VIRAL PROTEINASES – POSSIBLE TARGETS OF ANTIVIRAL DRUGS

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Summary. – Viral infections represent various types of human, veterinary and plant diseases with a significant economic, ethic and demographic impact. Over the years a significant effort has been made to develop various means of prevention and therapy of viral diseases. Proteinases play an important role in the process of virus replication as well as in the pathophysiology of many viral diseases. The aim of this review is to assess the prospects of the application of proteinase inhibitors in antiviral therapy and to characterize viral proteinases of various classes. Six Human immunodeficiency virus (HIV) proteinase inhibitors have been approved for therapeutic use and can serve as examples of prospective application of proteinase inhibitors to antiviral therapy.

Key words: viral proteinases; proteinase inhibitors; virus maturation; antiviral therapy

Introduction

Human, veterinary and plant diseases of viral origin have significant economic, ethic and demographic impacts (Boyer and Marcellin, 2000; Wennergren, 1996; Meissner, 1994; Prober, 1991). Each year, they cause enormous economical damage and claim millions of human lives, especially in the developing world.

During cell colonization and reproduction viral particles assume a specific biochemical status with metabolic activity that is intertwined with the cell metabolism. This fact greatly complicates antiviral therapy and has therefore drawn significant attention over the years (Pang, 1993; Hoofnagle, 1998; Riddell and Greenberg, 1995; Georgiades, 1993; Whitley 1987). One of the most successful therapeutic strategies so far employed has been the vaccination therapy of infections, such as chickenpox, diphtheria and others. Antiviral effects of various compounds of natural origin including scopadulic acid derivatives, fluoranthene derivatives, anthrachinones, bianthrones, hipericin, flavones, and flavanes (Hayashi *et al.*, 1989; Yi *et al.*, 1995; Cohen *et al.*, 1996; Selway, 1986) are well known. In the last two decades a strategy based on the employment of proteinase inhibitors has received considerable attention in antiviral therapy (Baboonian and Dalgleish, 1991; Bartenschlager, 1999; Watanabe *et al.*, 1998). In general, proteinases are responsible for all processes associated with protein digestion and turnover. In all viruses, serine, cysteine (thiol), aspartate (acid) proteinases and metalloproteinases containing serine, cysteine, aspartate and a metal at their catalytic site, respectively, participate in the viral life cycle and represent

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Abbreviations: AIDS = acquired immunodeficiency syndrome; BBScV = Blueberry scorch virus; BHV = Bovine herpes virus; BYV = Beet yellows virus; CMV = Cytomegalovirus; CPMV = Cowpea mosaic virus; CSFV = Classical swine fever virus; CSFV = Classical swine fever virus; EBV = Epstein-Barr virus; EHV = Equid herpesvirus; EMCV = Encephalomyocarditis virus; FMDV = Foot-and-mouth disease virus; GFLV = Grapevine fanleaf virus; HadV-22 = Human adenovirus 22; HAV = Hepatitis A virus; HCMV = Human cytomegalovirus; HCV = Hepatitis C virus; HHV-6 = Human herpesvirus 6; HRV = Human rhinovirus; HSV-1 = Herpes simplex virus 1; HSV-2 = Herpes simplex virus 2; HSV-1/HSV-2 = HSV-1 and/or HSV-2; HVS = Herpes virus saimiri; HIV = Human immunodeficiency virus; ILTV = Infectious laryngotracheitis virus; IN = integrase; MCMV = Murine cytomegalovirus; MHV = Murine hepatitis virus; PR = proteinase; PRV = Pseudorabies virus; PRRSV = Porcine respiratory and reproductive syndrome virus; PV = Poliomyelitis virus; RHDV = Rabbit hemorrhagic disease virus; RT = reverse transcriptase; RUBV = Rubella virus; SCMV = Simian cytomegalovirus; SinV = Sindbis virus; TEV = Tobacco etch virus; TYMV = Turnip yellow mosaic virus; VZV = Varicela-zoster virus

an attractive target for antiviral therapy (Saura *et al.*, 1999; Lowenstein, 1999; Grinde and Jonassen, 1996; Holwerda, 1997).

Serine proteinases

Herpesviruses, members of the *Herpesviridae* family are large enveloped double-stranded DNA-containing pathogens that infect many species throughout the animal kingdom (Roizman *et al.*, 1990). At least eight herpesviruses are responsible for infections in children, including roseola infantum, pneumotitis, encephalitis and lymphomas, caused by Human cytomegalovirus (HCMV), Herpes simplex viruses 1 and 2 (HSV-1, HSV-2), and Epstein-Barr virus (EBV) (Alford *et al.*, 1990; Whitley, 1990; Miller, 1990). HSV-1 contains genetic information for the synthesis of assemblin, a protein important in virus maturation, and constitutes as such a major challenge in antiviral therapy. This enzyme is essential for the production of infectious viral particles (Welch *et al.*, 1993; Burck *et al.*, 1994). The synthesis of assemblin starts with the production of the AC_{pra} precursor and successively, there are at least two activation cleavages in post-translational modification. The first one takes place at the maturation site (M), located near the carboxy end of AC_{pra}. As a result, AC_{pra} is converted to AC_{prb}. The second cleavage occurs at the release site (R), located near the middle of the precursor, leading to the formation of a catalytically active N-terminus and two catalytically inactive C-terminus fragments C_{pra} and C_{prb}. The assemblin undergoes a third cleavage at the internal site (I), near the center of the molecule, yielding an amino (An) and a carboxyl (Ac) fragment of comparable size (Fig. 1) (Gao *et al.*, 1994; Baum *et al.*, 1993). The evidence obtained by site-directed mutagenesis and affinity labeling with diisopropyl-fluorophosphate (Holwerda *et al.*, 1994; Stevens *et al.*, 1994; DiIanni *et al.*, 1994) indicates that the catalytically active nucleophile of the proteinase is an absolutely conserved serine residue (e.g. CD3' Ser118 in Simian cytomegalovirus (SCMV), Ser132 in HCMV[1], Ser129 in HSV-1). One of the two absolutely conserved histidines is a strong candidate for a catalytic diade (triade) (1993; Liu and Roizmen, 1992). Aspartic acid, the third predicted member of the catalytic site of the classical charge-transfer proton system has not been found until now. However, the results of site-directed mutagenesis indicate that glutamic acid may fulfil the function of aspartic acid (Cox *et al.*, 1995). Assemblins are the first of serine proteinase with glutamic acid involved in the catalytic mechanism. Interestingly, the cysteine chymotrypsin-like proteinases of positive-strand RNA viruses have been predicted to contain glutamate rather than aspartate as the third member of catalytic triade (Gorbalenya *et al.*, 1989). As assemblins do not contain classical motifs near the catalytic site of the chymotrypsin- or subtilisin-like proteinases (e.g. G-X-S/C-G-G or G-T-S-M/A), they appear to represent a new subclass in the serine proteinase superfamily. Based on these active site characteristics assemblins represent the S21 family, however, not a clan (Barrett, 1994; Barr, 1991; Bazan and Fletteric, 1989; Gorbalenya *et al.*, 1989). The homology in the amino acid sequences near the M, R and I sites of assemblins proves their related character and common phylogenetic origin. The homology involves sequences of the recognition and cleavage domains. The M site is strongly conserved, identical with the carboxy-terminus of pAF. Assemblins contain a general consensus sequence (V/I/L-X-A-S/A), where P3 and P3' are usually valine and serine, respectively, and X can be asparagine, aspartate, glutamine, glutamate or lysine (Schecher *et al.*, 1967). The only invariant amino acid among all of the known cleavage sites is the P1' alanine. The P4' tyrosine is absolutely conserved at the R site sequences with only two exceptions, while the P1' serine is absolutely conserved among all M and R site sequences. These findings indicate that P3, P1 and P1' are essential in recognition sequences. The I sites amino acid sequences are significantly less conserved. Table 1 presents the amino acid sequences near the M and R sites, namely (V/L/I-X-A↓-S, where X can be aspartate, glutamate, asparagine or glutamine.

The substrate specificity data obtained from *in vitro* studies on purified HSV-1 and HCMV assemblins indicates, that HSV-1 assemblin has substrate specificity. The exact substrate specificity was determined by conducting kinetic measurements and parameter evaluation for various oligopeptides. The values obtained for oligopeptides mimicking the HCMV and HSV-1 M sites, are summarized in Table 2. As it can be seen the rates ($k_m min^{-1}$) observed for HSV-1 assemblins are generally by about one order of magnitude lower than those observed for HCMV assemblins. Antiviral therapy based on the assemblins' inhibition is challenging and is of high interest to scientists and pharmacologists from all over the world.

Cysteine proteinases

More than 30 years have passed since the first reports on the human poliomyelitis virus (PV) protein production in the process of limited proteolysis (Summers and Maizel, 1968; Jacobson and Baltimore, 1968), namely by the proteolytic cleavage of a large precursor (250 K). Inhibition of this process can prevent virus replication. These observations have indicated for the first time that proteolytic enzymes play an important role in the process of the virus proteins formation and could therefore be an attractive target in antiviral therapy. PV is the best characterized picornavirus, the member of the *Picornaviridae* family. In general,

Table 1. Amino acid sequences recognition and cleavage domains of maturation (M) and release (R) sites in the serine proteinase-like assemblins (Gibson *et al.*, 1994)

Virus	M site		R site	
	Recognition domain	Cleavage domain	Recognition domain	Cleavage domain
HSV-1	NAEAGALVNA	SSAAHVDDVT	IAG-HTYLQA	SEKFKMWGAE
HSV-2	GAEAGALVNA	SSAAHVNVDT	IAG-HTYLQA	SEKFKIWGAE
VZV	VGQDVNAVEA	SSKAPLIQGS	IGMGHVYLQA	STGYGLARIT
EHV	PQASSQTVDA	SASTGLEFGR	GIAGHTYLQA	SAVFPLPTGG
BHV	EPGVAATVDA	SAMASLPPAQ	IEG-HTYLQA	SASFGITNGC
ILTV	QESARETVDA	SMPKRLKDAQ	AVYNPKYLQA	NEVITIGIKE
PRV	ASAPQPPVQA	SVSAPRPTES	GVRAHTYLQA	TMWAGLLPKS
HCMV	ERAQAGVVNA	SCRLTATASGS	VTERESYVKA	SVSPEARAIL
HHV-6	ASPKPSILNA	SLAPETV	VTARESYVKA	SVSPAEQETC
SCMV	KSAERGVVNA	SCRVAPLEA	VAGRESYVKA	SVSPREQEPC
MCMV	LITAGKLVNA	SCEPTPMEVG	LQSKETYVKA	SELPAEDDG
EBV	HHRGKKLVQA	SASGVAQSKE	NIPAESYLKA	SDAPDLQKPD
HVS	SQEPVHIDA	SFAQDPVSKL	KIKKLYTLKA	SEIGKPVTED

Table 2. Kinetic parameters of the cleavage of oligopeptides mimicking M and R sites of HCMV and HSV-1 by various herpesvirus assemblins (Gibson *et al.*, 1994)

Cleaved site	Oligopeptide	K_m (μmol/l)	k_{cat} (min^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)
HCMV				
M site	RGVVNA↓SSRLAK	586	16.0	455
M site	RGVVNA↓SSRLAK	625	12.4	331
M site	RGVVNA↓SSRLAK	144	9.8	1,134
M site	GVVNA↓SCRLA	510	12.0	392
M site	ac-GVVNA↓SSRL-NH_2	546	14.6	446
M site	VVNA↓SSRLATAS-NH_2	440	0.9	34
R site	SYVKA↓SVSPE	532	1.9	60
R site	SYVKA↓SVSPE	450	1.7	63
R site	SYVKA↓SVSPE	2,700	2.0	12
R site	SYVKA↓SVSPE-NH_2	380	0.07	3
HSV-1				
M site	ALVNA↓SSAAHVDV-NH_2	190	0.2	18
M site	ALVNA↓SSAAHVDV-NH_2	–	–	40
R site	HTYLQA↓SEKFKM-NH_2	1,360	0.43	5
R site	HTYLQA↓SEKFKMW-NH_2	880	2.0	38

picornaviruses infect primates, mammals, insects, plants, lower eukaryots and eubacteria. PV contains a single stranded RNA of positive polarity, i.e. it functions as mRNA (van Regenmortel *et al.*, 2000).

Table 3 lists the known cysteine proteinases of positive RNA viruses. In general, viral proteinases are an attractive potential target for antiviral chemotherapy (Gorabelenya *et al.*, 1996). The well-known cysteine proteinase inhibitor, E64 and its lipophilic derivatives have been shown to suppress replication of Foot-and-mouth disease virus (FMDV, L^{pro}) and Murine hepatitis virus (MHV, PLI^{pro}) in tissue culture (Kim *et al.*, 1995). As successful examples of proteinase inhibitor application to antiviral therapy elastase-like-enzymes inhibitors elastatinal and methoxysuccinyl-Ala-Ala-Pro-Val-chloromethylketone can be mentioned as inhibitors of PV, Human rhinovirus (HRV,-142 A^{pro}) and Hepatitis A virus (HAV, $3C^{pro}$) *in vitro* (Molla *et al.*, 1993). Of other inhibitors it is spiro-indolinon-β-lactam, calfungin, radicinin (fytotoxin), and citrinin hydrate (Jewell *et al.*, 1992; Skiles and McNeil, 1990; Singh *et al.*, 1991; McCall, 1994). It has been shown recently that two groups of HAV and HRV (3Cpro) inhibitors mimic the recognition oligopeptide sequence containing a glutamic acid chemically modified by a reactive functional group (Skiles *et al.*, 1990; Sham *et al.*, 1995). Another HAV ($3C^{pro}$) inhibitor, the peptidyl aldehyde ac-Leu-Ala-Gly-Gly (N,N-dimethylglutamin) has been shown to be 50 times more active against HRV ($143C^{pro}$) with a K_i value of 42 nmol/l (Malcolm *et al.*, 1995). Similarly P^4-P^1 oligopeptide Leu-Ala-

Table 3. Characteristics of viral cysteine proteinases (Gorbalenya and Snijder, 1996)

Virus family/genus	Virus	Host	Proteinase/Protein family	Reference
Picornavivridae				
Enterovirus	PV	Homo sapiens	$3C^{pro}$, $2A^{pro}$/CHL	Krausslich and Wimmer, 1988; Skern *et al.*, 1994
Rhinovirus	HRV	Homo sapiens	$3C^{pro}$, $2A^{pro}$/CHL	Krausslich and Wimmer, 1988; Skern *et al.*, 1994
Cardiovirus	EMCV	Mus musculus	$3C^{pro}$/CHL	Palmenberg, 1990
Aphtovirus	FMDV	Cattle	$3C^{pro}$, L^{pro}/PL	Grubman *et al.*, 1995
Hepatovirus	HAV	Homo sapiens	3C/CHL	Gauss-Muller *et al.*, 1991
Comoviridae				
Comovirus	CPMV	Vigna sinensis	P24(3CL)/CHL	Dessens *et al.*, 1991
Nepovirus	GFLV	Vitis vinifera	P23(3CL)/CHL	Margis *et al.*, 1991
Caliciviridae/Lagovirus	RHDV	Oryctolagus cuniculus	3CL/CHL	Boniotti *et al.*, 1994
Potyviridae/Potyvirus	TEV	Nicotiana clevelandii	NIa(3CL)/CHL HC^{pro}/PL	Dougherty and Semeler, 1993
Coronaviridae/Coronavirus	MHV	Mus musculus	3CL/CHL	Lu *et al.*, 1995
Arteriviridae/Arterivirus	PPRSV	Pig	Nsp11α, nsp11β/PL, Nsp2	Den Boon *et al.*, 1995; Snijder *et al.*, 1995
Flaviviridae				
Pestivirus	CSFV	Pig	N^{pro}/PL	Wiskerchen *et al.*, 1991; Strak *et al.*, 1993
Hepacivirus	HCV	Homo sapiens	NS2-3	Grakoui *et al.*, 1993
Togaviridae				
Alphavirus	SinV	Homo sapiens	NsP2/PL	Strauss *et al.*, 1992
Rubivirus	RV	Homo sapiens	P150/PL	Marr *et al.*, 1994
Closteroviridae				
Tymovirus	TYMV	Brassica rappa	P150/PL	Bransom and Dreher, 1994; Royanov *et al.*, 1995
Carlavirus	BBScV	Vaccinium myrsinites	P166/PL	Lawrene *et al.*, 1995
Closterovirus	BYV	Beta vulgaris	P65/PL	Agranovsky *et al.*, 1994

CHL = chymotrypsin-like cysteine proteinase; PL = papaine-like proteinase.

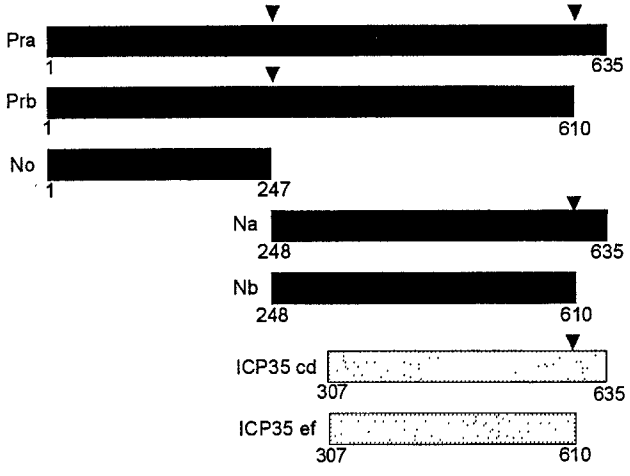

Fig. 1

Precursor protein and its proteolytic cleavage products in HSV-1 (Gao *et al.*, 1994)

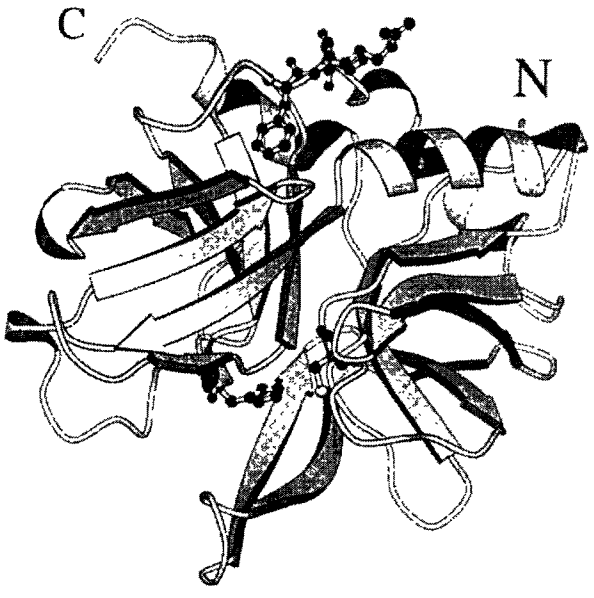

Fig. 2
The 3D model of the HAV $3C^{pro}$

The catalytic His-Cys diade is located in the cleft between two domains. The conserved Phe-Arg-Asp sequence, important for the interaction with RNA, is on the opposite site of the enzyme (Gorbalenya and Snijder, 1996).

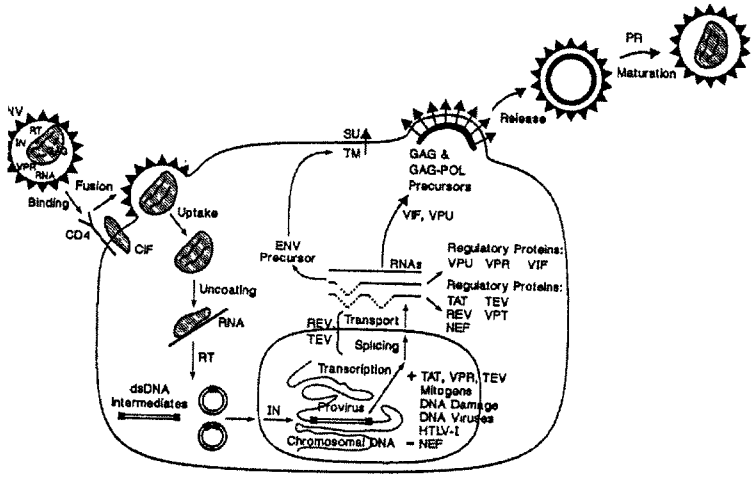

Fig.3

The mechanism of the process of HIV-1 particle maturation

(Rattner, 1993).

Gly-Gly with dimethylacetal terminus or nitrile has been found to be effective inhibitors of Human adenovirus 22 (HAdV 22) 3k proteinase (Alford *et al.*, 1990). A 3D model of HAV $3C^{pro}$ is shown in Fig. 2. Two important highlighted domains are the Cys-His catalytic diade and the Phe-Arg-Asp domain, essential for the interaction with RNA.

Aspartic proteinases

Aspartic proteinases also play a crucial role in the process of virus replication. HIV proteinases are important members of the aspartic proteinase family. Application of HIV proteinase inhibitors to clinical therapy constitutes a separate chapter of successful application of proteinase inhibitors to antiviral therapy. The introduction of HIV proteinase inhibitors as anti-AIDS drugs has been the answer to the acute requirement for the AIDS pandemia therapy. Up to date, plenty of papers on the essential role of proteinases in the maturation of viral particles have been published (Debouck and Metcalf, 1988; Tomasselli *et al.*, 1991; Debouck, 1992). An HIV virus particle is composed of the RNA genome, structural core (gag) proteins, enzymatic core (pol) proteins and the envelope (env) proteins. Gag proteins make up the inner nucleocapsid core of the virus particle. Pol proteins include three enzymatically active proteins, namely proteinase (PR), reverse transcriptase (RT) and integrase (IN). Env proteins make up the outer envelope of the virus particle. The proteinase in involved in the gag and gag-pol precursors processing and is essential for the virus maturation (Ratner, 1993). The process of maturation is outlined in Fig. 3. Fig. 4 shows the backbone of the complex of HIV-1 proteinase (aspartic family), a bilobal protein, with the inhibitor U85548E, in which a mutation replacing Asp25 by Ala leads to the loss of catalytic activity. Mutant proteinases lead to the formation of non-infectious virus particles. These, although fully developed, contain gag proteins, nucleocapsid and matrix – constitutive elements without the cleavage ability (Pearl and Taylor, 1987). An overview of the apparently very broad substrate specificity of the HIV-1 proteinase is outlined in Table 4 (Clare, 1993). Discovery and application of substrate analogs, peptidomimetics, has contributed to the development of low molecular mass inhibitors of the HIV-1 proteinase, perspective anti-AIDS drugs for combined therapy application (Galegov, 1997; Gazzard and Moyle, 1996). However, the development of such an inhibitors, as orally administered drugs of low toxicity, has proven problematic (Norbeck and Kempf, 1991; Plattner *et al.*, 1990; Roberts, 1995). Low oral bioavailability and the resulting effective *in situ* drug concentrations could lead to virus mutations, as demonstrated by the existence of an HIV-2 proteinase with changes in the primary structure of the viral genome making it resistant to any successive clinical applications of proteinase inhibitors (Plattner *et al.*, 1990). HIV-1/HIV-2 proteinase inhibitors can be grouped into the substrate based oligopeptide or peptidomimetic type and non-peptidic type (Kempf, 1994). Their leading structures have been discovered by random screening, but also using rational computer design. One of the most important features, used in the design studies, has been the C-2-homodimeric active site of the-proteinase. The structures designed include the symmetric, pseudo-symmetric and non-symmetric proteinase inhibitors. The design has been facilitated by the existence of a sufficient amount of generally

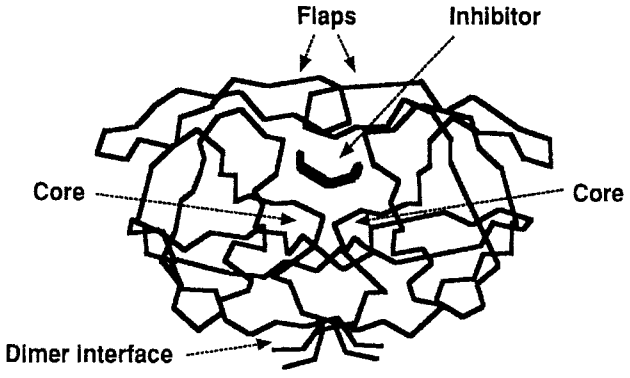

Fig. 4

The backbone of the complex of the HIV-1 proteinase with the inhibitor U85548E (Pettit *et al.*, 1993)

accessible X-ray (NMR) files of the HIV-1/HIV-2 proteinases and their complexes with different inhibitors (Kempf *et al.*, 1994). The structures of some pharmacologically effective HIV proteinase inhibitors are shown in Fig. 5, including the oligopeptide or peptidomimetic type of hydroxyethylamine, hydroxyethylene, and pseudo-symmetric structural subtypes and the examples of non-peptidic type HIV proteinase inhibitors as DMP-323 cyclic ureic subtype, UCSF8 and polyphenolic type U-96988. The inhibitors are products of various pharmaceutical companies, such as Abbott (A-77003, A-80987, and ABT-538/Ritonavir), Merck (L-735,524, L-748,496, and L-738872), Ciba-Geigy (CGP-533437), Upjohn (U-96988), Roche (Ro-31-8959), DuPont-Merck (DMP-323) and others. Development of biologically active and pharmacologically effective HIV proteinase inhibitors has proven feasible in *in vitro* systems, although difficulties have been encountered for several compounds in tissue culture studies. Moreover, there are a few problems regarding the inhibitors' oral bioavailability in humans. Table 5 presents the following pharmacological parameters: elimination half-life ($t_{1/2}$), the ratio of the percentage of the orally bioavailable inhibitor concentration to the bioavailable inhibitor concentration after intravenous administration (F), maximal value of the inhibitor concentration in plasma (c_{max}), and the ratio c_{max}/EC_{50} *in vitro* (Kempf, 1994). Higher values of the c_{max}/EC_{50} parameter for the compounds ABT-538 (Ritonavir) and L-735,524 indicate their sufficient peroral efficiency and make them strong candidates for application to clinical therapy. Compounds KNI-272, ABT-537, Ro-31-8959, and SC-52152 are known to bind to plasma proteins (Kageyama *et al.*, 1994).

The optimal bioavailability of HIV proteinase inhibitors (oligopeptides, peptidomimetics) depends on certain physicochemical properties and conditions: (i) a sufficient aqueous solubility; (ii) concentrations over 1 μg/ml (Hungate *et al.*, 1994); (iii) the final effect of molecular size remains unclear, although, evidently, its lower value promotes the bioavailability (Kempf, 1994); (iv) concerning lipophilicity the relationship between this property of HIV proteinase inhibitors and the oral bioavailability has not been studied systemically. In related peptidomimetics, rennin inhibitors, no overall relationship between the log $P_{(octanol/water)}$ values and absorption, following intraduodenal administration, was observed (Rosenberg *et al.*, 1993). In order to take advantage of hydrophobic interactions with the enzyme active site, the majority of HIV proteinase inhibitors are highly lipophilic compounds. However, a sufficient aqueous solubility is required for oral absorption (Rosenberg *et al.*, 1993). (v) Another important parameter is the hydrogen bonding potential of an inhibitor, estimated by its partition coefficient between octanol and ethylene glycol (Kempf *et al.*, 1991). (vi) As with any class of biologically active agents, the pharmacokinetic profiles of HIV proteinase inhibitors can be profoundly affected by the rates and extent of metabolic processes. From the structural features affecting the compound's metabolism, we can mention the example of the pyridine ring in the molecule as the target for N oxidation (Kempf *et al.*, 1995).

Up to date, five HIV proteinase inhibitors have been approved and introduced into clinical therapy as anti-HIV drugs. These are Ritonovir, Nelfinavir, Saquinavir, Delfinavir, and Indinavir (Grinde and Jonassen, 1996; Bragman, 1996; Pai and Nahata, 1999). The experience obtained from the application of HIV proteinase inhibitors to clinical therapy and their design and development show the way for future work in this field.

Metalloproteinases

Although not much has been published on the role of metalloproteinases in virus replication they should be mentioned as a possible future target in antiviral therapy. In general, protein processing, the formation of final proteins from their precursors, is a common feature of all proteins in living organisms. Specification of the type of proteolytic activity depends on the genetic and evolutionary relations. Proteinases of all types are capable of proteolytic activation, with the particular aspects depending on the substrate recognition and cleaved sequences. Hijikata *et al.* (1993) have reported involvement of metalloproteinases in the maturation of the hepatitis C virus (HCV). The cell transformation caused by Simian virus 40 (SV40) alters the actin cytosceleton, the expression of matrix metalloproteinases and inhibitors of metalloproteinases, and the invasive behavior of ataxia-telangiectasia of human skin fibroblasts (Hansell *et al.*, 1995). High levels of MMP-9 and MMP-2, as measured by ELISA,

Table 4. Cleavage sites of HIV-1 proteinase

Cleavage sites	Scissile bond		
P17/p24	Val-Ser-Gln-Asn-Tyr	↓	Pro-Ile-Val-Gln-Asn
p24	Lys-Ala-Arg-Val-Leu		Ala-Glu-Ala-Met-Ser
/p7	Thr-Ala-Thr-Ile-Met		Met-Gln-Arg-Gly-Asn
p7/p6	Arg-Pro-Gly-Asn-Phe		Leu-Gln-Ser-Arg-Pro
/PR	Val-Ser-Phe-Asn-Phe		Pro-Gln-Ile-Thr-Leu
PR/RT	Cys-Thr-Leu-Asn-Phe		Pro-Ile-Ser-Pro-Ile
p51/p15	Ile-Arg-Lys-Ile-Leu		Phe-Leu-Asp-Gly-Ile
RT/ENDO	Gly-Ala-Glu-Thr-Phe		Tyr-Val-Asp-Gly-Lys
Schechter-Berger notation	P5 - P4 - P3 - P2 - P1		P1'- P2'- P3'- P4'- P5'
		← critical span →	

PR = proteinase; RT = reverse transcriptase; ENDO = endonuclease.

Table 5. Pharmacokinetics of HIV proteinase inhibitors (Kempf, 1994)

Inhibitor	Type	EC_{50} (μmol/l)	Organism	$t_{1/2}$ (hr)	c_{max} (μmol/l)	F (%)	c_{max}/EC_{50}
Ro-8959	HEA	0,01	rat	–	0.15	<5	15
L-735,524	HEA,S	<0.01	rat	0.48	2.8	23	>28
			dog	0.58	11.4	72	>114
			monkey	1.24	0.71	14	>7
L-748,496	HEA,S	<0.01	rat	–	–	70	–
			dog	–	7.3	65	>730
L738,872	HEA	<0.1	dog	–	5	35	>50
CGP 533437	HE	0.05	mouse	–	1.5	–	30
KNI-272	NS	0.067	rat	2.86	1.35	42.3	20.1
A-77003	S	<0.2	rat	0.47	0.15	0.7	0.75
			dog	1 13	0.59	2.0	2.95
			monkey	3.19	0.16	2.5	0.8
A-80987	S	0.22	rat	1.91	4.11	26.4	18.7
			dog	154	4.2	23.2	19.1
			monkey	2.00	0.99	13.5	4.5
ABT-538	S	0.025	rat	0.66	2.62	77.6	105
			dog	1.07	23.0	100.0	921
			monkey	2.26	5.3	70.4	212
DMP-323	NP, S	0.057	rat	0.95	0.78	27	13.7
			dog	1,8	1,56	37	27,4
U-96988	S	3	rat	6.0	50	45	>17
			dog	4.0	–	76	–

HE = hydroxyethylene; HEA = hydroxyethylamine; NS = norstatine; S = symmetry-based; NP = non-peptidomimetic.

were associated with high proteolytic activity, detected in the cerebral fluid by zymography during viral meningitis (Kolb *et al.*, 1998). Maturation of vaccinia virus infectious particles appears to be dependent on the proteolytic processing of at least five viral proteins, each containing a conserved AG cleavage motif and each requiring proper association with a previrion particle. The site-directed mutagenesis of this consensus sequence suggests that the G1L protein may be a novel virus-encoded metalloendoproteinase (Whitehead and Hruby, 1994). Romanic and Madri (1994) have reported a relationship between metalloproteinase hyperactivity and viral encephalitis. Finally, it has been shown that the macrophage-secreted collagenase and metalloproteinases play a critical role in cell microenvironment regulation and cell movement. The HIV infection of macrophages might be capable of deregulating the expression of gelatinases (Chapel *et al.*, 1994).

Conclusions

Antiviral therapy constitutes a big challenge for scientists all over the world and there is a considerable potential for the application of proteinase inhibitors to this field. The

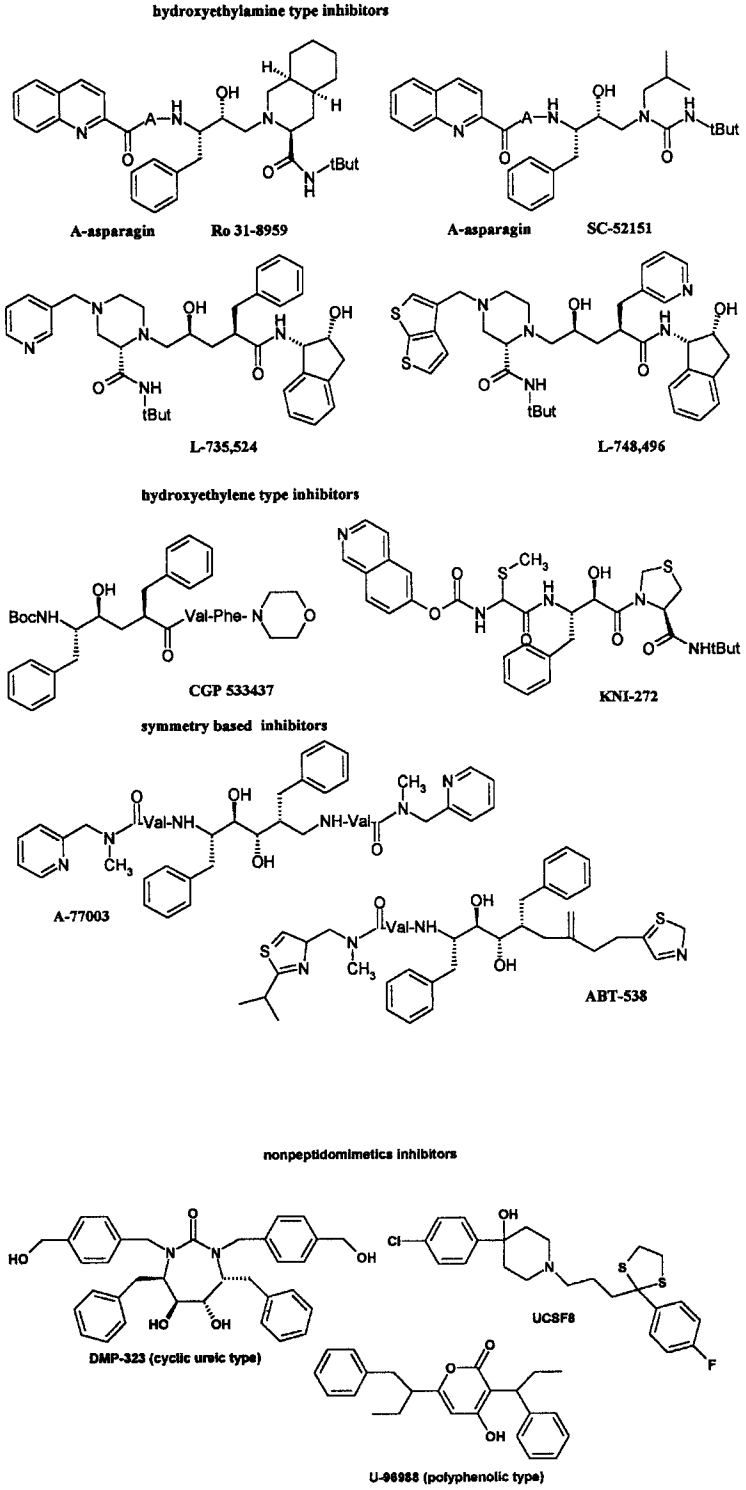

Fig. 5

The examples of structures of peptidomimetic and non-peptidomimetic types of HIV-1 proteinase inhibitors

experience gained in antiviral therapy of HIV/AIDS can provide an indication of possible future applications of proteinase inhibitors to the treatment of other viral infections and diseases. The therapy of the flu, various types of hepatitis, herpes and other viral diseases is only in the phase of biological evaluation. In the future, the development of compounds with proteinase-inhibitor activity will be facilitated by of the knowledge of and access to databases of X-ray files of viral proteinase inhibitor complexes, SAR and QSAR studies. New techniques of studying mechanisms of the inhibition on genetic and enzymatic levels will allow a very quick progress in the design and development of new antiviral agents. In general, the properties of anti-proteinase drugs relevant for antiviral therapy will be achieved with the second or third generation of the drugs.

Note of the Editor-in-Chief. The names of the viruses, abbreviated in this article as BHV, CMV, EHV, HIV, HRV, RHDV, and SCMV, are not compatible with the presently valid nomenclature of viruses (van Regenmortel *et al.*, 2000).

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