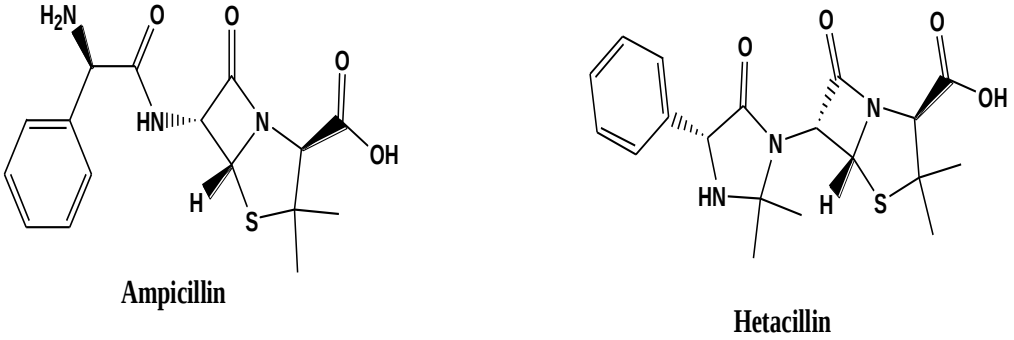

Figure 10: Chemical structures of hetacillin and ampicillin.

Another example of antibacterial predrug is clindamycin phosphate. Upon parenteral administration of clindamycin, an extremely irritating sensation at the injection site occurs.[30] However, an administration of clindamycin phosphate ester predrug overcomes this problem due to improved aqueous solubility with subsequent in vivo efficient release of clindamycin (Figure 11).[31] Consequently, clindamycin phosphate was approved for parenteral and topical administrations.[32]

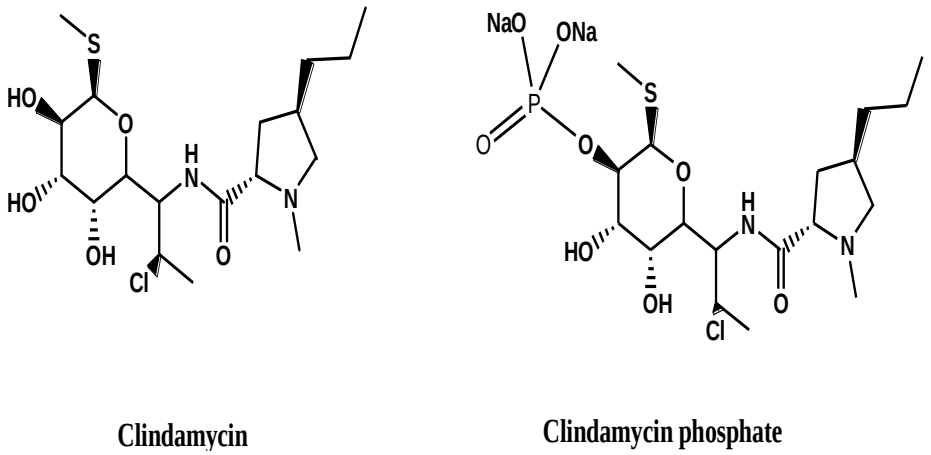

Figure 11: Chemical structures of clindamycin and its predrug, clindamycin phosphate.

As it is well known, ampicillin, acid resistant semi-synthetic penicillin, has a high polarity with a variable and low oral bioavailability. In order to achieve an efficient therapeutic activity, large oral doses are required, therefore considerable amounts of the antibacterial can reach the colon and modify colonic flora, and this generally leads to a high incidence of diarrhea.

In order to overcome this problem attempts were made to modify its structure by an addition of ester group to its carboxyl terminal thus obtaining a predrug with improved oral absorption.

This ester predrug remains in the gut in inactive form and it is less likely to cause diarrhea, however, after absorption, it undergoes a rapid hydrolysis to furnish its active parent drug.

There are three ester predrugs of ampicillin that are currently available (Figure 12): (1) Pivampicillin (pivaloyloxymethyl ester) releases formaldehyde and pivalic acid, (2) talampicillin (pthalidylthiazolidine ester) releases 2-carboxybenzaldehyde and (3) bacampicillin (ethoxycarbonvloxvethvl ester) releases acetaldehyde, carbon dioxide and ethanol. The Plasma concentrations of ampicillin attained with these esters are up to five times higher than those seen after oral administration of ampicillin. The advantages of pivampicillin over the other two ester predrugs that it undergoes the least hydrolysis in the intestine before absorption.[33]

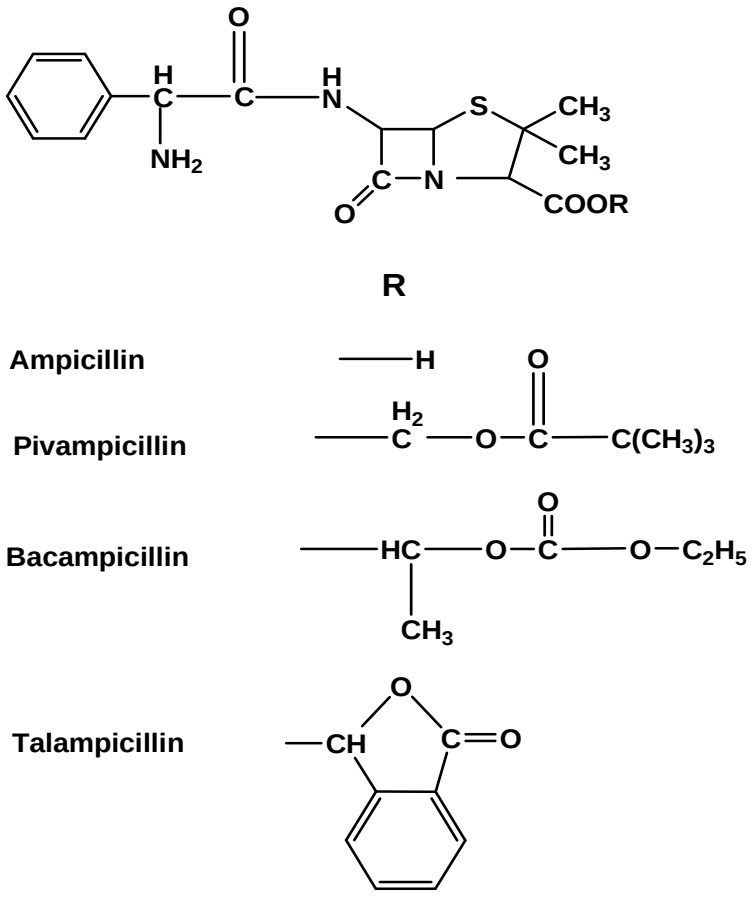

Figure 12: Chemical structures of ampicillin ester predrugs.

On the other hand, the orally administered cephalosporin predrugs have dual-esters. Those are fairly hydrophilic drugs of third and fourth generations that include aminotiazol and methoxamine groups in their structure, that responsible for their broad spectrum bactericidal activity and resistance to beta-lactamases.

For example cefuroxime axetil (Figure 13), cefpodoxime proxetil and cefetamet pivoxil (Figure 14) predrugs are more lipophilic, and administered orally with greater bioavailability than their parent active drugs. After absorption, enzymatic hydrolysis occur , followed by a chemical rearrangement due to the electronic characteristics of the intermediary formed, thus releasing their active parent drugs.[34]

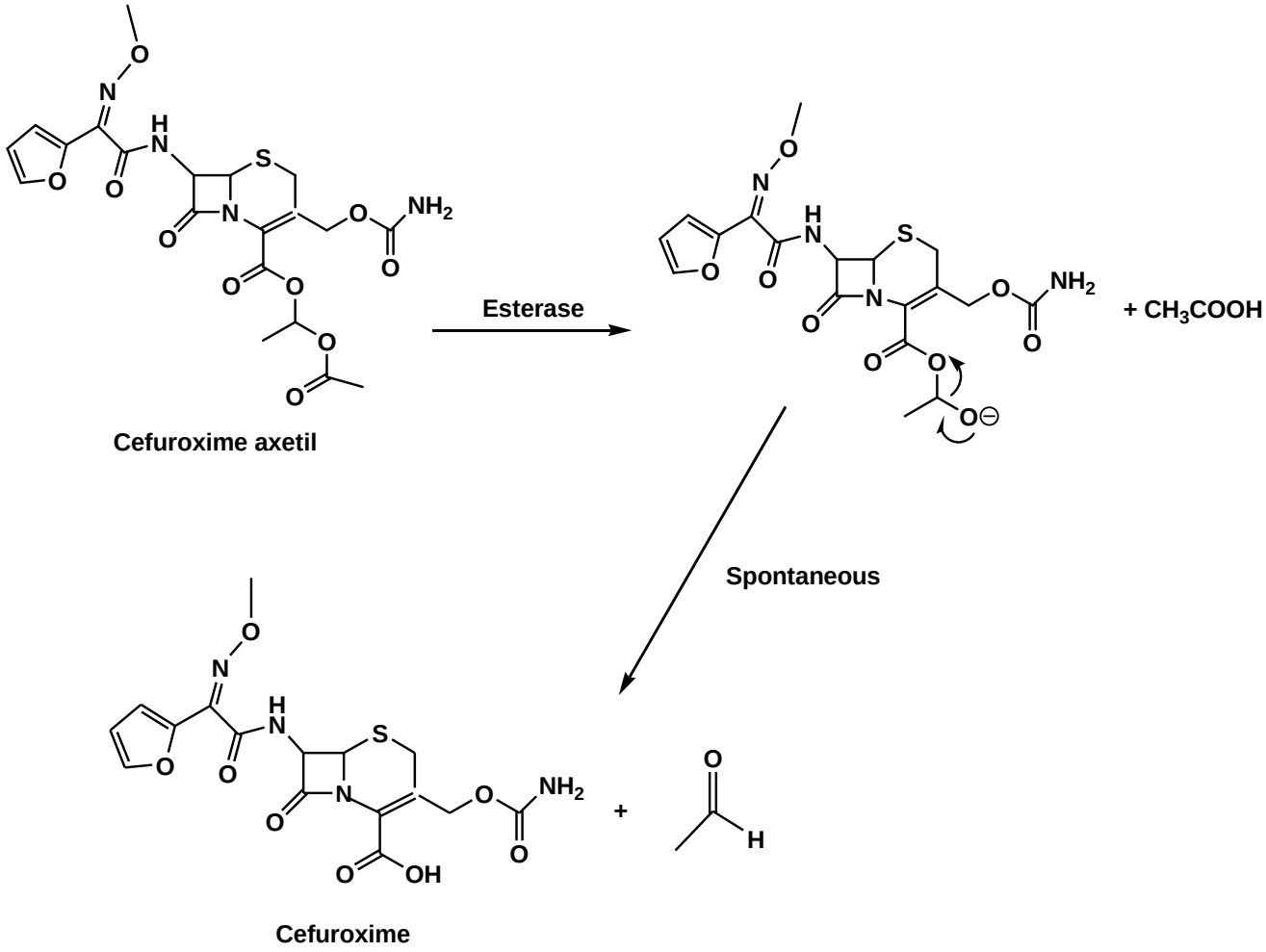

Figure 13: Cefuroxime axetil structure and its conversion into cefuroxime.

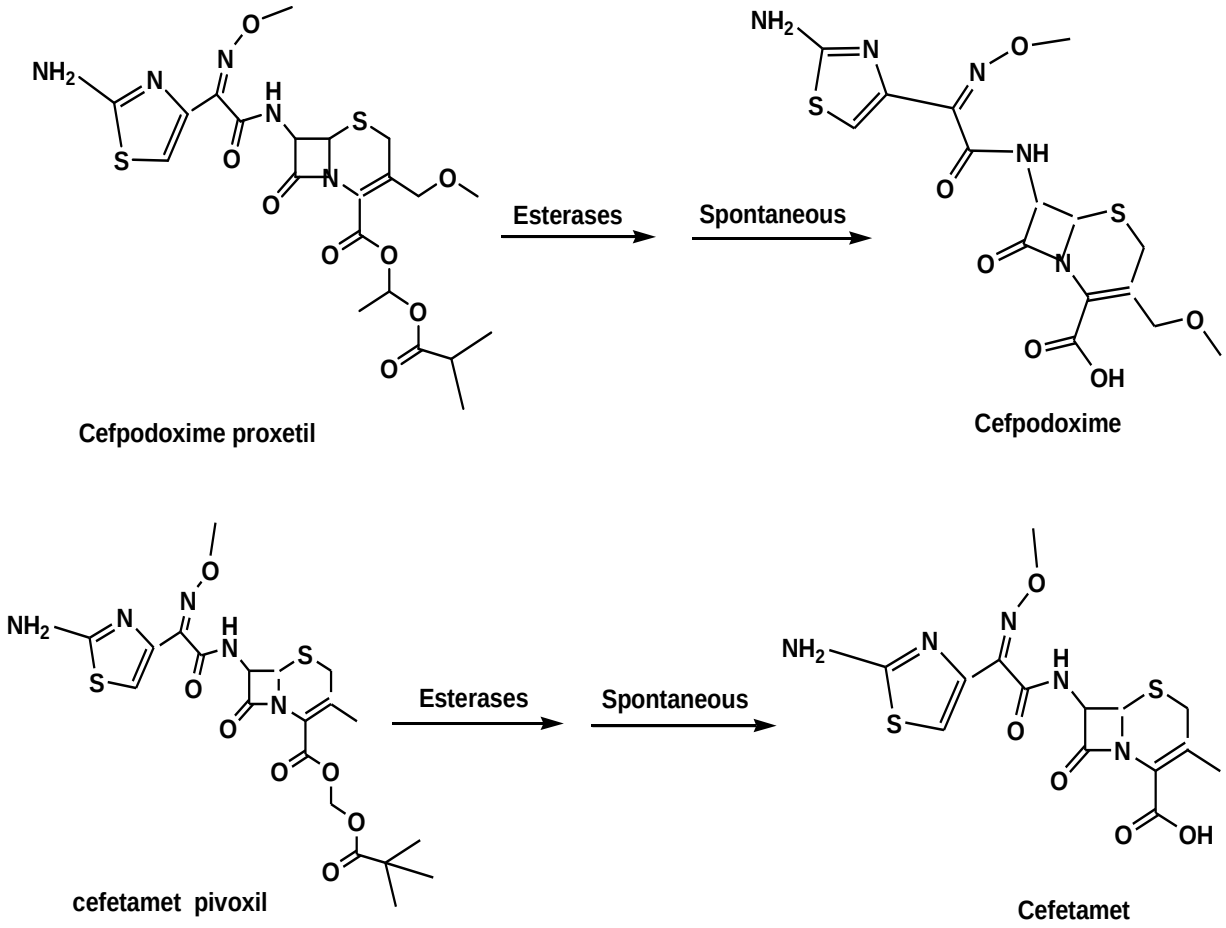

Figure 14: Cefpodoxime proxetil and cefetamet pivoxil structures being bioactivated into cefpodoxime and cefetamet.

Erythromycin is a macrolide antibiotic, consists of 14-atom lactone to which two sugars are bonded. One of these sugars features an amino group which gives the drug the characteristic of a weak base that enables the formation of salts with organic acids. Although this drug is effective against a wide variety of microorganisms its bitter taste and instability in an acidic medium prevented its therapeutic use.

Erythromycin stearate is a salt able to reduce the bitterness and, especially, increases the stability of the drug in an acidic medium. The lauryl sulfate salt of propionyl erythromycin ester, more commonly known as erythromycin estolate, is the well-known erythromycin predrug. This predrug is obtained from an esterification between the hydroxyl group of the amino sugar present in erythromycin and propionic acid carboxyl group. The bitterness of erythromycin is reduced by the esterification process and the oral absorption of the drug is increased. The active parent drug, erythromycin, is released into the bloodstream or muscle tissue after the action of esterases on its ester predrugs (Figure 15).[34]

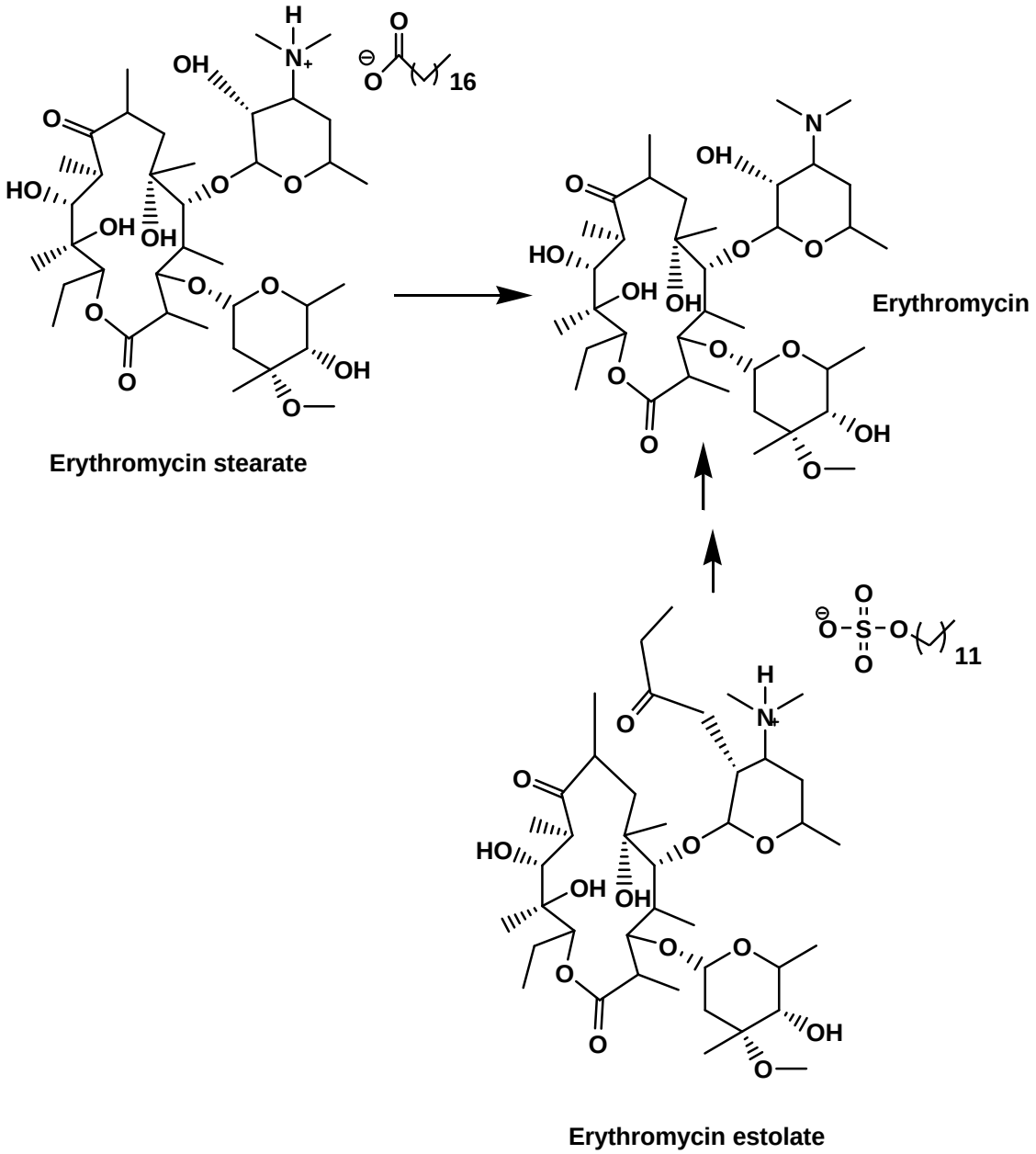

Figure 15: Conversion of erythromycin stearate and erythromycin estolate into erythromycin.

The Desire to develop a 'dual-action cephalosporin' antibiotics that intended to expel potent antibacterial agents when acted upon by βL producing bacterial strains set the phase for designing cephalosporin-containing anticancer predrugs.[35] Cephalosporin nitrogen mustard derivatives are an example of one of the first reported cephalosporin-based anticancer predrug that is activated by broad scale βL enzymes from Enterobacter cloacae. Extension of this work includes predrugs of other nitrogen mustards, doxorubicin, mitomycin C, vinca alkaloid, and paclitaxel and carboplatinum analogues. A diverse array of βL enzymes from E.

cloacae, E. coli, and B. cereus were used to activate these predrugs.[35, 36] The chemical structures of some of the predrugs activated by βL enzymes are shown in Figure 16.

One of the first reported in vivo activities of mAb- βL system was cephalosporine- vinca alkaloid predrug using a βL enzyme from E. cloacae; the predrug was linked to mAb Fab' fragment such that recognition of CEA, TAG-72 and KS1/4 antigens on tumor tissues was observed. Therapeutic effects of mAb- enzyme conjugate in combination with vinca predrug were studied in models of human colorectal carcinoma in nude mouse and it was found in all cases to be superior to naked drug therapy.[35]

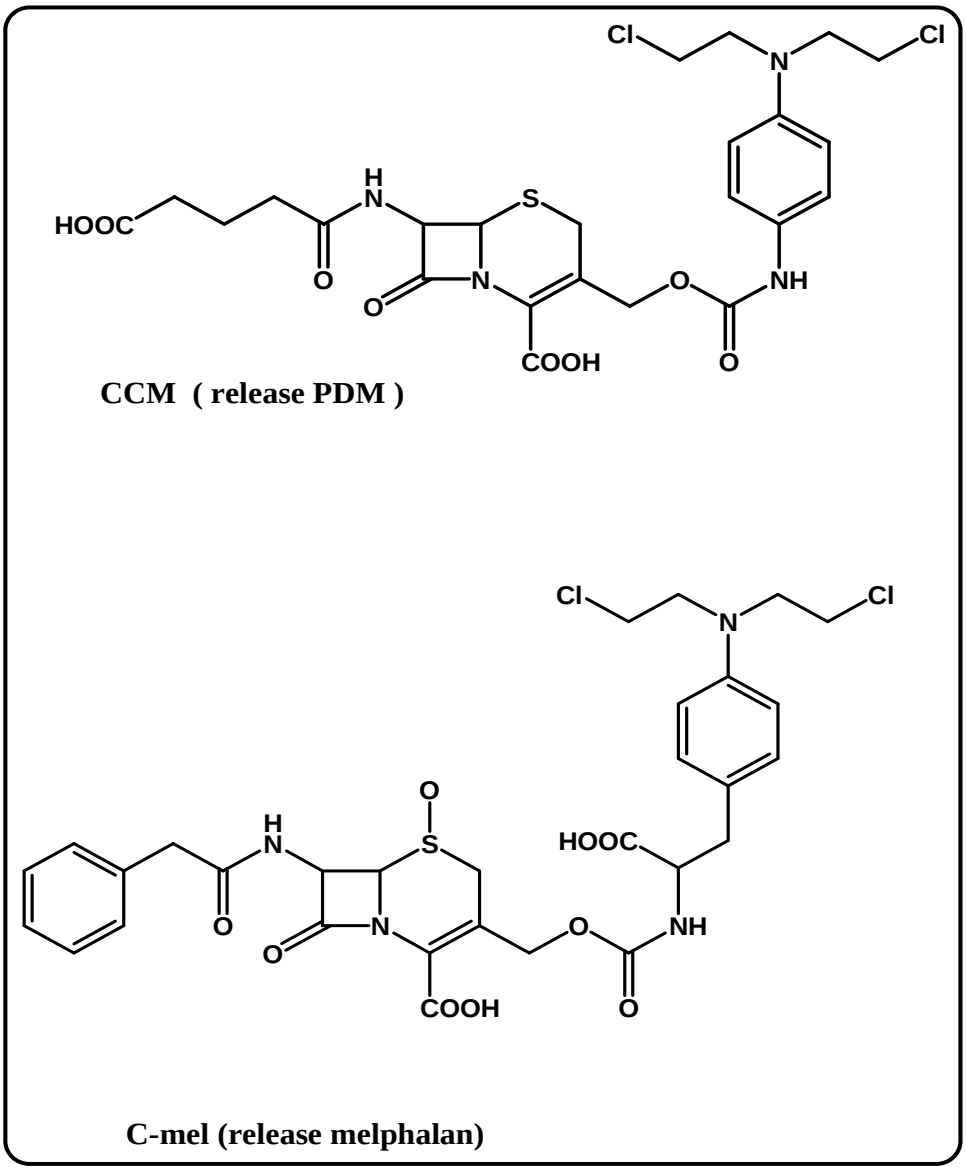

Figure 16: Chemical structures of CCM and C-mel predrugs that are activated by βL enzymes.

Bitterless amoxicillin and cephalexin predrugs

A large number of commonly used antibiotics suffer unpleasant taste and some of them are characterized with bitter sensation. For instance, the β –lactam antibacterial agents such as amoxicillin, cephalexin and cefuroxime axetil have extremely bitter taste which is hard to eliminate. The bitter sensation of drugs has a negative effect on the patient compliance especially pediatric and geriatric patients who have difficulty in swallowing whole tablets or when small doses are given. Moreover, it is also even difficult for those patients to administer antibacterial suspensions due to their better and unpleasant sensation.[37-49]

It is believed that the bitter taste of those antibacterials is as a result of the binding of the antibacterial agent to the active site of a bitter taste receptor via intermolecular forces These forces are most likely due to the formation of hydrogen bonding or ionic bonding between the amido (in cefuroxime) or amino (in amoxicillin and cephalexin) group to the active site/s of the bitter taste receptor/s.

In the past few years we have investigated a large number of intramolecular processes that were advocated to understand enzyme catalysis. Using different quantum molecular mechanics methods we have unraveled the mechanisms of a large number of intramolecular processes. Continuing our studies on how to use these enzyme models as linkers in predrugs design we have utilized the acid-catalyzed hydrolysis of N-alkyl maleamic acids (Kirby's enzyme model).[50-63] to design and synthesize predrugs of amoxicillin, cephalexin and cefuroxime axetil. Our goal was to provide drugs with relatively good antibacterial activities and lacking the bitter sensation associated with the use of their parent drugs.

Based on our previously reported DFT calculations and on experimental data for the acid-catalyzed hydrolysis of several N-alkylmaleamic acid amides.[64-102] two amoxicillin and cephalexin predrugs were proposed (Figures17 and 18, respectively). As shown in Figures17 and 18, the antibacterial predrugs, 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 predrug molecule (a lipophilic moiety).

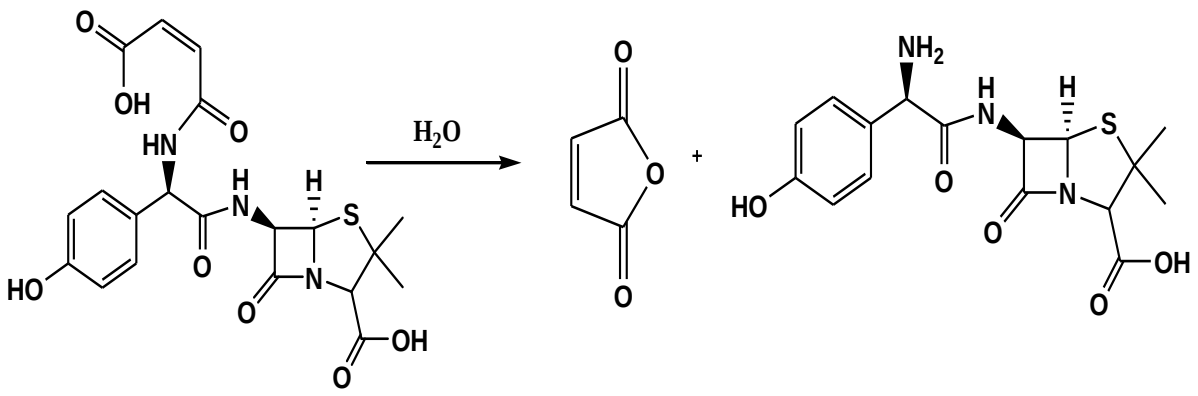

Figure 17: Acid-catalyzed hydrolysis of amoxicillin ProD 1.

The combination of both, the hydrophilic and lipophilic groups provides a predrug entity with a moderate HLB and potentially with a high permeability. The HLB value of the predrug is determined upon the pH of the target physiological environment. In the stomach where the pH is in the range of 1-2, it is expected that amoxicillin **ProD1** and cephalexin **ProD1** will be in a free carboxylic acid form whereas in the blood circulation where the pH is 7.4 a carboxylate anion 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.

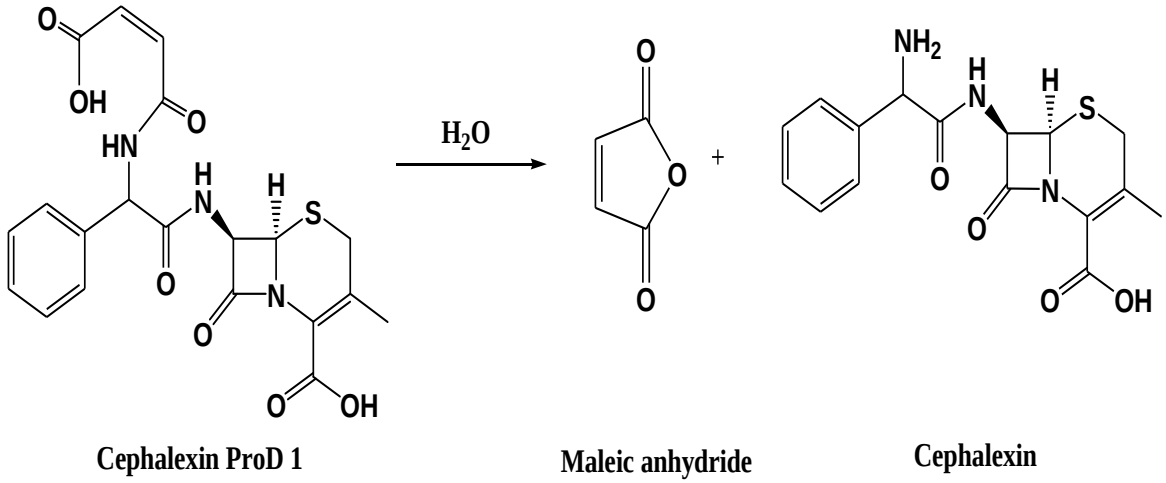

Figure 18: Acid-catalyzed hydrolysis of cephalexin ProD 1.

The conversion of amoxicillin ProD 1 and cephalexin ProD 1 to their parent drugs 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 predrugs intraconverted to

release the parent drugs as was determined by HPLC analysis. For both amoxicillin and cephalexin predrugs, 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 predrug verves time. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$) for amoxicillin ProD 1 and cephalexin ProD 1 in the different media were calculated from the linear regression equation obtained from the correlation of log concentration of the residual predrug verses time. The kinetic data for amoxicillin ProD **1**and cephalexin ProD 1 are listed in Tables 1 and 2, 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 cephalexin 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 predrugs in blood circulation system. Acid-catalyzed hydrolysis of both, amoxicillin ProD 1 and cephalexin ProD 1 was found to be much higher in 1N HCl than at pH 2.5 and 5. On the other hand, at pH 7.4, both predrugs amoxicillin ProD 1 and cephalexin ProD 1 were quite stable and no release of the parent drugs was observed. At pH 5 the hydrolysis of both predrugs amoxicillin ProD 1 and cephalexin 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 predrug 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 predrug will exist as the free acid form and at pH 7.4 most of the predrug will be in the anionic form. Thus, the discrepancy in rates at the different pH buffers.

Table 1: Kinetics of amoxicillin ProD 1 in 1N HCl and at pH 2, 5 and 7.4

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

Table 2: Kinetics of cephalexin ProD 1 in 1N HCl and at pH 2, 5 and 7.4

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

SUMMARY AND CONCLUSIONS

In this mini review we have covered most of antibacterial predrugs which were designed and synthesized during the past few decades aiming to provide compounds with potent antibacterial activity but without the side effects associated with their parent drugs.

Almost all antibacterial predrugs documented in this mini-review were obtained by the classic predrug approach, by which the active antibacterial agent is linked to a linker or another active drug directly, or via a spacer and upon in vivo administration is cleaved by enzyme-catalyzed reaction to liberate the active parent drug. The enzyme-catalyzed activation has many disadvantages due to many intrinsic and extrinsic factors that can affect the rate of the predrug cleavage. For example, the activity of many predrug-activating enzymes may be varied due to genetic polymorphisms, age-related physiological changes, or drug interactions, leading to variation in clinical effects. Therefore, there is a necessity to invoke a new approach in which the predrug's cleavage is independent on the source and nature of the metabolic enzyme.

The novel computational approach which has been utilized by Karaman's group considers linking a designed linker to an active drug, such as amoxicillin, that has poor bioavailability and/or bitter sensation, which upon exposure to physiologic environment releases the parent drug via intramolecular chemical reaction in the absence of metabolic enzymes. With the possibility of designing predrugs with a variety of linkers, the cleavage rate of the predrug can be controlled and the disadvantages associated with the metabolic enzymes will be eliminated.

Advances must be made and achieved in comprehending the chemistry of many organic processes that can be effectively used to enable the development of even more types of predrugs. The understanding of organic reaction mechanisms of certain processes, particularly intramolecular reactions, will be the next major milestone in the field of predrug design.

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