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REVIEW

Exploring Alkaloids as Inhibitors of Selected Enzymes

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Living organisms possess complex networks of chemical reactions which are controlled by enzymes. Enzymes are responsible for almost all processes in a biological cell to occur at significant rates. Sometimes the functions of these enzymes need to be checked and hence requires isostructural molecules called enzyme inhibitors that can be cellular metabolites, or foreign substances. In this article, literature has been surveyed to provide comprehensive coverage of alkaloids being used as enzyme inhibitors.

Key Words: Alkaloids, Enzyme Inhibitors, Oxidoreductases, Transferases, Hydrolases.

INTRODUCTION

Living organisms possess complex networks of chemical reactions which are controlled by enzymes. Enzymes are mainly proteins that catalyze chemical reactions[1-3]. In enzymatic reactions, the molecules at the beginning of the process are called substrates and the enzyme converts them into different molecules, called the products through enzyme-substrate complex (**Scheme-I**). Enzymes are responsible for almost all processes in a biological cell to occur at significant rates. They are very selective towards their substrates and hence speed up only a few reactions from among many possibilities. The set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Enzyme + Substrate → [Enzyme-substrate complex] → Enzyme + Product

Scheme-I

Sometimes the functions of these enzymes need to be checked and hence requires isostructural molecules that can be cellular metabolites or foreign substances. These molecules are called enzyme inhibitors. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. The inhibitors bind to the active sites of enzymes and do not allow the proper formation of enzyme-substrate complex (ES). Sometimes they bind with the enzyme-substrate complex and prevent the formation of the products. In other words formation of enzyme-substrate-inhibitor complex (ESI) takes place. Due to the formation of this ESI complex, ES complex is not available to break down to product.

Enzyme inhibitors have been classified into two major groups *i.e.*, irreversible and reversible. Irreversible are those which combine with or destroy a functional group on the enzyme molecule that is necessary for its catalytic activity. The phenomenon of reversible is characterized by equilibrium between enzyme (E) and inhibitor (I). There are three main groups of reversible inhibitors exist: **(i) Competitive inhibitor (C):** Substances usually similar with the structure of the substrate, competes with the substrate for binding to the active site but, once bound cannot be transformed by the enzyme. In this type of inhibition only two types of complex formation takes place: enzyme-inhibitor (EI) and ES. This inhibition can be reversed by increasing the substrate concentration, **(ii) Non-competitive inhibitors (NC):** The inhibitors binds at a site on the enzyme other than the substrate binding site, changing the conformation of the enzyme molecule show that reversible inactivation of the catalytic site results. In this type of inhibition three-complex formation takes place: ES, EI and ESI and **(iii) Uncompetitive inhibitor (UC):** Substances that only combined with the ES complex and not with the free enzyme are uncompetitive. In this type of inhibition two type of complex formation takes place: ES and ESI. Other forms of inhibitor include product inhibitor, substrate inhibitor and mixed type inhibitor. For inhibition, the avalability of protein structure is important and this is important also for drug design process[4].

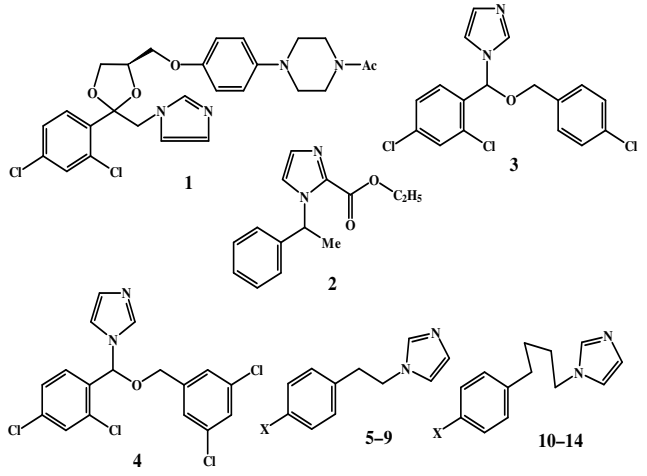
Enzyme inhibitors are important for a variety of reasons: (i) they can be used to gain information about the shape on the enzyme active site and the amino acid residues in the active site, (ii) they can be used to gain information about the chemical mechanism, (iii) they can be used to gain information about the regulation or control of a metabolic pathway and (iv) they can be very important in drug design.

Alkaloids are the largest and most exotic class of secondary metabolites biosynthesized from amino acids. Most of the amino acids are utilized in the biosynthesis of essential enzymes and proteins; whereas selected amino acids are metabolized to alkaloids generally in flowering plants. Alkaloids have been detected and isolated by various methods from animals, insects, marine organisms, microorganisms and lower plants[5-9].

Alkaloids as inhibitors of selected enzymes

Oxido-reductase

Cytochrome $P450_{17\alpha}$: This enzyme controls androgen biosynthesis and hence inhibitor of this used for the treatment of prostatic disease. The enzymology of the reactions catalyzed by cytochrome $P450_{17\alpha}$ has widely been discussed[10]. Ketoconazole **1** is an imidazole antifungal agent that acts by inhibiting the cytochrome P450 mediated 14β-demethylation of lanosterol, disrupting fungal membranes[11]. The *cis*-isomer is the most potent stereoisomer against pig testicular cytochrome $P450_{17\alpha}$ (IC_{50} = 0.05 μM) but had undesirable activity against stoidal 11β-hydroxylase. Other imidazole derivatives include the etomidat **2**[12], econazole **3**[13], myconazole **4**[13] and other compounds whose extent of activity summarized in Tables 1 and 2. The benzimidazole derivative liarozole **18** is a hydroxylase/lyase inhibitor. The triazole derivative **19** is potent inhibitor of aromatase (IC_{50} = 2.6 nM) but possesses moderate inhibition of rat testicular 17,20-lyase. Novel pyrrolidine-2,5-diones, structurally similar to the aromatase inhibitor aminoglutethimide inhibited cytochrome $P450_{17\alpha}$[14].



Several compounds that had potencies comparable with that of ketoconazole are shown in Table-3.

5α-Steroid reductase: Human 5a-steroidreductase (5α-SR) is a membrane bound enzyme requiring NADPH as co-enzyme. There are two distinct human isozymes: Type I (5α-SR I) which predominates in skin and type II (5α-SR II), which

TABLE-1
INHIBITION OF RAT TESTICULAR CYTOCHROME $P450_{17\alpha}$ BY CERTAIN IMIDAZOLE DERIVATIVES

Compd.	X	IC_{50} (μM)	Compd.	X	IC_{50} (μM)
1	-	12.1	**10**	H	171
5	H	46	**11**	NO_2	25
6	NO_2	30.3	**12**	NH_2	22
7	NH_2	27.6	**13**	Cl	29
8	Cl	184	**14**	F	39
9	F	50.6	–	–	–

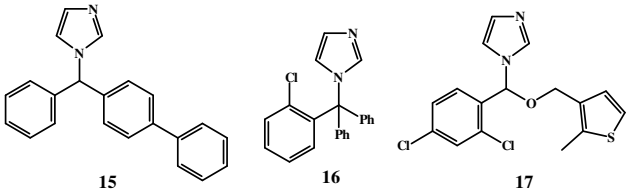
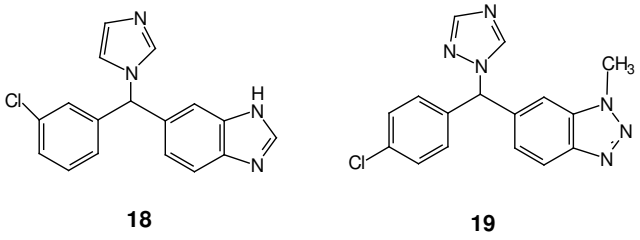


TABLE-2
INHIBITION OF RAT TESTICULAR MICROSOMAL 17α-HYDROXYLASE AND 17, 20-LYASE BY VARIOUS IMIDAZOLE DRUGS

Compd.	Ki/nMol/L	
	17α-Hydroxylase	17,20-Lyase
1	160	84.0
3	668	325.0
4	599	243.0
15	86	56.5
16	170	81.5
17	901	505.0



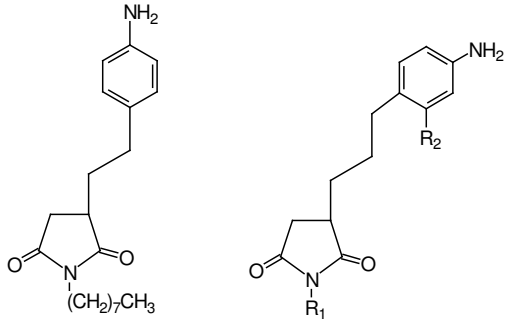
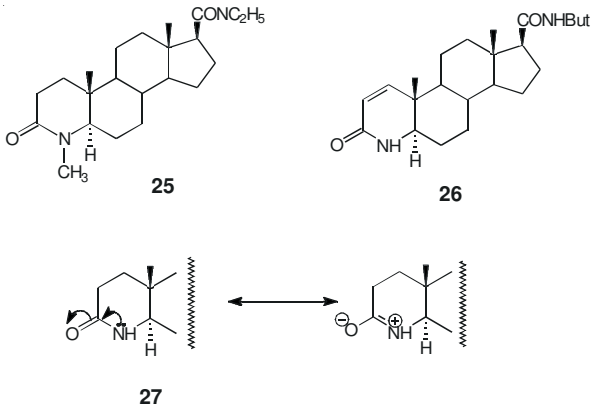


TABLE-3
INHIBITION OF RAT TESTICULAR CYTOCHROME $P450_{17\alpha}$ BY PYRROLIDINEDIONES

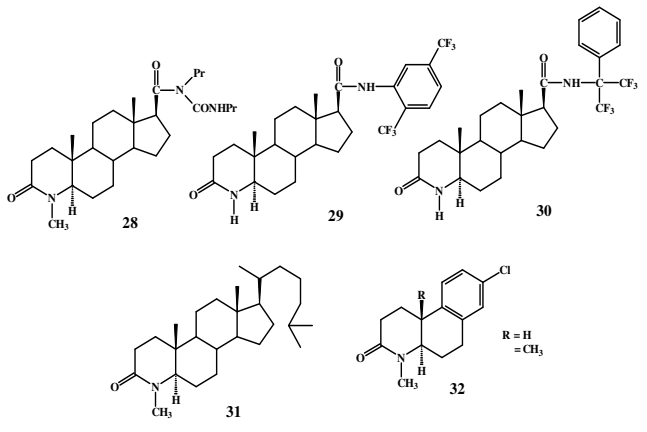
Compd.	R_1	R_2	% Inhibition
20	-	-	88
21	H	NH_2	95
22	Me	NH_2	81
23	$(CH_2)_3CH_3$	NH_2	75
24	$(CH_2)_4CH_3$	N_2	89

predominates in prostate and genital skin[15]. The first reported 5α-SR inhibitor was the 4'-azasteroids developed by Merck from early 1980's onwards. **25** (4MA) was described as a potent competitive reversible inhibitor of rat prostetic enzyme (human 5α-SR I and 5α-SR II, Ki = 5 and 0.23 nM, respectively) but had undesirable agonist activity at the rat androgen receptor and also lacked specificity towards other oxidoreductase[16]. Modification of 4MA with optimization of the c-17 side chain to contain a mixture of hydrogen bonding accepters and hydrophobic residues led to the $\Delta^{1,2}$-steroids Finasteride **26**. This is a potent inhibitor of 5α-SR II (IC_{50} = 9.4 nM) with much lower activity towards type I (410 nM)[17]. The azasteroids **27**, are considered to mimick the enolate intermediate for the enzyme reaction (equation 1).



Equation 1

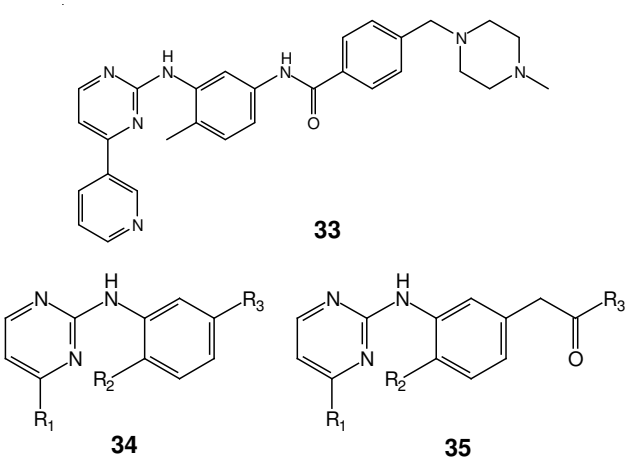
Finasteride used for the treatment of BPH (benign prostetic hypertropy) that lowers BHT in the plasma to 20-35 % of its baseline lable[18-20]. Another agent in the clinical trial for BPH is related acylurea derivative turosteride **28**, which inhibits 5α-SR II (IC_{50} = 55 nM) with little effect on 3β-HSD (IC_{50} = 2.5 mM)[21,22]. The anilide **29** is the potent irreversible inhibitor of both forms[23,24]. After a single oral dose which reduces DHT levels in man to less than 90 % of the base line levels[24]. Another anilide **30** has IC_{50} value 36 and 3.3 nM for 5α-SR I and 5α-SR II respectively[25]. Compound **31**, which is a potent, selective recombinant 5α-SR I inhibitor (IC_{50} = 0.9 nM) with decreased SR II activity (IC_{50} = 150 nM)[26]. The benzoquonolinones **32** based on the 4-azasteroids are potent inhibitors of human 5α-SR I and the activity being enhanced by the substituent present at C-8 position (Cl > Me > F > H).



where R = H, it is a selective competative inhibitor of human 5α-SR I (Ki = 4.6 nM)[27] with little effect on 5α-SR II (1750 nM).

Transferase

Protein kinase: Chronic myelogenous leukemia (CML) is a hematological stem cell disorder associated with a specific chromosomal translocation known as the philadelphia chromosome detected 95 % of patients with CML and 20 % with acute lymphocytic lieukemia (ALL)[28]. The molecular result of the traslocation is the fusion of the abl protooncogene to the bcr gene resulting in the production of the activated form of abl tyrosine protein kinase[29]. The bcr-abl protein induce leukemia in mice[30]. Hence, a well tolerated inhibitor of the abl tyrosine protein kinase is needed for these disorders. There were so many inhibitors prepared or isolated[31-35]. A pyrimidine derivatives inhibited four tyrosine kinases[36]. (V-abl, PDGFR-kinase, EGFR-kinase and C-Src) and three serine/threonine kinase[36,37]. The most active compound being tested was derivative **33** with IC_{50} value 38 nM. The structural modifications of **34** and **35** were done and their inhibitory activity were checked at different concentrations[37].

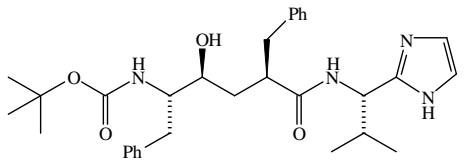


Hydrolases

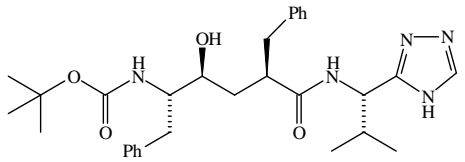
Protease enzymes

HIV-1 Protease: Protease (proteinase) enzymes catalyze sequence selective amide hydrolysis. Based on the mechanism, there are two broad classes of protease enzymes. The zinc metalloproteinases and the aspartase proteinases. Extensive work has been done on two members of the aspartic proteases *i.e.*, renin and HIV-1 proteases. Renin inhibitors have potential as anti-hypertensive agents[38] and HIV-1 protease controls AIDS[39,40]. The first reported inhibitor of HIV-1 protease was the natural product pepstatin[41]. Later the Smith Kline group has reported on using an imidazole ring as an amide bond replacement (**36**, **37**, **38**)[42,43]. The inhibition and structural data indicate that an imidazole or a triazole ring, can be useful amide bond surrogate. The Smith Kline has also reported constant inhibitor **39**[44], which is competitive in nature.

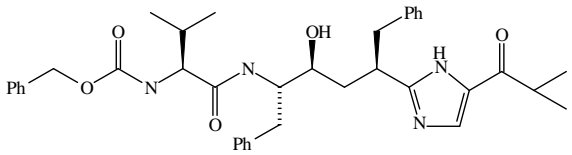
Another inhibitor **40**, observed by the Seale group contained the tertiary urea functionality[45] while inhibitor **41**, contains a hydroxy ethyl amine isostere replacement for the phe-pro cleavage site[46].



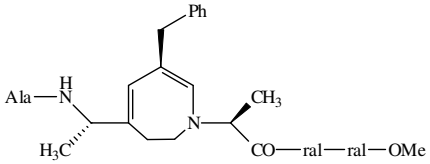
36 Ki = 18.0 nM



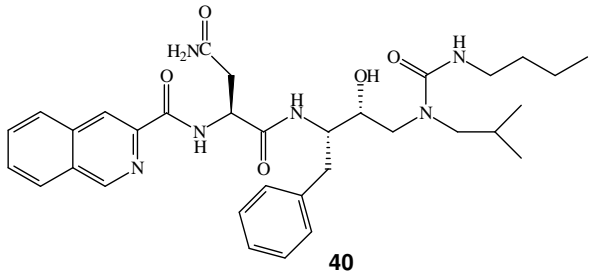
37 Ki = 4.2 nM



38 Ki = 0.6 nM



39 Ki = 430 nM



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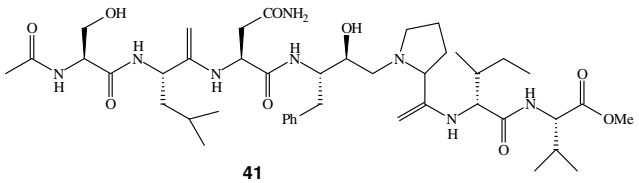
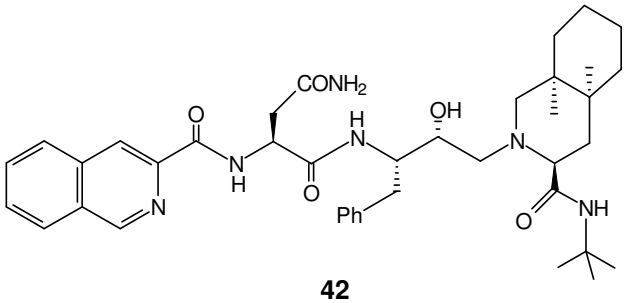


41

A potent HIV-1 protease inhibitor **42**, was reported in 1990 which contained a novel decahydroisoquinoline hydroxy ethyl amine isostere replacement for phe-pro cleavage site[47,48]. This compound is now marketed as anti-AIDS drug saquinavir. Its R-isomer was thousand times more potent than S[22]. The structure of the related inhibitor **43**, has been reported[49]. Another inhibitor of HIV-1 protease based upon the phe-pro cleavage site includes **44**[50]. Compound **45**, is very potent inhibitor and was reproducible 4-fold more potent than **42** as a competative inhibitor. The epemeric **46**, was 100-fold less effective as **45**[51]. Molecule **47**, is an orally bioavailable anti HIV compound[52].



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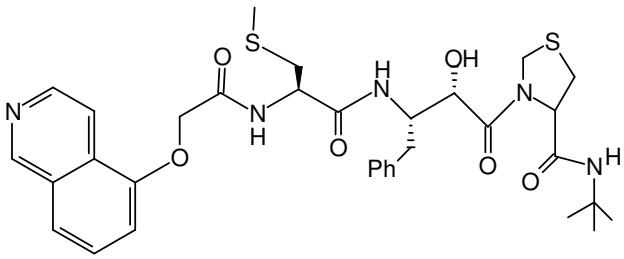
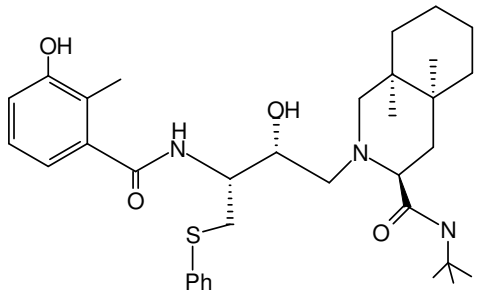


43 Ki = 5.0 pM



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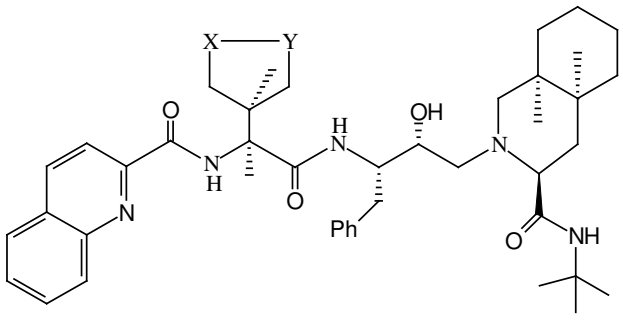
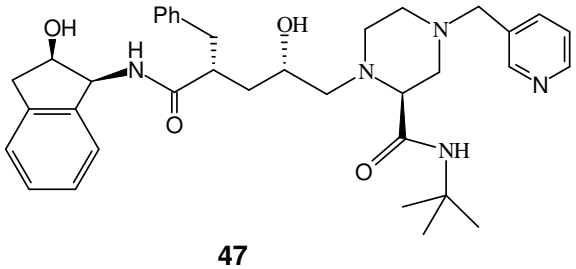


45 X = CH_2, Y = O; **46** Y = CH_2, X = O



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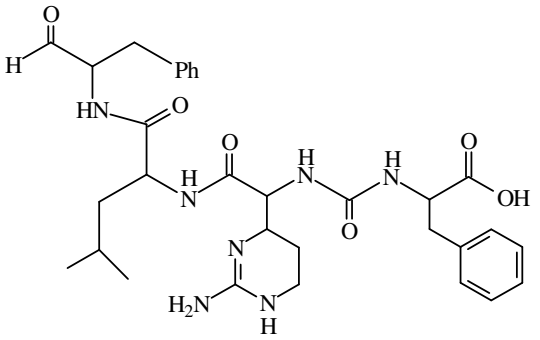
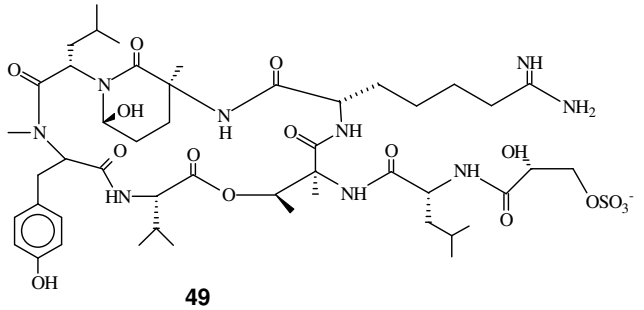
Serine protease: There are several different classes of serine proteases have been examined. Of these the trypsin/ chymotrypsine class was thoroughly characterized. Chymostatin **48**, a natural product, inhibit various serine protease[53]. This is the reversible inhibitor.



48

A 19-membered cyclic depsipeptide (**49**), is a marine natural product which inhibit bovine trypsin (IC_{50} = 10 nM), bovine thrombin (IC_{50} = 275 nM) and human plasmin (IC_{50} = 30 nM)[54]. Another macrocyclic marine natural product cyclotheonamide A, **50** has been reported to be slow-binding inhibitor of both thrombin (Ki = 1.0 nM) and trypsin (Ki = 0.2 nM)[55,56]. Another marine natural product nazumamide A (NAZA), **51** has IC_{50} value 4.6 μM against thrombin but doesn't inhibit trypsin at concentrations up to 165 μM[57].



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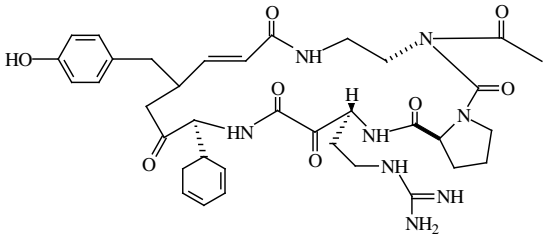

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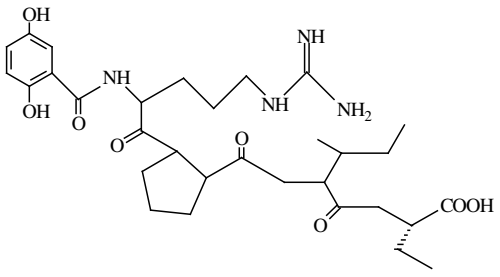

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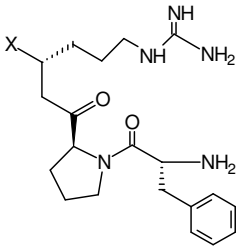
Thrombin: Thrombin is a serine protease responsible for the cleavage of soluble fibrinogen into insoluble fibrine in the last protease-mediated step of the coagulation cascade. Compounds that inhibit thrombin are effective inhibitors of blood clotting and called anti-coagulants[58].

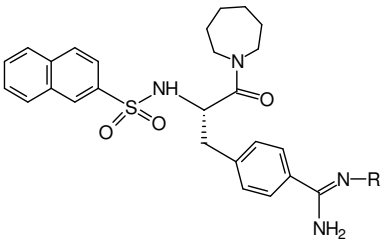


X = $-C(=O)-CH_2-Cl$ **52**

= $-C(=O)-H$ **53**

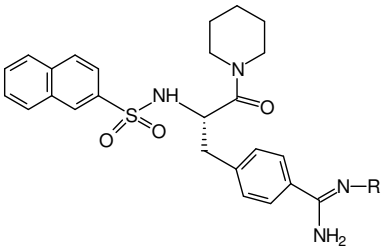
= $-H$ **54**

The α-chloromethyl ketone **52**, is the potent irreversible inhibitor of thrombine. Other similar compound includes **53** and **54**[59]. Two other series (series A and B) of compounds have been used as thrombin inhibitors[60].



Series A

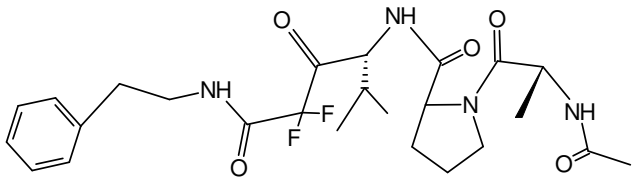
R = H, OH, NH_2, CH_3, C_2H_5



Series B

R = H, NH_2, CH_3

Elastase: Human neutrophile elastse (HNE), human leukocyte elastse, is a serine protease of the chymotrypsin class. In general, they are one of the most destructive enzymes in the body having the ability to destroy connective tissue components. Hence, their inhibition is important. The peptide based inhibitors having alkaloid characteristic are given (**55-60**). The other inhibitors have been reported in the series of papers[61-63].



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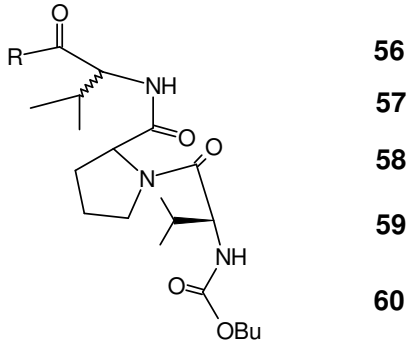
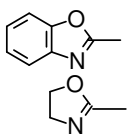


	R
56	H
57	CH_3
58	CF_3
59	
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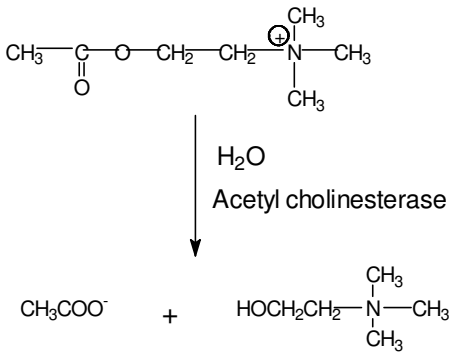
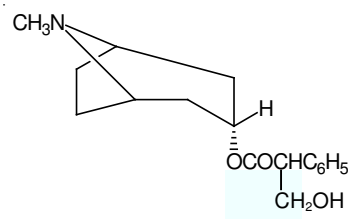
Acetyl cholinesterase (AChE): The enzyme acetyl cholinesterase catalyzes the hydrolysis of acetylcholine (ACh), a neurotransmitter substance functioning in certain portion of the nervous system (**Scheme-I**).



Scheme-I

The alkaloid inhibitors of this enzyme have been reported. Atropine, **61** a tropane alkaloid has got acetylcholine antagonist activity. Nicotine, **62** also acts as cholinergic site. This mimics acetylcholine (Ach) by binding with the appropriate receptor at the synapse of insectan CNS. Litebamine N-homolouge, **63**[64] also have anti-AChE activity. These compounds exhibited moderate activity (IC_{50} = 7.0 mM) while the corresponding N-methosalt of N-propyl-norlitebamine showed potent activity (IC_{50} = 2.7 μM).



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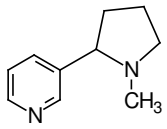

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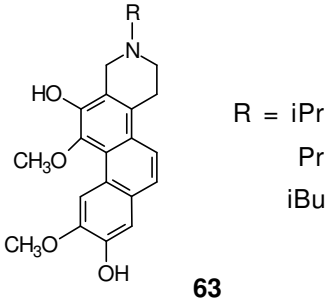
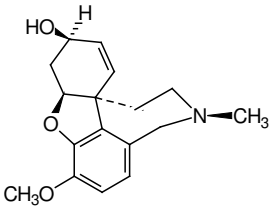


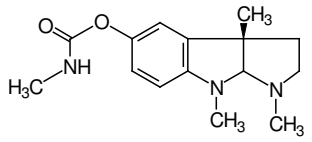
63

Galanthamine, **64**, an amaryllidaceae alkaloid has powerful cholinergic activity and used in healing Alzheimer's disease (AD)[65,66]. Alzheimer's disease is an age-related, chronic neurodegenerative disorder occurring in middle or late life and is characterize by a progressive dementia *i.e.* associated with both a severe disability in performing the activity of everyday life and a reduced life expectancy. The well known cholinergic hypothesis[67] of Alzheimer's disease was based on evidence suggesting that the break down of central cholinergic transmission is intimately associated with cognitive deficits and other symptom of the disease and resulted in efforts to treat Alzheimer's disease with various cholinomimetic agents.

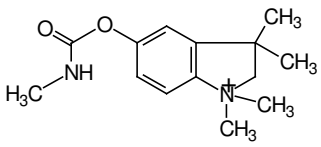
Physostigma venenosum Balf. (Leguminosae) contains an important alkaloids physostigmine, **65**. Several synthesis of this compound has appeared[68-70]. It is a reversible cholinesterase inhibitor[71]. The compound like **66** was found to be 100 times more active than **65** as a AChE inhibitor. One of the tetrahydroisoquinoline alkaloid derivatives has been used as AChE inhibitor[72].



64

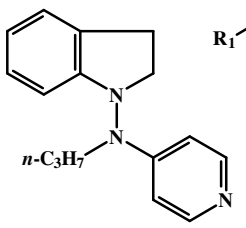


65

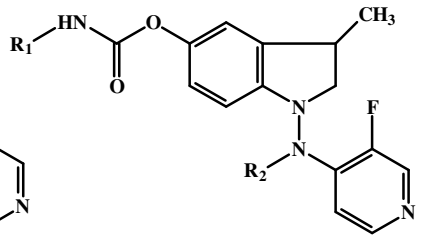


66

A compound (HP 749)[73] has failed to inhibit rat brain AChE or stimulate muscarine or nicotinic receptors. Based on SAR to carbamate type of AChE inhibitors, a series of carbamate derivatives of besipirdine was investigated and the compound **67**[72] emerged as a potent centrally active reversible AChE inhibitor.

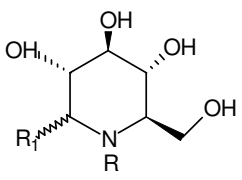


HP 749



67

Glycosidase: The alkaloidal inhibitors of these enzymes includes piperidine nucleus and obtained special name, called azasugar[74-76]. An azasugar derivative, deoxynojirimycin (DNJ) **68** and a series of derivative found to be slow binding inhibitors of intestinal surcase and isomaltase[77-79]. Deoxynojirimycin is the deoxy derivative of nojirimycin **69**.

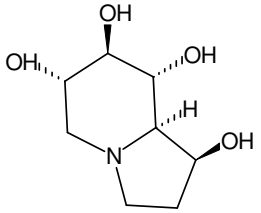


68 R = H, R_1 = OH

69 R = R_1 = H

Another natural compound castanospermine **70**, structure of which is related with deoxynojirimycin, was assayed for slow binding inhibition of surcase and isomaltase and the K_{off}

values, that is the rate of dissociation of the inhibitor from the enzyme for sucrase and isomaltase were found to be respectively 16 and 22, fold smaller than the corresponding K_{off} values of deoxynojirimycin[80].



70

Future prospects: Many drugs produce their pharmacological effects by inhibiting enzymes. To produce a successful drug, it is essential that selective inhibitors be identified, which requires that each candidate be evaluated for potency against a plethora of potential targets. With the increase in drug-related research in academia and with the need for medicinal chemists and pharmacologists to understand enzyme inhibition to facilitate drug discovery, alkaloids find themselves as a potential candidate.

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