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Intramolecular Processes and Their Applications in Prodrugs Approaches- Experimental and Computational Studies

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Abstract: This review supplies the reader with a detailed overview on the utilization of intramolecular processes for a design and synthesis of prodrugs. It is well known that a respected number of drugs suffer from low bioavailability, toxicity, unpleasant taste and presystemic first-pass metabolism which result in drug inactivation. The classical prodrug approach in which the linkage attaching the parent drug to its non-toxic linker and cleaved by *in vivo* enzyme's catalyzed reactions has proven its success in solving toxicity and bioavailability related issues. On the other hand, prodrugs based on chemical interconversion in which the prodrug releases the corresponding active parent drug *via* inter or intramolecular chemical process in the absence of an enzyme is considered as a better alternative approach since the prodrug cleavage is not dependent in the efficiency or quantity of the enzyme catalyzes the interconversion of the prodrug. Examples of successful prodrugs using the chemical approach *via* intramolecular processes such as cyclization reactions are illustrated as well.

In addition, another part of this review is devoted to cover reported studies on enzyme models and their utilization for the design and synthesis of a variety of novel prodrugs. In this approach, computational calculations using DFT and MM methods were exploited and correlations between experimentally determined and computed values of the rate-limiting step in the studied intramolecular processes were utilized in the prodrugs design. Selected examples of the designed prodrugs include aza-nucleosides for the treatment of myelodysplastic syndromes, the anti-Parkinson's agent dopamine, the anti-viral acyclovir, the anti-malarial atovaquone, and statins for lowering cholesterol levels in the blood, the antihypertensive atenolol, the antibacterial cefuroxime, the anti-bleeding tranexamic acid, the decongestant phenylephrine, and the pain killer paracetamol.

Keywords: Intramolecular, cyclization, prodrug, trimethyl lock, intramolecular Activation, Quantum Mechanics Calculations, lactonization cyclization.

1. INTRODUCTION

The development of prodrugs has the potential to overcome many pharmacokinetics barriers. For example, several drugs undergo first pass metabolism which leads to drug inefficiency and poor bioavailability, and sometimes to toxicity. The classical prodrug approach by which the linkage attaching the active parent drug to a non-toxic linker and cleaved by enzyme's catalyzed reactions has proven its success in solving toxicity and bioavailability related issues. On the other hand, prodrugs based on chemical interconversion by which the prodrug releases the corresponding active parent drug *via* inter or intramolecular process without the involvement of enzymes is considered as a better alternative approach since it does not have inter- and intra-individual variability (differences in the quantity and efficiency of the metabolic enzyme) that might affect the efficiency of the metabolic enzymes.

Among the most studied activation strategy in the prodrug chemical approach is cyclization elimination reactions that have been used in providing many prodrugs from analgesics to HIV protease inhibitors [1].

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2. INTRAMOLECULAR ACTIVATED ESTER PRODRUGS

One of the problems that drugs may suffer is a lack of specificity for their target. The prodrug approach was successful in overcoming this problem and improving the delivery of drugs to their targets, especially for ester prodrugs, involving substitution of carboxyl and hydroxyl groups of their active drugs, which are readily hydrolyzed *in vivo*, either by chemical or enzymatic means to provide their active drugs [2].

Drug delivery can be improved by modulating the drug's physiochemical properties which affect their absorption and delivery to various membranes and tissues. The prodrug approach by which the activation is catalyzed by enzymes is useful for parent drugs containing hydroxyl or carboxyl groups. On the other hand, phenol esters when activated by metabolic enzymes provide the corresponding active drugs in fast rates, whereas, amides show little chemical reactivity towards enzyme's activation. Therefore, intramolecular activated amide prodrugs had been considered as an alternative approach for enzymatic activation that bypasses inter- and intra- individual variability that might affect the metabolism by enzymes. In addition, this approach provides a fine tuning of the cleavage rate of the prodrug which can be accomplished by selecting the functional groups involved in the cyclization process.

An example for such approach is the pilocarpine prodrugs based on diesters of pilocarpic acid. These prodrugs upon *in vitro* and *in vivo* administration (eliminate comma) undergo specific

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base- catalyzed cyclization to pilocarpine which consists of a first step by which hydrolysis of the ester yields a hydroxyl which acts as a nucleophile and attacks the phenyl ester group that initiates for cyclization to finally give the parent drug, pilocarpine (Fig. **1**) [3].

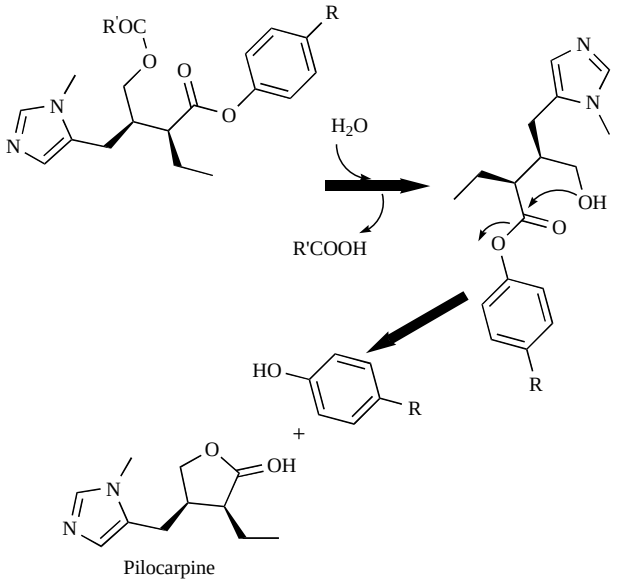


Fig. (1). Cyclization of diester of pilocarpic acid to release pilocarpine, R=H, R' = OH.

The prodrug approach has been utilized to enhance the ocular delivery of pilocarpine.

A number of lipophilic alkyl and aryl alkyl esters of pilocarpic acid were synthesized and evaluated as prodrugs of pilocarpine. The results obtained suggested that the pilocarpic acid esters may be potentially useful prodrugs with enhanced drug delivery and bioavailability properties, especially when further derivatized to give non-labile, easily formulated pilocarpic acid diesters that are readily converted to the active parent drug, *in vivo*. In aqueous solution the ester prodrugs undergo a quantitative and apparent specific base-catalyzed lactonization to pilocarpine.

The diester prodrugs were of special interest, since they combine an enhanced bioavailability and greatly prolonged duration of action with high stability in eye drops formulations for the treatment of glaucoma. The corneal permeability of pilocarpine is increased by the prodrug derivatives.

Several monoesters were capable of maintaining durations of action of 1.5 times longer than that of pilocarpine while the diesters were active for up to 2.25 times longer [4].

Some of the lipophilic pilocarpine prodrugs exhibit amphiphilic properties, which may contribute to the ocular irritation of these compounds [5].

α-Amino acid prodrugs of camptothecins (CPTs) are another example of intramolecular ring closing activation. The camptothecin ester prodrugs converted to their active lactones by intramoleul-

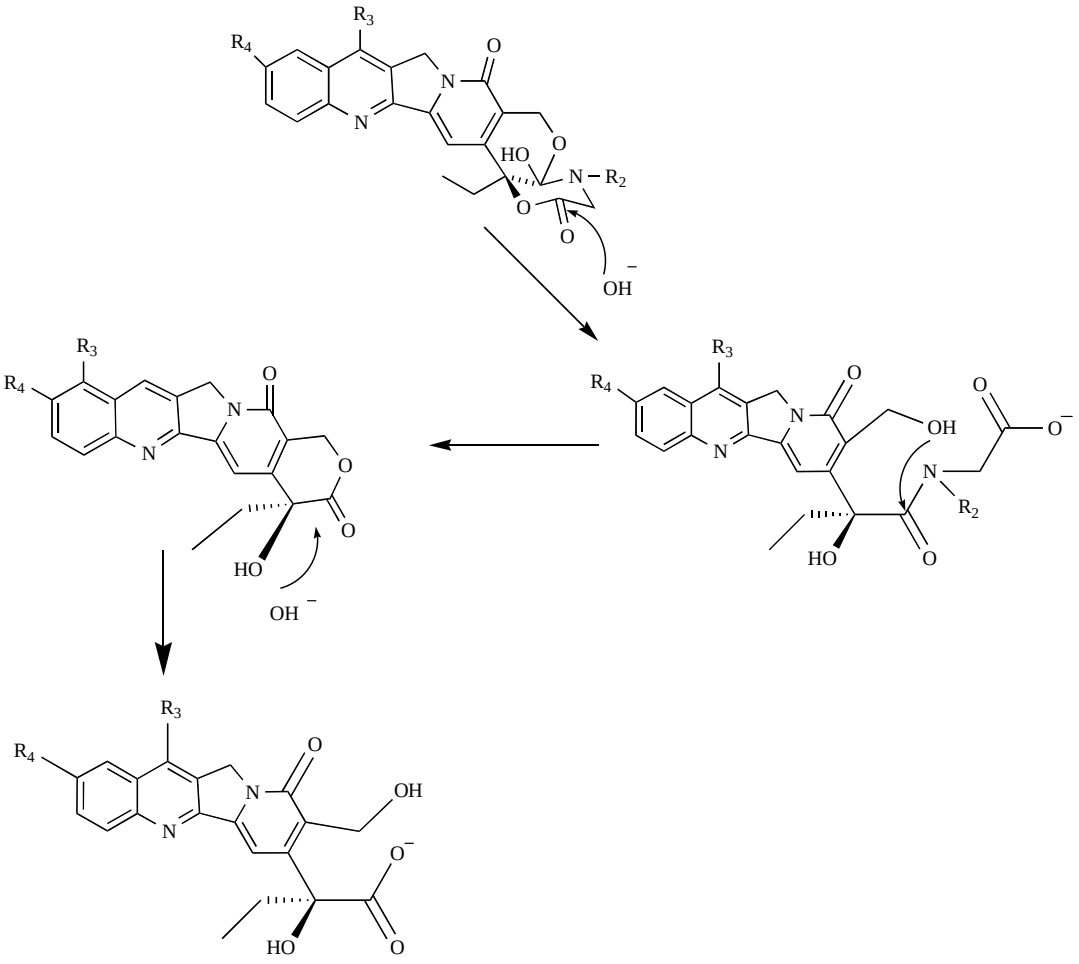


Fig. (2). A mechanism for the conversion of camptothecin α-amino acid esters to their carboxylates. R_2= H or CH_3, R_3= H or TBS, R_4= H or OH.

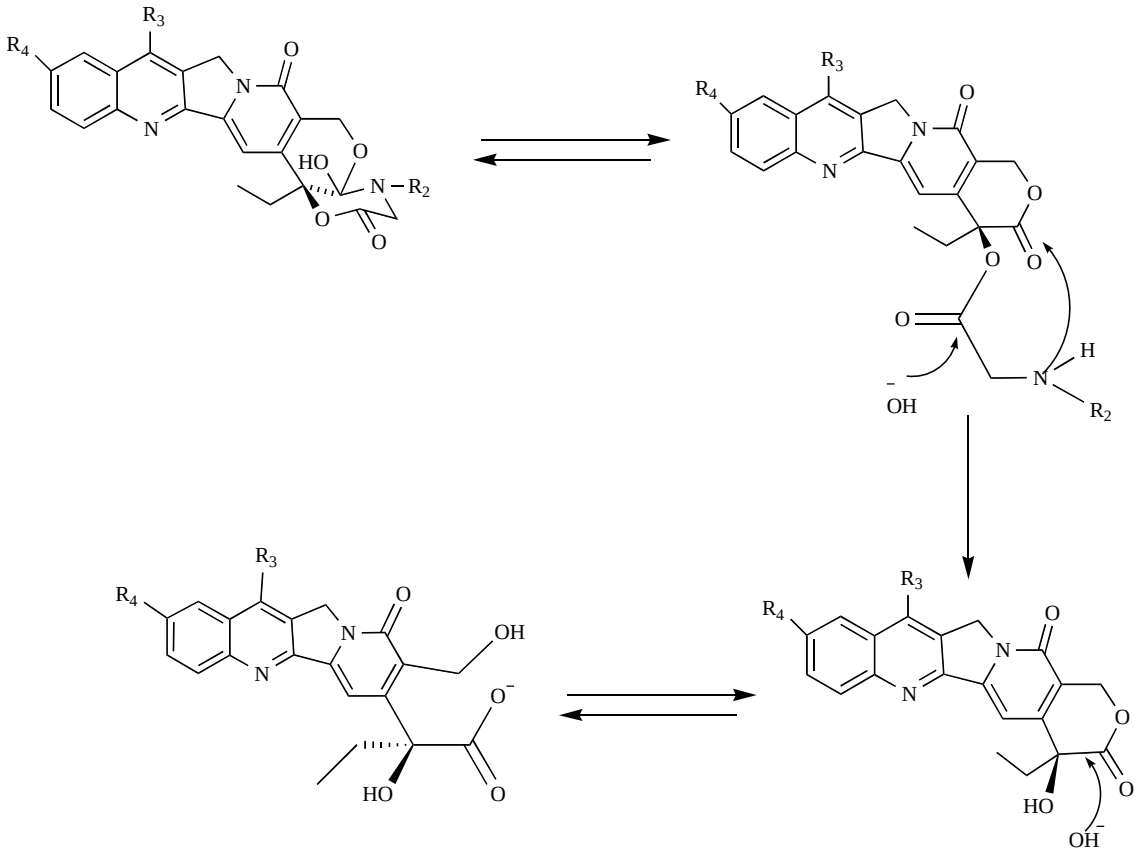


Fig. (3). An alternative route in which camptothecin prodrugs convert to their active lactones. R_2= H or CH_3, R_3= H or TBS, R_4= H or OH.

car cyclization reaction that is preferable over hydrolysis followed by intramolecular cyclization reaction at pH 7.4 [6].

Camptothecins are antitumor agents that work on S-phase by binding to the topoisomerase and I-DNA complex during DNA replication causing double strand breaks and cell death (Fig. **2**) [6].

α-Amino acid ester prodrugs reactivity is relatively high due to the fact that at pH values below the their pK_a values their amino terminal is positively charged. Recently, an alternative route that facilitates the production of reactive lactone from camptothecin α-amino acid ester prodrugs was suggested as shown in Figure **3** [6].

The most common problem associated with CPT is the instability of the 20-hydroxy lactone, which is easily hydrolyzed at neutral pH to yield the inactive carboxylate anion. It was shown that both the lactone ring stability and the 20-hydroxy group of CPT are critical for its antitumor activity. Further, it was demonstrated that masking the 20- hydroxyl group of CPT, results in reduced activity of the drug and increased stability of the lactone ring. The hydroxyl in position 20 generates an intramolecular hydrogen bond with the carbonyl moiety of the lactone, which accelerates the hydrolysis of the otherwise stable lactone ring. Therefore, masking this hydroxyl by a chemical linker that can be selectively removed, is a convenient approach for a generation of CPT prodrug [7].

In addition, cytotoxicity studies performed with several cancer cell lines clearly demonstrated a significant reduce in toxicity of the prodrugs of CPTs compared with their active parent drug [7].

2.1. Prodrug Activation *Via* Diketopiperazine Formation

Dipeptides as carriers for delivering parent drugs *via* non-enzymatic pathway (formation of DKP) have been suggested as good candidate to deliver drugs to their target sites. Dipeptides are carriers used to optimize drug delivery especially for hydroxyl-containing drugs. Dipeptide esters of paracetamol (Figure 4) have shown to undergo hydrolysis to the corresponding parent drug and DKP at pH 7.4 and temperature of 37˚C. Higher half-life values were observed with C-terminal bulky amino acids. The latter is believed to cause the peptide linkage to reside in the cis form, which is easily can cyclize to a DKP, thus leading to very reactive substrates. Hepatotoxic effects of paracetamol was decreased or even eliminated by paracetamol esterification with dipeptides [8].

The esterification approach of paracetamol with amino acids was proposed to obviate the sever hepatotoxicity of the drug at high doses as well as to increase its aqueous solubility [8].

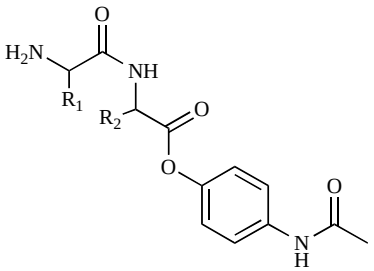


Fig. (4). Paracetamol esterification with dipeptides, $R_1 = R_2 = H$

2.2. Coumarin-Based Prodrug System

Prodrugs of amine and alcohol containing drugs based on coumarin derivatives as a linker with low toxicity have been developed in order to increase the bioavailability and reduce the toxicity of certain drugs. This approach was advocated by Wang and co-

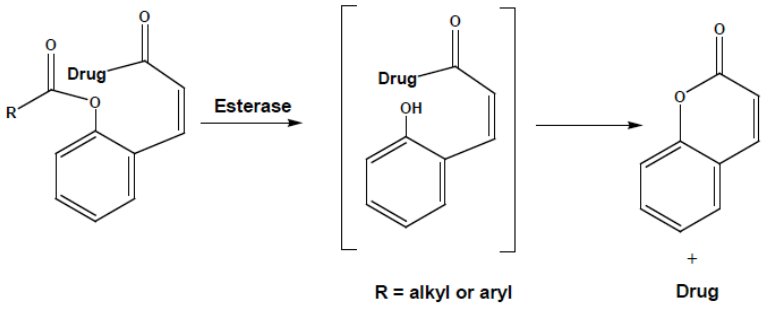


Fig. (5). Coumarin based prodrugs.

workers and is based on the fact that coumarin derivatives attached to active drugs undergo a rapid lactonization at a physiological environment of pH 7.4 (Fig. **5**).

The coumarins are of great interest due to their biological properties. In particular, their physiological, bacteriostatic and antitumor activity makes these compounds attractive for further backbone derivatisation and screening as novel therapeutic agents. Weber and co-workers have shown that coumarin and its metabolite 7-hydroxycoumarin have antitumor activity against several human tumor cell lines. Both coumarin and coumarin derivatives have proved to be potential inhibitors of cellular proliferation in various carcinoma cell lines. In addition it has been shown that 4-hydroxycoumarin and 7-hydroxycoumarin inhibited cell proliferation in a gastric carcinoma cell line [9].

One example for such approach is coumarin based prodrug of DADLE (H-D-Leu-Phe-Gly- D -Ala-Tyr-OH) which is capable to undergo cyclization after hydrolysis catalyzed by esterases. This prodrug releases the active drug after incubation in the plasma or with porcine liver esterase.

One unique feature of this prodrug system is that the final product of the promoiety is coumarin, the toxicity of which has been extensively studied. Coumarin was found to be relatively nontoxic. The known toxicity profile of coumarin clears one major hurdle in developing a generally applicable prodrug system [10].

2.3. Propranolol Prodrugs

Propranolol is a sympatholytic nonselective beta blocker. It is used to treat high blood pressure, a number of heart dysrhythmias, thyrotoxicosis, and essential tremors. It is used to prevent migraine headaches, and to prevent further heart problems in those with angina previous heart attacks. Propranolol highly variable and low oral bioavailability due to high first pass metabolism. Therefore, the prodrug approach was used to reduce the extensive effect of the liver on the propranolol before reaching the systemic circulation.

A cyclic oxazolidinones derivative and esters of propranolol (7) have also been investigated as good and novel prodrug candidates [11].

There are 3 possible different mechanisms for the degradation of propranolol prodrugs [11]:

(a) a mechanism that involves an intra-nucleophilic attack of the free amine group on the carbonyl of the ester group, (b) a mechanism consists of a nucleophilic attach of a water molecule on the carbonyl ester group catalyzed by a base (free amine group) (c) a mechanism by intramolecular general acid (protonated amine) catalysis in which a hydroxyl ion attacks the carbonyl ester. Both mechanisms (b) and (c) are hydrolysis processes that yield propranolol whereas, mechanism (a) represents an intramolecular aminolysis giving rise to the formation of stable N-acylated propranolol derivatives (Fig. **6**).

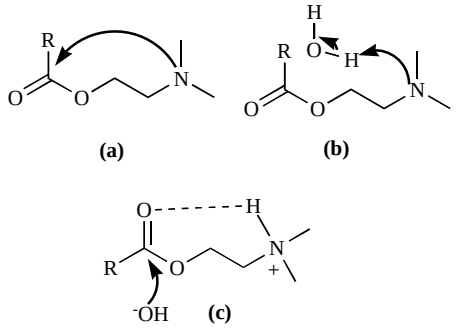


Fig. (6). Mechanisms (a), (b) and (c) for the degradation of propranolol prodrugs with increasing the pH to >7 the aminolysis becomes predominant.

Figure **7** illustrates seven different esters that were synthesized and studied with the aim to investigate the kinetics of their degradation in aqueous solution and in human plasma which might reflect the behavior of propranolol prodrugs in such media. This discrepancy between the different esters might be related to steric hindrance of the tert-butyl group that hinders the nucleophilic attack taking place in the O→N acyl transfer reaction. At pH > 11.5 the main reaction of esters **2-6** is intramolecular aminolysis.

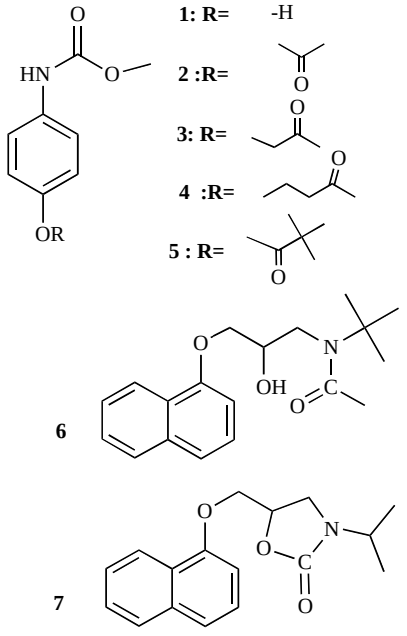
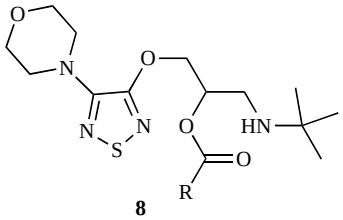


Fig. (7). Propranolol prodrugs and similar other esters **1-7**.

At pH 7.2 the O →N acyl transfer reaction of the propranolol esters is < 7% of the reaction yield. Similar alkyl esters of timolol (**8**) (Fig. **8**) were found to be completely hydrolyzed in aqueous media.

The presence of the bulky tertiary butylamino group in timolol esters is the main cause for steric hindrance that limits the ability of these esters to undergo intramoleuclar aminolysis. At pH 7-10, propranolol esters have showed greater tendency to hydrolyze than to undergo intramolecular aminolysis. The predominant factor that determines the mode of the reaction whether to proceed with hydrolysis or intramolecular aminolysis mechanism is the bulkiness of the amine moiety in the esters of β-aminoalcohols. Another factor that affects the ratio hydrolysis vs. aminolysis is the steric properties within the structure, as it is seen with the low tendency of the O -pivaloyl ester (**5**) to react *via* aminolysis compared to alkyl esters **2**-**4** (Fig. **8**).



8

Fig. (8). Timolol ester **8**.

Timolol prodrugs are more lipophilic than their active parent drug, timolol, thus their permeation through the cornea is higher than their parent drug. Timolol ester prodrugs increase timolol concentration in aqueous humor and decrease timolol systemic concentration by 10 fold. Additionally, they increase the therapeutic index of timolol by 15-fold and also showed prolonged duration of action [12].

Moreover, various aliphatic esters of timolol, a non-selective P-adrenergic receptor blocker, have been developed as prodrugs to potentially diminish the systemic absorption and therefore side-effects of topically applied timolol through increased corneal absorption [2].

2.4. Cyclization-Elimination Due to a Carboxylate Group

Intramolecular cyclizations are not restricted to attack by nucleophilic nitrogen (basic amino or acidic amido group). They can also be catalyzed by nucleophilic oxygen, as in a carboxylate group, a phenol or an alcohol. These reactions can also be proceeding by catalysis of nucleophilic oxygen (phenol or an alcohol) such as the hemiester prodrugs of phenol or paracetamol shown in Figure 9. Three different mechanisms were proposed for these hydrolysis reactions: acid- catalyzed, base-catalyzed, and an intramolecular nucleophilic process resulting in a cyclization elimination reaction (Fig. **9**). In buffer solutions, the mechanism is pH dependent. At pH 7.4 and 37° C, the cyclization- elimination reaction was the dominant, with $t_{½}$ of 1 - 350 minutes. The number of promoiety substituents and their shape and the pK_a of the phenol derivative influence the reactivity of these prodrugs. The reactions of the succinate esters at this physiological pH were about 150 times more potent than their corresponding glutarate esters. In addition, it was found that branching with methyl of the promoiety (X= CH2CH(CH3)CH2) has increased the reactivity of the ester. The cleavage rate for paracetamol esters (R = NHCOCH3) was found to be 2-fold higher than that of phenol esters (R = H). Generally, no or little enzyme's activity was seen in human plasma. This is in accordance with the fact that hemiesters are known to be inert towards cholinesterase enzyme [13].

2.5. Two Step Activation Prodrugs

2.5.1. General

In two-step activation of prodrugs the nucleophile is formed *in situ*. Otherwise, it remains until the prodrug undergoes cleavage by metabolism. Examples for such nucleophile are a phenol or an alcohol masked with a carboxylate ester. These kinds of prodrugs release the corresponding active parent drugs *via* enzymatic and/or nonenzymatic hydrolysis of their ester moiety which is followed by an intramolecular nucleophilic attack.

An example for such approach is pilocarpine prodrugs that aimed at improving ocular delivery (Figure 1). These prodrugs are pilocarpic acid diesters characteristic with high lipophilicity. The first activation step is removing the acetyl linker by an enzymatic

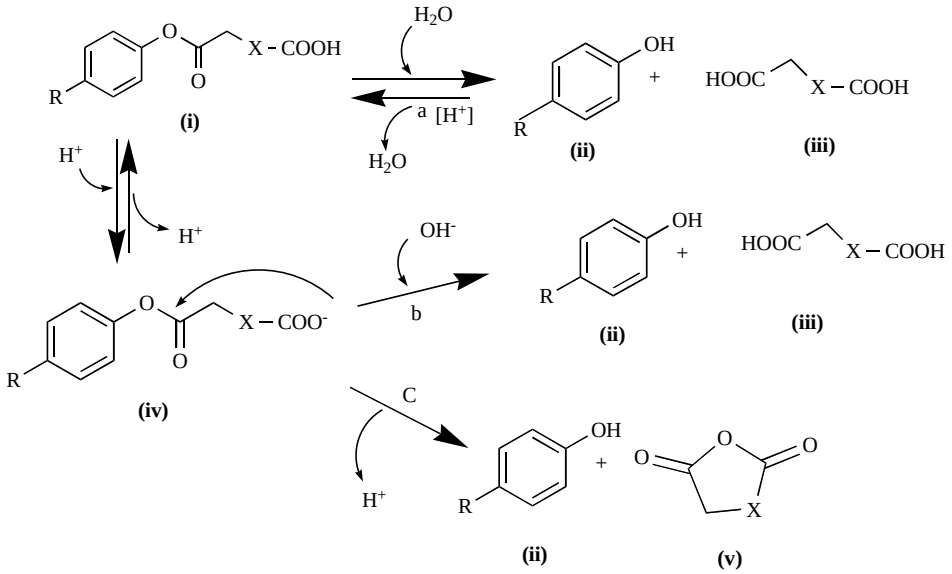


Fig. (9). Mechanism of carboxylic acid hydrolysis, X=$CH_2CH(CH_3)CH_2$, $CH_2CH_2CH_2$,$CH_2C(CH_3)_2CH_2$, R=H or $NHCOCH_3$.

hydrolysis of ester which is followed by a second step in which an intramolecular nucleophilic attack is leading to a loss of the alcohol linker and to a ring closing to yield pilocarpine [14].

p-Acetamidophenol amino acid esters (Figure 10) have been suggested as useful and efficient prodrugs for p-acetamidophenol. Stability and solubility in water for these prodrugs can be achieved by selecting the appropriate amino acid group with the desired hydrophilic lipophilic balance value (HLB value) [13].

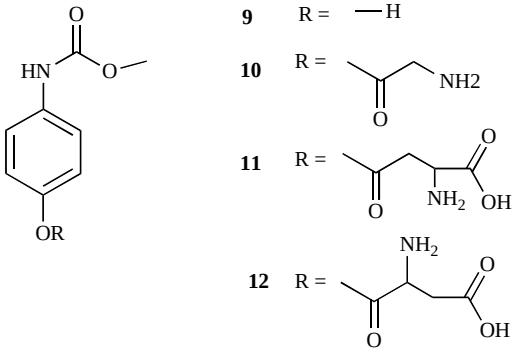


Fig. (10). p-acetamidophenol amino acid esters.

Investigations have shown that the pH-rate profile for ester **12** hydrolysis (Fig. **10**) is totally different from that of esters **10** and **11**. At pHs above the pK_a of the carboxyl group of **12** its decomposition rate was found to be unaffected by the pH of the medium and the concentrations of the zwitterions **12a** or the anion **12b** were essentially constant (Fig. **11**). This phenomenon can be attributed to the fact that the dominant reaction is intramolecular involving the zwitterion of **12** (**12a**) at pH 2-6 and its anion (**12b**) when the pH of the medium is 6 - 11.

At pH values slightly above the pK_a of the carboxyl group of **12** (between pH 2 and 10), the hydrolysis of **12** is subjected to intramolecular nucleophilic catalysis, and its rate value is higher than that of ester **10**. The main hydrolysis of aliphatic alcohol esters is intermolecular reaction such as in the case of esters of α- and β -aspartic acid and the intramolecular hydrolysis is rarely occurring. The ethanolic esters of β -aspartic acid undergo slower hydrolysis than the glycinate esters and the shapes of the pH-rate profiles for the three systems are similar [13].

2.5.2. Therapeutic Advantages of Amino Ester Prodrugs

Amino acid ester prodrugs of the dopamine D2 agonist 5-OH-DPAT L-valine and β-alanine prodrugs of 5-OH-DPAT may meet

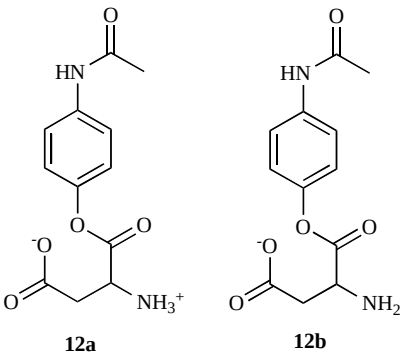


Fig. (11). p-Acetamidophenol amino acid esters.

the requisites for improved transport across the skin by iontophoresis for treatment of Parkinson disease [15].

Most striking effect was observed with the levels of glutathione, GSH, as the glycine-acetaminophen prodrug significantly protected the depletion of GSH when compared to its parent drug. Glycine-acetaminophen prodrug showed remarkable potential in the field of gastric and hepatic complications [16].

An ester prodrug approach for NSAID's by which the carboxyl group of the NSAID is blocked is another approach to reduce the GI toxicity associated with NSAIDs without affecting their activity. Because of the low toxicity of the amino acids, they are considered the best choice to be utilized as linkers for NSAIDs prodrugs. For example, the basic α-amino group such as in prodrug **13** (Figure 12) was found to enhance the water-solubility of the parent drug (Figure 12), however, it decreased the chemical stability. It is believed that such behavior is a result of the presence of the protonated amine group which enhances water solubility whereas the acceleration in the hydrolysis rate of the prodrug is mainly due to the unprotonated amine group which serves as a nucleophile resulting in intramolecular rearrangement of the prodrug to yield the amide drug. However, a number of prodrugs containing a secondary carbamate such as **14** ($R_1 = R_2 = H$), tend to form the corresponding N acylamine *via* a rearrangement process (Fig. **13**). This rearrangement process is inhibited by the presence of bulky amino acids such as Phe or Val and a carrier such as N- methyl glycine (sarcosine). Figure **12** illustrates a number of aminocarbonyloxymethyl derivatives of flufenamic acid and diclofenac containing amino acid amides as carriers. This carrier replacement decreases the carbamate pK_a in 1.2 units and therefore it inhibits the O→N rearrangements when compared with **14**.

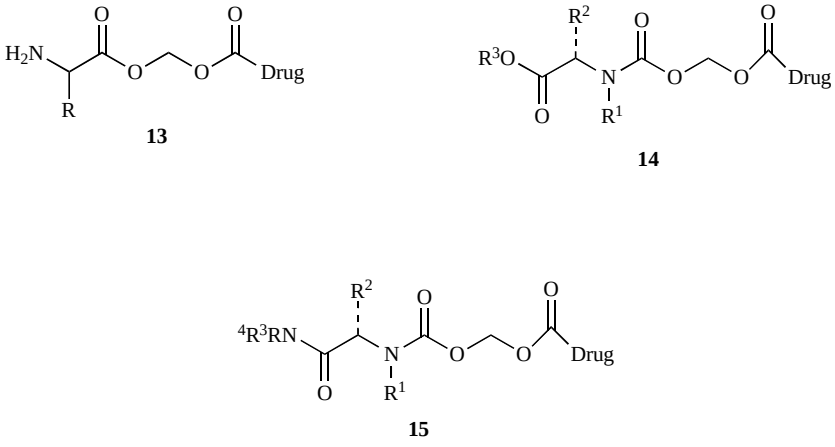


Fig. (12). Chemical structures of amino acid ester **13** and amino acid amides **14** and **15**.

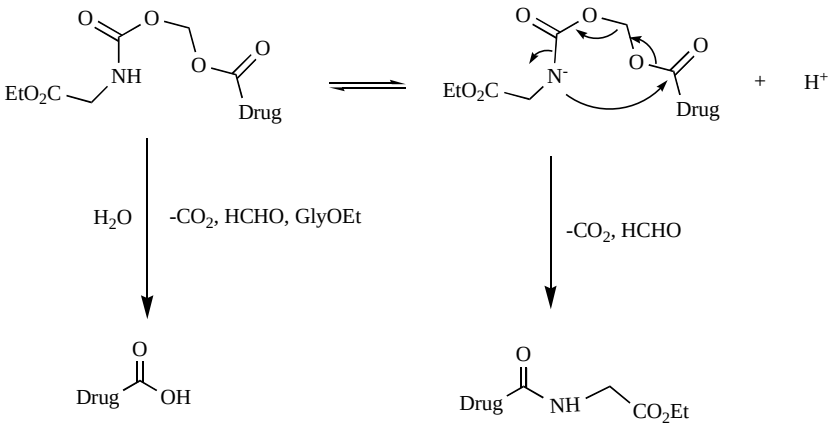
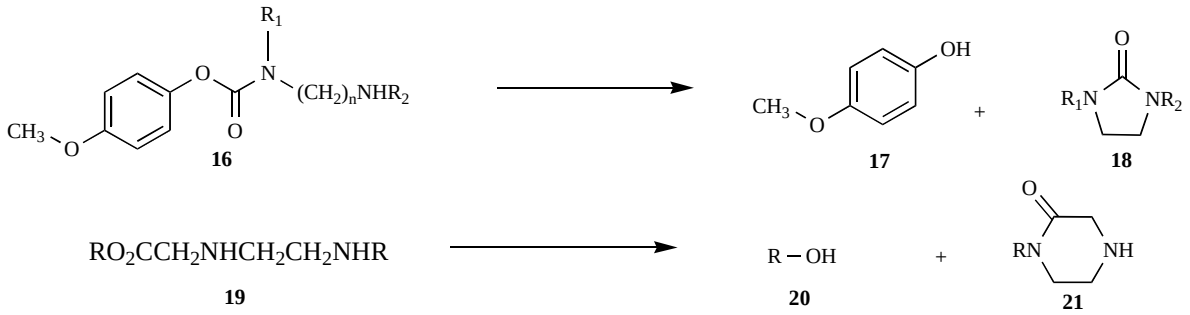


Fig. (13). Rearrangement of prodrugs such as **14** in pH 7.4 to yield N-acylamine.



1.	**n = 2**	**R_1= CH_3**	**R_2 = CH_3**	**R_3= H**	**5.**	**n = 2**	**R_1= H**	**R_2 = CH_3**	**R_3= H**
2.	**n = 2**	**R_1= CH_3**	**R_2 = CH_3**	**R_3= CH_3**	**6.**	**n = 2**	**R_1= H**	**R_2 = H**	**R_3= H**
3 .	**n = 2**	**R_1= C_2H_5**	**R_2 = C_2H_5**	**R_3= H**	**7.**	**n = 2**	**R_1= H**	**R_2 = H**	**R_3= H**
4.	**n = 2**	**R_1= CH_3**	**R_2 = H**	**R_3= H**	**8.**	**n = 3**	**R_1= CH_3**	**R_2 = CH_3**	**R_3= H**

Fig. (14). Carbamate prodrugs **16** and **19** and their cleavage reactions to phenol **17** and alcohol **20**, respectively.

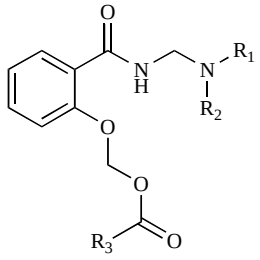


Fig. (15). *N*-Mannich basses of salicylamide.

The differences in the pK_a and bulkiness of the terminal amino acid amide and ester groups lead to a large difference in the chemical reactivity of both amino acid ester **13** and amide **14** [17].

In this approach, the prodrug is intraconverted to its active parent drug by an intramolecular cyclization-elimination and the intermolecular cleavage of the ester bond by hydrolysis is not observed. By this approach, the prodrug releases its corresponding active parent drug in rates that are solely determined by the intramolecular ring-closing reaction.

Studies have shown that carbamate prodrugs of alcohols such as **19** undergo cyclization reactions in slower rates than carbamate prodrugs of phenols such as **16** (Fig. **14**) [18]. On the other hand, N-Mannich bases of salicylamide (Fig. **15**) as prodrugs for amines undergo cleavage into their corresponding parent drugs in much slower rates than their corresponding N-Mannich bases of alkyl amides (Fig. **15**) [17].

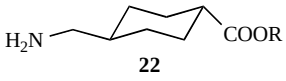
2.6. Basic Esters

Basic esters are esters involving a basic group in their alkyl part or in their acyl moiety and are considered important for the prodrug approach. These esters are characterized with good water solubility and are ready to be chemically hydrolyzed at physiological pH. In addition, their binding to carboxylesterase is very weak. Examples for such compounds are the amino esters (compounds **22-24** in Fig. **16**) which their hydrolysis rates are largely affected by steric effects.

For instance, a study has showed that aryl esters of 2-aminocarboxylic acid such as compound **23,** 4- nitrophenyl 2-aminocyclohexanecarboxylate, aryl = 4-NO2-C6H4, Figure 16) underwent hydrolysis in pH 7 in higher rates than their corresponding lacking the amine moiety [19].

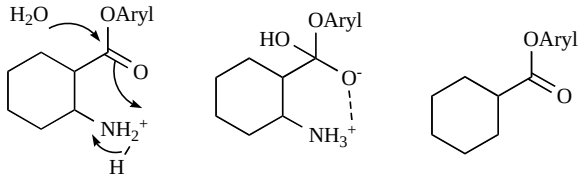
Various promising applications of α-amino acid esters have been published in recent years, such as valaciclovir, the L-valyl

ester of acyclovir, anti-viral agent, which showed encouraging pharmacokinetic results in the rat and monkey. For example, there was significant improvement in the oral bioavailability of acyclovir using the prodrug approach over the limited bioavailability of the drug itself. Interestingly, the rat liver enzyme that hydrolyzes valaciclovir, showed high selectivity for various amino acid esters of acyclovir, differently from typical esterases and peptidases. Recently, the L-aspartate β-ester, and L-lysyl and L-phenylalanyl esters of acyclovir were prepared and examined as prodrugs for nasal absorption. The aspartate β -ester was the most stable, and was also the only one to be detectably absorbed, seemingly by active uptake [19].



22

Trans-4-(aminomethyl) cyclohexanecarboxylates



23 **24**

Fig. (16). Hydrolysis of basic esters, R=aryl or aryl methyl.

3. AMIDE PRODRUGS

Prodrug design has been used to improve physicochemical characteristics of amine drugs, by improving the metabolic stability and decreasing ionization of the amine parent drugs resulting in improvement of its membrane penetration. Intramolecular cyclizations have been proposed to release the parent drug [2].

Vigroux and coworkers reported the synthesis of mutual prodrugs of benzoxazolones and oxazolidinones. In the cases of prodrugs of chlorzoxazone, 6-benzoylbenzoxazolone and 5-benzoylbenzoxazolone with paracetamol, the activation of these prodrugs involves cyclization reaction that is independent on enzymes catalysis (Fig. **17**). While the activation of prodrugs of oxazolidinones (metaxaloned and mephenoxalone) with paracetamol involve two steps, the first depends on plasma enzymes to form the intermediate 2-hydroxypropyl isocyanate, which is then converted spontaneously by intramolecular cyclization to the active oxazolidinones drugs (Fig. **18**) [20].

Fredholt and Bundgaard synthesized and studied 13 phenyl carbamate prodrugs to determine the effect of R_1, R_2 and R_3 on the rate of intramolecular regeneration of the parent phenol (Figure 19) [21]. They concluded that when R_1 is hydrogen the rate is much higher than when it is methyl or ethyl. Bulky R_2 groups decreased the reactivity of these prodrugs since the amide attack on the carbamate becomes difficult, while increasing the polarity of R_2 group increased the prodrug reactivity. The decrease in the pK_a of R_3 increases the intramolecular cyclization rate by making the phenolate ion a better leaving group [21]. Carbamate prodrugs were further

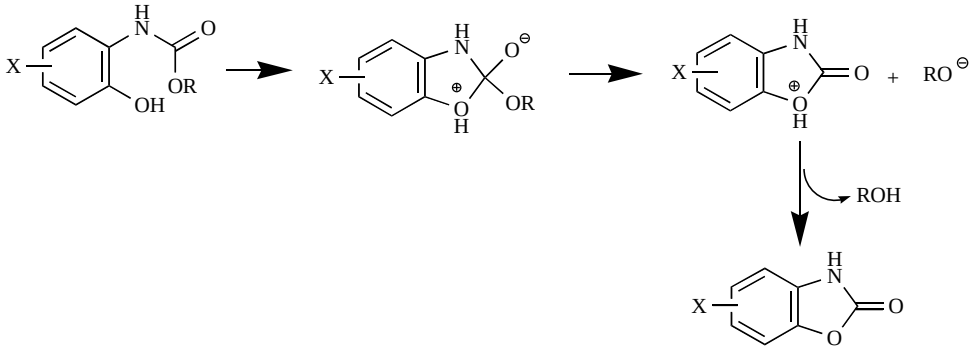


Fig. (17). Intramolecular activation of benzoxazolones prodrugs: chlorzoxazone, X = 5-Cl; 6-Benzoylbenzoxazolon, X= 4-benzyl; 5-benzylbenzoxazolone, X= 5-benzyland R= paracetamol in all three prodrugs.

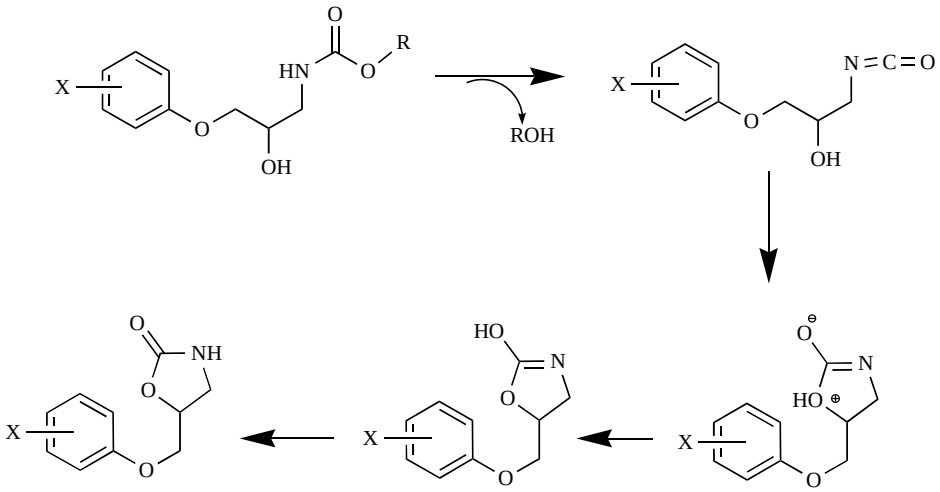
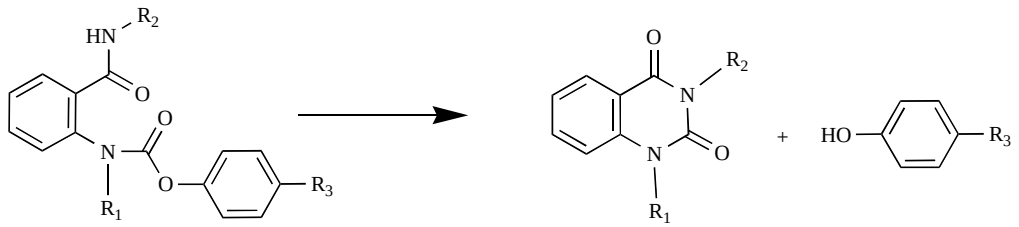


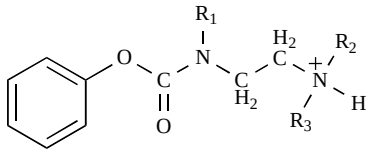
Fig. (18). Intramolecular activation of oxazolidinones prodrugs: metaxalone, X = 3, 5(Me)$_2$;mephenoxalone, X = 2-OMe;R= paracetamol in both prodrugs.



Compound	R1	R2	R3
A1	CH_3	**H**	**H**
A2	CH_3	**H**	**Cl**
A3	CH_3	**H**	**OCH3**
B1	CH_3	CH_3	**H**
B2	CH_3	CH_3	**Cl**
B3	CH_3	CH_3	**OCH3**
C	CH_3	CH_2CH_3	**H**
D	CH_3	$CH_2CH_2CH_3$	**H**
E	CH_3	$CH(CH_3)_2$	**H**
F	CH_3	$CH_2CH_2CH_2CH_3$	**H**
G	CH_3	$CH_2COOC_2H_5$	**H**
H	CH_3	CH_2CONH_2	**H**
I	CH_3	$CH(CH_3)_2CONH_2$	**H**
J	CH_3	$CH_2CH_2COOC_2H_5$	**H**
K	C_2H_5	CH_3	**H**
L	C_2H_5	CH_2CONH_2	**H**
M	**H**	**H**	**H**

Fig. (19). Carbamate prodrugs activation.

studied using phenol as a model for phenol containing drugs having low bioavailability due to first pass metabolism. A Study of 9 compounds with different substituents at the nitrogen atoms (Fig. **20**) demonstrated that the carbamate prodrugs were stable in acidic conditions and were not affected by liver or plasma enzymes. While the rate of intramolecular cyclization was not affected by enzymes it was greatly influenced by the steric effect of the substituent groups at the amine nitrogen [22].



	R1	R2	R3
1	CH_3	CH_3	CH_3
2	CH_2CH_3	CH_2CH_3	CH_2CH_3
3	CH_3	CH_2CH_3	CH_2CH_3
4	CH_2CH_3	CH_3	CH_3
5	CH_3	CH_3	**H**
6	CH_2CH_3	CH_2CH_3	**H**
7	**H**	CH_3	CH_3
8	**H**	CH_3	**H**
9	**H**	**H**	**H**

Fig. (20). Chemical structure of phenyl -N-(2- aminoethyl) carbamates.

Basic carbamate prodrugs were developed in which the mechanism of the release of the parent drug is intramolecular and not enzymatic (Fig. **15**). A study on these prodrugs confirmed that 4-hydroxyanisole is formed by cyclization and that the nonprotonated amine is essential for the reaction, which is fast at pH 7.4 compared to lower pHs. This difference in reaction rates is dependent on the environmental pH and structure and can be utilized for the development of prodrugs [18].

Doxorubicin hydrazone and hydrazine carboxylate prodrugs were developed in order to be targeted to tumors. Hydrazine carboxylate and hydrazone designed to be linkers between doxorubicin and a polymer. The study of these prodrugs confirmed that the product of cyclization is due to intramolecular reaction in which the hydroxyl on C14 attacks the carbonyl group in the hydrazone (Fig. **21**) [23]. Activity of the cyclization product has not been studied neither *in vivo* nor *in vitro*, since doxorubicin itself is ineffective in inhibition of tumor growth rate in murine B16F10 melanoma animal model without a carrier [24]. It is expected that this conjugate will be less active than doxorubicin and the free doxorubicin will not be highly active [23].

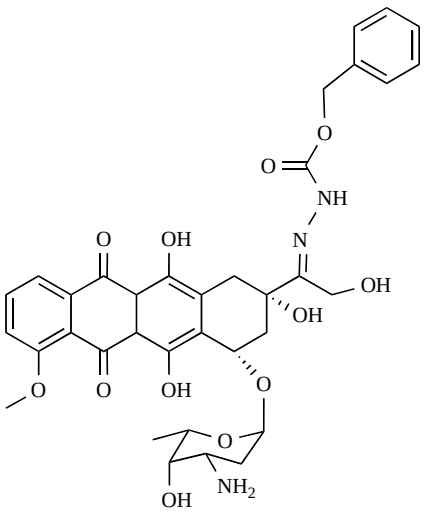


Fig. (21). Intramolecular cyclization of doxorubicin prodrug.

Prednisolone prodrug was designed as prednisolone 21-hemisuccinate β-cyclodextrin. This prodrug is converted in-

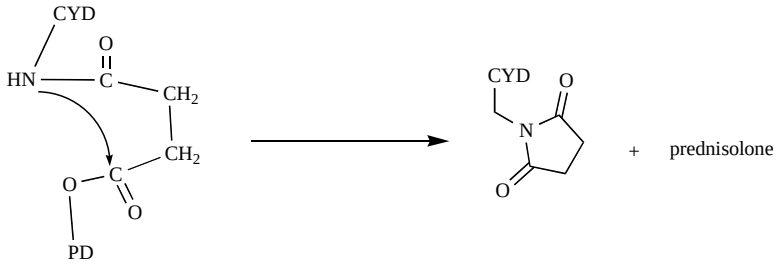


Fig. (22). Intramolecular hydrolysis mechanism for prednisolone 21-hemisuccinate β-cyclodextrin, CYD = cyclodextrin, PD = Prednisolone.

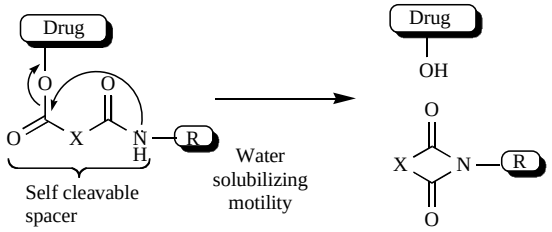


Fig. (23). A strategy of water soluble prodrugs that provide the active parent drug *via* an intramolecular reaction.

tramolecularly to prednisolone (Fig. **22**). The rate of the reaction is increased with an increase in the pH. This indicates that the nucleophilic amide group attacks the ester group and causes the release of prednisolone [25]. Prednisolone succinate α-cyclodextrine prodrug was also synthesized and studied *in vitro*, this prodrug showed slower hydrolysis compared to the prednisolone succinate β-cyclodextrin, therefore it was used for *in vivo* studies. *In vivo* studies in irritable bowel syndrome rat model indicated that Prednisolone succinate α-cyclodextrine alleviated systemic side effects, since the amount of prednisolone absorbed from the upper gastrointestinal tracts was much smaller than that absorbed when prednisolone administered orally alone. In addition, this prodrug also maintained the therapeutic anti-inflammatory effects [26].

4. INTRAMOLECULAR ACTIVATION OF WATER-SOLUBLE PRODRUGS OF HIV-PROTEASE INHIBITOR

A strategy of water soluble prodrugs of HIV-protease inhibitor was developed to release the active parent drug *via* an intramolecular process [2, 27, 28].

These prodrugs have two useful unites, a water solubilizing group and self-cleavable spacer, which are linked in tandem to the parent drug (Fig. **23**). The intraconversion of these prodrugs to their active drugs is *via* a chemical degradation at the spacer. This cleavage is based on intramolecular cyclization-elimination involving the formation of an imide formation upon exposure to physiological environment [2, 27, 28].

A number of water-soluble KNI-727 prodrugs having different conversion times (4 minutes to 34.3 hours) were synthesized by structural alteration on the solubilizing moiety (Fig. **24**) [2,28]. It was found that introducing bulky cyclic tertiary amines with different electronic properties (**25a-c** in Fig. **25**) enhanced the intraconversion, due to the fact that bulky cyclic structures force the *tert*-amino group into a suitable conformation for a proton abstraction on the neighboring-group rather than providing a steric hindrance [1]. It was reported that the half-life value of compound **25a** is about 10 times higher than that of prodrug **25b**, which could be explained by the high electron- inductive effect that might be caused by the morpholine oxygen. Furthermore, introduction of propyl morpholine (**25c**) displayed a further increase in the half-life value because an addition of methylene increases the electron donating effect. Possibly more difficult to make six membered transition state for deprotonation compared to five membered TS which would be more plausible for increasing half-life (Fig. **24**) [1, 28].

In addition, It was demonstrated that an increase in the water solubility of these prodrugs resulted in improvement of their gastrointestinal absorption.

It was found that the biological activity of the AZT-KNI prodrug was 920 and 62 times more potent than that of KNI-727 and AZT, respectively [1, 28].

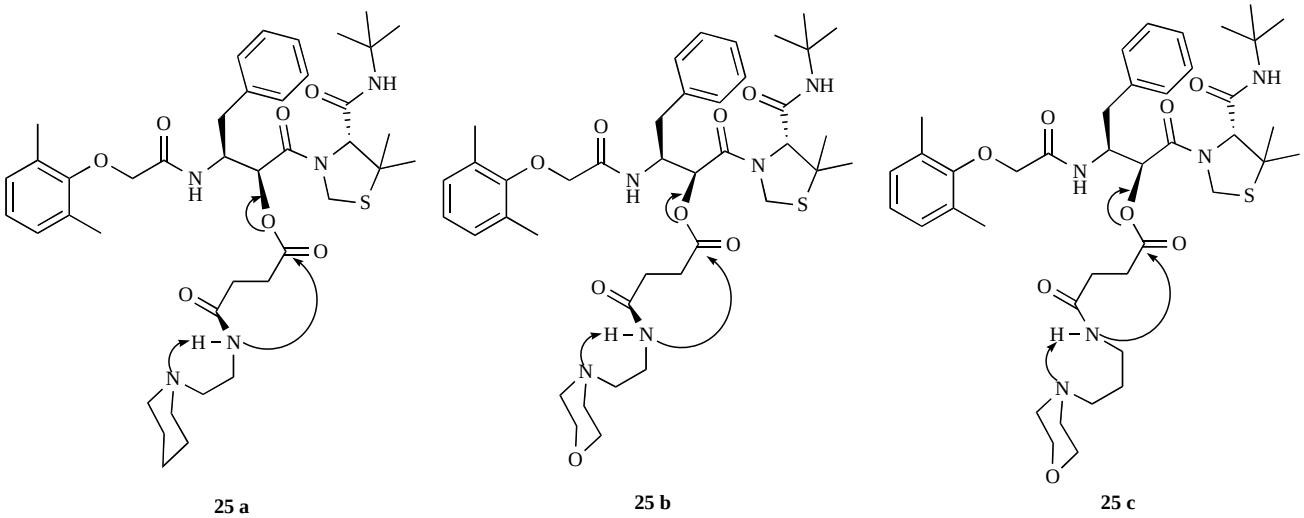


Fig. (24). Activation of water-soluble prodrugs of KNI-727, **25a-c** *via* Intramolecular process.

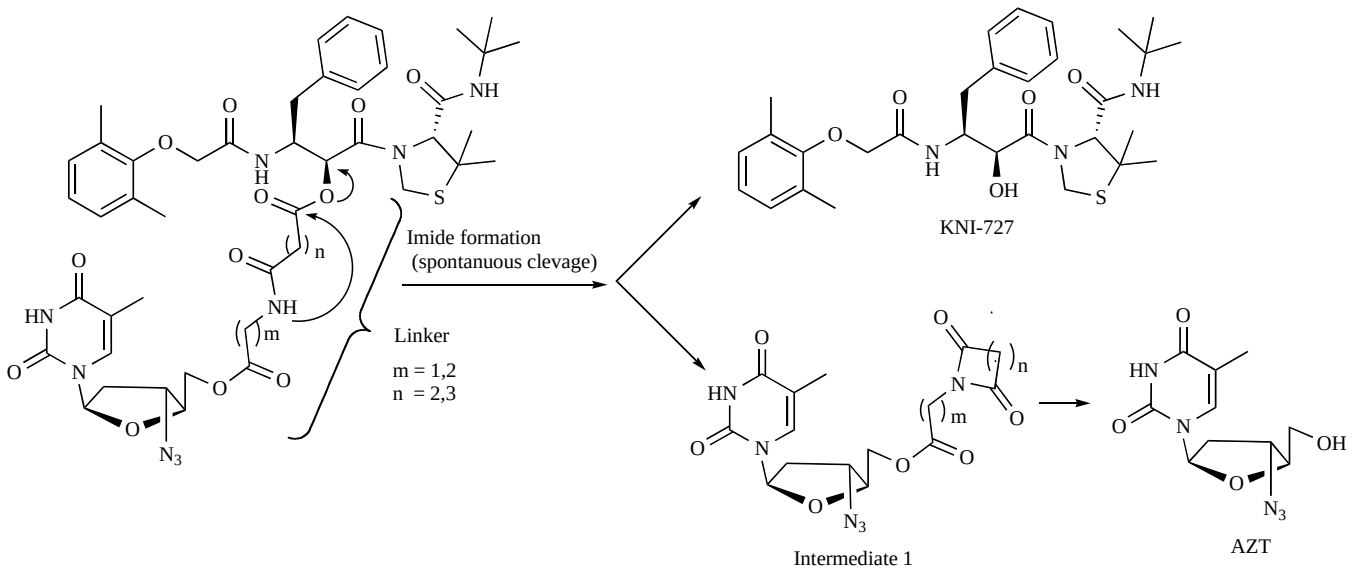


Fig. (25). Conversion of double-drug to KNI-727 and AZT *via* intramolecular cyclization.

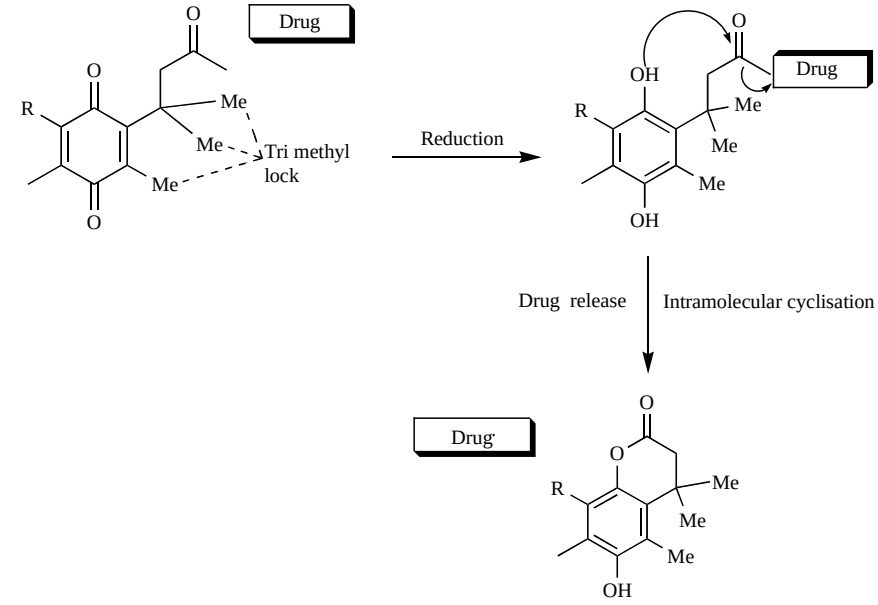


Fig. (26). Bioreductive delivery of quinone propionic delivery system.

A number of promoieties connecting the hydroxyl of KNI-727 to 3-azido-3-deoxythimidine (AZT) were also studied by the same research group. Under mild alkaline conditions, these linkers were found to provide the active parent drug, KNI-727, through intramolecular cyclization involving an imide formation (Fig. **25**) [29, 30].

5. THE ACTIVATION OF BIOREDUCTIVE PRODRUGS THAT UNDERGO INTRAMOLECULAR CYCLIZATION FOLLOWING CHEMICAL REDUCTION

Hypoxia appears to be a popular and unique feature of cells in solid tumors [31-36] and it has been reported to exist in a variety of other diseases [31]. Bioreductive prodrugs were designed to selectively target the hypoxic cells in malignant tumors and release cytotoxic species after metabolic reduction [31-36]. This section will focus on the activation of bioreductive prodrugs that undergo intramolecular cyclization following chemical reduction.

The three main classes of bioreductive drugs are quinones, N-oxides and hetero-aromatic nitro groups. However, activation of bioreductive prodrugs that undergo intramolecular cyclization following chemical reduction has only tested with nitroaromatic and quinone compounds [33, 36].

5.1. Quinone as Bioreductive Prodrugs Targeting the Hypoxic Cells in Tumors

An early approach for hypoxia-selective delivery agents is the one utilized the quinone propionic delivery agent; the benzoquinone proceeds as a trigger and the propionic carrier works as the linker while a trimethyl lock provides the steric hindrance necessary to optimize a ring closure [37, 38]. Steric hindrance arises from the methyl groups (termed 'trimethyl lock') has been represented to greatly enhance lactonization processes [39, 40]. Metabolic reduction of the benzoquinone triggers intramolecular cyclization to generate a lactone and release the drug (Fig. **26**) [41]. A comprehen-

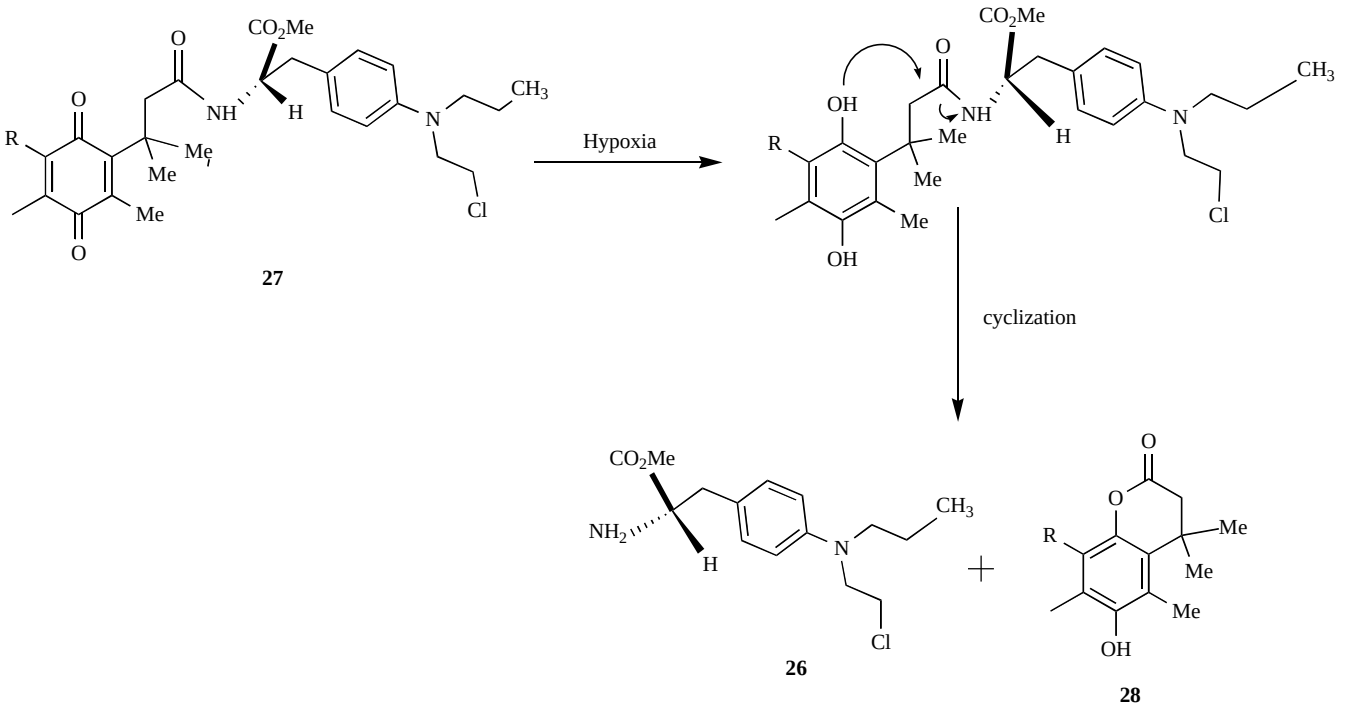


Fig. (27). Activation and cyclization of a quinone based hypoxia prodrug.

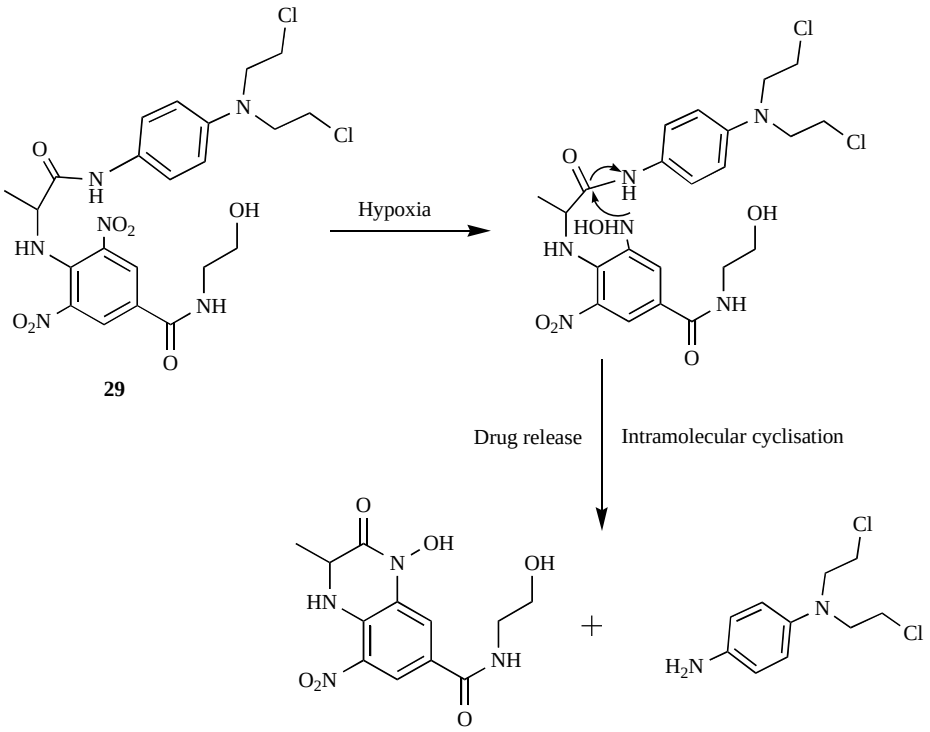


Fig. (28). Reduction, cyclization and releasing of aromatic mustard.

sive study of the trimethyl lock facilated lactonization has been represented [39].

Prodrug **27** was developed to release the parent drug **26** after a reduction of the quinone system under hypoxic conditions, followed by formation of bioreduction product (lactone) **28** and a release of the parent drug, melphalan methyl ester; MME, **26** (Fig. **27**) [33, 42-43].

Upon chemical reduction with sodium borohydride in phosphate buffer (pH = 7.4) a formation of lactone and a release of the drug was indeed observed. No biological data of these compounds has been reported yet [33].

5.2. Nitroaromatics As Hypoxia-Selective Delivery Agents

Developing anticancer drugs that could be selectively activated under hypoxic conditions was attempted by Denny and coworkers who developed few nitro aromatic mustard prodrugs which *in vivo* were converted to the corresponding hydroxylamino derivatives (**29** in Fig. **28**). In the hypoxic cells in malignant tumors, nitro groups

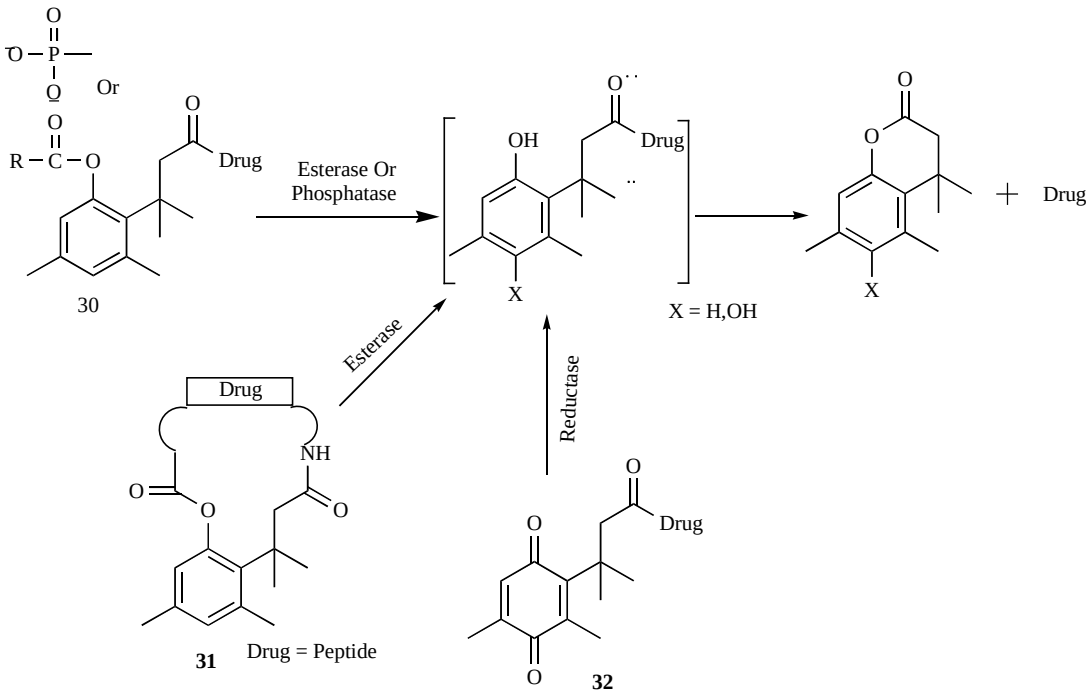


Fig. (29). Trimethyl lock-based prodrugs.

are reduced into amino groups to trigger cyclization and result in a release of these cytotoxic aromatic mustards (Fig. **28**) [1, 32, 33].

5.3. Trimethyl Lock-Based Prodrugs

The accepted definition of the Thorpe-Ingold or *gem*-dialkyl effect is the increase in both rate and equilibrium constants of cyclization reactions resulting from placing groups in the linking chain. The effect of *gem*-dialkyl substitution is explained in terms of strain enhancement and reduced entropy of rotation in the open-chain moiety. It is well documented that the Thorpe-Ingold effect on the formation of normal rings varies considerably, and depends on both the type of reaction and the position of the group(s) in the chain.

A "trimethyl lock" system has been known to facilitate lactonization reactions of *o*-hydroxylphenylpropionic acid and its derivatives with an effective molarity of 10 [44]. These facile lactonization reactions have been used by Borchardt and co-workers to develop redox-, esterase-, [45-47] and phosphatase-sensitive prodrug systems [48] and an esterase sensitive cyclic prodrug system for peptides (Fig. **29**) [49, 50].

The esterase-sensitive prodrug system was adjusted with anisidine as an amine model. The prodrug was found under all conditions to degrade with half-life values of 12 minutes under exposure of porcine liver esterase, 54 minutes upon exposure to plasma and 475 minutes in plasma containing diisopropylfluorophosphate [47].

The redox sensitive prodrug concept was studied with both aromatic and aliphatic amines [45, 51, 52]. The reduction of quinone portion of prodrugs **30-32** (Fig. **29**) generates the intermediate hydroquinone which rapidly lactonizes, yielding the corresponding parent drug. Following reduction to hydroquinone (X=OH) the half-life values of these prodrugs were found in the range 1.4 to 3.4 minutes [45].

The pharmaceutical properties of several drugs were improved by using the two-step prodrug approach based on ''trimethyl lock" concept. For example, the "trimethyl lock" prodrug process has been shown to increase ganciclovir (antiviral) oral bioavailability. This prodrug increased oral bioavailability by four folds [53, 54]. In an effort to increase the oral water solubility of the anticancer agent paclitaxel, its phosphatase-sensitive prodrug was made by using the "trimethyl lock" approach. This prodrug was shown to hydrolyze quickly to taxol upon exposure to alkaline phosphatases (within 25 minutes) [53, 55].

In another study, the efficacy of daunorubicin against ovarian cancer was increased upon conjugation of the drug with PEG that functions *via* a trimethyl lock lactonization [53, 56]. More recently, Raines and co-workers designed latent fluorophores based on the trimethyl lock approach. These fluorophores are essential probes for basic research in biochemical and biological sciences [57, 58].

6. ENZYME MODELS

Over the past six decades pioneer studies by Bender, Jencks, Bruice, Benkovic, Menger, Kirby, Walesh and others have contributed for und Understanding the most important question in biochemistry "how enzymes catalyze biochemical transformations" ?

Generally, enzymatic reactions rates are about 10^{10} - 10^{20}-fold higher than their non-enzymatic counterparts. For example, biochemical transformation catalyzed by orotidine monophosphate decarboxylase are enhanced by 10^{17}-fold whereas those involving the enzyme cyclophilin are accelerated by only 10^{5}-fold. It is believed that the extraordinary rates enhancement achieved by enzymes is due to the substrate binding onto the active site of the enzyme [59-63].

The resemblance between intramolecularity and enzymes has encouraged scientists to design and synthesize chemical devices having two reactive centers that undergo intramolecular reactions. Unraveling the mechanisms of these intramolecular processes has helped in comprehending the efficient catalysis by enzymes [64-66]. During the past sixty years proposals were emerged from trials to interpret variation in efficiency versus alteration in the chemical features in such intramolecular devices. Among those chemical devices or enzyme models: (1) ''orbital steering'' proposal by

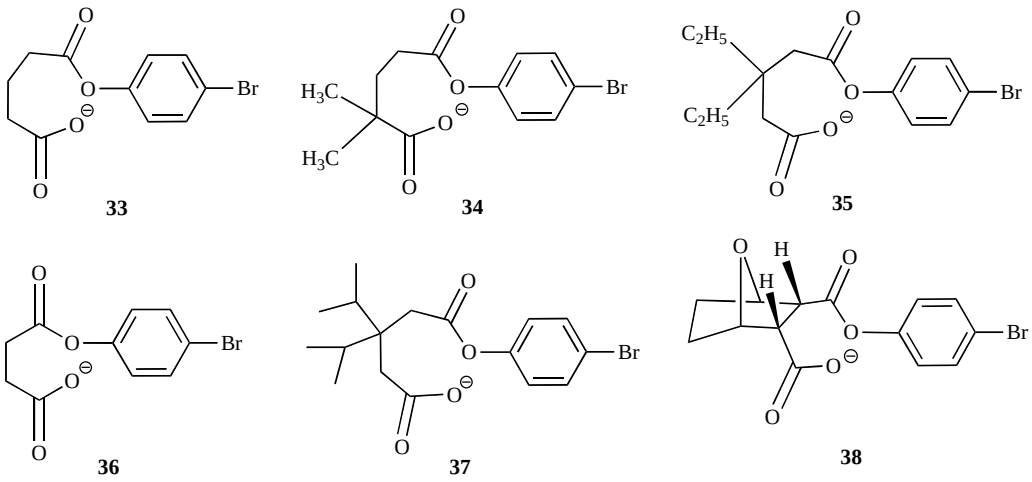


Fig. (30). Di-carboxylic semi-esters **33-38**.

Koshland which relates accelerations in intramolecularity rate with the attack angle of the nucleophile onto electrophile such as in the acid-catalyzed lactonization of hydroxy acids [67]; (2) near attack conformation or ''proximity'' in intramolecular reactions as suggested by Bruice and illustrated in the ring-closing of di-carboxylic semi-esters [68-70]; (3) Cohen's ''stereopopulation control'' proposed accelerations in rate as a result of freezing a molecule into a productive conformation [71-73], (4) ''spatiotemporal hypothesis'' proposed by Menger which postulates that the interatomic distance between the nucleophile and electrophile is linearly correlated with the reaction rate of intramolecular process [74-78] and (5) proton transfer models advocated by Kirby on the acid-catalyzed hydrolysis of acetals and N-alkylmaleamic acids which attribute the enhancement in rates to hydrogen bonding formation in the transition states and their products [79-86].

Investigations on the role of chemical functional groups participate in intramolecular reactions have been conducted in order to comprehend the importance of such groups in enzyme catalysis. The mechanisms revealed from these studies have provided a clear picture on the relationship between intramolecular reactivity and chemical structure which consequently explains related aspects involving in enzyme catalysis.

In this section, the mechanisms of a number of some enzyme models that were proposed to comprehend the catalysis by enzymes were explored using computational methods. The computational approach used in this study involves calculations of ground and transition states energies by different methods such as *ab initio*, AM1 and density functional theory (DFT), and molecular mechanics (MM), and correlations between experimental and calculated rates. The computational methods used in this study are widely used due to their precise prediction results and are considered as reliable tools to predict potential drugs and prodrugs [87].

6.1. Bruice's Enzyme Model Based on Ring-closing of Di-carboxylic Semi-Esters

The hydrolysis of di-carboxylic semi-esters **33-38** (Fig. **30**) was investigated by Bruice and Pandit and their study demonstrated that the relative rate (k_{rel}) for **38**>**37**>**36**>**35**>**34**>**33**. This result was explained on the basis that variation in rate is due to proximity factors; alkyl substituents on succinic acid derivatives affect the distribution of conformations, the reactive gauche to unreactive anti ratio. Based on this hypothesis, they suggested that *gem*-dialkyl groups magnify the formation of the more reactive conformer. Thus, for cyclization to take place, both electrophile and nucleophile must reside in the gauche conformation. In the reactant without substituents, the electrophile and nucleophile are totally in the anti-conformer for overcoming steric interactions [68-70].

Menger and Bruice attributed the phenomenon of rate enhancements in intramolecularity to the important role of the proximity between the two reacting centers in the reactants [68-70, 74-78]. The "spatiotemporal" hypothesis advocated by Menger is summarized in an equation combining activation energy and interatomic distance of the nucleophile and electrophile and based on that, he proposed that significant rate enhancements in enzymatic reactions are obtained by close proximity of the enzyme and substrate reacting centers [74-78]. Differently, Bruice and Pandit have ascribed the rate accelerations in biotransformation catalyzed by enzymes to favorable "near attack conformations". Based on this hypothesis, molecules reside in reactive conformations will have higher rates than those with unreactive conformers. Bruice's idea combines both the interatomic distance between the nucleophile and electrophile and the angle of attack [68-70].

Contrary to the proximity orientation proposal, some other chemists proposed that high rate enhancements observed in intramolecular processes is due to steric factor [88, 89]. For testing whether this high enhancements in rate obtained in the cyclization of di-carboxylic semi-esters **33-38** is a result of proximity or strain factors, we have calculated, using DFT at B3LYP/6-31G (d,p) and HF/6-31G (d,p) levels, the energy profiles for the cyclization reactions of **33-38**. Our study revealed that the rate-limiting step is the collapse of the tetrahedral intermediate and not its formation (Fig. **31**). In contrast to Bruice's and Pandit's conclusion, our study demonstrated that the rate enhancements are due to strain and not proximity effects, "rotamer effect" [90].

Further, to investigate whether the differences in rates of processes **33-38** (Fig. **30**) is due to proximity or strain energy, we have calculated using MM2 method the strain energy values (Es) [91] for the entities involved in **33-38.** The strain energy values were plotted against the experimental relative rate values (log k_{rel}) [68-70] and the results revealed a good correlation between both parameters (equations 1 and 2). No correlation was observed between log k_{rel} and the interatomic distance between the nucleophile and electrophile (r_{GM}). This result strongly suggests that the determining factor for the enhancements in the ring-closing reaction rate is strain ef-

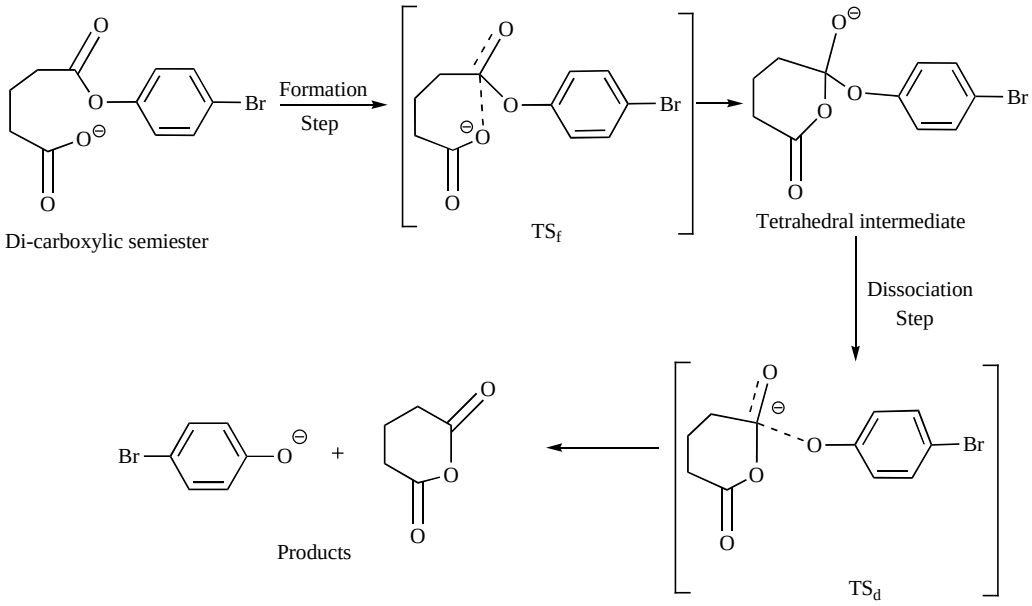


Fig. (31). A diagram showing the suggested mechanism for the cyclization of di-carboxylic semi-esters **33**-**38**.

fects and not Bruice's near attack proximity orientation [68-70]. Additional support to this conclusion was evident by the strong correlation obtained from the plot of the activation energy values ($\Delta G^{\ddagger}_{H2O}$ and $\Delta G^{\ddagger}_{GP}$) for **33**-**38** versus both log k_{rel} and the MM2 strain energy values, ΔEs (TS - AN) (equations 3 and 4).

1 $\Delta G^{\ddagger}_{H2O}$ B3LYP/6-31G (d,p) = -2.0657logkrel + 17.653 0.95

2 $\Delta G^{\ddagger}_{GP}$ B3LYP/6-31G (d,p) = -1.7747logkrel + 14.729 0.90

3 $\Delta G^{\ddagger}_{H2O}$ B3LYP/6-31G (d,p) = -1.0467ΔEs (TS - AN) + 3.262 0.99

4 $\Delta G^{\ddagger}_{GP}$ B3LYP/6-31G (d,p) = -0.9101ΔEs (TS -AN) + 3.647 0.98

The main conclusions emerged from our computational study include (i) the activation energy in **33**-**38** is a function of ΔEs, and no correlation was found between the ring-closing rate and the interatomic distance O1-C2. (ii) The transition states of the rate-limiting step were found to be with an open cyclic ring. This provides support to the conclusion that ΔEs is the sole factor causing the discrepancy in rates of **33**-**38**. (iii) Reactants having high strain energy values such as **38** are more reactive than the ones with less strain energy values, and the reaction rate is proportionally correlated with the transition state and the reactant strain energies (ΔEs). (iv) The activation energy for the reaction of reactants having bulky substituents in their alkyl chains (strained reactants) is less than that of the ones without substituents (unstrained reactants) [90].

6.2. Cohen's Stereopopulation Control

About five decades ago, Cohen *et al.* have researched the lactonization reaction of a number of hydroxyhydrocinnamic acids, **39**-**45** (Fig. **32**). The study demonstrated reaction rates in the range of 1 to 10^{15}. The discrepancy in rates for the structurally different hydroxyhydrocinnamic acids was attributed to the so called ''stereopopulation control" [61-63]. Later, Cohen's ''stereopopulation control" theory was criticized by others who suggested that the high acceleration in rates determined by Cohen is due to a relief in strain energy upon the lactonization process and not a result of stereopopulation control of the trimethyl lock system [88-89].

The controversy regarding the determining factor for the significant accelerations in the reaction rates of hydroxyhydrocinnamic acids **39**-**45** and hydroxy acids **46**-**50** (Fig. **32**) has continued for many years. For the verification of whether Cohen's stereopopulation control [61-63] or the relief in strain as proposed by Wilcox and Winans [92] and confirmed by Houk's calculations [88-89] is in fact the determining factor for the acceleration in rates, we have executed a comprehensive computational study using AM1 semi-empirical, HF/6-31G(d,p) and DFT at B3LYP/6-31G(d,p) methods. In this study we studied the following: (1) calculations of ground state geometries and energies (ΔG°, ΔH° and ΔS°) of all the entities involved in the reactions of **39**-**45**, (2) calculation of the rotation barriers of the side chain in **39**-**45** for determining whether the conformational restriction is crucial in the rate acceleration of the lactonization process, (3) calculations of the activation energies ($\Delta G^{\ddagger}$) for all steps involved. The calculation results revealed the following: (1) contrary to Houk *et al.* [88-89] proposal, the lactonization's rate-limiting step of hydroxy acids studied is the formation of the tetrahedral intermediate and not its dissociation (Fig. **33**). The rate-limiting step involves: (i) an attack of the hydroxyl (nucleophile) on the carbon of C=O (electrophile), (ii) a proton transfer from the ether oxygen to O^- of the carboxylic group. The activation energy in (ii) is dependent on the structural features of the hydroxy acid and its value is a sum of the changes in entropy and enthalpy. Entropy changes are predominant in the initial stage of the approach (C-O distance is 4-2.5 Å) and enthalpy changes are in the approach's second stage (C-O distance is 2.5-1.5 Å). For example, in systems **41** and **42**, the entropy change from the ground (C-O distance is around 3 Å) to the organized ground states (C-O distance is around 2.5 Å) is less than that in **39** and **40** due to proximity. In a similar manner, in the second stage, the enthalpy change for **41** and **42** is less than that for **39** and **40** as a result of the proximity found in the tri-methyl lock system. In step (i), the activation energy is almost constant and not affected by the nature of the reactant.

(2) The computational methods utilized in this study provide a tool for activation energy prediction if the attack angle and the interatomic distance between the nucleophile and electrophile are known. Using the equation of the calculated and experimental en-

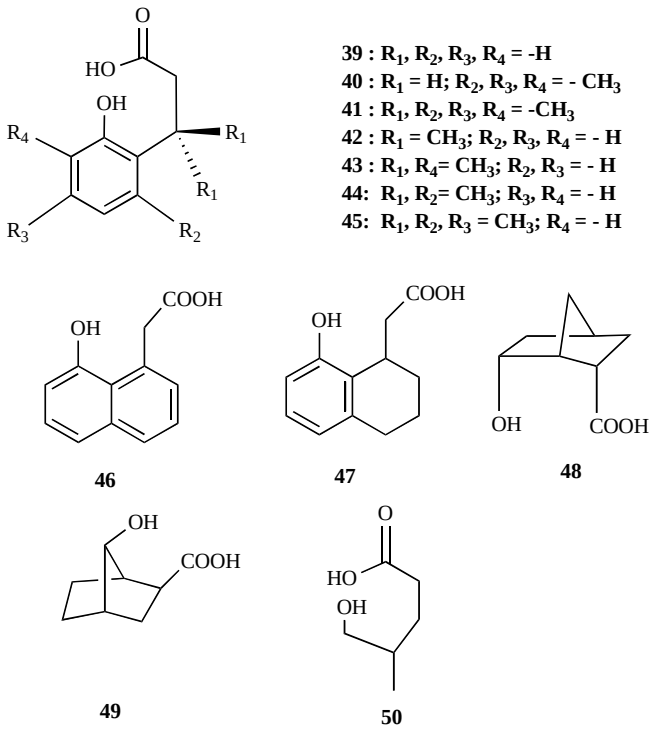


Fig. (32). Chemical structures for hydroxyhydrocinnamic acids **39**-**45** and hydroxy acids **46**-**50**.

ergy values potential hydroxy acids having high lactonization rates can be predicted, (3) contrary to that proposed by Wilcox and Winans [92] ground state energies alone are not sufficient to estimate rates, and it is a must to find a transition state along the reaction path to calculate its energy (barrier).

As mentioned before, there are two different explanations for rate enhancements in intramolecular processes and enzymes: Menger [74-78] and Bruice [68-70] proximity hypothesis and Jencks and others entropy driven process [88-89, 92, 93]. Our calculations revealed that Jencks's entropy proposal [93] does not apply to this studied case, since our calculations demonstrate that there is no change in entropy from GS_p to TS (Fig. **34**) and most entropy changes happen during the pre-organization step. In addition, the calculated frequencies revealed a constant ratio of the number of frequencies < 1000 cm^{-1} to the sum of frequencies from the GS_p to the TS (C-O distance between 2.5 - 1.5Å, X and Y in Fig. **34**). Furthermore, our calculations revealed that the system studied is in a perfect fit to the proximity proposal. The calculated ground state energies indicate that **41** possesses a near attack conformation (NAC), while **39** requires about 7 kcal/mol in order to reach the NAC. This difference in energy is equal to 10^6-fold in the rate ratio of **41**/**39**. In addition, we have demonstrated that in the approach's second stage the energy required for **39** to reach the transition state is 7 kcal/mol higher than for **41** (10^6-folds in **41**/**39** ratio rate). This is due to the fact that the pre-organized structure of **41** is much more favorable conformation for achieving efficient overlapping than the pre-organized conformation of **39**, which was found to possess conformational changes during the second stage of the approach because of its high flexibility [94-96].

6.3. Menger's Enzyme Model Based on Intramolecular Proton Transfer in Kemp's Acid Amides

Menger's singularity model and spatiotemporal hypothesis predict that a good and efficient enzyme model could be made using

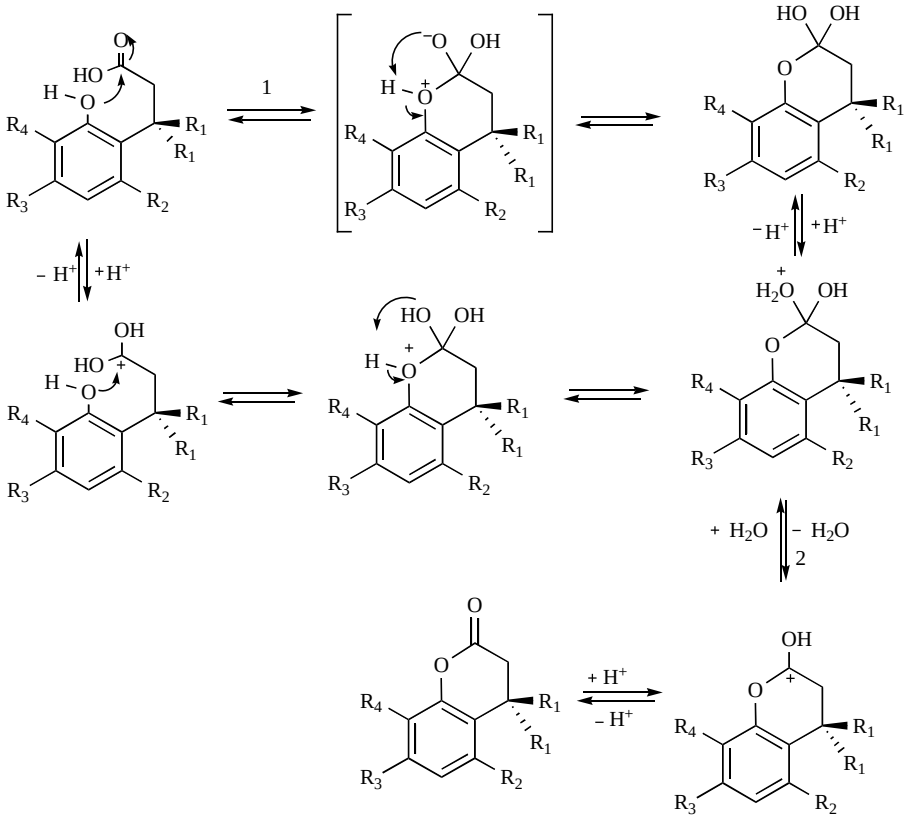


Fig. (33). Suggested mechanism for the lactonization of hydroxyhydrocinnamic acids **39**-**45** and hydroxy acids **46**-**50**.

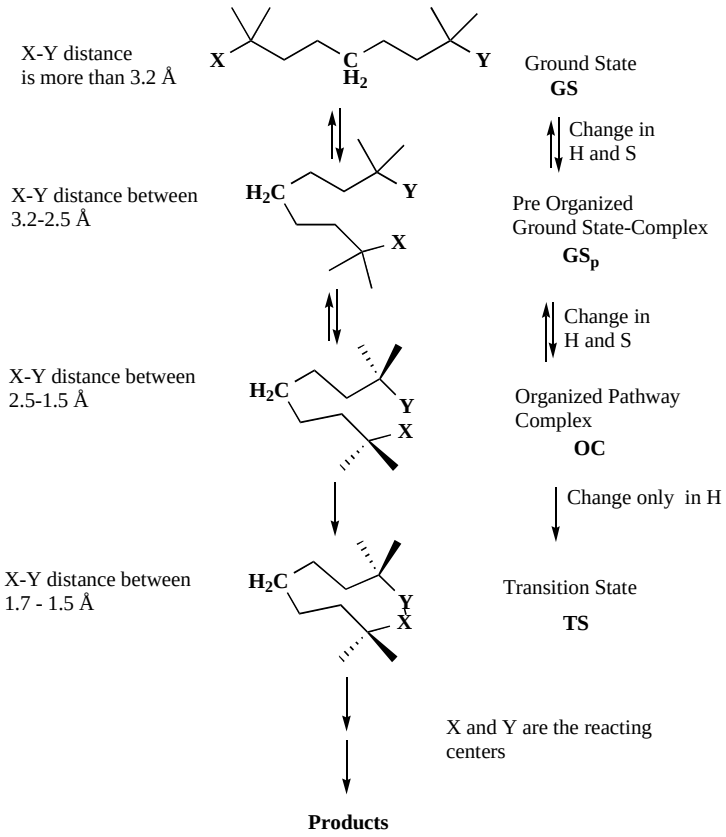


Fig. (34). Suggested mechanism for the approach of the hydroxyl O to the carboxylic C in the lactonization of hydroxy acids. X and Y are the reactive centers.

rigid organic moieties having the two reacting centers at or near the critical distance. One of the most striking enzyme models that meet this requirement is the intramolecular-catalyzed hydrolysis of an aliphatic amide (Fig. **35**) [74]. Noting that an aliphatic amide is very difficult to be hydrolyzed (more than 10-hour reflux in concentrated hydrochloric acid is needed for such hydrolysis). On the other hand, when a carboxyl group is placed in positions 1 and 3 diaxial to the amide, the $t_{1/2}$ value for the amide cleavage is 8 minutes at pH 7 and 21.5 °C. Although the structure shown in Figure **35** has two carboxyl groups, only a single carboxyl is necessary for achieving an enzyme-like rate enhancement. It is worth noting that the reaction of this aliphatic amide occurs through the more stable structure B rather than structure A (Fig. **35**).

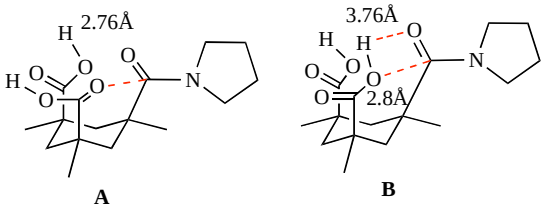


Fig. (35). Conformations A and B for Menger's reactive amide where B is presumably the most reactive conformer.

The fundamental question of how enzyme catalysis is achieved is yet to be unraveled [59-65]. This kind of understanding needs a proven mechanism for the reaction, and a comprehensive knowledge on the energy components, enthalpy and entropy of all entities along the reaction profile [64-65]. One prominent example of enzyme models that were advocated for understanding enzyme catalysis is the breakdown of Kemp's di-acid amide **51** that was synthesized by Menger and Ladika to explore the mechanism by which chymotrypsin enzyme catalyzes biotransformation processes [74]. Enzymes such as chymotrypsin catalyze the cleavage of peptide bonds *via* a nucleophilic attack of serine 195 of the enzyme onto the amide unreactive carbonyl group resulting in covalently linked substrate-enzyme intermediate. It was suggested that this breakdown of the peptide bond with chymotrypsin enzyme involves two stages: ''burst'' and ''ping-pong''. In other words, this enzymatic reaction occurs in two steps; an acylation of the peptide which involves a transfer of a proton to yield an acyl-enzyme intermediate which subsequently followed by deacylation to allow the enzyme to be freed to its original state [97, 98]. In the chemistry laboratory, peptide cleavage rate is very slow; at pH 7 and room temperature, the $t_{1/2}$ is around 500 years, with the absence of strong acid [99, 100], whereas in the small intestines (pH 6.8), all proteins are hydrolyzed rapidly. This acceleration in rate is as a result of peptidases catalysis through a proton transfer process [101].

HF/6-31G (d,p) and DFT at B3LYP/6-31G (d,p) level thermodynamics and kinetics calculations for the amide breakdown of Menger's enzyme model, **51-61** (Fig. **36**) were conducted to comprehend the significant rate acceleration brought about when these amides stand in neutral aqueous solution at ambient temperature. The aims of our computational study were: (1) to determine whether those amides undergo rapid cleavage as a result of a proton transfer reaction driven by proximity of the nucleophile (O6, see Scheme **1**) to the electrophile (H5, see Scheme **1**), as proposed by Menger [74], or whether it is a result of pseudoallylic (pseudo-A) strain relief upon the breakdown of the distorted amide bond to unstrained tetrahedral intermediate that is evolved upon the carboxylic oxygen approach (O4, see Scheme **1**) towards the amide car-

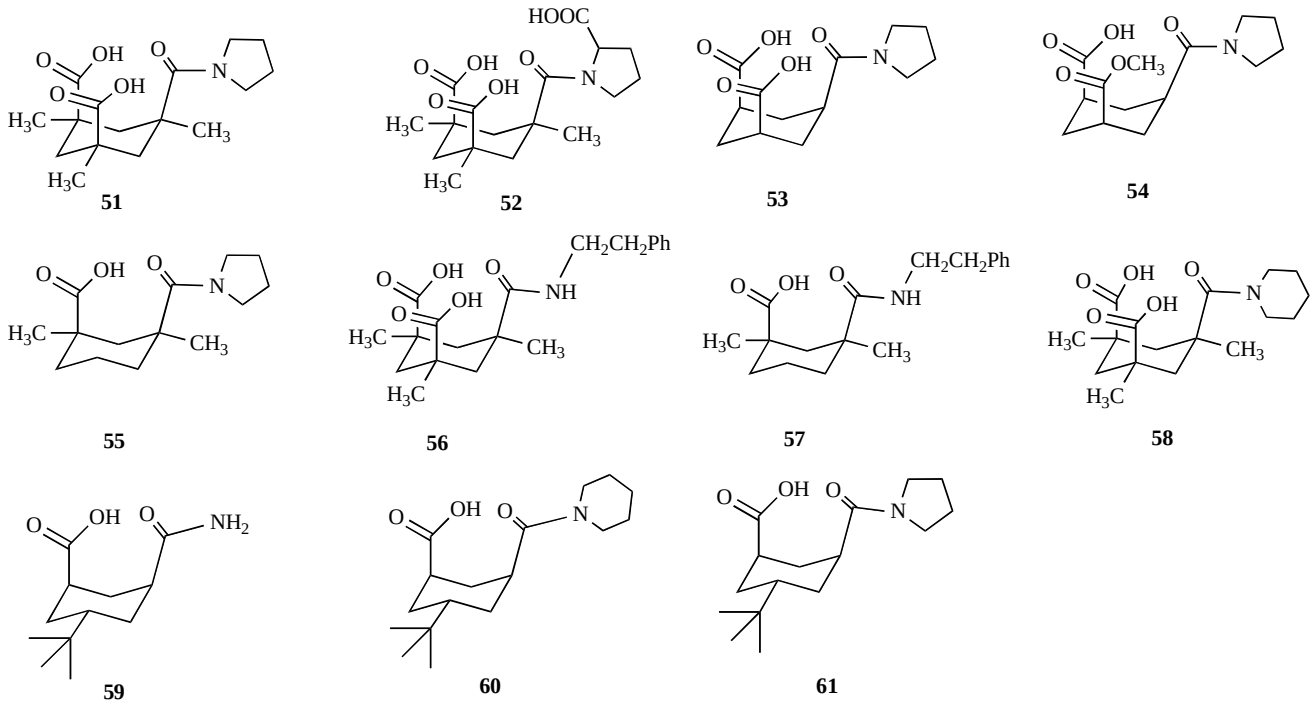
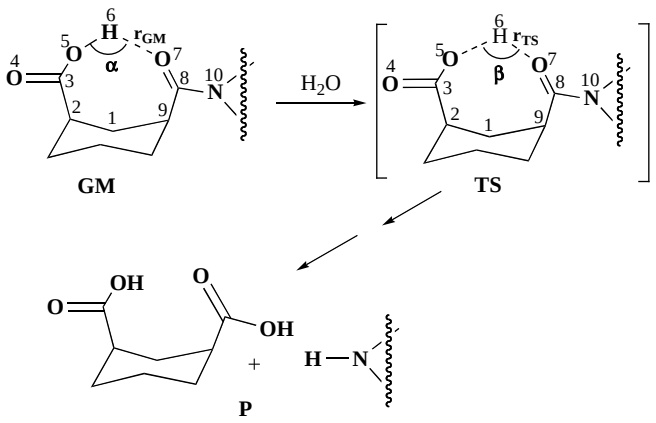


Fig. (36). Chemical structures of aliphatic amides **51-61**.

bonyl carbon (C7, see Scheme **1**), as was suggested by Curran and others [102, 103].

The mechanistic study results illustrated in Figure **37** revealed the following: (1) The barriers for the cleavage of amides **51- 61** at a pH range where the carboxylic group is in its free acid form (unionized) are much smaller than that through a proton transfer (route b) from the carboxylic oxygen onto the amidic carbonyl carbon (route a). For example, the B3LYP/6-31G (d,p) calculated activation energy in the gas phase for the hydrolysis of amide **51** *via* a proton transfer (route b) is 45.18 kcal/mol less than that *via* route a. (2) The HF and DFT calculated activation energy values for the cleavage reactions of amides **51** and **52** are almost the same. This result revealed that the presence of only one carboxylic group (amide **52**) is necessary for achieving the same enhancement as for the reaction of amide **51**. The calculation results are in agreement with Menger's experimental results [74]. (3) The calculated activation energy in water for the breakdown of amide **53** was ~3 kcal/mol less than that of amide **51**. This slight difference in energy might be due to the difference in the interatomic distances **51** and **53**; H5—O6 distance (Scheme **1**) in **51** is 2.9 Å and in **53** is 3.0 Å. This is in agreement with the''spatiotemporal hypothesis" and our calculations of other enzymes models [104-109].

In addition, the DFT calculations for the breakdown of Kemp's mono- and di-acid amides **51-61** demonstrate that a proton transfer from the carboxyl into the amide carbonyl oxygen is the rate limiting step in the acylolysis. It is suggested that enhancements in rate are dependent on r and α (Scheme **1**). In addition, it was found that the activation energy ($\Delta G^{\ddagger}$) is strongly correlated with r^2 x sin (180 - α). Further, contrary to previous reports the pseudoallylic factor has a negligible contribution if any to Kemp's triacid tertiary amides cleavage rates. Furthermore, our calculations propose a change in the mechanism when changing the reaction pH. Therefore, peptidase enzymes are too reactive at neutral pH while their efficiency is drastically decreased at basic pHs.



Scheme 1. Schematic representation for the cleavage of **51-61** showing the interatomic distance between the reacting centers (r) and the attack angle (α).

6.4. Kirby's Enzyme Model - Acid-Catalyzed Hydrolysis of N-alkylmaleamic Acids

Among the most common biotransformation that enzymes catalyze is the proton transfer reactions catalyzed by acid or base such as in the cases of triose phosphate isomerase and Δ5-3-ketosteroid isomerase. The findings that enzyme substrate interactions occur between functional groups held closely encouraged scientists to utilize intramolecularity in modeling the extremely high efficiency of enzymes.

Kirby *et al*. have studied the acid catalyzed hydrolysis of N-alkylmaleamic acids **62-68** to their corresponding maleamic acids and amines (Fig. **38**). The study found that the reaction is remarkably sensitive to the nature of the alkyl groups on the alkene bond. In addition, it demonstrated that the range of the hydrolysis rates for the studied dialkyl-N-methylmaleamic is more than 10^{10} s^{-1}, and the "effective concentration" of the carboxyl group of

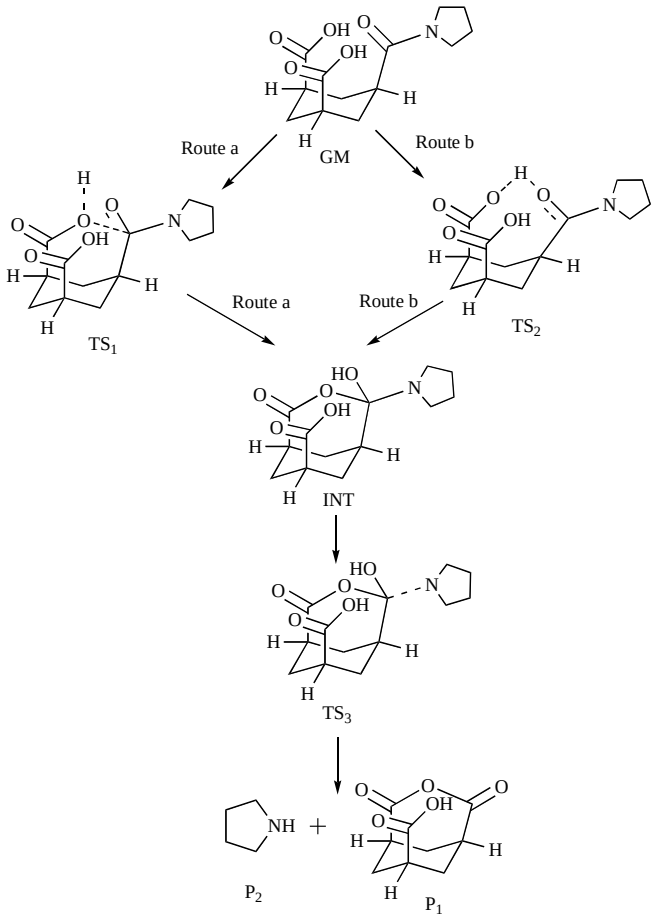
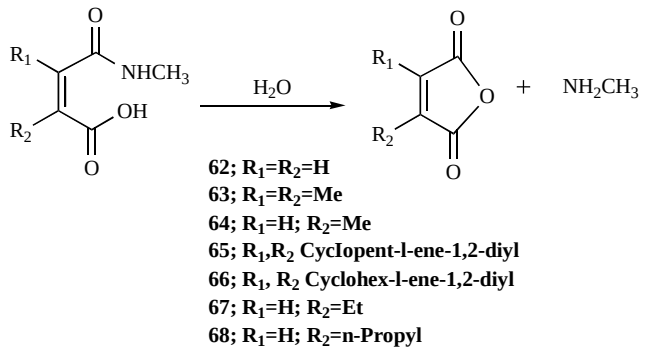


Fig. (37). Suggested mechanism for the hydrolysis of aliphatic amides **51**-**61**.

the most reactive amide, dimethyl-N-n-propylmaleamic acid, is greater than 10^{10} M. In addition, the study revealed that dimethyl-N-n-propylmaleamic acid underwent cleavage to the more stable dimethylmaleic anhydride in less than 1s at 39 °C < pH 3 [80]. In addition, the study by Kirby revealed that the neighboring carboxyl group is involved in the intramolecular nucleophilic catalysis which causes the breakdown of the amide bond. Moreover, based on that the isomaleimide was formed as intermediate and totally was converted to into N-methylmaleamic acid (Fig. **39**); Kirby concluded that the collapse of the tetrahedral intermediate is the rate-limiting step of the reaction [80]. On the other hand, Kluger *et al*. investigated the intramolecular hydrolysis mechanism of a number of N-alkylmaleamic acids having alkyl amine moieties with different basicities [110]. Their study demonstrated that the factor affecting the rate-limiting step is the leaving group basicity of the leaving group and the solution acidity. In the early nineties, Katagi has calculated the reaction mechanism using AM1 semiempirical method and based on his study he concluded that the tetrahedral intermediate formation is the rate-limiting step and not its collapse [111]. For unraveling the factors playing dominant role in proton transfer processes we have computationally studied the hydrolysis of Kirby's N-alkylmaleamic acids, **62**-**68**.

The goals of our computational study were to: (i) examine if the rate-limiting step in **62**-**68** is due to the tetrahedral intermediate association or collapse, and to determine the factors responsible for the extremely high rates achieved in the acid-catalyzed hydrolysis of **63** and **66**, (ii) assign the structural features associated with the



62; R_1=R_2=H
63; R_1=R_2=Me
64; R_1=H; R_2=Me
65; R_1,R_2 CycIopent-l-ene-1,2-diyl
66; R_1, R_2 Cyclohex-l-ene-1,2-diyl
67; R_1=H; R_2=Et
68; R_1=H; R_2=n-Propyl

Fig. (38). Chemical structures of N-alkylmalic acids **62**-**68**.

significant reactivity in the hydrolysis for considering such factors when investigating biotransformation catalyzed by enzymes.

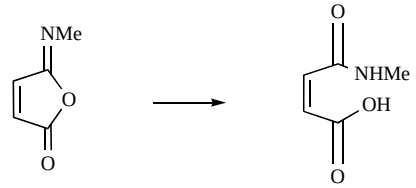


Fig. (39). Conversion of isomaleimide to N-methylmaleamic acid.

Using DFT calculation methods at B3LYP/6-31G (d,p), B3LYP/311+G (d,p) levels and hybrid GGA (MPW1k) we have computed the acid-catalyzed hydrolysis of maleamic (4-amino-4-oxo-2-butenoic) acids (Kirby's N-alkylmaleamic acids) **62**-**68** (Fig. **38**) and the calculations confirmed that the reaction proceeds in three steps: (a) a transfer of a proton from the carboxylic acid moiety to the neighboring amide carbonyl followed by, (b) an attack of the carboxylate anion onto the protonated amide carbonyl carbon and the final step of the reaction involves (c) the tetrahedral intermediate collapse to provide products (Fig. **40**). In addition, the calculation results revealed that reaction medium is a crucial factor in determining the rate-limiting step of the reaction. The calculations in the gas phase revealed that the rate-limiting step was the tetrahedral intermediate formation, whereas the calculations in water demonstrated that the tetrahedral intermediate collapse was the rate-limiting step. In addition, when CH_3NH_2 in **62**-**68** was replaced with an amine having a low pK_a the rate-limiting step was the formation of the tetrahedral intermediate, such as in the case where CH_3NH_2 was replaced with CF_3NH_2 (see Fig. **40**).

Furthermore, the calculation results demonstrated that the hydrolysis rate is dependent on the substituents on the C=C bond. It was found that the rate of hydrolysis is a function of the tetrahedral intermediate or product strain energies. High rates were found for systems with unstrained tetrahedral intermediates or products whereas systems with strained intermediates or products were associated with low hydrolysis rates [112-115].

The fact that the calculated EM values were found to linearly correlate with the experimental EM values establishes a credibility to use DFT methods for calculating similar systems to that studied herein [112-115].

6.5. Kirby's Enzyme Model Based on Intramolecular Proton Transfer in Acetals

Another important enzyme model that was computationally investigated by us is Kirby's intramolecular proton transfer in acetals.

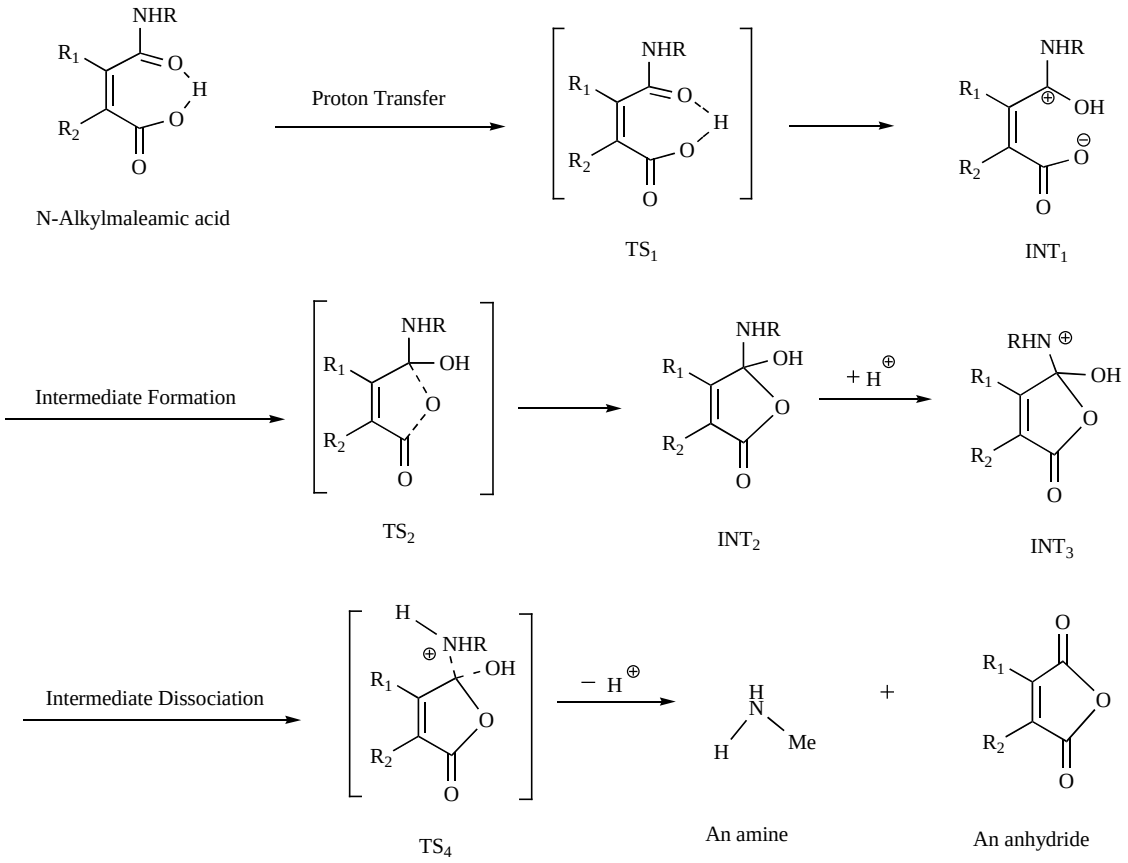


Fig. (40). Suggested mechanism for the hydrolysis of **62-68**.

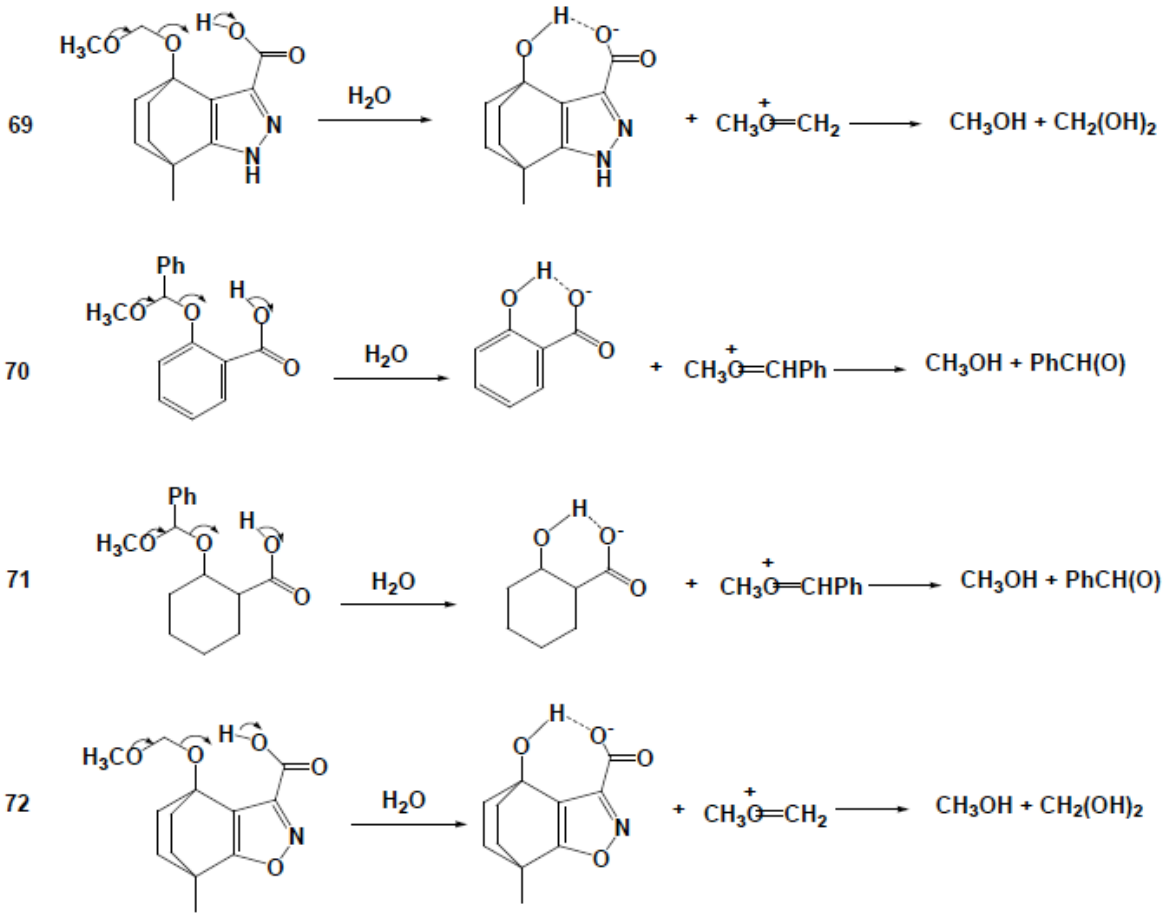
The efficiency of proton transfer in Kirby's enzyme models **69-76** (Figs. **41-43**) were studied by computing the thermodynamic and kinetic parameters for all entities involved in the reaction [65-66, 79, 81-86, 116-120].

The aim of this computational investigation was to: (a) determine the nature of the driving force(s) for the intramolecular general acid catalysis (IGAC) in **69-76** (Figs. **41** and **42**), and (b) to assign the hydrogen bonding along the reaction and to determine their role in the process.

Using HF/6-31G (d,p) and DFT B3LYP/6-31G (d,p) methods the kinetic and thermodynamic parameters for the IGAC in processes **69-76** (Figs. **41** and **42**) were calculated. **77** and **78** were chosen to be used as intermolecular proton transfer processes for calculating the effective molarities (EM) of the intramolecular processes **69-72** and **73-76**, respectively. Process **79** was studied to represent a proton transfer process driven by "classical" general catalysis (GAC) for comparisons with IGAC. The calculations revealed that the following structural features should be included in a system for an efficient proton transfer: (i) a short interatomic distance between the nucleophile and electrophile (r_{GM}, see Scheme **2**) in the GM which results in strong intramolecular hydrogen bonding, and (ii) the attack angle α (see Scheme **2**) in the GM should be close to 180°. Among systems **69-72** that were investigated, systems **69** and **72** were the most efficient since both comply with the two requirements (α= 170° and r_{GM} = 1.7 Å). System 74 is the most inefficient since the attack angle and interatomic distance are quite different from the optimal values, α=48° and r_{GM} = 3.7 Å.

In order to test if the reaction mechanism for systems such as **69** occurs *via* efficient IGAC or GAC calculations for processes **77** and **79** were conducted; where process **77** involves intermolecular proton transfer from acetic acid to an acetal, and process **79** is similar to that of **69** where acetic acid was replaced with a water molecule as a proton donor to the acetal (Fig. **43**). When the calculated DFT activation energies for processes **77** and **79** were compared with that of **69** it was found that IGAC for **69**is much more efficient than that of **77** and **79** by GAC ($\Delta G^{\ddagger}$ value for **69** is 24.15 kcal/mol, and for **77** and **79** are 38.55 kcal/mol and 53.14 kcal/mol, respectively). This result confirms that the proton that catalyzes the breakdown of the acetal group of **69** must be provided by the carboxyl group. Thus the mechanism in systems such as **69** is *via* IGAC and not GAC [116-120].

The EM values obtained indicates that **69** and **72** are the most efficient processes among **69-76** (log EM 10-13) and among the least efficient processes are **71, 75** and **76** with log EM <1. In addition, the calculations demonstrated that the proton transfer rate in systems **69-76** is largely affected by geometrical parameters, particularly r_{GM}, and α. The following requirements are necessary to achieve efficient intramolecular proton transfer: (1) small values of r_{GM} which could result in obtaining strong intramolecular hydrogen bonds, and (2) α_{GM} values at or near 180˚ for maximizing orbitals overlapping. The equation $\Delta G^{\ddagger}$ vs. r^2_{GM} x (1 + sin (180 - α) which correlates calculated activation energy values with geometrical parameters establishes an efficient and fruitful tool for the prediction of reactions rates. The equation slope value is an indication to



Scheme 41. Chemical structures of **69**-**72**.

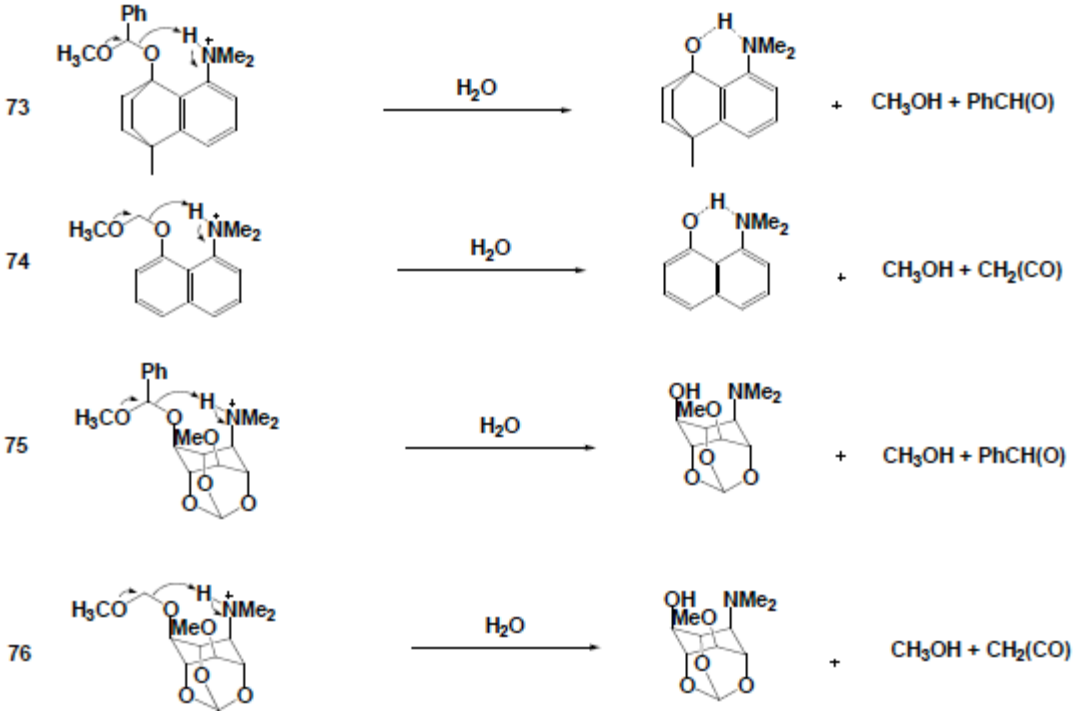


Fig. (42). Chemical structures of **73**-**76**.

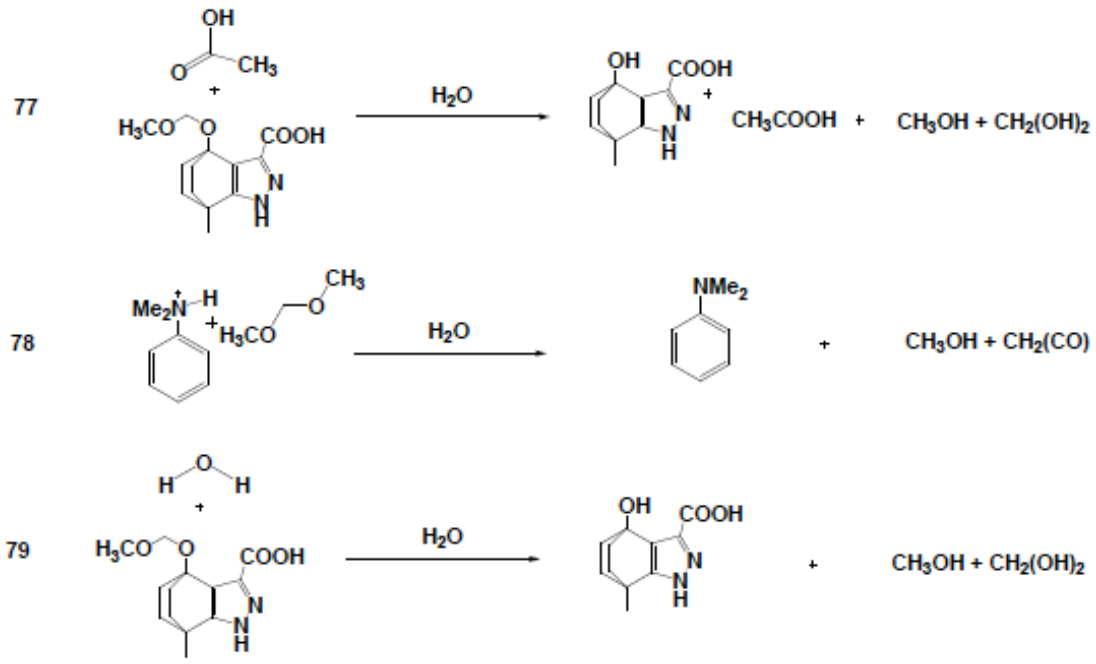
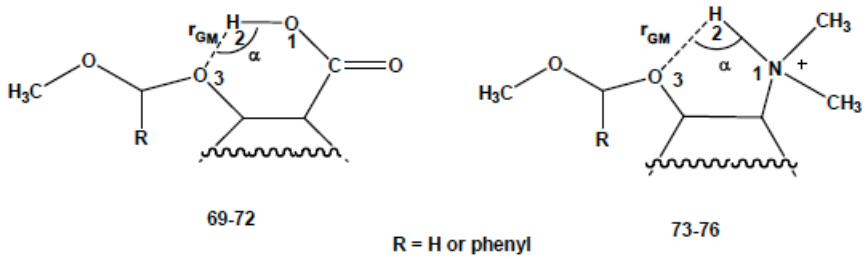


Fig. (43). Chemical structures of **77-79**.



Scheme 2. Schematic representation for the cleavage of **69-72** and **73-76** showing the interatomic distance between the two reacting centers (r_{GM}) and the attack angle (α).

the mode by which the two reactive centers orchestrate in an intramolecular process. In extrapolation of the calculation results to enzymes we suggest that enzymes are very efficient in catalysis due to their ability to hold the reacting centers in a range of contact distances at the enzyme's active site [64-66, 74-79, 81-86, 116-120].

7. NEW PRODRUGS BASED ON THE ABOVE DISCUSSED ENZYME MODELS [121-165]

The above mentioned computational studies on intramolecularity have demonstrated that these chemical devices (enzyme models) have the potential to be utilized as efficient prodrug linkers to be attached to parent active drugs and upon *in vivo* administration they will release the active drugs in a programmable manner by a chemical and not enzymatic process.

For instance, the aza-nucleoside azacitidine is administered by SC injection and reached peak plasma concentration immediately after administration. If a sustained release prodrug can be synthesized, then C_{max} associated adverse effects may be eliminated and the drug's duration of action will be longer resulting in potentially more effective clinical profile. Another example of the same family, decitabine is given by continuous IV infusion. If a prodrug that can be degraded in programmable manner can be provided by SC injection this could provide a very good option for MDS maintenance patients on treatment [121, 122].

Improvement of the pharmacokinetic properties and hence the effectiveness of the marketed aza-nucleosides such as azacitidine, cytarabine and decitabine may enhance the permeation of the the aza-nucleoside through various administration routes, such as SC injection route. This can be obtained by using the prodrug approach in which the aza nucleoside drug is attached to a carrier moiety to yield a chemical device capable of partitioning through the membrane tissues and releasing the aza nucleoside drug in a controlled manner.

Statins are a family of pharmaceuticals used for lowering cholesterol levels in the blood. They are commonly applied for the reduction of cardiovascular-related morbidity and mortality in patients with or at risk of coronary heart diseases. In the long and complex biosynthesis of cholesterol in humans, the first and rate-determining step is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonic acid. Clinical studies have demonstrated that statins are potent and competitive inhibitors of HMG-CoA reductase for cholesterol synthesis, thereby limiting the hepatic production of low-density lipoprotein (LDL). The absorption of satins such as simvastatin, atorvastatin and rosuvastatin is very rapid and reaches peak plasma concentration within 4 hours after administra-

tion, and all of statins have the liver as their target organ [123, 124]. Statins in general have a relatively low bioavailability mostly due to the extensive first pass effect and/or bad solubility.

Increasing the usefulness of therapeutics can be achieved utilizing the prodrug approach strategy, because one can optimize any of the absorption, distribution, metabolism and elimination (ADME) properties of potential medicines to reach a satisfactory bioavailability. Simvastatin solubility is the limiting factor for its bioavailability as a result of slow tissue penetration therefore, it undergoes a significant first pass effect; hence, improving its solubility and its dissolution rate is a crucial for enhancing its bioavailability. Thus, development of more hydrophilic prodrugs that have the potential to liberate the active parent drug in physiological environments is a significant challenge.

Aza-nucleosides prodrugs for myelodysplastic syndromes [125-132] and statins prodrugs to reduce cholesterol levels in the blood circulation [133-137] were designed and made utilizing the proton transfer reaction in Kirby's acetals. In these examples, the linker was covalently bonded to the hydroxyl group of the parent drug such that upon exposure to stomach, intestine, or blood circulation the prodrug degrades in a rate that is only dependent on the structural features of the linker (Kirby's acetal). Kinetics studies are underway to determine the interconversion rates of the synthesized prodrugs into their parent active drugs in different physiologic media and plasma [125-137].

On the other hand, tranexamic acid, a synthetic lysine amino acid derivative. was synthesized to reduce and prevent hemorrhage in hemophilia patients and decrease the frequency for replacement therapy during tooth extraction. In addition, it is used in excessive bleeding conditions. The availability of blood and fluid replacement in most of third world countries is scarce. Among the approaches to decrease the risk of maternal hemorrhage is to improve the availability of blood and fluid replacement. Another approach is to decrease the likelihood of maternal hemorrhage. Moreover, the above mentioned treatments are intended for I.V. administration which may not be a viable option in the third world counties. Hence, the possibility of using a cheaper oral treatment will be better suited for such circumstances [138, 139].

The oral bioavailability of the amino acid tranexamic acid is relatively low due to its ionization in physiologic environments which results in poor absorption through membranes tissues. The log P (partition coefficient) value for tranexamic acid is -1.6. Thus, it is a necessity to design and synthesis more lipophilic prodrugs of tranexamic acid that can improve the permeation of the drug and consequently its oral bioavailability. In addition, tranexamic acid prodrugs with certain linkers can provide a chemical device which upon exposure to physiologic environment is able to release the active parent drug in a slow release manner which might result in better clinical outcome, more convenient dosing regimens and potentially less adverse effects than the tranexamic acid.

Another important drug that suffers poor oral bioavailability is acyclovir, a synthetic acyclic purine nucleoside derivative that is the first anti-viral medicine to be licensed for the treatment and prevention for viral infections result from herpes simplex (HSV), varicella zoster (chicken pox) and herpes zoster (shingles). The water solubility of acyclovir is very low and has a poor oral bioavailability of 10-20%; therefore, I.V. administration is a must if large doses are required. Aciclovir reaches a peak plasma concentration after 1-2 hours from its administration. It has a high distribution rate with about 30% is protein-bound in plasma. Aciclovir elimination half-life is about 3 hours. It is renally excreted by both glomerular filtration and tubular secretion [140, 141].

Similarly, a number of tranexamic acid prodrugs for the treatment of bleeding conditions [142] and acyclovir prodrugs as antiviral drug for the treatment of Herpes Simplex [113] were designed and synthesized using Kirby's N-alkylmaleamic acids enzyme model as a linker.

In vitro kinetic studied on these prodrugs have shown that the interconversion rate of a prodrug can be programmed according to the nature of the linker (Kirby's N-alkylmaleamic acid). Strained linkers provided interconversions with higher rates than those with unstrained linkers.

Another example by which the prodrug approach utilizing intramolecularity has achieved successes is the design and synthesis of dopamine prodrugs. As it is well known, the death of dopamine neurons in the nigrostriatal pathway results in shortage of dopamine which causes Parkinson's disease. This disease is accompanied by a loss of the ability to execute smooth and controlled movements. Dopamine can be provided as a drug that acts on the sympathetic nervous system to produce effects such as increased heart rate and blood pressure. However, because dopamine cannot cross the blood-brain barrier (BBB), when administered as a drug it does not directly affect the central nervous system (CNS). For increasing the concentrations of dopamine in the brains of Parkinson's disease patients and dopa-responsive dystonia, levodopa (L-DOPA), the precursor of dopamine, is administered since it can cross the blood-brain barrier.

Levodopa is usually is given in combination with an inhibitor of peripheral decarboxylation (DDC, dopa decarboxylase), such as carbidopa or benserazide. Although this combination has shown some enhancement in the bioavailability of dopamine, further modification on dopamine structural features still to be made in order to provide a medication with very effective clinical profile [143, 144].

Based on the computational calculations done on the previously mentioned enzyme models, prodrugs of dopamine to treat Parkinson's disease [145] were made utilizing Menger's Kemp acid enzyme model as the prodrug promoiety. In addition, dimethyl fumarate prodrugs for psoriasis treatment were designed, synthesized and work in progress to determine their *in vitro* and *in vivo* kinetic parameters [146]. It is worth noting that high oral doses of dimethyl fumarate is needed to efficiently treat psoriasis, however, obtaining a chemical device that can release the active drug in a sustained release manner will be a good option to eliminate the adverse side effects associated with the treatment of dimethyl fumarate in high dosing and improve the therapeutic profile of the drug.

Following this approach we have succeeded to make bitterless prodrugs to replace their corresponding bitter active drugs. Examples for this approach include antibacterial drugs, amoxicillin, cephalexin and cefuroxime, and the pain killer, paracetamol [147,148]. The role of the linker in these prodrugs examples is to block the amine or hydroxyl group responsible for bitter sensation. In the antibacterial prodrugs the amine group is replaced with an amide, thus inhibiting any interaction between the drug and the bitter taste receptor.

In the case of paracetamol prodrugs the hydroxyl group on the phenyl ring of paracetamol is believed to be the main contributor for the bitter sensation of the drug presumably through hydrogen bonds between the phenolic hydroxyl and the bitter taste receptor's active site. Masking hydroxyl in paracetamol with a linker is ex-

pected to prevent any binding between paracetamol and the receptor's active site [147-165].

CONCLUSION

In order to avoid the disadvantages associated with the use of enzymatic prodrug activation strategy, intramolecular activated prodrug strategy has been developed. Cyclization activated prodrugs are widely used in ester and amide prodrugs. Ester prodrugs are derived from carboxyl or hydroxyl containing drugs, including pilocarpine acid double esters that are activated by base-catalyzed lactonization, paracetamol dipeptide esters that intramolecularly release diketopiprazine and paracetamol *in vitro*, ester prodrugs of propranolol that were developed to increase parent drug bioavailability and amino esters, an important class of prodrugs in which the release rate of the parent drug is dependent on steric effect.

Amide prodrugs were also designed to improve physicochemical characteristics of amine drugs, these prodrugs include carbamate prodrugs, quinone propionic acid prodrugs, doxorubicin hydrazone and hydrazine carboxylate prodrugs, prednisolone 21-hemisuccinate β-cyclodextrin prodrugs and HIV-protease inhibitor prodrugs that were designed to increase water solubility, and to be intramolecularly converted into their active drugs.

Furthermore, ab initio and DFT have proven to be effective in predicting energies and release rates of prodrugs to their parent drugs. Using this approach, tranexamic acid, atenolol, statins, phenylephrine, atovaquone, aza-nucleoside, aciclovir, cefuroxime and paracetamol prodrugs were designed, synthesized and their *in vitro* kinetics were studied.

ABBREVIATIONS

HAS	=	Human serum albumin.
CPT	=	Camptothecin.
DPK	=	Diketopiprazine.
Leu	=	Leucine.
Phe	=	Phenylalanine.
Gly	=	Glycine.
Ala	=	Alanine.
Tyr	=	Tyrosine.
HIV	=	Human Immunodeficiency Virus
AZT	=	3-azido-3-deoxythimidine
MME	=	Melphalan Methyl Ester

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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