Chapter 26. Selective Enzyme Inhibitors in Medicinal Chemistry

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Introduction - Many old drugs have inhibitory properties on one or another enzyme, but only a few were conceived with that goal in mind. The knowledge of the structure and mechanism of action of enzymes has progressed tremendously over the past two decades so that the search of specific enzyme inhibitors as potential new drugs became more attractive and promising. In theory, if the primary and tertiary structure of an enzyme is known, for instance by X-ray crystallography, it should be possible to design compounds having optimal binding properties. This however is only rarely the case so that one has to rely on empirical methods to find structural requirements for a good binding, and this method has yielded some good competitive inhibitors.[1] This type of inhibitors is not always satisfactory because they are short-acting and high doses are often needed. The specificity of competitive inhibitors depends only on the recognition by an enzyme, so that different enzymes performing different reactions on the same substrate might be inhibited by such a compound. Additional selectivity can be gained if one takes into account what happens to the substrate after binding, and this has led to two classes of more potent and selective inhibitors: the transition state analogs[2] and the enzyme activated irreversible inhibitors, also called k_{cat} or catalytic inhibitors[3] or suicide enzyme inactivators.[4] The first example of an enzyme activated irreversible inhibitor was provided by K. Bloch's discovery of the interaction of β-hydroxydecanoylthioester dehydrase with the β,γ-unsaturated substrate analog CH_3-$(CH_2)_5$-C≡C-CH_2-CO-SCoA.[5] Nature has often developed potent and specific enzyme inhibitors and the systematic screening of extracts from mammalian tissue, bacterial or fungus culture has yielded enzyme inhibitors of various types of action.[6,7] Selective examples of the different kinds of enzyme inhibitors which have led or are likely to lead to biological applications, are presented. The review is divided into sections dealing with classes of enzymes metabolically linked or performing similar functions.

Enzymes Involved in Amino Acid Metabolism

Ornithine decarboxylase: α-Methylornithine,[8] α-hydrazino δ-amino valeric acid,[9] β,γ-dehydro-ornithine,[10] are potent competitive inhibitors of ornithine decarboxylase, the rate-limiting enzyme of polyamine biosynthesis. These compounds have been found to inhibit the proliferation or differentation of several cell lines in cultures[9,10,11]. The inhibition has been attributed to the depletion of spermidine. The competitive inhibitors have the disadvantage of increasing the amount of ornithine decarboxylase, probably by slowing down its degradation, which results in an increased ability to produce polyamines after the inhibitor has disappeared. Two irreversible inhibitors, α-difluoromethylornithine(1) and 5-hexyne 1,4-diamine (2), have been described.[12] Compound (1) blocks the proliferation of rat hepatoma tumor cells and mouse leukemia cells in culture.[13] The respective mechanisms of action are described in Scheme 1.

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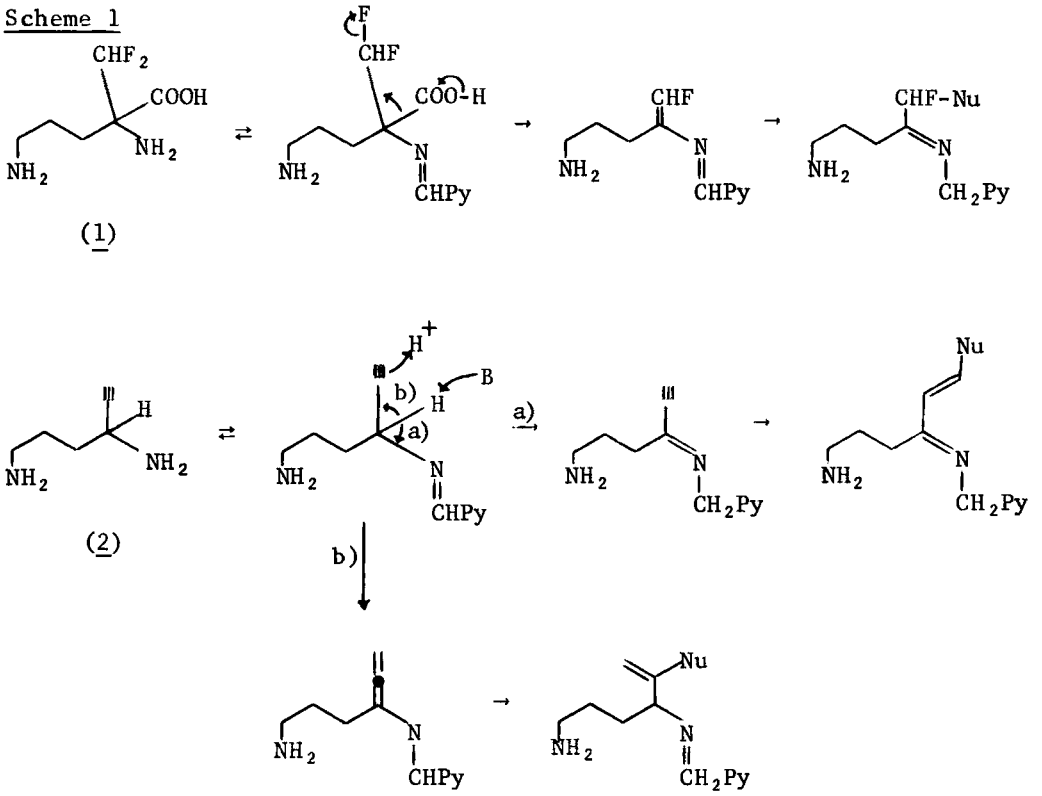
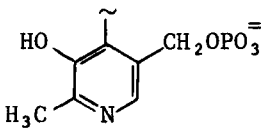
Scheme 1

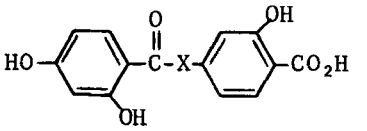


Py=



Nu= nucleophile

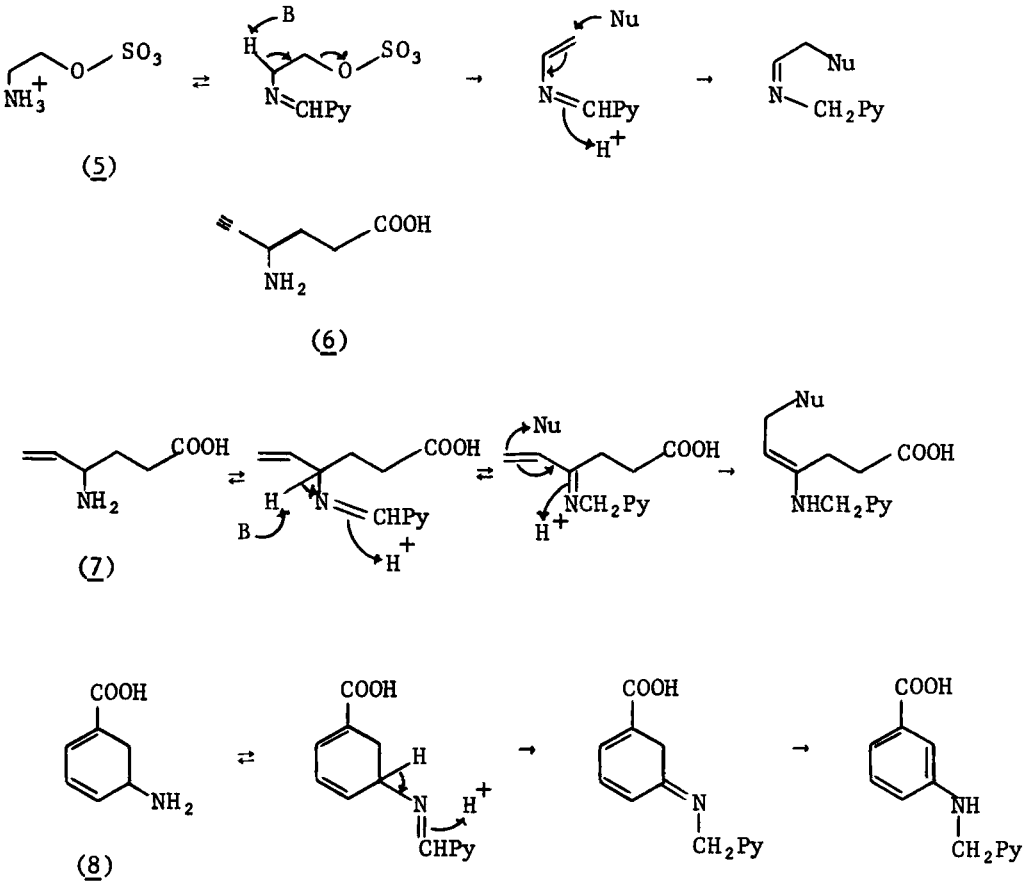
Histidine decarboxylase: Both hydrazinohistidine and tetrahydroisoquinolylhydrazino butyric acid are inhibitors of rat stomach histidine decarboxylase. They lower the histamine levels in stomach and reduce gastric secretion.[14] Structurally unrelated to histidine, lecanoric acid (3), a natural product isolated from a fungus broth, is an excellent reversible inhibitor of fetal rat histidine decarboxylase in vitro.[15] In vivo, the compound inhibits the development of rat paw edema induced by carrageenin, it remains, however, to be proved that these two actions are related. The synthetic amide analog (4) is more stable and is a stronger inhibitor of the enzyme and of edema formation.[15]



(3) X = O
(4) X = NH

4-Aminobutyric acid aminotransferase: The concentration of 4-aminobutyric acid (GABA), an inhibitory neurotransmitter in mammalian brain, can be elevated by inhibiting GABA-aminotransferase, the enzyme responsible for its degradation. As a result there is protection against electroshock, audiogenic and certain chemically induced seizures in animals. Until recently, inhibitors available were pyridoxal scavengers like aminooxyacetic acid,[16] hydrazino-propionic acid,[17] γ-glutamylhydrazone.[18] In recent years, four enzyme activated irreversible inhibitors have been described for this enzyme: ethanolamine-O-sulfate[19] (5), 4-aminohex 5-ynoic acid[20] (6), 4-aminohex 5-enoic acid[21] (7) and 5-amino-1,3-cyclohexadienyl carboxylic acid[22] (8). All of these compounds produce marked elevations of brain GABA levels and possess anticonvulsant action.[23-26] Their respective mechanisms of action are described in Scheme 2.

Scheme 2

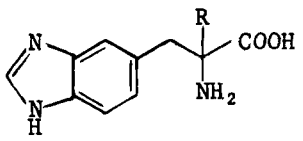


In addition, (6) inhibits irreversibly bacterial and mammalian glutamate decarboxylase,[27] probably by a mechanism similar to the one described for the inhibition of ornithine decarboxylase by (2) in Scheme 1.

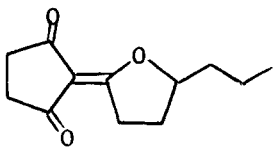
Other pyridoxal-dependent enzymes: Much has been written during recent years about the irreversible inhibition of different pyridoxal-phosphate-dependent enzymes by mono or poly haloalanines[28,29] and propargyl,[30] allyl,[31] or vinyl[32] glycine. The mechanisms implied involve in all cases abstraction of the α-hydrogen from the pyridoxal-Schiff base by the enzyme, followed by elimination of a halide atom in the case of halo-alanines, tautomerisation of the conjugated Schiff base in the case of vinyl glycine, yielding reactive Michael-acceptors in the enzyme active site. The irreversible inhibition by propargyl and allyl glycine is more complex as it involves a second hydrogen abstraction to yield a conjugated Schiff base. This Michael acceptor can alkylate the enzyme which results in either a fully inhibited enzyme or an enzyme having modified properties. It can also be hydrolysed in either chemically reactive or unreactive species.[33] These compounds, although of limited utility in animal studies due to their lack of selectivity, helped to promote the search for more specific enzyme activated irreversible inhibitors of pyridoxal-dependent enzymes. Two compounds deserve a special mention, these are 3-fluoro-D-alanine and its 2-deutero analog.[34,35] Both compounds are irreversible inhibitors of alanine racemase and possess a broad spectral antibiotic activity. Animal toxicity is low with the protio compound and even lower with the deuterated compound. The anti-bacterial activity of D-carbamylserine and D-cycloserine, two natural antibiotics, is also due to the enzyme activated irreversible inhibition of alanine racemase.[36]

Enzymes involved in the synthesis or degradation of biogenic amines

Tyrosine hydroxylase: α-Methyl-p-tyrosine is a strong competitive inhibitor of tyrosine hydroxylase,[37] the key enzyme of catecholamine biosynthesis, and lowers dramatically the concentrations of brain, heart and adrenal catecholamines in animals, without any significant effect on serotonin levels.[38] Although this compound was promising in the treatment of pheochromocytoma,[39] it was not developed beyond the clinical phase because there was marked drug crystalluria[40] in experimental subjects. Benzimidazole alanine[41] (9) and its α-methyl analog[42] (10) are also good competitive inhibitors of tyrosine hydroxylase and block the synthesis of catecholamines in reserpinized animals.[43] Oudenone (11), a microbial product, inhibits tyrosine hydroxylase by competing with the pteridine cofactor,[44] and reduces catecholamine levels in mammalian brain and adrenal glands; the compound has hypotensive activity[45] in spontaneous hypertensive rats.



(9) R = H
(10) R = CH_3



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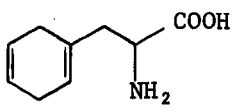
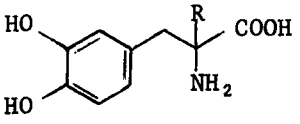


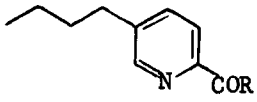
(12)

Tryptophan hydroxylase: p-Chlorophenylalanine, a competitive inhibitor of tryptophan hydroxylase in vitro, depletes serotonin levels in vivo by a more complex mechanism.[46] The serotonin depleting activity of α-methyl-tryptophan in vivo is probably due to its conversion to α-methyl serotonin rather than to tryptophan hydroxylase inhibition.[47] The Umezawa group has reported recently that 2,5-dihydrophenylalanine (12), a natural product isolated from a streptomyces strain, is a competitive inhibitor of tryptophan hydroxylase in vitro.[48]

Aromatic amino acid decarboxylase: Benserazide and Carbidopa are very potent inhibitors of peripheral decarboxylase, probably by forming hydrazones with the enzyme-bound pyridoxal-phosphate, and both compounds increase the amount of dopamine formed in brain after peripheral administration of Dopa to an animal.[49] The combinations of Dopa and Carbidopa or Dopa and Benzerazide are used clinically for the treatment of Parkinsonism.[50] Recently, the synthesis of α-vinyl Dopa (13) and α-ethynyl Dopa (14), two potential suicide inactivators of Dopa-decarboxylase, has been described by two independent groups.[51a,b]



(13) R = /═
(14) R = -≡

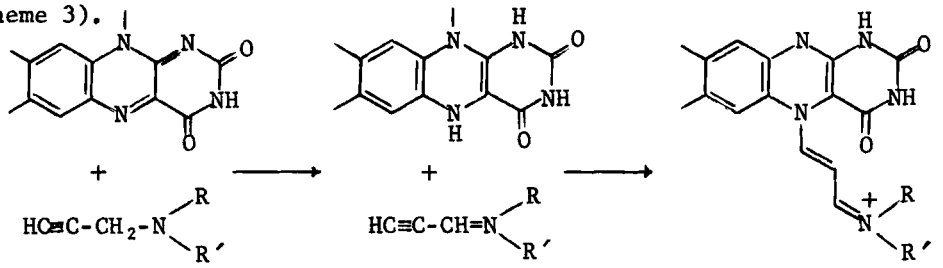


(15) R = OH
(16) R = NH_2

It was claimed without experimental details that (14) is a very potent inhibitor of this enzyme.[51a]

Dopamine-β-hydroxylase: This copper enzyme is inhibited by copper chelator. However, the only inhibitors, useful in vivo, are picolinic acids substituted in position 5 by aliphatic groups. The first member of this series is fusaric acid[52] (15), obtained from filtrates of microbial cultures; synthetic analogs have modified side chains,[53] or other functions in position 2[54] (16). All of these compounds have blood-pressure lowering effects.

Monoamine-oxidase inhibitors: The design of monoamine oxidase inhibitors is probably one of the fields in which medicinal chemists have been most active in the last decades. Some of the compounds are still in use to treat severe depression and hypertension. Mechanistically the most interesting are the propargylamine derivatives, like pargyline, clorgyline and deprenyl, which inhibit the flavin dependent enzyme irreversibly.[55] Very thorough investigations, including the characterization of the flavin adduct, have allowed Abeles and coworkers to propose the following reaction mechanism for the inhibition of mitochondrial MAO by a tertiary propargylamine[56] (Scheme 3).



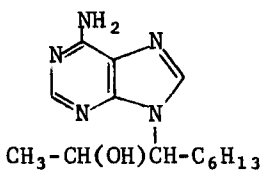
Plasma monoamine oxidase, a pyridoxal phosphate-dependent enzyme is inhibited irreversibly by aminoacetonitrile[57] and some esters of glycine.[58] The mechanisms implied, involve the formation of a keteneimine and a ketene by enzyme catalysis, followed by the alkylation of the enzyme by these reactive species.

Other flavin-dependent enzymes

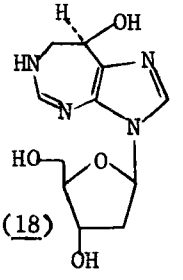
Other flavin-dependent enzymes are also inhibited irreversibly by substrate analogs, suitably substituted by vinyl or acetylenic groups. The inhibition of sarcosine oxidase and dimethyl glycine oxidase, in vitro and in vivo by N-allyl or N-propargylglycine[59,60] may be of relevance with the long-known, prolonged pharmacological activity of N-allyl amines. Flavin dependent α-hydroxy acid and α-amino acid dehydrogenases are inhibited by H-C≡C-CH(OH)-COOH and propargylglycine, respectively.[33,61,62]

Enzymes involved in purine and pyrimidine synthesis or degradation

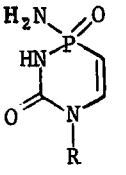
Adenosine deaminase: The anti-tumor activity of arabino-furanosyl adenine in animals can be increased by coadministration of tight-binding adenosine deaminase inhibitors[63] such as erythro-9-(2-hydroxy-3-nonyl) adenine (17) ($K_I = 10^{-9}M$)[64] and deoxycoformycin (18) which can be looked at as a transition state analog of adenosine during deamination.[65] Along this line, compounds (19, 20 and 21) have been synthesized and will be tested against the corresponding deaminases.[66]



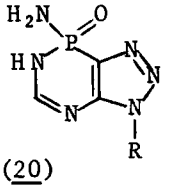
(17)



(18)



(19)



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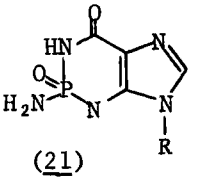
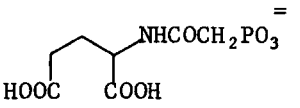
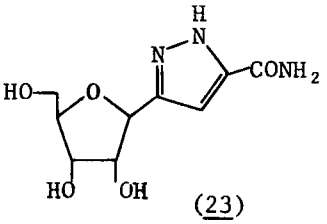


(21)

Aspartate-transcarbamylase: An early step in the de novo pyrimidine biosynthesis is the carbamylation of aspartate. Aspartate transcarbamylase can be effectively blocked by N-(phosphono-acetyl) aspartate (PALA) (22), a transition state analog of carbamyl aspartate.[67] This compound is effective in the mg/kg range in blocking this enzyme and the isoproterenol stimulated DNA synthesis in mouse submaxillary glands.[68] It is active against certain experimental tumors.[69]



(22)

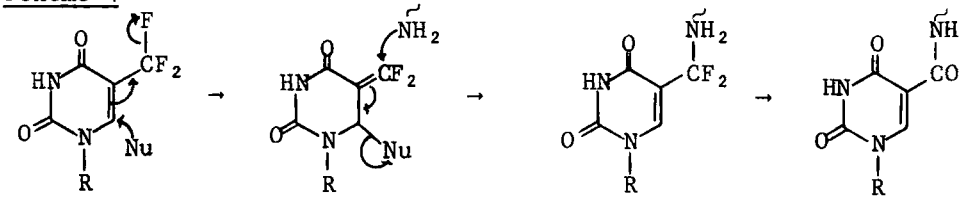


(23)

Orotate decarboxylase: Pyrazofurin (23), a natural antibiotic, is a very effective inhibitor of pyrimidine biosynthesis by blocking orotate decarboxylase. In combination with arabinosylcytosine, it increases greatly the cell toxicity of the latter compound.[70]

Thymidylate synthetase: Another key enzyme for DNA synthesis is thymidylate synthetase. This enzyme is irreversibly inhibited by fluorodeoxyuridine monophosphate formed in vivo from 2-fluorouracil, which had been used for about 20 years in cancer chemotherapy. The reader is referred to Ref. 71 for an excellent discussion on its mechanism of action. 5-Trifluoromethyl 2'-deoxyuridylic acid (24), formed from trifluoromethyl-uracil in vivo, is also an irreversible inhibitor of thymidylate synthetase.[72] The mechanism suggested by Santi and Sakai[73] is described in Scheme 4.

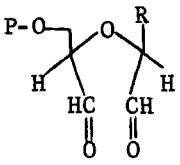
Scheme 4



(24)

Ribonucleotide reductase: Since it has been shown that ribonucleotide reductase activity is increased dramatically in rapidly growing tissues and viral infection, much work has been done in understanding the mechanism[74] and designing inhibitors of this enzyme.[75] The antitumor action of hydroxyurea, a radical scavenger has been attributed to inhibition of this enzyme, and analogs of hydroxyurea have been found to increase life span of leukemic mice.[76] The dialdehyde derivatives of purine nucleosides (25) are inhibitors of ribonucleotide reductase, they inhibit DNA synthesis in cell cultures.[77]

2'-Deoxy-2'-azido (or 2'-chloro) ribonucleotide diphosphates (26, 27) are irreversible inhibitors of ribonucleotide reductase from E. Coli and (26) also inactivates the calf thymus enzyme.[78] In addition, azidocytidine inhibits cell growth in vitro,[79] however the inhibition was not related to a depletion of the deoxynucleotide pool and may be due to an interaction at the initiation stage of DNA synthesis.



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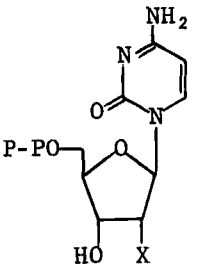
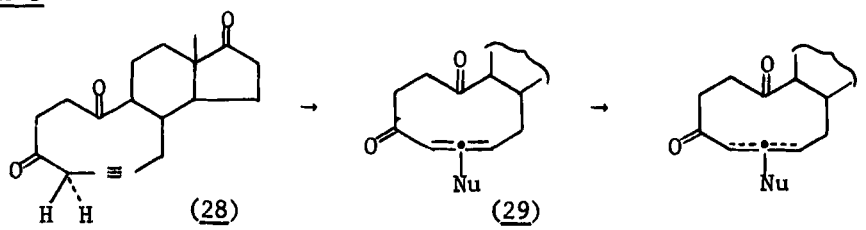


X = N_3 (26)

X = Cl (27)

Inhibitors of steroid biosynthesis: The cytotoxic effects of several hydroxylated sterols against rat hepatoma cells in culture seem to be at least partly due to inhibition of 3-hydroxy-3-methyl-glutaryl CoA reductase[80] which catalyses an early step in cholesterol biosynthesis. These sterols are extracted from drugs used in chinese folk medicine for the treatment of cancer. The hypocholesterolemic action of clofibrate was attributed to the inhibition of mevalonic acid synthesis at an unspecified step[81] while citrinin, another hypocholesteremic agent, inhibits hydroxymethyl-glutaryl CoA reductase in a time-dependent irreversible manner.[82]
There are a number of competitive type inhibitors of steroid hormone biosynthesis. Recently enzyme activated irreversible inhibitors of bacterial Δ^5-3-ketosteroid isomerase and of Δ^4-3-ketosteroid reductase, namely β,γ-acetylenic 3-oxo-5,10-secosteroids (28) and allenic 3-oxo-5,10-secosteroids (29) have been described.[83,84] These compounds act also selectively on the mammalian enzymes from rat epididymis.[85] Their mechanism of action is described in Scheme 5.

Scheme 5

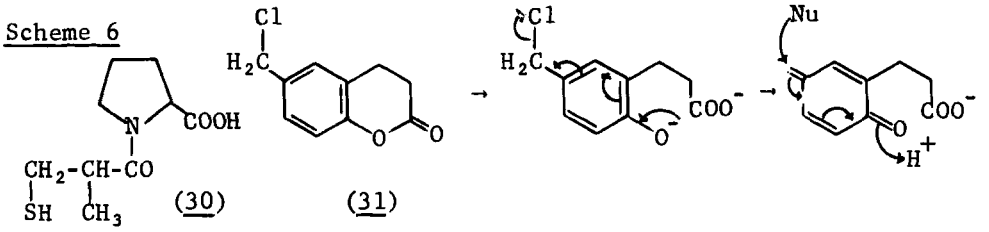


(28) (29)

Inhibitors of hydrolytic enzymes: There is no space to name the numerous proteolytic enzyme inhibitors found either in animals and plants[6] or in fungi and bacterial cultures.[7] Some of them have been tested clinically, such as pepstatine for the treatment of stomach ulcers, or leupeptin as an anti-coagulant. Leupeptin and antipain have terminal aldehyde functions which probably form hemi-acetals with a serine residue in the enzyme active site. It has been proposed that the unusual terminal amino acid of pepstatine, 4-amino-3-hydroxy-6-methylheptanoic acid, is an analog of the transition state in amide bond hydrolysis catalysed by acid proteases.[86] Inhibitors of angiotensin converting enzyme have a great potential as antihypertensive agents in renal hypertension. Active site mapping of such enzymes has lead to the design of a very powerful inhibitor, 3-mercapto-2-D-methylpropanoyl-L-proline (30) (K_I = 1.7 x 10^{-9}M).[87] This compound is orally active and prevents angiotensin-induced hypertension in normotensive humans

with little side effects.[88]
α-Chymotrypsin and other acid proteases are inhibited in a time-dependent manner by halomethylated derivatives of dihydrocoumarins (31).[89] The mechanism suggested is described in Scheme 6.[90]

Scheme 6



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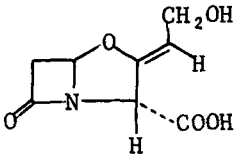

(32)

Penicillin G. inhibits cell wall biosynthesis in bacteria by several mechanisms one of which is irreversible acylation of D Ala-D Ala transpeptidase,[91] so that penicillin could be considered as a suicide enzyme inactivator.[3] Bacteria develop resistance to penicillin by developing specific β-lactamases. Medicinal chemists in turn, have tried to inhibit these β-lactamases. Over 1000 semi-synthetic penicillins have been screened for that purpose. Let us just mention clavulanic acid (32), a compound isolated from a streptomyces strain, which inhibits β-lactamases from both gram + and gram - bacteria in a time-dependent irreversible manner.[92]

Conclusion - Different possibilities to find selective enzyme inhibitors have been evaluated: The classical antimetabolic approach (like α-methyl analogs of amino acids), the systematic screening for active compounds (applied to microbial cultures) followed by chemical modifications to make stable and more potent products, (fusaric acid, lecanoric acid, etc.•••), the active site mapping of enzymes (angiotensin converting enzyme), and when the enzymatic mechanism is known the design of transition analogs (PALA, adenosine deaminase inhibitors) or of enzyme activated irreversible inhibitors (GABA-transaminase inhibitors, seco-steroids). It often happens that the molecular mechanism of action is not understood until long after the compound is known (acetylenic monoamine oxidase inhibitors, penicillins, clavulanic acid).
It can be expected that with the advent of the potent, selective, long-lasting enzyme inhibitors like the enzyme activated irreversible inhibitors or transition state analogs, the usefulness of enzyme inhibitors in therapy will increase.

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