Mechanism-based enzyme inactivators in rational drug discovery: Curing diseases with biochemical Trojan Horse reagents

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Mechanism-based inactivation of target enzymes is a fascinating area of research at the interphase of chemistry, biology and medicine. Since the first paper by Endo *et al.* in 1970, mechanism-based inactivation of enzymes has interested academic and industrial researchers alike. It is increasingly becoming a leading approach in the discovery of novel, potent and effective drugs in pharmaceutical R & D laboratories. This review article summarizes the recent advances made in mechanism-based enzyme inactivators and their applications in medicine.

An effective collaboration between chemists and biologists is the cornerstone of research on design and discovery of new pharmaceutical candidates. The union of organic chemistry with molecular biology seems tailor-made for rational design of drugs. The understanding of biological events at the molecular level coupled with the electronic and mechanistic interpretation of chemical reactions is the basis for a fruitful collaboration[1]. The merging of the two disciplines has resulted in a better understanding of the structure of enzymes, their active sites, and the mechanism by which they catalyse chemical reactions. The intervention or blockade of specific enzymatic processes by natural and unnatural substrates has potential applications in medicinal research.

The single most important goal in drug design is increased specificity. The three major approaches for the enhancement of specificity in enzyme inactivation are: (1) transition state analogues, (2) mechanism-based enzyme inactivators, and (3) multi-substrate analogue inhibitors. The idea that 'rate enhancement by enzymes depends on the affinity for the transition state or the activated complex, and that a stable mimic of the transition state might be used to derive potent inhibitors of the enzyme' is the basis for the design of selective enzyme inhibitors on the transition state analogue principle[2]. Mechanism-based inactivation mandates that the inhibitor molecules react with the target enzyme analogous to the catalytic process. The unveiling of a latent electrophilic group in the active site of enzyme and its acylation or alkylation with a suitably placed nucleophilic residue inactivates the enzyme. Since the enzyme unwillingly and mistakenly causes its own demise by processing the inhibitor molecule midway through the catalytic cycle, these molecules are also referred to as 'suicide substrates'. The pioneering paper in 1970 by Endo *et al.*[3] at Harvard University on the inactivation of β-hydroxydecanoylthioester dehydrase by 3-decynoylthioesters marked the beginning of research on mechanism-based enzyme inactivators. Contributions from the laboratories of Rando[4], Walsh[5], Abeles[6], Silverman[7] and Knowles[8] during the 1970s and 1980s resulted in the blossoming of research activity in this exciting area. Multi-substrate analogue inhibitors[9] offer an opportunity for specificity by combining two or more structural elements required for recognition by the target enzyme into a single molecule. The obvious advantage of this approach is that neither of the components will be recognized by other enzymes using either of the substrates, and so a very high order of specificity is expected.

Medicinal chemistry is basically an empirical science and an element of rationality in the search for new pharmaceuticals has been achieved through more rigorous approaches during the last two decades. Some of these are mechanism-based enzyme inactivation[10], (which is the topic of this review), peptide combinatorial libraries[11], 3D-structure-based leads[12], molecular modelling on computers[13], cellular adhesion molecules[14], and immune regulation[15].

Mechanism-based enzyme inactivators and drug discovery

The advantage of mechanism-based inactivators as potential drug candidates is because of their high specificity and potency, coupled with low toxicity and side-effects. For example, α-difluoromethylornithine, which is a specific mechanism-based inhibitor of ornithine decarboxylase, causes only minor side-effect symptoms when administered for protozoal infections in doses of 30 g per day for several weeks! The older-generation affinity-labelling agents not only modify the enzyme protein but also undergo unselective reactions in the biochemical milieu, resulting in toxicity normally associated with these drugs.

The chemical specificity of enzymes is derived through a binding term (K_D) and a catalytic term (k_{cat}) (eq. 1). Most enzymes do not bind their natural substrates very tightly ($K_D \sim 10^{-4}$–10^{-6} M) since exceedingly tight binding in the ground state is counterproductive to catalysis:

$$E + S \underset{K_D}{\rightleftharpoons} E \cdot S \xrightarrow{k_{cat}} E \cdot P \rightleftharpoons E + P. \quad (1)$$

Here E denotes enzyme, S substrate and P product. The chemical basis for the specificity of mechanism-based enzyme inactivation is schematized as follows (I denotes inhibitor):

$$E + S \underset{K_D}{\rightleftharpoons} E \cdot S \xrightarrow{k_{cat}} \begin{matrix} E \cdot I \\ \rfloor\lceil \\ E + I \end{matrix} \xrightarrow{k_{inh}} E\text{-}I. \quad (2)$$

The inherent specificity and efficiency of mechanism-based inhibitors is derived from a high k_{cat} term and $k_{inh} > K_D$. The rate-determining step is often k_{cat} because these inhibitors have turnover numbers 10^3–10^5 times smaller than their natural substrates for the target enzyme. A high k_{inh} term preferentially partitions the E·I complex towards deactivation of the enzyme (E–I) over dissociation (E + I). Mechanism-based inhibitors are also aptly referred to as 'k_{cat} inhibitors'.

The number of times a suicide substrate is processed to the released product without harm to the target enzyme per inactivation event is called the partition ratio. Under ideal conditions of zero partition ratio, the inactivator molecule will be a strong drug candidate because it will be highly specific and low in toxicity.

To summarize, the cardinal features of mechanism-based enzyme inactivation are: latent reactivity, catalytic processing of inhibitor molecule to a reactive intermediate, and formation of an irreversible adduct prior to release from the active site of enzyme.

Mechanism-based inhibition of target enzymes is more than just a tool for rational drug design. It provides an indirect method to understand the factors that determine the binding specificity of enzymes to substrates. The contribution of various forces that control this specificity such as hydrogen bonding, hydrophobic and electrostatic interactions, Π-stacking and van der Waals contacts, etc., can be evaluated with reversible inhibitors. For example, boronic acid analogues were investigated as inhibitors to probe active-site binding with serine proteases, subtilisin and α-chymotrypsin[16].

Rational design of mechanism-based enzyme inactivator molecules as potential drugs is a recent approach in the pharmaceutical industry. Merril Dow Pharmaceuticals and Merck & Co. have adopted this strategy in their research programme for about two decades now. Two leading rationally designed mechanism-based enzyme inactivator drugs are 4-amino-5-hexenoic acid (γ-vinyl GABA; vigabatrin; inactivates γ-aminobutyric acid aminotransferase; treatment of seizures), and α-difluoromethylornithine (eflornithine; inactivates ornithine decarboxylase; treatment of protozoal infections). Vigabatrin is an effective anticonvulsant drug and eflornithine is in clinical use for the treatment of African sleeping sickness. The fact that there are only two rationally designed enzyme inactivator drugs in the world market today should not be construed as a drawback of this approach. A patented drug molecule has to clear a series of toxicological tests and clinical trials before it reaches the medicine counter, and this process takes about 12–15 years (Table 1). The remaining decade should reap dividends of research in this area with the launch of enzyme inhibitor drugs for at least a few major diseases.

Time is not the only issue in launching new drugs. High cost and low success rate are equally formidable factors. The total cost of introducing a new drug molecule into the world market, from synthesis through patenting to approval for marketing, is in the region of US $ 250 million. The risk factor is extremely high. Only 5 in 4000 molecules screened in pre-clinical testing make it to human trials, and only 1 out of these 5 gets approved. The hit rate has, in fact, significantly improved because of rational drug discovery. In the hit-and-trial random screen approach of the 1960s and 1970s, the success rate was anywhere between 1 in 25,000–30,000.

Inhibitors of some medicinally important enzymes

Some enzymes with potential in medicine which are targeted for mechanism-based enzyme inactivation are listed in Table 2. In the following sections, the basis for inactivation of some pharmaceutically important enzymes are presented. The first example on the development of clinically useful anticancer agents by inhibition of dihydrofolate reductase and thymidylate synthetase enzymes is explained in greater detail and depth to familiarize the reader with this fascinating topic. Subsequent discussions, though brief, do underscore the usefulness of this interdisciplinary research area in discovering new pharmaceutical leads.

Table 1. Typical time requirements in each stage of drug development

Chemical laboratory	1 year
Pre-clinical trials	3–4 years
Phase I, II, III clinical trials	6–7 years
Drug approval	2–3 years
Total time required	12–15 years

Table 2. Mechanism-based inactivation of enzymes with potential in medicine

Enzyme	Therapeutic goal
S-adenosylhomocysteine hydrolyase	Antiviral agent
Alanine racemase	Antibacterial agent
D-Amino acid aminotransferase	Antibacterial agent
γ-Aminobutyric acid aminotransferase	Anticonvulsant agent
Arginine decarboxylase	Antibacterial agent
Aromatase	Anticancer agent
L-Aromatic amino acid decarboxylase	Synergistic with antiparkinsonian drug
Dihydrofolate reductase	Anticancer, antibacterial and anti protozoal agent
Dihydroorotate dehydrogenase	Antiparasitic and anticancer agent
DNA polymerase I	Antiviral
Dopamine β-hydroxylase	Antihypertensive and pheochromo, cytoma agent
Histidine decarboxylase	Antihistamine and antiulcer agent
β-Lactamase	Synergistic with antibiotics
Monoamine oxidase	Antidepressant, anthypertensive and antiparkinsonian agent
Ornithine decarboxylase	Anticancer and antiprotozoal agent
Serine proteases	Treatment of inflammation, emphysema, respiratory, digestive and degenerative skin disorders; anticoagulant and antiviral agent
Testosterone 5α-reductase	Anticancer agent
Thymidylate synthetase	Anticancer agent
Xanthine oxidase	Uricosuric agent
Thyroid peroxidase	Antithyroid agent

Dihydrofolate reductase and thymidylate synthetase

Cancer cells grow more rapidly than cells of normal healthy tissues and thus they have greater demand for nucleotides as precursors to DNA and RNA synthesis. Consequently, cancer cells are more sensitive to inhibitors of nucleotide biosynthesis than normal cells. Out of the four bases in DNA and RNA, three are common to both the nucleic acids. Thymine is unique to DNA and its equivalent in RNA is uracil. In the *de novo* pathway to thymine, the final step to thymidine monophosphate (dTMP) **2** is methylation of deoxyuridine monophosphate (dUMP) **1**. The one-carbon homologation of dUMP to dTMP is catalysed by thymidylate synthetase. In this biochemical process, a one-carbon unit is transferred from N^5,N^{10}-methylenetetrahydrofolate (MetTHF) **3** to dUMP at the 'wrong' hydroxymethyl (CH_2OH) oxidation level, then reduced to a methyl group (CH_3) at the expense of oxidising 5,6,7,8-tetrahydrofolate (THF) **5** to 7,8-dihydrofolate (DHF) **4**. The enzyme dihydrofolate reductase regenerates the coenzyme THF, which is converted to MetTHF by serine hydroxymethyl transferase (Scheme 1). Thus, DNA precursors are derived from the metabolic cycle of three enzymes and the inhibition of any one will block the growth of DNA, thereby inducing cell death. In practice, selective inactivation of dihydrofolate reductase and thymidylate synthetase are targeted clinically in cancer chemotherapy.

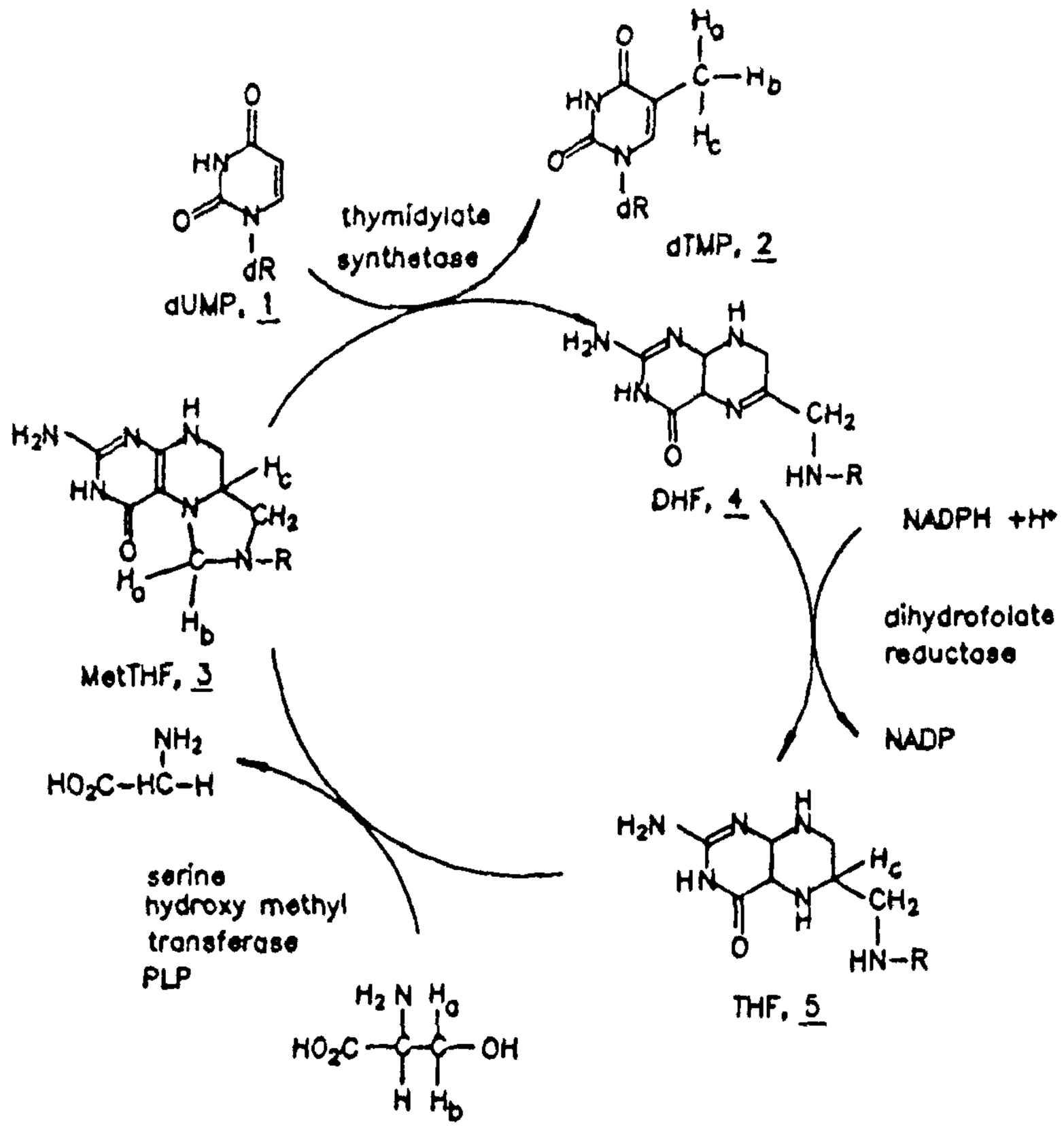
Methotrexate **6**, a folate analogue, is an inhibitor of dihydrofolate reductase. It acts as a competitive inhibitor and binds to the enzyme about 100 times stronger than DHF. The first mechanism-based inhibitor of dihydrofolate reductase, 2-amino-7,8-dihydro-6-hydroxymethyl-7-spirocyclo-propylpteridin-4 (3H)-one **7**, is activated by protonation[17], which is similar to the catalytic mechanism of the enzyme (Scheme 2). The reduction of DHF to THF by dihydrofolate reductase is initiated by protonation (ξ–BH^+) of pteridine N-5, followed by hydride reduction at C-6 by NADPH. In case of inhibitor **7**, opening of cyclopropane on protonated pteridine by active-site nucleophile (ξ–X:) leads to the dead-end adduct **8** (E–I complex). The structurally related molecule **9** is a competitive reversible inhibitor of the enzyme.

As the van der Waals radius of fluorine (1.35 Å) and hydrogen (1.20 Å) are similar, fluorinated molecules are processed analogous to their natural substrates. Therefore, in cell salvage pathways fluorouracil **10** is converted to 5-fluoro-2′-deoxyuridine monophosphate (FdUMP) **11**, which is the actual inactivator of thymidylate synthetase. In the presence of MetTHF cofactor a 1 : 1 complex **12** forms with FdUMP, similar to the enzyme reaction with dUMP. The electrophilic complex **12** easily tautomerizes to neutral **13** in case of the natural substrate dUMP (X = H). However, complex **12** with FdUMP (X = F) cannot tautomerize and is attacked by an enzymatic thiol residue to generate the dead-end covalent complex **14**, thereby inhibiting the enzyme. The ternary covalent complex **14** has an *in vivo* half-life for decomposition of about 14 h, long enough to curtail DNA synthesis in the affected cells. FdUMP is a time-dependent inactivator of thymidylate synthetase, with MetTHF cofactor controlling the rate and extent of inactivation (Scheme 3)[18, 19]. 5-Ethynyl-2′-deoxy-uridylate **15** is also a time-dependent inhibitor of thymidylate synthetase.

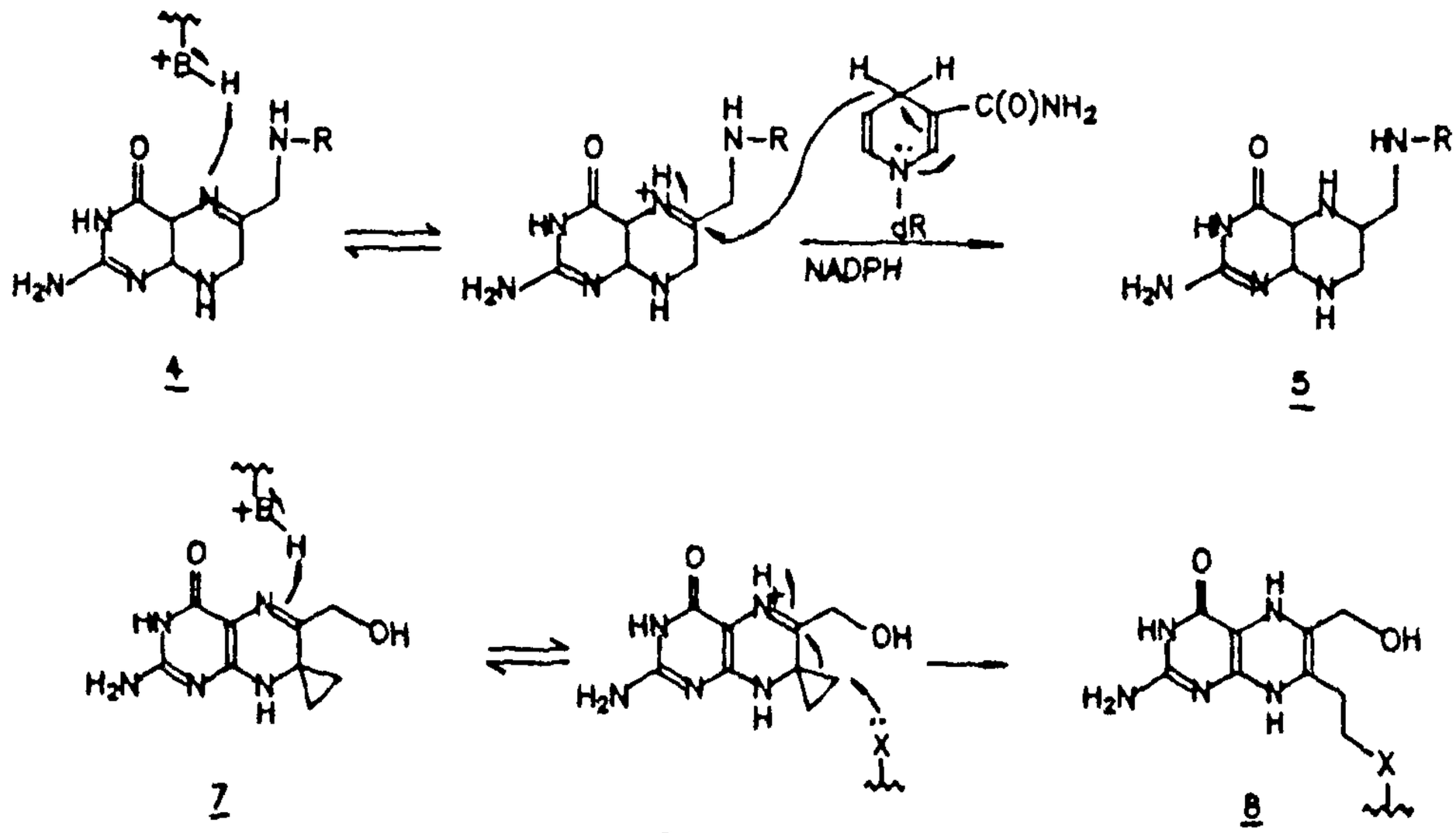
The essential hallmark of mechanism-based enzyme inactivation is elegantly demonstrated in the above examples. The inhibitor is benign until the target enzyme itself converts it to a reactive species which eventually becomes the cause of its own demise. The enzyme is, in a sense, committing suicide mistakenly by processing the Trojan Horse reagent.

Alanine racemase

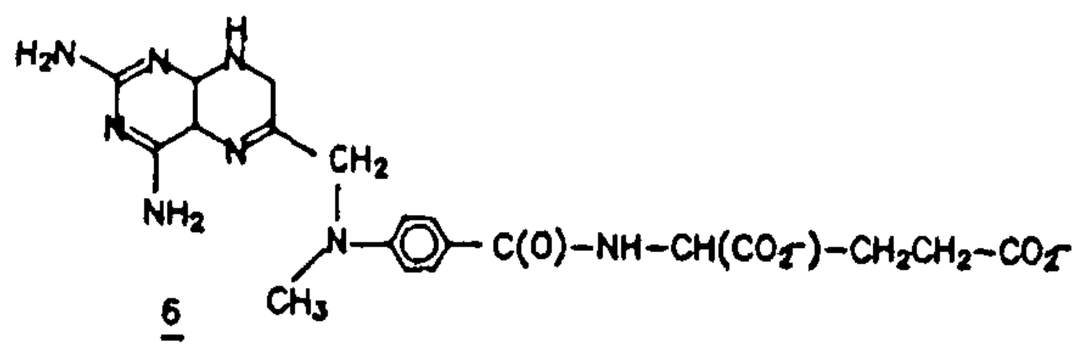
The amino acids found in proteins have the natural L-configuration, contrary to D-alanine residues found in bacterial cell walls. Bacteria biosynthesize the required D-alanine from its natural L-configuration using alanine



Scheme 1.



Scheme 2.



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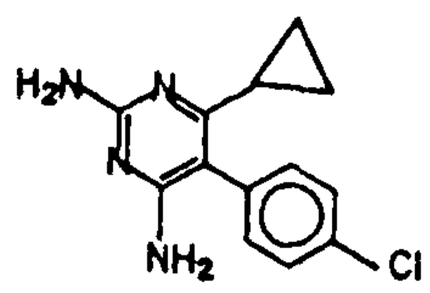

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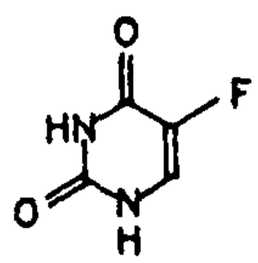

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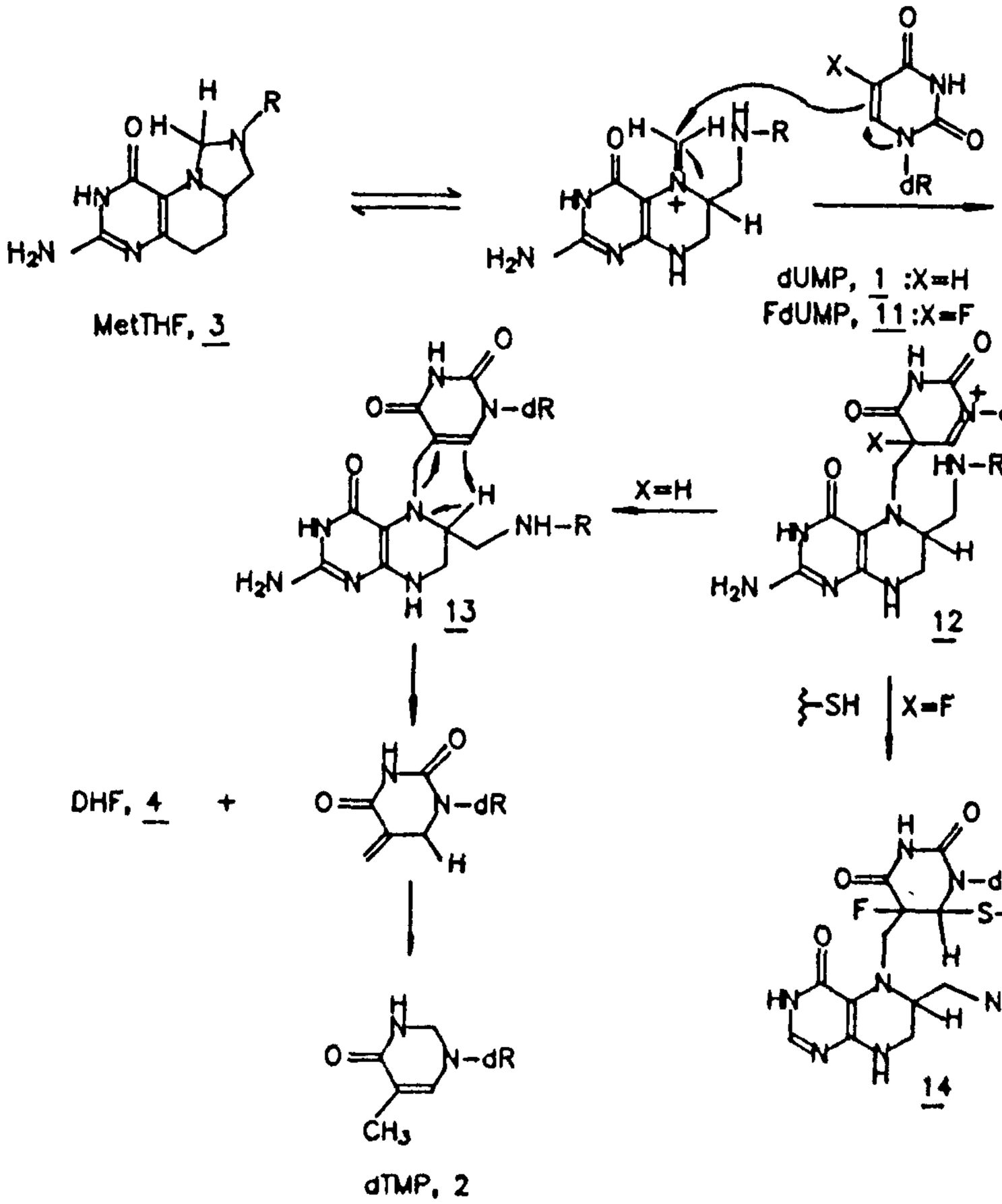


Scheme 3.

racemase, an enzyme not found in mammalian sources. The inhibition of alanine racemase is important for the design of antibacterial agents.

Kollintsch *et al.*[20] and Wang and Walsh[21] synthesized D- and L-isomers of β-fluoroalanine **16** as inhibitors of alanine racemase, a pyridoxal phosphate-dependent (PLP) enzyme. The inhibitor compound **17** partitions between hydrolysis (pathway a) and inactivation (pathway b) (Scheme 4). The efficacy of enzyme inactivation is evidenced from the complete lack of racemization of alanine. The chemical basis for inactivation is the isosteric C–F bond, which undergoes facile β-elimination to generate **17**, but sluggish displacement.

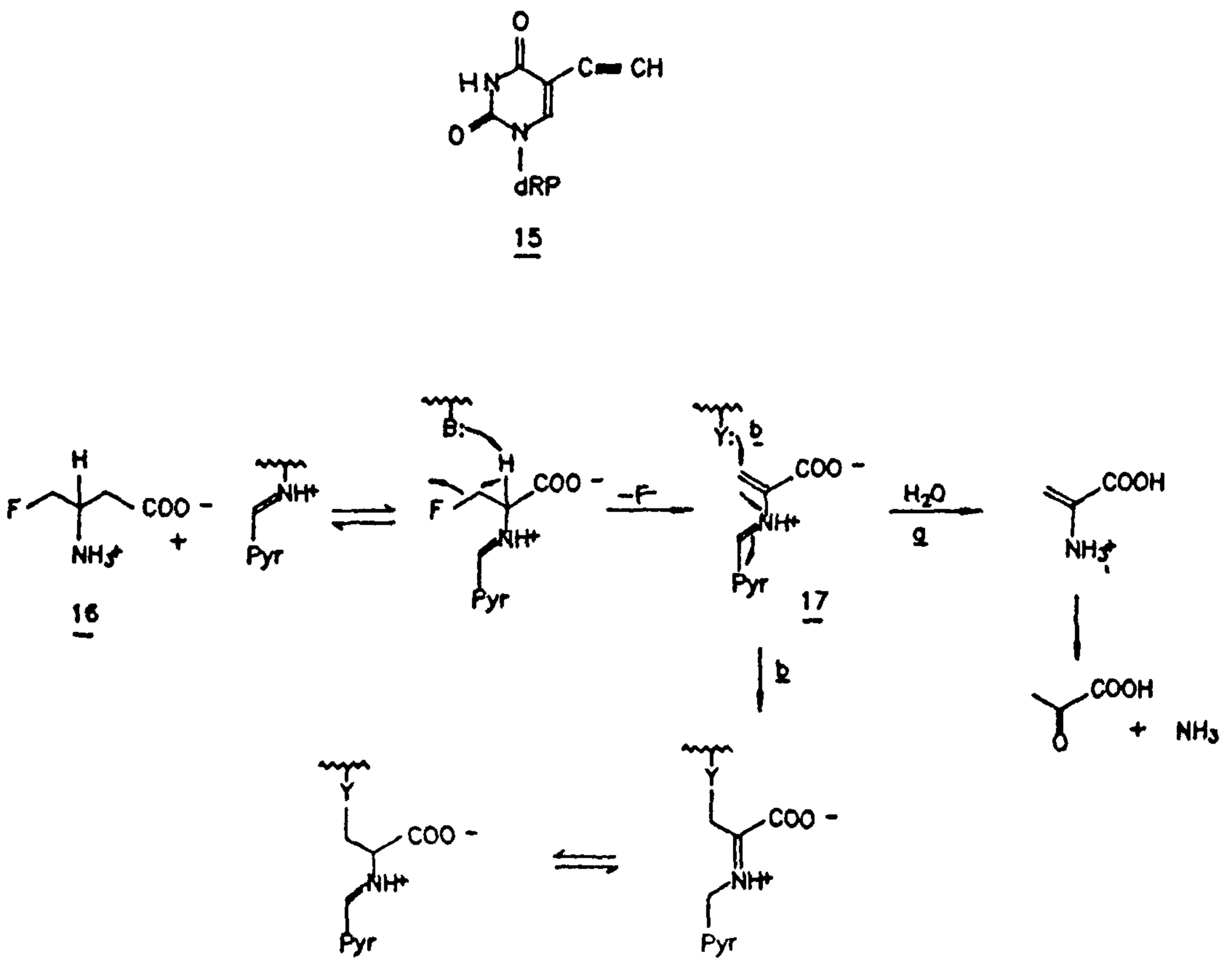
D-Amino acid aminotransferase

Racemization of L- to D-amino acid is not the only route that bacteria rely on for synthesizing their building blocks. Certain D-amino acids (e.g. D-glutamic acid) essential for the formation of peptidoglycan layer are biosynthesized by transamination of the corresponding α-keto acids rather than by racemization. Inactivation of D-amino acid aminotransferase blocks the formation of these essential cell wall constituents.

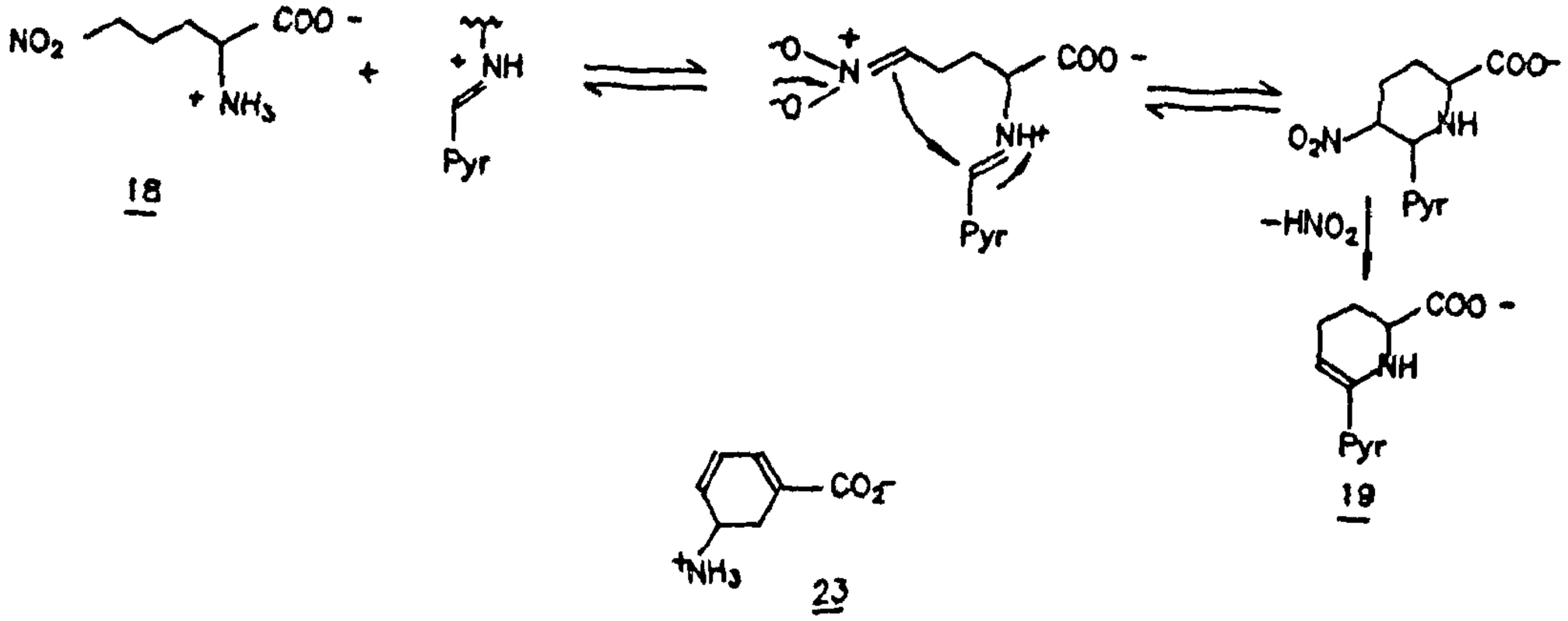
5-Nitro-L-norvaline **18** is a time-dependent inactivator of alanine, aspartate- and 4-aminobutyrate aminotransferase[22] according to the mechanism postulated in Scheme 5. Instead of the enzyme, the pyridoxal cofactor (PLP) is trapped as **19** during inactivation. Since the cofactor is essential for enzymatic transamination, D-amino acids are no longer biosynthesized.

γ-Aminobutyric acid aminotransferase

Certain convulsions arise from an imbalance in two neurotransmitters in the brain: glutamate, an excitatory neurotransmitter, and γ-aminobutyric acid (GABA), an inhibitory neurotransmitter. In test experiments, GABA effectively controls convulsions when directly injected into the brain. However, peripherally administered GABA is ineffective because it cannot cross the blood–brain barrier. A compound that can cross the blood–brain barrier and inactivates GABA aminotransferase, the enzyme that degrades GABA, should raise GABA levels in the brain and act as an anticonvulsant agent.



Scheme 4.



Scheme 5.

Incubation of rat brain GABA aminotransferase with 4-aminohex-5-enoic acid **20** ($R = CH_2CH_2CO_2H$; γ-vinyl GABA) results in the time-dependent pseudo first-order loss of enzyme activity[23]; dialysis does not restore the activity. Dithiothreitol and β-mercaptoethanol do not protect the enzyme from inactivation. Two possible mechanisms for inactivation, isomerization-addition[23] (Scheme 6) and enamine pathway[24] (Scheme 7), are shown. The pathway in Scheme 6 leads to covalent inactivation of enzyme through adduct **21**, whereas in Scheme 7 trapping of pyridoxal cofactor as **22** causes loss of enzyme activity. γ-Vinyl GABA is a promising drug for the treatment of convulsions and seizures. Gabaculine **23** (5-amino-1,3-cyclo-hexadienylcarboxylic acid), a natural product, is an irreversible inactivator of mouse and pig brain GABA aminotransferase.

Aromatase

Human placental aromatase catalyses the conversion of androgens to estrogens, which are essential hormones for growth and reproduction. High estrogen levels have been linked with various breast cancers and therefore inhibition of aromatase is an effective approach to cancer chemotherapy.

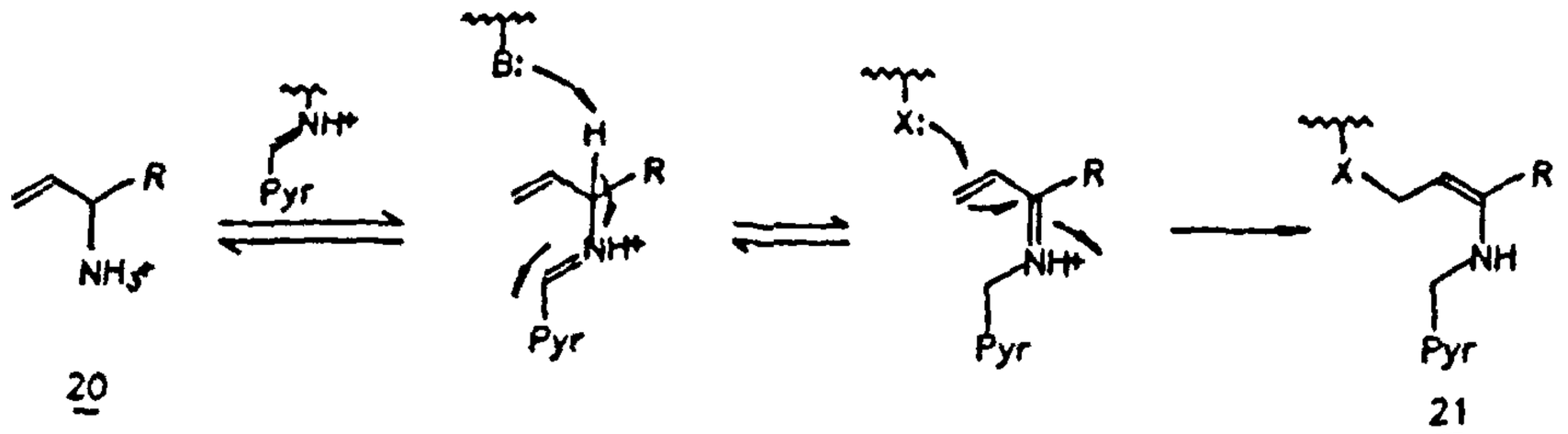
Aromatase is irreversibly inactivated by several 10β-propynyl-substituted steroids **24** in the presence of NADPH and O_2. Oxidation of the propynyl side-chain to α, β-acetylenic ketone **25** followed by Michael addition is the mechanism for inactivation[25] (Scheme 8). Alternatively, epoxidation of the acetylene to **26** results in inhibition[26] (Scheme 9).

Some other important inhibitors of cytochrome P-450 aromatase are allenic steroid **27**, 17β-hydroxy-10-methyl thioestra-1,4-dien-3-one **28**, and 19,19-difluoroandrost-4-ene-3,17-dione **29**.

L-Aromatic amino acid decarboxylase

Parkinson's disease is a neurological disorder resulting in tremors, rigidity and akinesia. These symptoms are caused by degeneration of dopamine receptors and reduced levels of L-aromatic amino acid decarboxylase, both of which result in low concentrations of inhibitory neurotransmitter dopamine in the brain. L-Dopa is an effective antiparkinsonian drug which is actively transported to the brain, where it is converted to dopamine by decarboxylases. However, much of the L-dopa administered is decarboxylated peripherally in the liver and kidney, much before it can reach the brain. Therefore L-dopa administered in combination with an inactivator of peripheral L-aromatic amino acid decarboxylase will act synergistically and enhance the efficacy of this drug.

α-(Monofluoromethyl) dopa **30** is an irreversible inactivator of pig kidney dopa (L-aromatic amino acid) decarboxylase[27]. The K_i for α-(CH_2F) dopa (0.04 μM) is much lower than the K_m for dopa (200 μM). Only one equivalent of fluoride ion is liberated, corresponding to a turnover of one inhibitor molecule per inactivation event. In other words, the ideal partition ratio of 0 is observed. The mechanism for inactivation is shown in Scheme 10 (pathway a). There are other PLP-dependent decarboxylase enzymes that are inactivated through similar mechanistic pathways (a and b, Scheme 10). For example, (+)-α-fluoromethylhistidine **31** inactivates histidine decarboxylase[28], and D,L-α-(difluoromethyl) ornithine (DFMO) **32** inactivates ornithine decarboxylase[29]. DFMO has proven highly effective against African sleeping sickness in clinical trials. Similar to pathway a in Scheme 10, loss of CO_2 and F^- generates β-fluorovinyliminium cation **33**; the β-fluorine is displaced with nucleophilic residue on enzyme to give E–I adduct **34** (Scheme 11).



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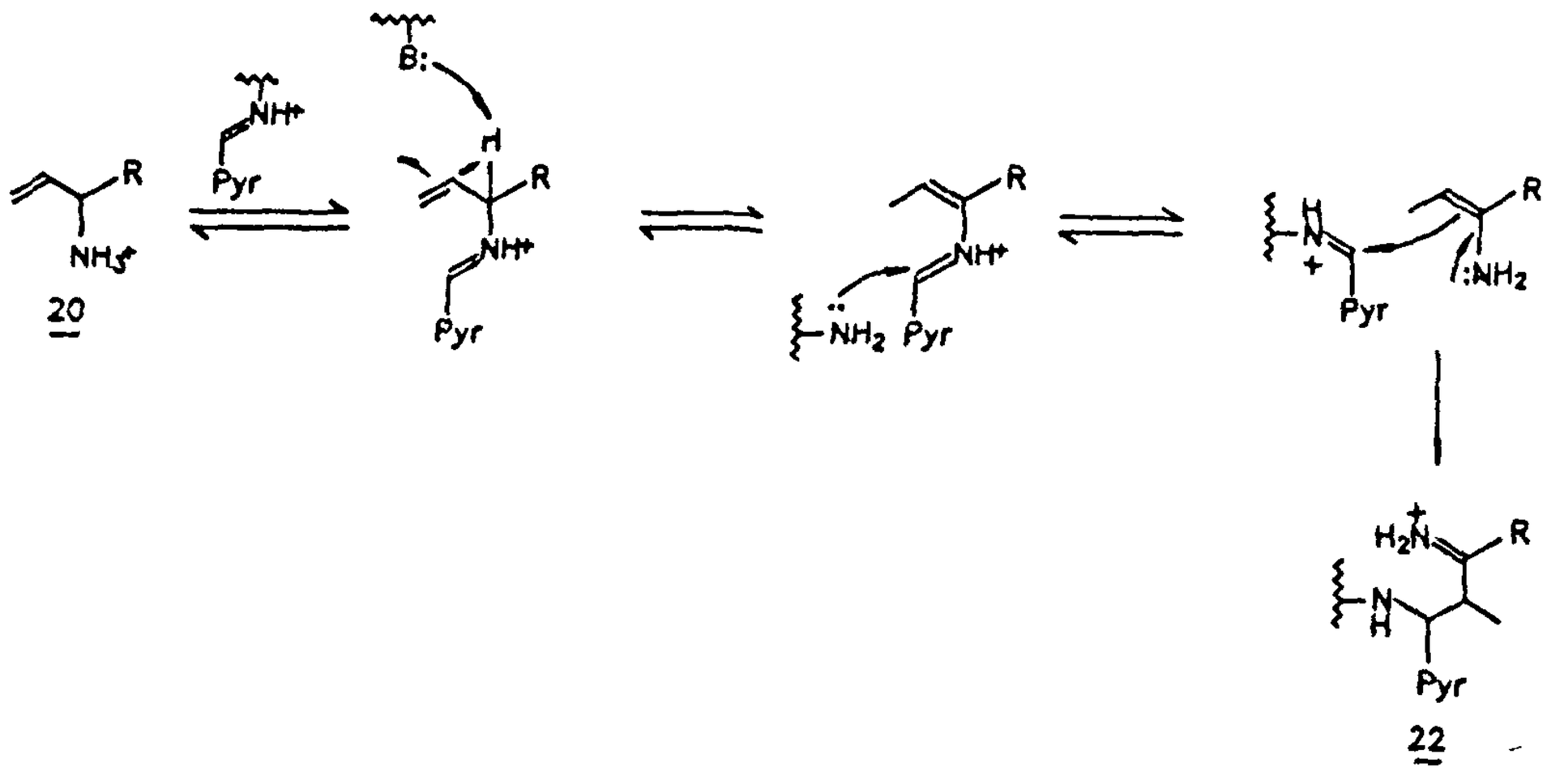
Scheme 6.



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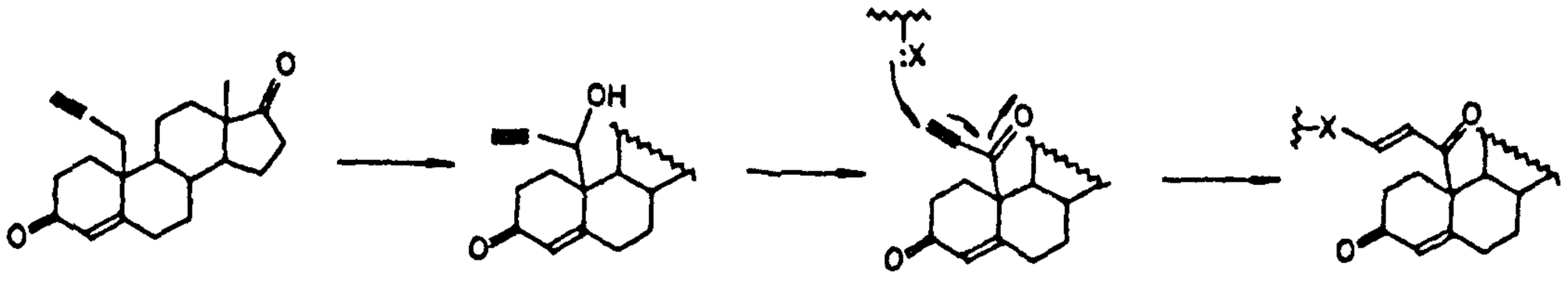
Scheme 7.



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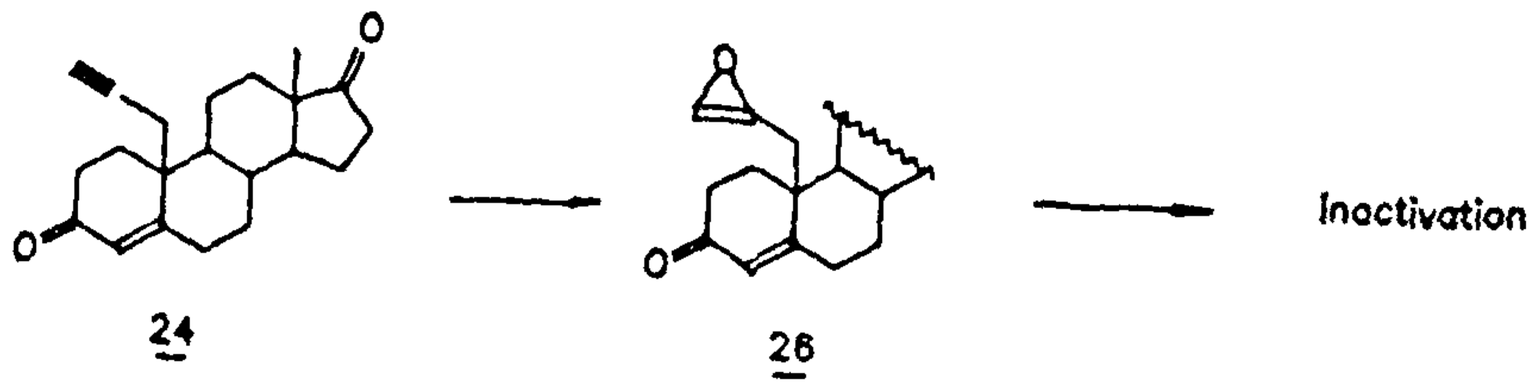
Scheme 8.



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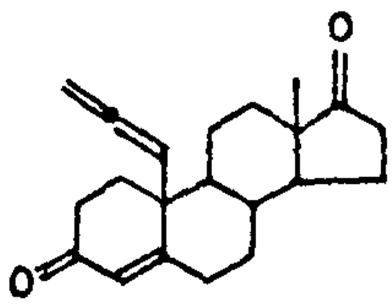
Scheme 9.



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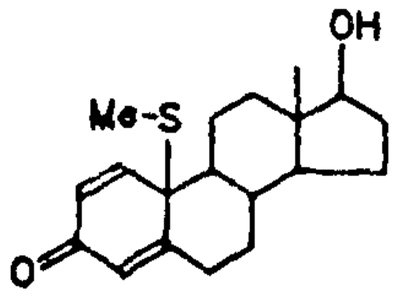

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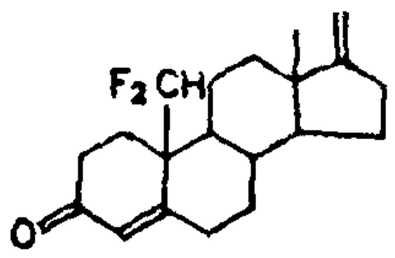

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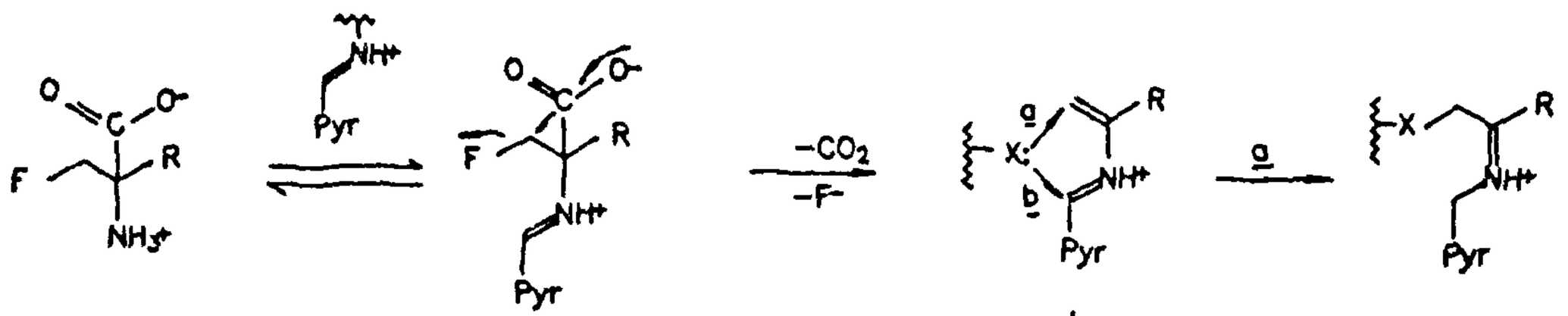
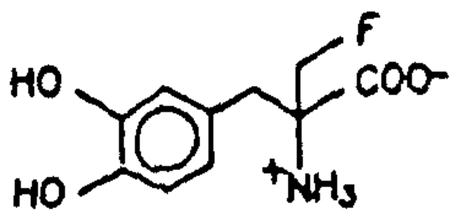


Scheme 10.



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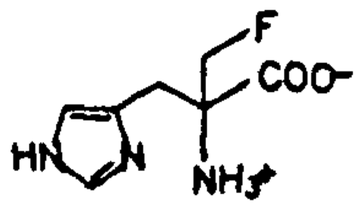

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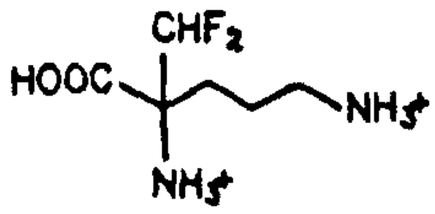

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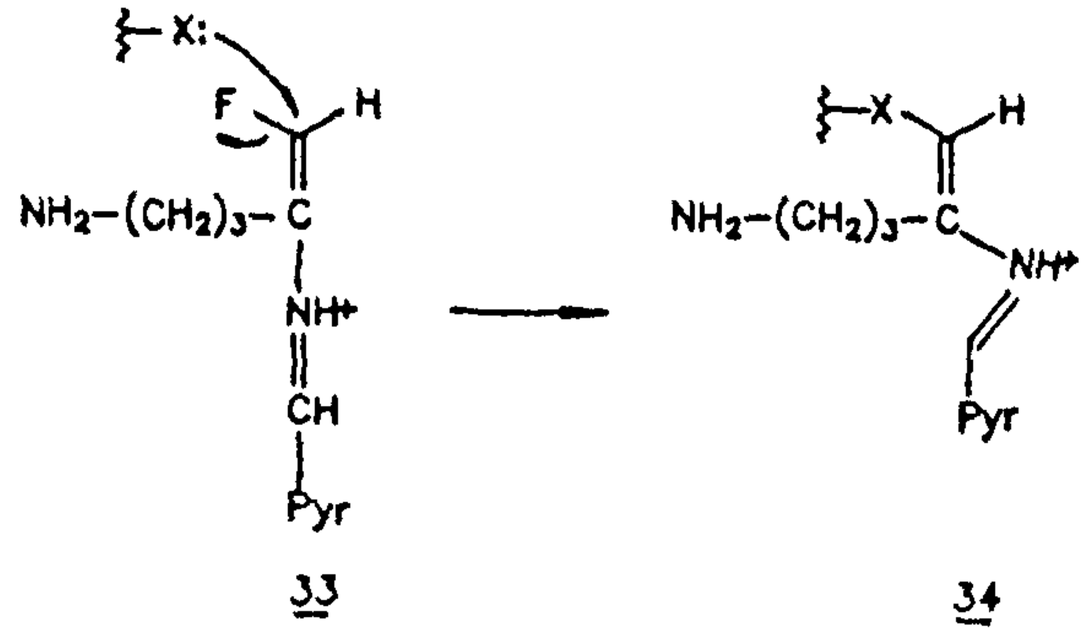


Scheme 11.

Histidine decarboxylase

Histamine interacts in the human body with H_1 and H_2 receptors. Interaction with H_1 receptor causes allergic or hypersensitivity reactions by stimulation of sensory nerve endings. Interaction with H_2 receptor stimulates gastric acid secretion, leading to ulceration. Since most of the histamine in mammalian tissues is produced by decarboxylation of histidine, inhibition of histidine decarboxylase appears to be a viable approach to decreasing histamine levels.

Metcalf *et al.*[30] synthesized α-trifluoromethyl histamine **35**, which exhibits nonpseudo-first-order kinetics. The inactivation of histidine decarboxylase occurs via an intermediate similar to **34** generated by displacement of fluoride ion from **35**.

β-Lactamase

The penicillins are potent antibiotics for the treatment of a wide variety of bacterial infections. The acylation of transpeptidase serine hydroxyl with β-lactam antibiotics inactivates the enzyme. Transpeptidase are bacterial enzymes that catalyse the cross-linking of cell wall peptidiglycan strands. Various resistant strains of bacteria have now evolved with β-lactamase enzymes which hydrolyse the active β-lactam drugs to inactive forms. β-Lactamase inhibitors are synergistic with penicillins and expand their armoury against these resistant strains of bacteria.

The first step in the reaction of cephalosporins, such as cefoxitin **36**, with β-lactamase, is acylation of β-lactam carbonyl with serine OH via a tetrahedral intermediate[31]. Cephalosporins containing a 3′-leaving group undergo facile elimination to a stable acyl–enzyme intermediate (E–I complex) with the 1,3-thiazine structure **37** (Scheme 12). Other potent β-lactamase inhibitors from the laboratories of Charnas and Knowles[32], and Brenner and Knowles[33] are clavulanic acid **38** (natural) and sulbactam **39** (semi-synthetic).

Compound **40** is a multienzyme-activated inactivator involving simultaneous participation of β-lactamase, alanine peptidase and alanine racemase. This dipeptide antibiotic was evaluated for prodrug antibacterial properties[34]. The β-lactamase-catalysed hydrolysis of cepham **40** releases β-chloro-L-alanyl–β-chloro-L-alanine; this dipeptide is hydrolysed by alanine peptidase to β-chloro-L-alanine, which is a mechanism-based inactivator of alanine racemase.

Lipoxygenase

Arachidonate is parent to the eicosanoids, a family of very potent biological signalling molecules. The enzyme 5-lipoxygenase catalyses the first step in the cytochrome P-450 oxidation of arachidonic acid to leukotrienes, which are involved in a variety of biological responses such as smooth muscle contraction, increased vascular permeability, and chemotaxis. The fatty acid hydroperoxides generated by lipoxygenases are important in the development of atherosclerotic lesions.

7-Thiaarachidonic acid and related compounds[35] **41** are time-dependent inactivators of 5-lipoxygenase in the presence of oxygen. The polarizable sulphur atom interacts with the active-site iron to form a radical cation-like species **42**, which reacts covalently with active-site nucleophile to the E–I adduct **43** (Scheme 13).

Monoamine oxidase

Monoamine oxidases (MAO isozyme forms A and B) are responsible for the catabolism of biogenic amines. Compounds that inhibit MAO increase the concentration of biogenic amines and exhibit an antidepressant effect. As mentioned earlier, L-aromatic amino acid decarboxylase inactivation increases the brain concentration of dopamine and is a goal for antiparkinsonism. MAO inhibitors can be used in conjunction with L-dopa therapy for treatment of Parkinson's disease.

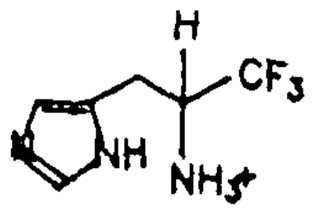
A series of pargyline analogues **44** are time-dependent inactivators of MAO[36], an enzyme containing a covalently bound flavin (Fl) **45**. Based on products from ^{14}C-labelled studies and photochemical experiments, the following mechanisms for inactivation are likely. Attachment of the propargyl amine **44** to N-5 position of flavin and reduction to dihydroflavin initiates the inactivation cascade[37], leading to flavin–amine adduct **46** (Schemes 14 and 15).

Symptoms of Parkinson's disease were observed by administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine **47** (MPTP) in man and animal test studies. Pretreatment of animals with known MAO inhibitors, such as deprenyl and pargyline, protects the animals from neurotoxicity of MPTP[38]. This indicates that MPTP is a substrate of MAO.

Ornithine decarboxylase

Ornithine decarboxylase catalyses the conversion of ornithine to putrescine, the rate-limiting step in polyamine biosynthesis. The shut-down of polyamine production is effective in the treatment of parasitic infestations.

D,L-α-Monofluoromethylputrescine **48** is a potent time-dependent inactivator of ornithine decarboxylase[39]. Because of PLP–imine and fluorine, the pK_a of β-hydrogen in **49** is low enough for deprotonation and subsequent elimination of fluoride ion to occur at physiological pH (Scheme 16). Azadiene **50** is the actual inhibitor species which reacts with active-site nucleophile.



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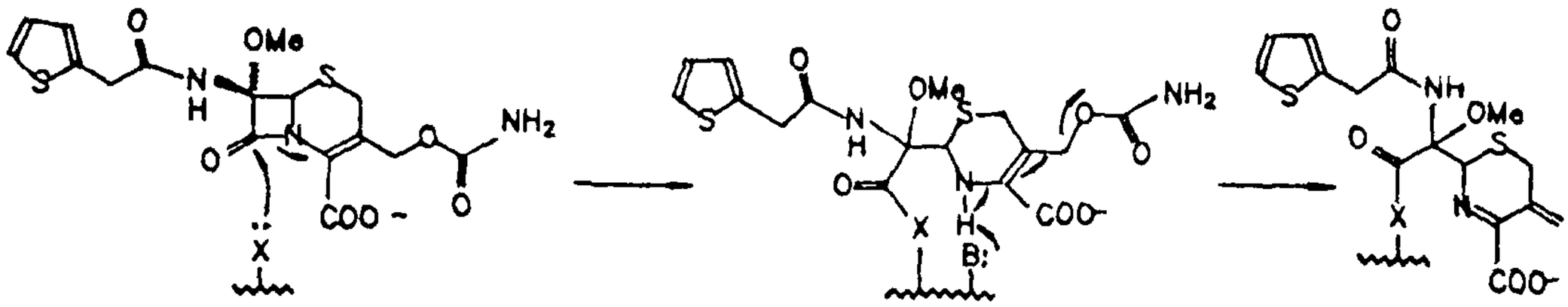
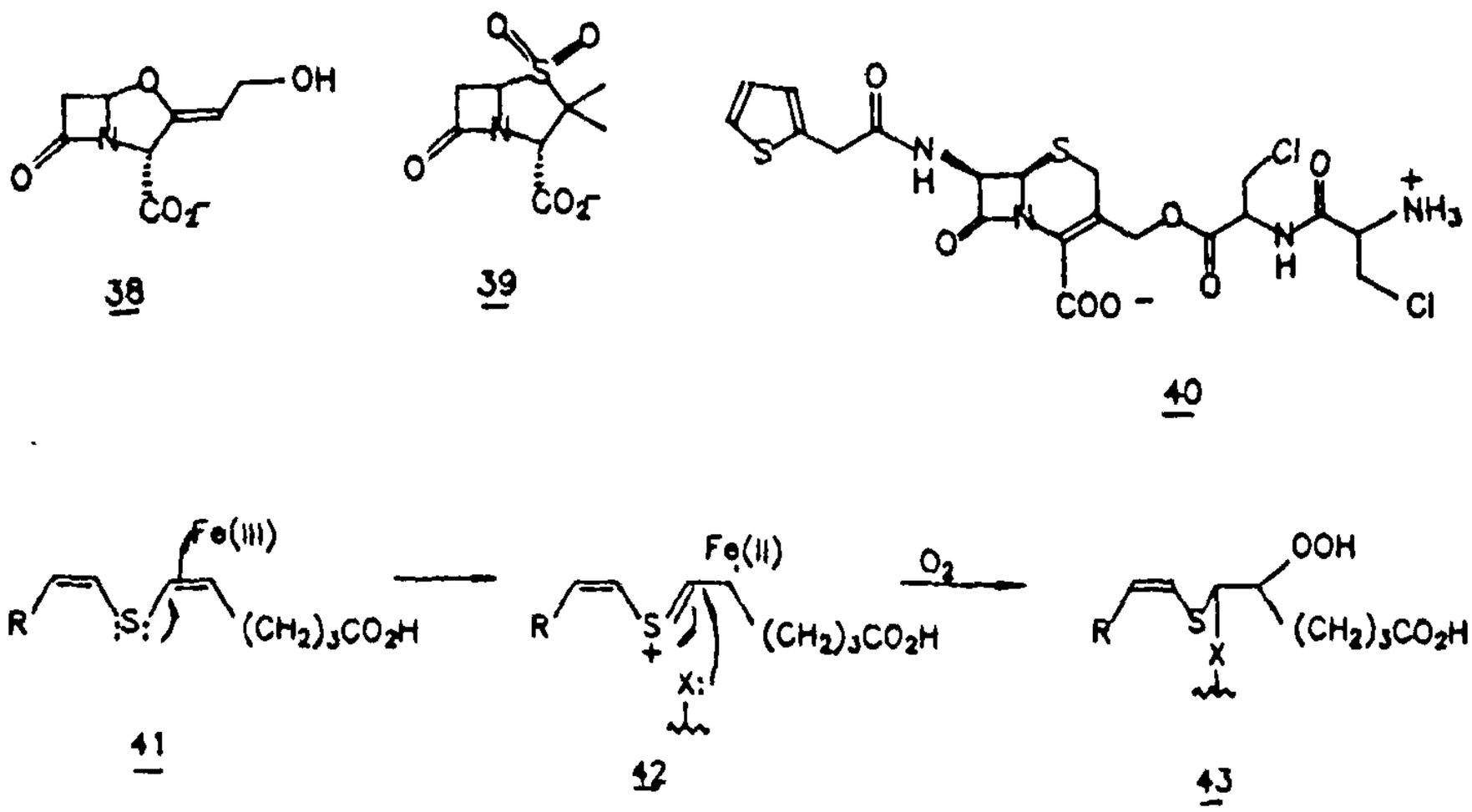


36 37

Scheme 12.



38 39 40

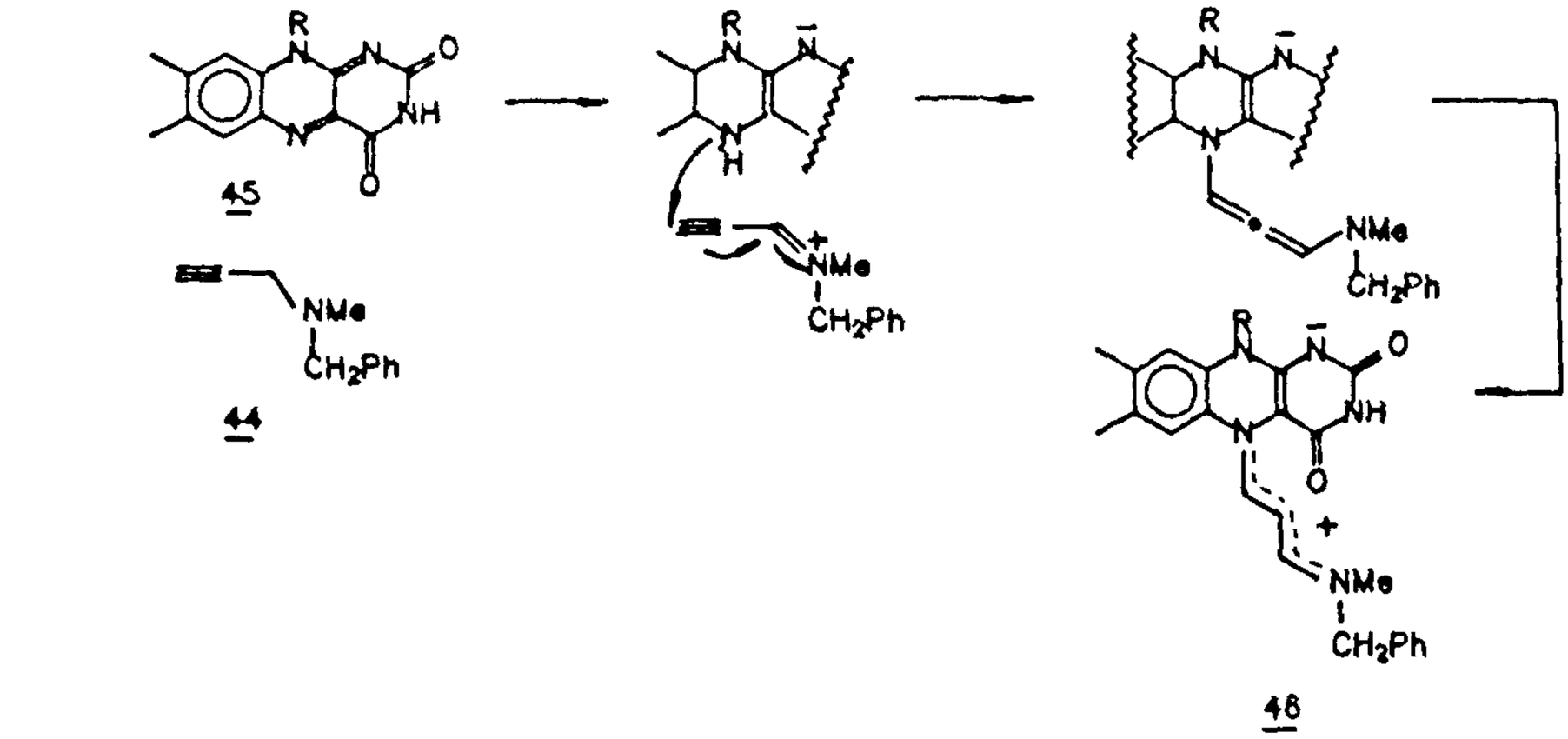
41 42 43

Scheme 13.

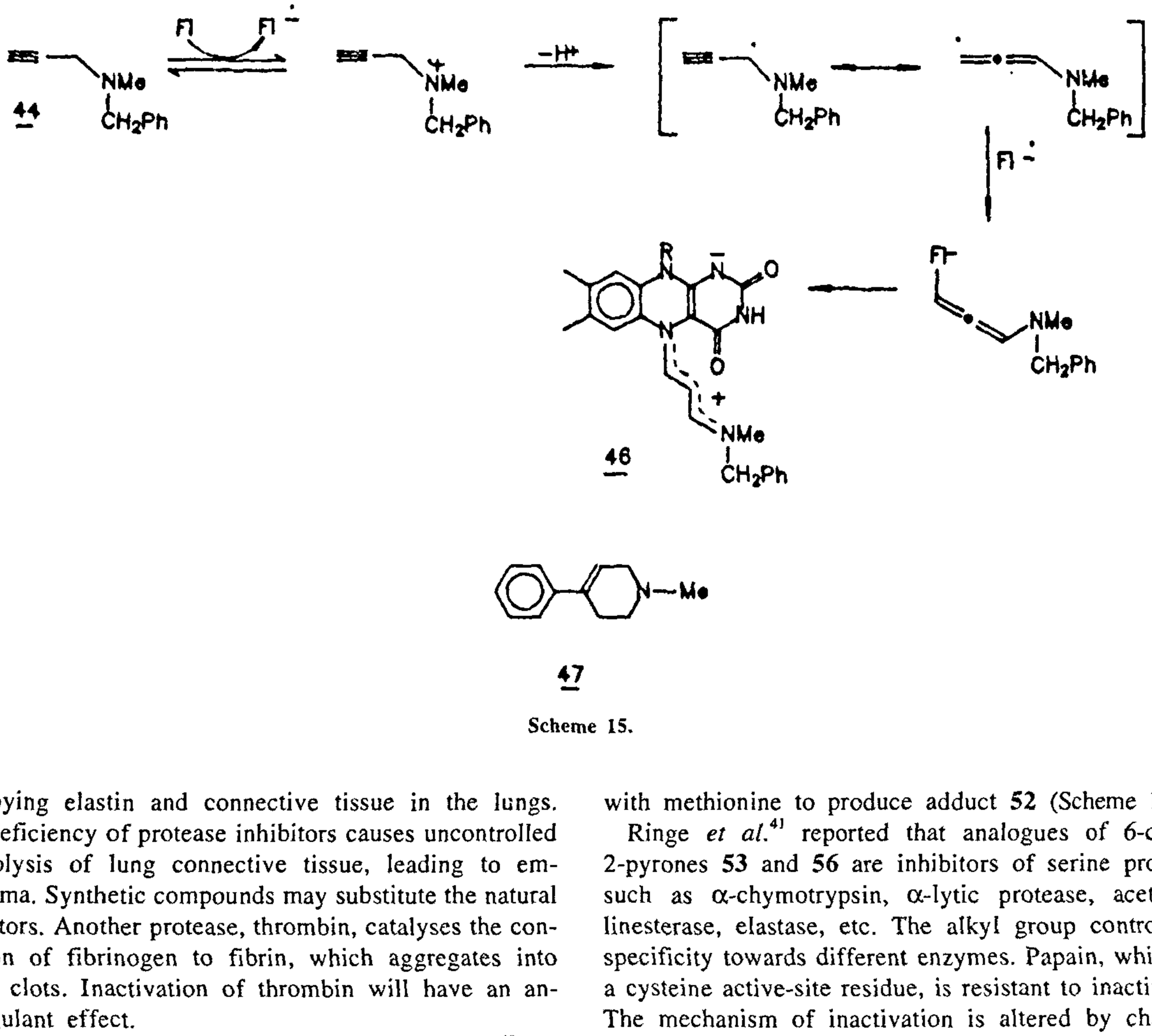
Serine protease

The optimal activity of certain pancreatic enzymes, such as trypsin, chymotrypsin and carboxypeptidase, occurs at pH 7–8. The exocrine cells of pancreas synthesize these enzymes as their inactive zymogens to protect themselves from destructive proteolytic attack. Additionally, the pancreas prevent premature production of proteolytic enzymes by maintaining a balance between the concentration of the enzyme and its inhibitor. During acute pancreatitis condition, the catalytically active proteolytic enzymes are produced prematurely inside the pancreas. As a result, the pancreatic tissues are attacked, causing a painful and serious destruction of the organ. The inhibition of proteases by added concentrations of inactivator compounds is an effective treatment for acute pancreatitis.

The imbalance in the concentration of certain proteases and their inhibitors in the lungs causes pulmonary emphysema. Human leukocyte elastase and cathepsin G digest dead lung tissue and foreign bacteria. The role of natural inhibitors is to prevent these enzymes from



Scheme 14.

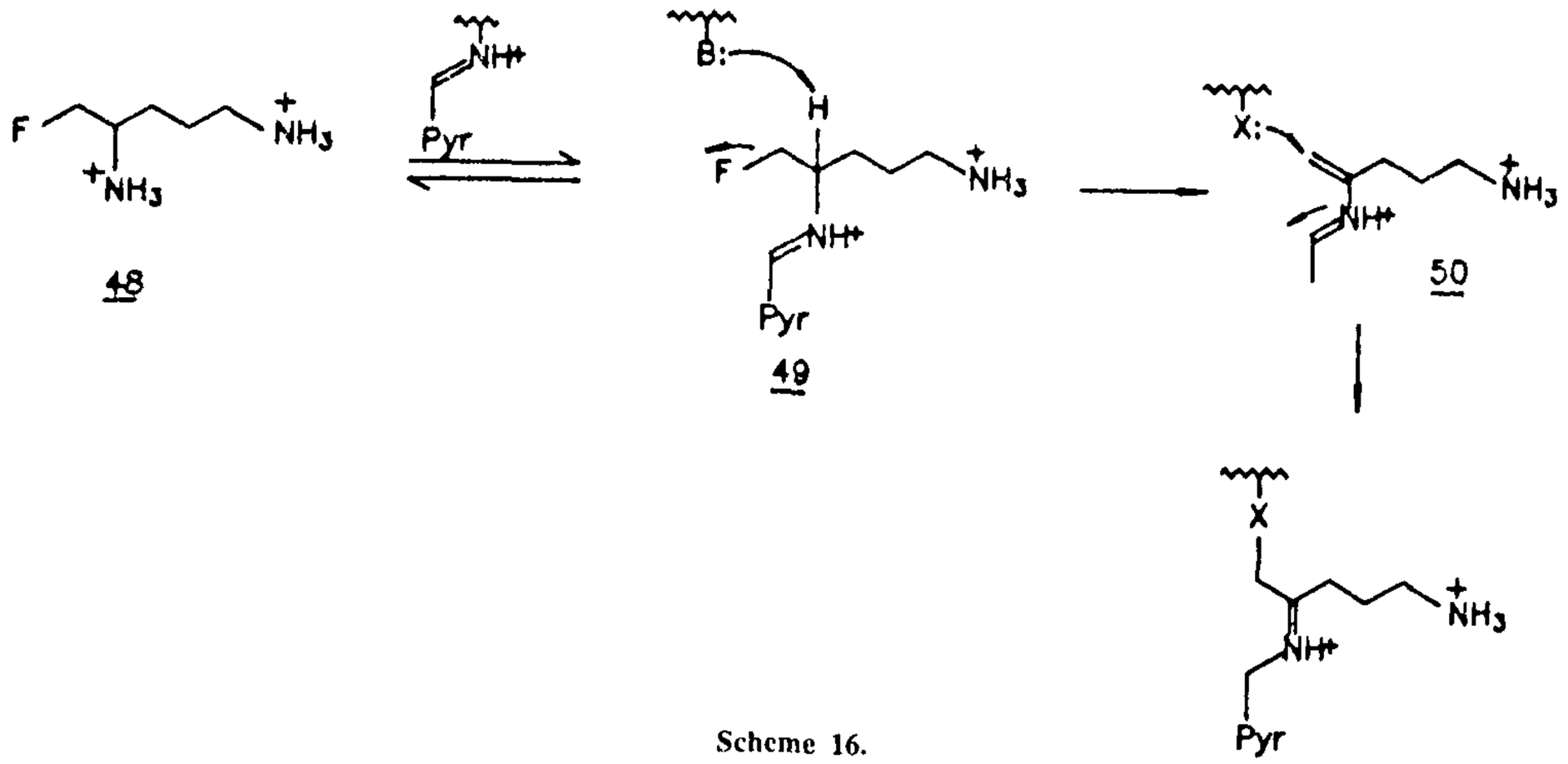


Scheme 15.

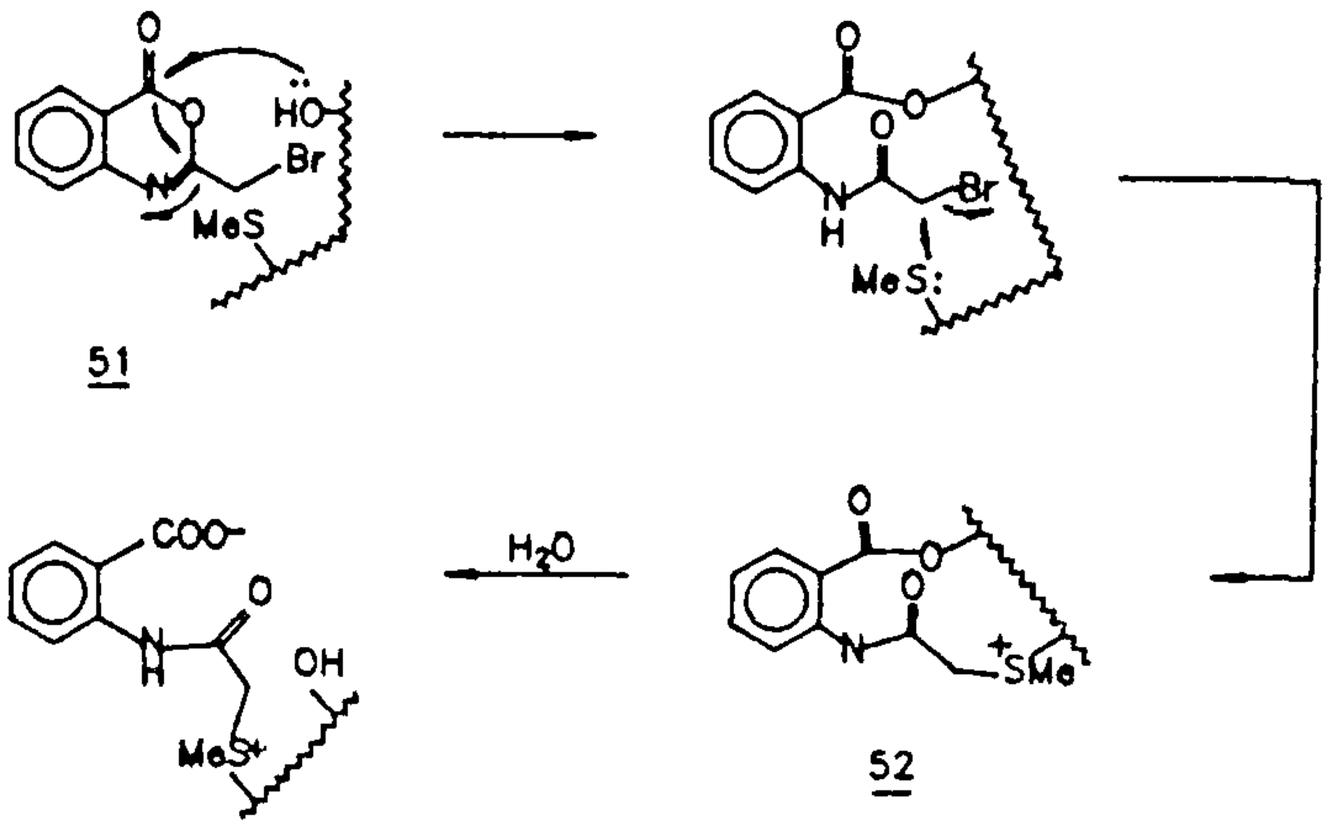
destroying elastin and connective tissue in the lungs. The deficiency of protease inhibitors causes uncontrolled proteolysis of lung connective tissue, leading to emphysema. Synthetic compounds may substitute the natural inhibitors. Another protease, thrombin, catalyses the conversion of fibrinogen to fibrin, which aggregates into blood clots. Inactivation of thrombin will have an anticoagulant effect.

The irreversible inactivation of α-chymotrypsin[40] by 2-bromomethyl-3,1-benzoxazin-4-one **51** occurs by acylation of active-site serine residue, followed by alkylation with methionine to produce adduct **52** (Scheme 17).

Ringe *et al.*[41] reported that analogues of 6-chloro-2-pyrones **53** and **56** are inhibitors of serine proteases such as α-chymotrypsin, α-lytic protease, acetylcholinesterase, elastase, etc. The alkyl group controls the specificity towards different enzymes. Papain, which has a cysteine active-site residue, is resistant to inactivation. The mechanism of inactivation is altered by changing the position of benzyl group, which recognizes the hydrophobic pocket of the enzyme. For 3-benzylpyrone **53**, addition to tetrahedral intermediate **54** and its collapse



Scheme 16.



Scheme 17.

to acyl–enzyme intermediate **55** is the mechanistic pathway (Scheme 18). However, for 5-benzylpyrone **56**, conjugate addition of serine OH and elimination of chloride ion produces the inhibitor species **57** (Scheme 19).

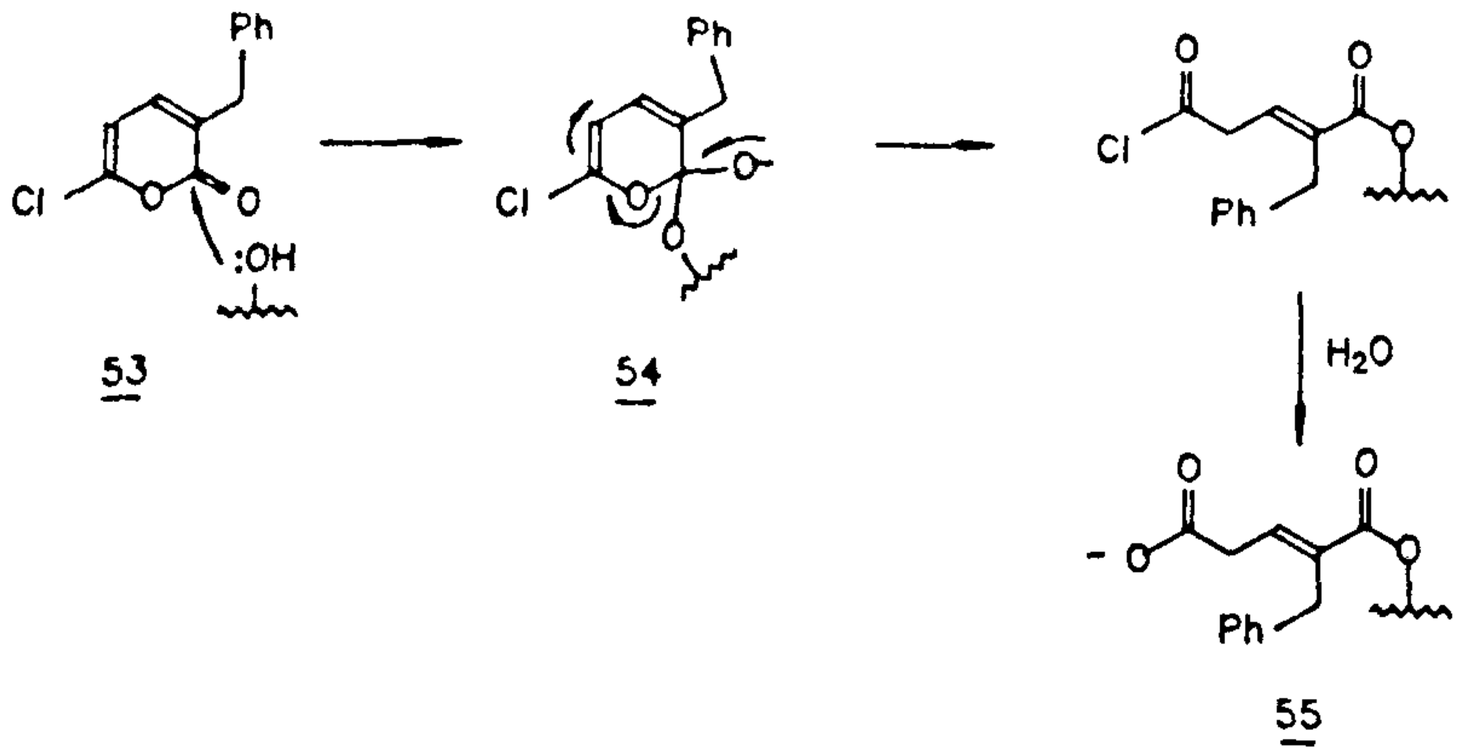
Testosterone α-reductase

Testosterone is reduced to the more active androgen 5α-dihydrotestosterone, which mediates androgenic activity in organs and glands. Inactivators of testosterone 5α-reductase will be useful in averting diseases such as acne, hirsutism, baldness and prostrate cancer.

(5α, 20R)-4-Diazo-21-hydroxy-20-methylpregnan-3-one **58** is a time-dependent inactivator of rat prostrate microsomal testosterone 5α reductase[42]. This compound has a great affinity for the enzyme with an apparent K_i of 35 nM. The mechanism for inactivation involves protonation of diazoketone to **59**, followed by active-site nucleophile attack (Scheme 20).

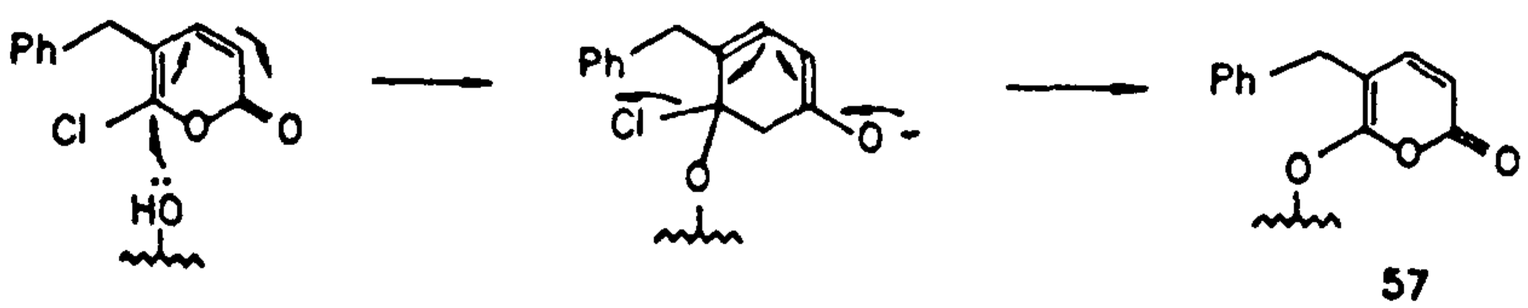
Xanthine oxidase

The disease gout is caused by elevated concentration of uric acid in blood and tissues. The joints become inflamed, painful and arthritic due to the abnormal deposition of sodium urate, which is fairly insoluble at physiological pH. The kidneys are also affected because excess uric acid is deposited in the kidney tubules. Although the exact causes of gout are not clearly



53 54 55

Scheme 18.



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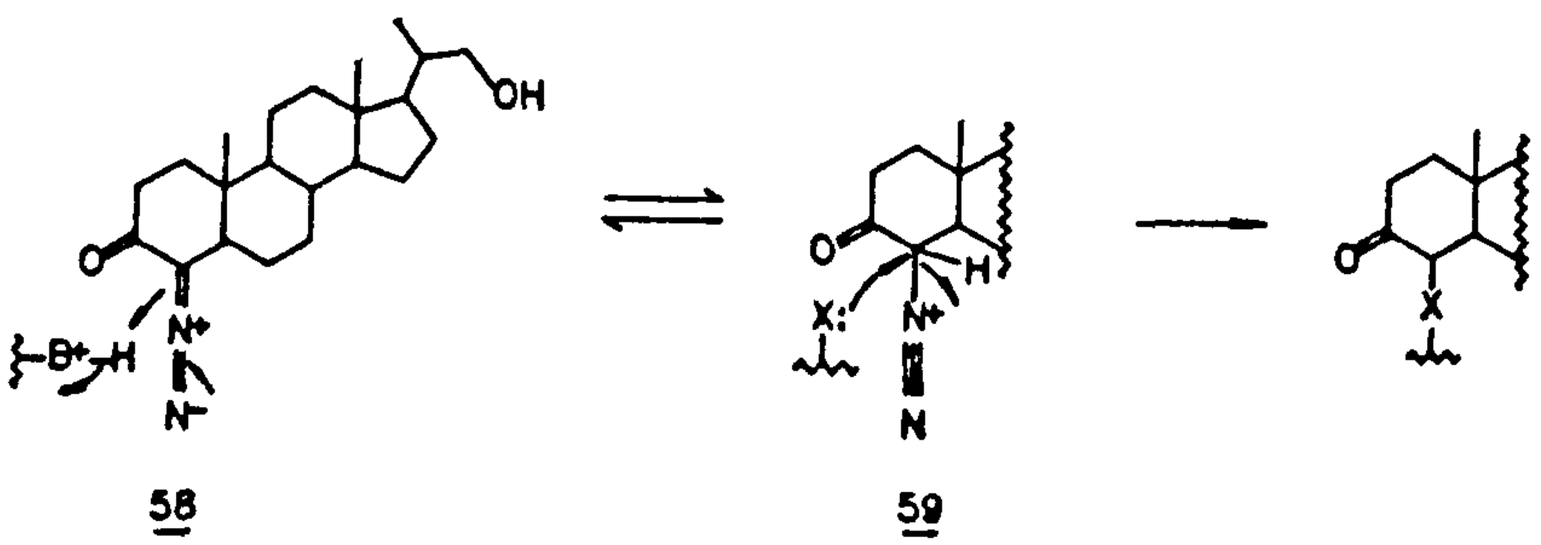
Scheme 19.



58 59

Scheme 20.

understood, a halt in the concentration of uric acid alleviates the pain. Xanthine oxidase is responsible for converting purines (xanthine) into uric acid.

The drug allopurinol **60** is a non-covalent mechanism-based inactivator of xanthine oxidase[43]. A slight alteration in the structure of hypoxanthine **61** (enol form) yields the useful drug allopurinol. When xanthine oxidase is inhibited, the products of purine metabolism are xanthine and hypoxanthine, both of which are more soluble in water than uric acid and less likely to form crystalline deposits.

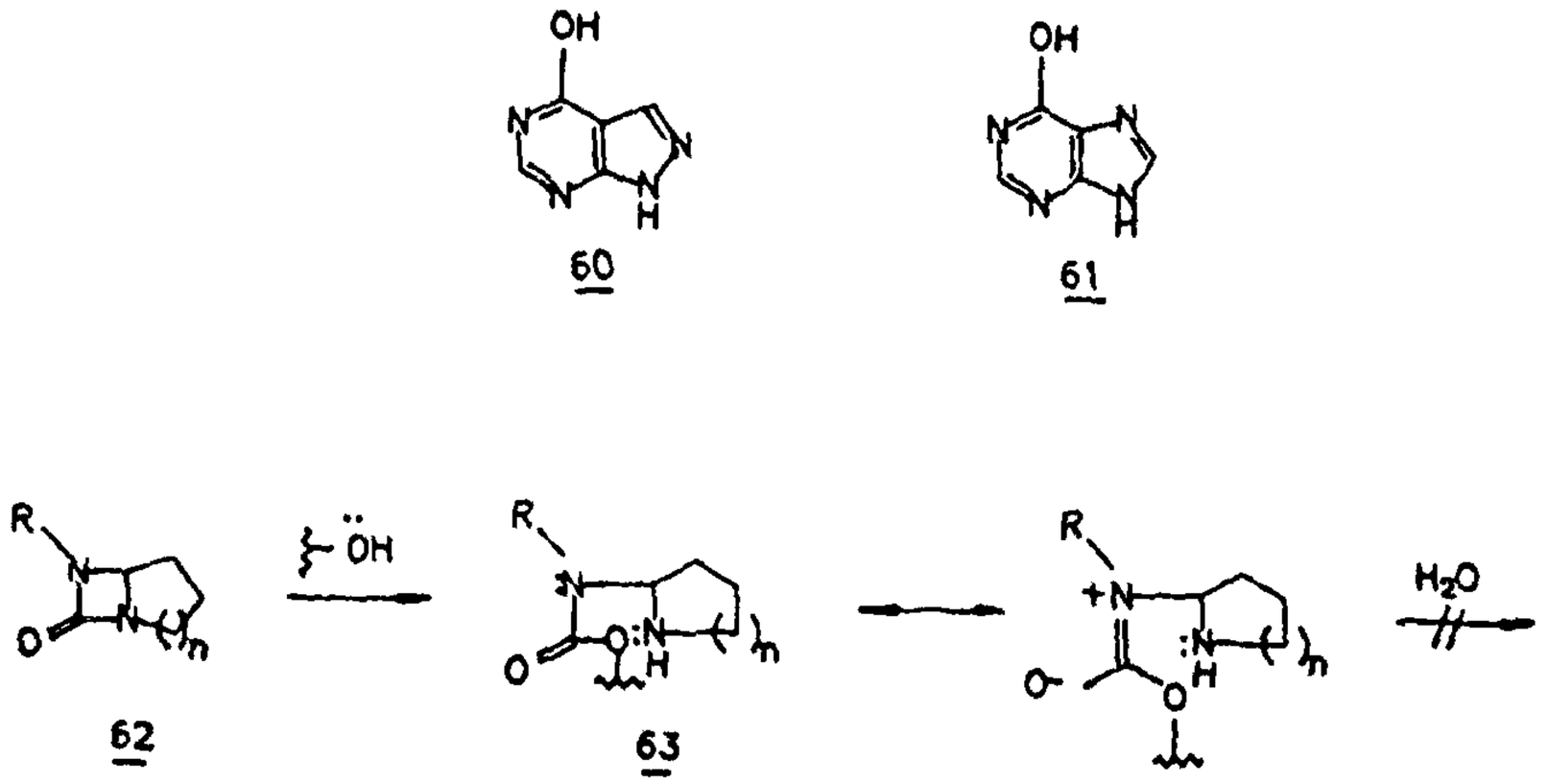
Tail piece

In this brief section, some ongoing studies in our research laboratory on the design and synthesis of mechanism-based inhibitors of two medicinally important enzymes, β-lactamase and aromatase, are presented.

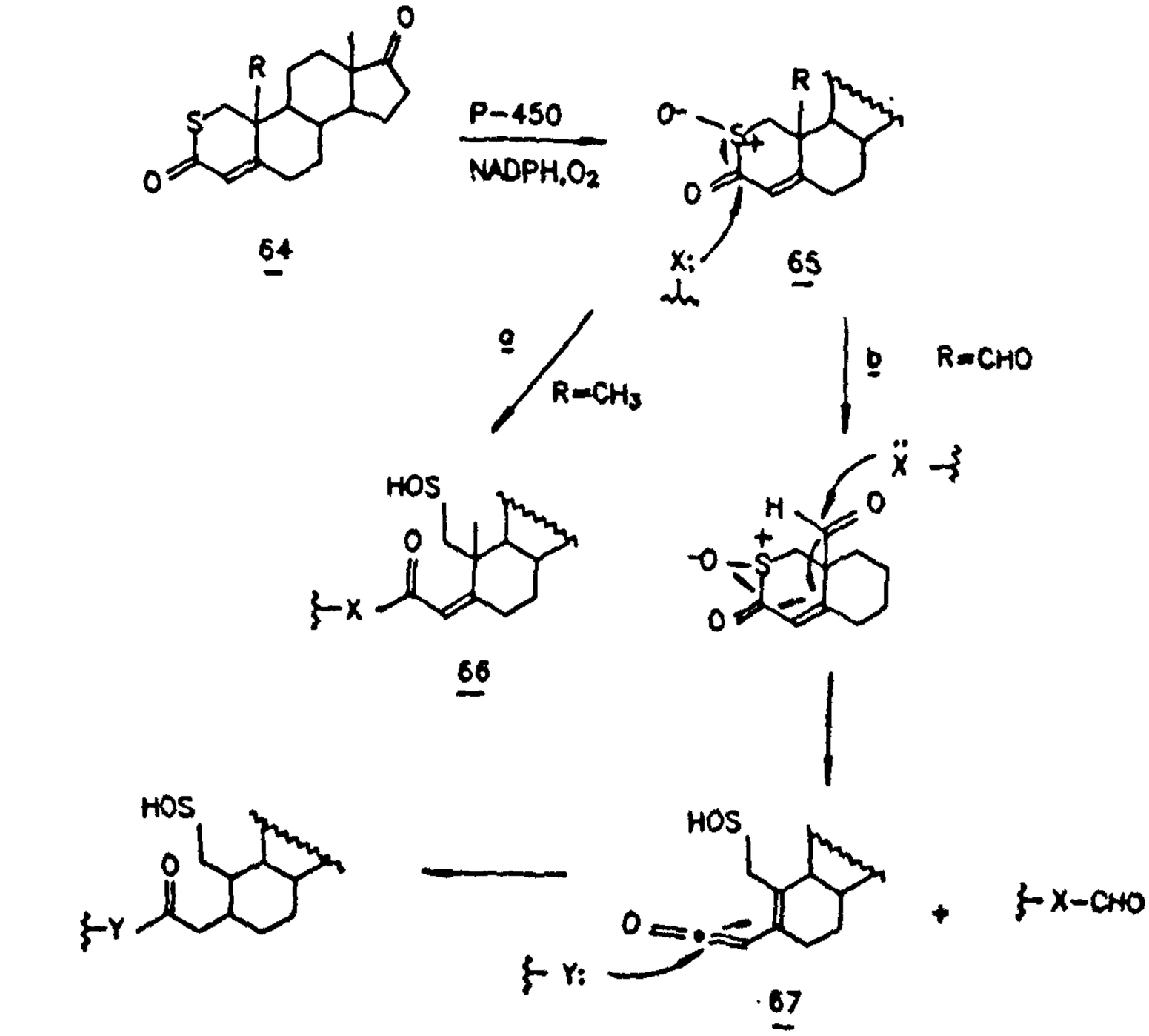
The synthesis of model bicyclic 1,3-diazetidin-2-ones **62** (R = Ph; $n = 1,2$) as anticipated inhibitors of β-lactamase enzyme is currently under study. The rationale for the expected inactivation is the higher hydrolytic stability of carbamoyl–enzyme intermediate **63** because

of amide resonance (Scheme 21). Preliminary results from semi-empirical calculations[44] on the putative inhibition appear encouraging.

The facile oxidation of steroidal A-ring thialactone **64** ($R = CH_3$) to acyl–sulfoxide **65** by aromatase P-450 enzyme[45] will open two possible pathways for mechanism-based inactivation: one via trapping of acyl–sulfoxide **65** by active-site nucleophile to E–I adduct **66** (pathway a), and the other via covalent inactivation of reactive ketene species **67** (pathway b) (Scheme 22).



Scheme 21.



Scheme 22.

Conclusions and future directions

Some recent advances made in mechanism-based inactivation of medicinally important target enzymes are summarized in this article. The design of new drugs by mechanism-based inactivation of target enzymes requires a detailed knowledge of the three-dimensional enzyme structure, its mechanism of action on the substrate, the active-site residues, and models for interaction between enzyme protein and substrate. In addition, computational data from high-speed computers and X-ray crystal structures of many important enzymes fuel the drug discovery process. A close collaboration between biologists, crystallographers, medicinal, computational and organic chemists is a crucial ingredient in the design of new enzyme inhibitor drugs with high potency and low toxicity. The success of initial research efforts is reflected in the recently launched drugs vigabatrin and eflornithine. Approval of some more mechanism-based enzyme inactivator drugs which are in advanced stages of clinical trials will be a great impetus for pharmaceutical companies to employ this approach in the coming years.

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