Zn Finger Containing Proteins as Targets for the Control of Viral Infections

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Abstract: The zinc finger proteins have fascinated many research groups because of their modular assembly, broad range of biological functions and more recently because they are attractive targets for antiviral therapy. The zinc finger domain is a very stable structural element whose hallmark is the coordination of a zinc ion by several amino acid residues, usually cysteines and histidines. These structural motifs are associated with protein-nucleic acid recognition as well as protein-protein interactions. The biological function of the zinc finger proteins is strongly dependent on the zinc ion, which assure integrity and stability. Thus, the disruption of critical zinc finger viral proteins represents a fundamentally new approach to inhibit viral replication in the absence of mutations leading to drug resistance phenotypes. This review summarizes the drug design and potential therapeutic applications of viral zinc fingers disrupting agents for the control of viral diseases.

Keywords: Structural domains, zinc fingers, antiviral, oxidizing compounds, human immunodeficiency virus, arenavirus.

ZINC ION, AN ESSENTIAL COMPONENT FOR ESSENTIAL PROTEINS

The natural diversity provided by the 20 amino acids is not sufficient to ensure all the functional and structural flexibility required in nature, but proteins had managed to recruit co-factors as helpers for performing chemical functions and achieving their structural integrity and stability. The smallest but probably most widely used co-factors are metal ions. Proteins frequently need to bind the metal ion either because it is involved in the catalytic mechanism or because it stabilizes the protein tertiary or quaternary structure. These proteins are hence called metalloproteins.

Among metal ions, zinc is particularly important as it is the second most abundant biological transition metal ion in humans and is used for diverse functions [1]. The intracellular concentration of metal ions as well as their distribution among the various cell compartments is tightly controlled, so there is a strict regulation of cellular zinc levels, since this nutrient is essential but also toxic if accumulated to excess. Thus, a proper balance of these ions is absolutely necessary for a healthy cellular phenotype.

Protein structures are built from domains. Each domain constitutes an independent module and has a separate function to perform for the protein. Small zinc-containing domains are termed 'fingers', and zinc fingers containing proteins are called "zinc finger proteins".

In the world of chemistry, the mutual affinity of zinc and sulfur is well-recognized [2, 3]. However, the earliest detected modes of zinc coordination in biology were different. The ligands associated with catalytic zinc ions in hundreds of enzymes proved to be nitrogen and oxygen (histidine and glutamate/aspartate), while the sulfur of cysteine served as a ligand less frequently [4, 5]. The discovery of the zinc finger domains in the early 1980s changed this balance [6, 7]. Their coordination of zinc involved two cysteine and two histidine ligands (ZnS_2N_2 coordination).

At the present, "zinc finger" is a generic term that includes motifs with different combinations of histidine and cysteine ligands. All known zinc-binding domains (excluding those in metalloenzymes) ligate the zinc ion through four amino acid side chains [8]. Since 3% of the ~32000 genes in the human genome encode proteins with zinc fingers [9], the Zn-S interaction is the predominant mode of binding in proteins [10]. Thus, the frequency at which Zn-S bonds occur in biology does not seem to differ from chemistry. Zn-S bonds serve as structural braces in protein domains that participate in all kind of biomolecule interactions. The Zn-S interaction offers specific mechanisms for enzyme catalysis, establishes reactivities of zinc sites that were believed to have only a structural role, allows zinc to be tightly bound and available, and, importantly, generates redox-active coordination environments for the redox-inert zinc ion.

As zinc finger domains are extremely widespread in nature, they have become extremely important. Their functions are extraordinarily diverse and include DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly, and lipid binding [1, 11]. Zinc finger structures are as diverse as their functions. More than 30 different classes of zinc binding motifs, differing in the number of zinc ions and the arrangement of the ligands have been described [11, 12, 13, 14, 15], many other have been classified into small families and the relationships between them remain largely uncertain, and there are more still to be uncovered.

STRUCTURAL CLASSIFICATION OF ZINC FINGERS

The first zinc finger domain was recognized 22 years ago as a repeated zinc-binding motif, containing two cysteine and two histidine conserved ligands, in *Xenopus* transcription factor IIIA (TFIIIA) [16, 17]. Since that time, numerous other zinc-binding motifs have been identified and designated as zinc fingers. We now recognize the classical CCHH zinc finger as the first member of a rapidly expanding family of zinc-binding modules. At the present, a "zinc finger" is any small, functional, independently folded domain that requires coordination of one or more zinc ions to stabilize its structure [14].

All of these 20–60 amino acid domains have zinc in their structural cores and form compact and stable structures in which the presence of the zinc ion is essential for the formation of that structure. These particular ions facilitate folding and increase protein stability, which is not easily achievable for small polypeptide chains. The great variety of functions is dependent on the existence of a stable structure, but not on the presence of the zinc ion itself.

In the last decade, genome sequencing projects have provided a huge number of protein primary sequences, and several different elaborate analyses have been enabled by available bioinformatic tools; however, protein-metal binding properties remain difficult to predict as well as to investigate experimentally. The experimental detection of an uncharacterized metalloprotein brings along typical problems, ie. proteins may be obtained in the demetalated form or nonmetalloproteins may be purified in association to a spurious

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metal ion. Consequently, the present knowledge about zinc finger proteins is only partial.

Many attempts have been made to classify zinc-binding domains in proteins based on ligand geometry [11, 18, 19, 20, 21, 22, 23] or on the type, number and spacing of zinc-ligating residues [13, 24, 25] that constitute these particular domains. More recently, Krishna *et al*. [26] have presented a comprehensive classification of available zinc finger structures concentrating on the protein backbone similarity around the zinc ligands, in other words, the spatial arrangement of secondary structural elements that contribute zinc ligands. Consequently, they have found that each available zinc finger structure can be placed into one of eight fold groups constituted by proteins that share common structural features and are frequently functionally related, but are not necessarily homologous [26].

The eight fold groups are: CCHH like, gag knuckle, treble clef, zinc ribbon, Zn_2/C_6, TAZ_2 domain like, zinc binding loops and metallothionein. We agree that this structural classification of zinc finger domains helps to link the structural properties of these proteins with their biological functions.

Three of these fold groups comprise the majority of zinc fingers, namely, CCHH-like finger, treble clef finger and the zinc ribbon.

1) CCHH-Like Finger

Domains from this group are composed of two short β-hairpins followed by an α-helix that forms a left-handed ββα-unit with the zinc ion sandwiched between them in a tetrahedral array to yield a finger-like projection. The first two zinc ligands (cysteines) are contributed by a zinc knuckle [27, 28] at the end of the β-hairpin and the other two ligands (histidines) come from the C-terminal end of the α-helix. Zinc knuckles are unique turns, having the consensus sequence CPXCG, where the two cysteines function as zinc ligands.

The CCHH zinc finger is the best characterized zinc finger domain and constitutes the "classical zinc finger". The domain is composed of 25 to 30 amino-acid residues, and this structure can be observed repeated many times in a protein sequence. The finger-like projection interacts with nucleotides in the major groove of the nucleic acid. The residues separating the second cysteine and the first histidine are mainly polar and basic, implicating this region in particular in the DNA binding. In contrast to DNA interactions, CCHH-protein interactions are poorly observed and characterized. More than 6500 different sequences with this motif are known. Despite most of transcription factors and other DNA- binding proteins present the classical CCHH, others combinations of zinc chelating residues, as CCHC, are also possible.

2) The Treble Clef Finger

Treble clef finger structures are present in several families of proteins that display virtually undetectable sequence similarity to each other [27]. Treble clef motifs are usually incorporated in larger structures, multi-domain proteins or occur in doublets. In some proteins, tandem or overlapping treble clefs are present possibly due to duplication events (i.e LIM and FYVE domains) [29, 30]. The major groups encompass RING fingers [31, 32, 33], nuclear receptor-like fingers, phosphatidylinositol-3-phosphate-binding domains, protein kinase cysteine-rich domains and His-Me finger endonucleases. The treble clef is 25-60 amino-acid residues long and consists of a zinc knuckle followed by a loop, a β-strand and an α-helix, which are folded around a zinc ion, and is characterized by the distinct structural spatial arrangement of these elements. Unlike most globular proteins, the treble clef domain does not have a pronounced hydrophobic core and is strongly stabilized by the zinc ion. Consequently, the most structurally conserved part of the treble clef motif is the zinc ion-binding site. The short 2–4 residue sequence segments preceding and following the knuckle turn usually to adopt an extended conformation. These segments typically form two to three hydrogen bonds with each other and comprise a short β-hairpin. The loop following this knuckle β-hairpin varies in length (0–9 residues) and conformation; thus, the treble clef motif can be described as a left-handed superhelix of the five secondary structural elements. These features are unique to this motif and allow for its easy detection in protein structures. The functional diversity of treble-clef proteins is greater than that of many larger domains, encompassing binding to other proteins, DNA, RNA, and small ligands and a role in enzymatic catalysis [32].

3) The Zinc Ribbon

The zinc ribbons are arguably the largest fold group of zinc fingers. The zinc ribbons are found in a diverse family of proteins and frequently display limited sequence similarity, which is mainly restricted to the zinc ligands and the zinc-knuckle motifs. This limited sequence conservation is reflected in the structural variability of zinc ribbons. Most zinc-ribbon domains are actually folded as three-stranded antiparallel β-sheets in their structure instead of finger-like helices. Zinc ribbon domain functions as interaction modules binding to nucleic acids and is frequently found inserted in larger proteins. The zinc ribbon proteins are involved in a broad range of processes such as DNA replication, repair, transcription, translation and transcriptional repression.

In the zinc ribbon fold group, the ligands for zinc binding are contributed by two zinc-knuckles. The core of the structure is composed of two β-hairpins forming two structurally similar zinc-binding sub-sites. The primary β-hairpin contains the N-terminal zinc sub-site in classic zinc ribbon proteins, such as the transcription initiation factor TFIIB [34, 35] and transcriptional elongation factor SII [36]. The other β-hairpin contains the C-terminal zinc sub-site in classic zinc ribbons. Typically, an additional β-strand forms hydrogen bonds with the secondary β-hairpin, thus most zinc-ribbon domains contain a three-stranded antiparallel β-sheets in their structure. The length of the β-strands in the primary β-hairpin is usually about 2-4 residues. The β-strands in the three-stranded sheet vary in length, but are frequently longer (4–10 residues). The distance between the two sub-sites can vary considerably and there could be additional domains inserted in between.

Structures that possess two knuckles in their zinc-binding sites and thus belong to this fold group fall into two distinct sub-groups defined by the geometry of zinc ligands. There exist two possible mutual orientations of the four zinc ligands placed on a tetrahedron: left-handed and right-handed. The majority of the zinc ribbon structures contain a site with left-handed geometry; however, a few structures belong to a right-handed sub-group.

Due to the significant sequence and structural variability of zinc ribbons [37], this classification into families is provisional and more work is required to clarify evolutionary relationships within this fold.

THE PRESENCE OF ZINC-FINGER PROTEINS IN HUMAN VIRAL PATHOGENS

In recent years, zinc finger motifs have been reported in structural proteins of several viruses and have been shown to play critical roles in the respective multiplication cycles (Fig. **1**). Since the improper folding of these domains is mostly associated with the loss of correct protein function, the disruption of viral zinc finger structures may result in interference with virus replication. Given the high conservation of these metal-chelating sequences in different viral strains and species within a family, they have turned very attractive candidates as new targets for antiviral therapy. The current status of antiviral studies performed with proteins containing Zn finger motifs in human pathogenic viruses are presented in this section.

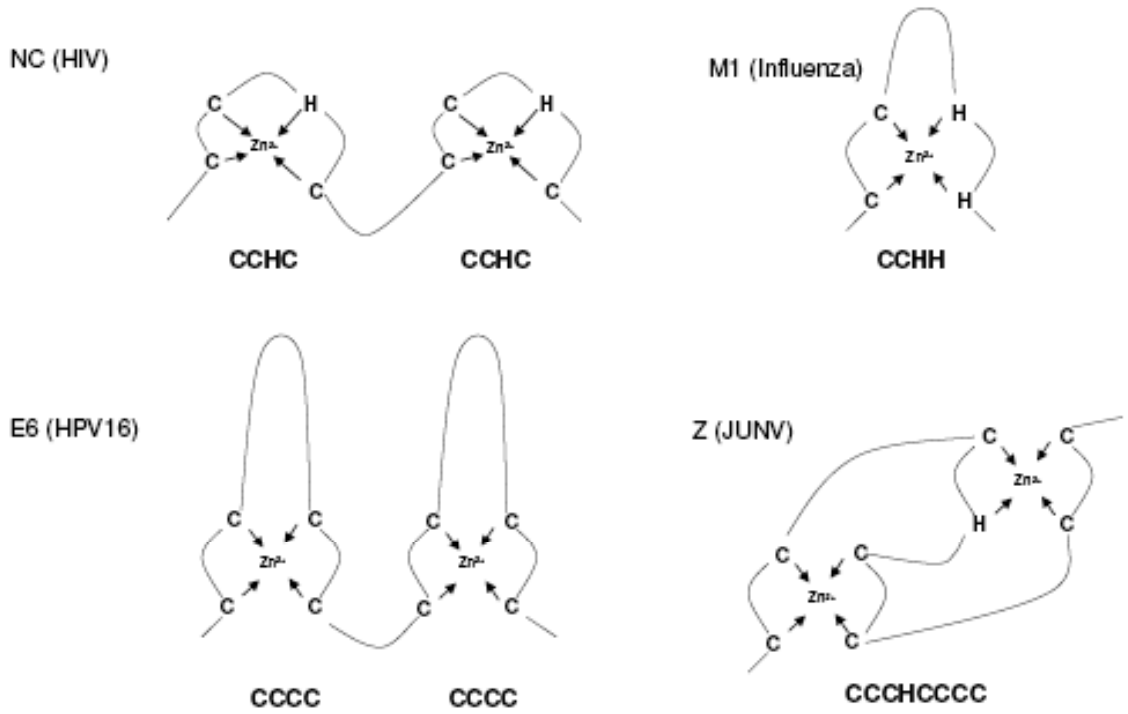

Fig. (1). Schematic structures of viral zinc finger target motifs.

RETROVIRUSES: HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Treatment of HIV infection with the multidrug combination known as highly active antiretroviral therapy (HAART) has been successful to delay onset of acquired immunodeficiency syndrome (AIDS) and to prolong patient survival. However, it is evident that the combined use of reverse transcriptase and protease inhibitors can still fail and, particularly, the emergence of drug-resistant strains is not totally abolished. Given the continued need to develop new effective drugs specific for HIV and without cross-resistance with existing drugs, the nucleocapsid protein (NC) of retroviruses is the zinc finger-containing viral protein more throroughly studied and characterized as a potential antiviral target. This protein is synthesized as part of a precursor polyprotein named GAG and, after cleavage by the viral protease during the virion budding process, NC remains in the internal core bound to the dimeric RNA genome. In all known retroviruses, except spumaviruses, the NC protein contains one or two copies of highly conserved zinc finger structures that consist of 14 amino acids arranged in a $C(X)_2C(X)_4H(X)_4C$ array, where X represents any amino acid, or CCHC box (Fig.**1**). A zinc ion is tetrahedrally coordinated by the three C-thiolate groups and the H-imidazole group [38]. The chelating residues and the spacing of the zinc finger array are absolutely conserved among NC proteins of mammalian type C retroviruses, which contain one zinc finger, and all other oncoretroviruses and lentiviruses, including HIV, which have two zinc fingers per NC molecule.

Site-directed mutagenesis studies have shown that the CCHC array within the NC protein is essential in several steps of the virus replication cycle. Mutations in any of the zinc chelating and a number of non-chelating residues in the zinc finger loop that disrupt the capacity of NC protein to coordinate zinc in avian, murine and human retroviruses give rise to particles which encapsidate reduced amounts of genomic RNA and have no detectable infectivity [39, 40, 41, 42, 43, 44, 45]. In addition to its essential function in genome packaging and infectious virion assembly, more subtle mutations have also shown a role for NC in early infection events acting as a nucleic acid chaperone to assist viral nucleic acids in obtaining the most thermodynamically stable structure to perform reverse transcription and integration [46, 47, 48, 49].

Based on these studies, the chemical disruption of the retroviral NC zinc finger domains was intended with chemical electrophilic reagents targeted to modify the nucleophilic sulfur atoms which are coordinating the zinc ions. Cysteine thiols are the most nucleophilic groups among all amino acids, and can be chemically modified with a large library of compounds through a variety of reaction mechanisms [50]. The wide range of zinc finger reactive compounds evaluated for anti-HIV-1 activity include disulfide-based compounds such as aromatic and aliphatic intermolecular disulfides [51, 52, 53, 54, 55, 56] and dithianes [57], aromatic C-nitroso compounds [58], azoics [59, 60], maleimides [61, 62] and others. Independently of the chemical composition of the compounds, most of these molecules can stoichiometrically and covalently modify the cysteine residues of the zinc fingers by oxidation of the thiolates, resulting in the ejection of zinc ions bound within the finger motifs of NC protein or its GAG precursor. This electrophilic attack is followed by formation of intra- and intermolecular disulfide crosslinking between the cysteines (Fig. **2**). The destabilization of the protein leads to a severe decrease of its affinity for nucleic acids and the consequent loss of its functional properties for the virus.

The disulfide-based molecules of the type R-SS-R, where R can be aromatic or aliphatic residues, represent one of the most extensively studied series of zinc finger-reactive HIV-1 inhibitors. Among them, the disulfide-substituted benzamides (DIBAs) (**1**) (Fig. **3**) were one of the first groups of chemotypes identified by the National Cancer Institute's drug screening program as inhibitors of HIV-1 and also a wide range of retroviruses in a variety of tumor cell lines and primary cells. The antiviral effective concentration 50% (EC_{50}) was in the range 1-10 μM whereas no cellular toxicity was observed at 200 μM [51]. The DIBAs evoked the release of zinc from the fingers and the cysteine thiolates became intra- or

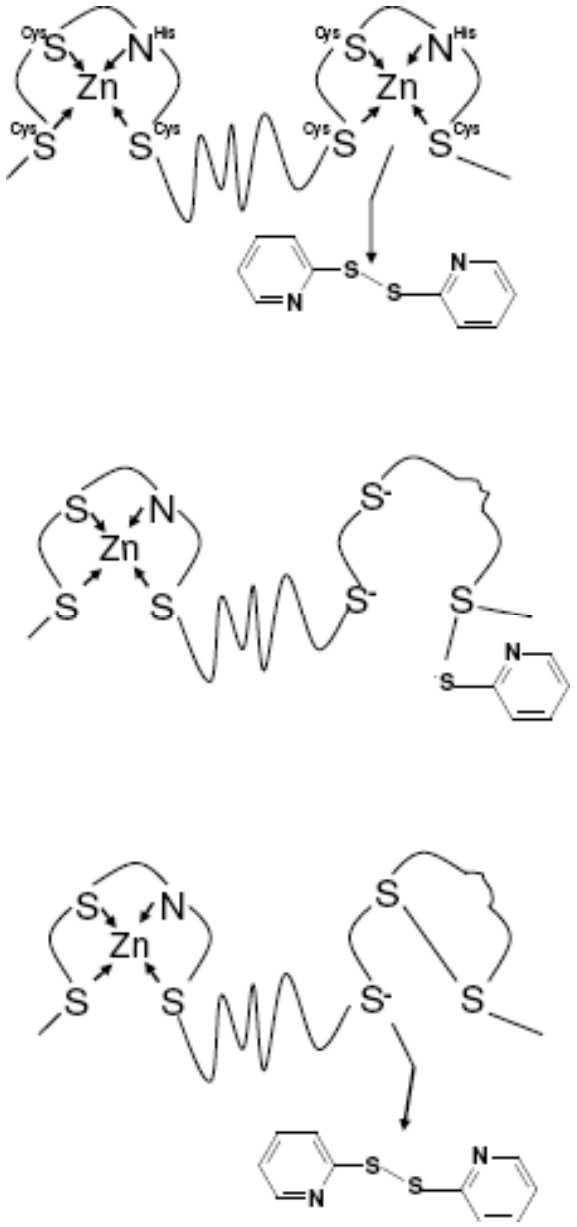

Fig. (2). Mechanism of action proposed for disulfide zinc finger disrupting compounds.

intermolecularly crossed-linked by disulfide bonds, causing the NC to resolve in non reducing gel electrophoresis as a ladder of multimeric aggregates [53, 63, 64]. The alterations in viral core structure lead to further inhibition of proviral DNA synthesis [56, 64]. Furthermore, attempts to isolate mutants resistant to DIBAs from HIV-1 infected cultures were unsuccessful even after years in passage illustrating that no mutations in the reactive motif are tolerated by the virus. When HIV-1 virions were treated with the compounds, cross-linkage of NC occurred within the virion and correlated with the ability of the compounds to inactivate HIV-1 infectivity [51, 63, 64].

After the above summarized promising results, research was focused to develop more stable and effective disulfide-based zinc finger inhibitors. A wide range of bis disulfides were evaluated and lead compounds with selective activity were identified [52]. But, a common inconvenience for all these compounds was the loss of zinc finger reactivity and antiviral activity by cellular reducing agents like glutathione. A series of dithianes consisting in dithiaheterocycles in which the disulfide moiety is tethered in a ring structure, such that reduction of the sufurs would not cause separation of two portions of the molecule and loss of reactivity to the zinc fingers, was developed. The lead compound of this series, 1,2-dithiane-4,5-diol, 1,1-dioxide,*cis* (NSC624151) (**2**) (Fig. **3**), showed broad antiretroviral activity, blocked production of infectious virus from infected cells and directly inactivated virions, similarly to the intermolecular disulfides [57]. Further development of the disulfide chemotype lead to the pyridinioalkanoyl thioesters (PATEs) (**3**) (Fig. **3**), based on 2-mercaptobenzamides as the thiol component and with its amide nitrogen initially substituted with various phenylsulphonyl moieties [65, 66], and subsequently modified with additional classes of ligand structures [67]. A significant improvement in antiviral activity was not obtained with PATEs (EC_{50} 1-5 μM) over other zinc finger inhibitor chemotypes, but reduced *in vitro* cytotoxicity, improved solubility, and lack of susceptibility to glutathione reduction represented significant improvements in this class inhibitor. To optimize the thioester properties, a library of several hundred of N-susbtituted *S*-acyl-2-mercaptobenzamide thioester derivatives was prepared and assayed, revealing that there was no significant correlation between serum stability of the compound and antiviral activity [68].

Then, it exist the possibility that the thioester may act as a prodrug and antiviral activity may be the result of a complex interaction with the host cell via a thioester intermediate. Thus, it must be further experimented if the mechanism of reaction of thioesters with the zinc coordinating cysteines of NC is highly differential from the other sulfide-based zinc finger inhibitors.

THE MAIN PROBLEM: SELECTIVITY

The main inconvenience found in the development and usage of zinc finger-reactive compounds (Fig. **3**) for human therapeutics is related to the selectivity of these agents. The presence of zinc finger motifs in numerous cellular proteins, as above summarized, has arise great concern about the real perspective to use this type of agents against HIV infection without affecting cellular zinc finger proteins. Four prototypic agents representing different chemotypes, 3-nitrosobenzamide (NOBA, C-nitroso compound), DIBA-1 (disulfide benzamide), dithiane NSC 624151 (dithiaheterocycle) and azodicarbonamide (ADA, azoic derivative) (**4**) (Fig. **3**) were evaluated for their ability to react with HIV NC and cellular zinc fingers by biochemical and computational studies. NOBA was found relatively promiscuous in its reactivity towards various cellular zinc finger proteins, such as poly-(ADP-ribose) polymerase, a nuclear enzyme containing two CCHC zinc finger motifs, and the transcription factors Sp1, with three classic CCHH motifs, and GATA-1, which uses two CCCC type domains, whereas the other three agents did not significantly affect the cellular protein properties neither produced zinc ejection from them [69].

The ability of different agents to exert discriminating reactivity between different zinc finger proteins depends on the interplay of multiple factors which may include ligand binding affinity and reactive proximity between the ligand molecule and the sulfur atoms of the zinc fingers as well as the requirement of sufficient redox properties in the chemical reagent to drive the reaction. For example, the sulfur atoms of zinc fingers in any protein may be sterically excluded from the reactive ligand contact by surrounding amino acid residues, as it was demonstrated by modeling analysis of the motif of GATA-1 [69]. A more recent study analyzed also the reactivity of two 2-mercaptobenzamide thioesters against diverse zinc-binding domains of viral and cellular proteins by determination of zinc ejection from the protein [70]. A variable level of reactivity was found for the different zinc finger motifs apparently determined by several factors such as the first and second zinc coordination shell interactions and the protein binding to its target DNA sequence.

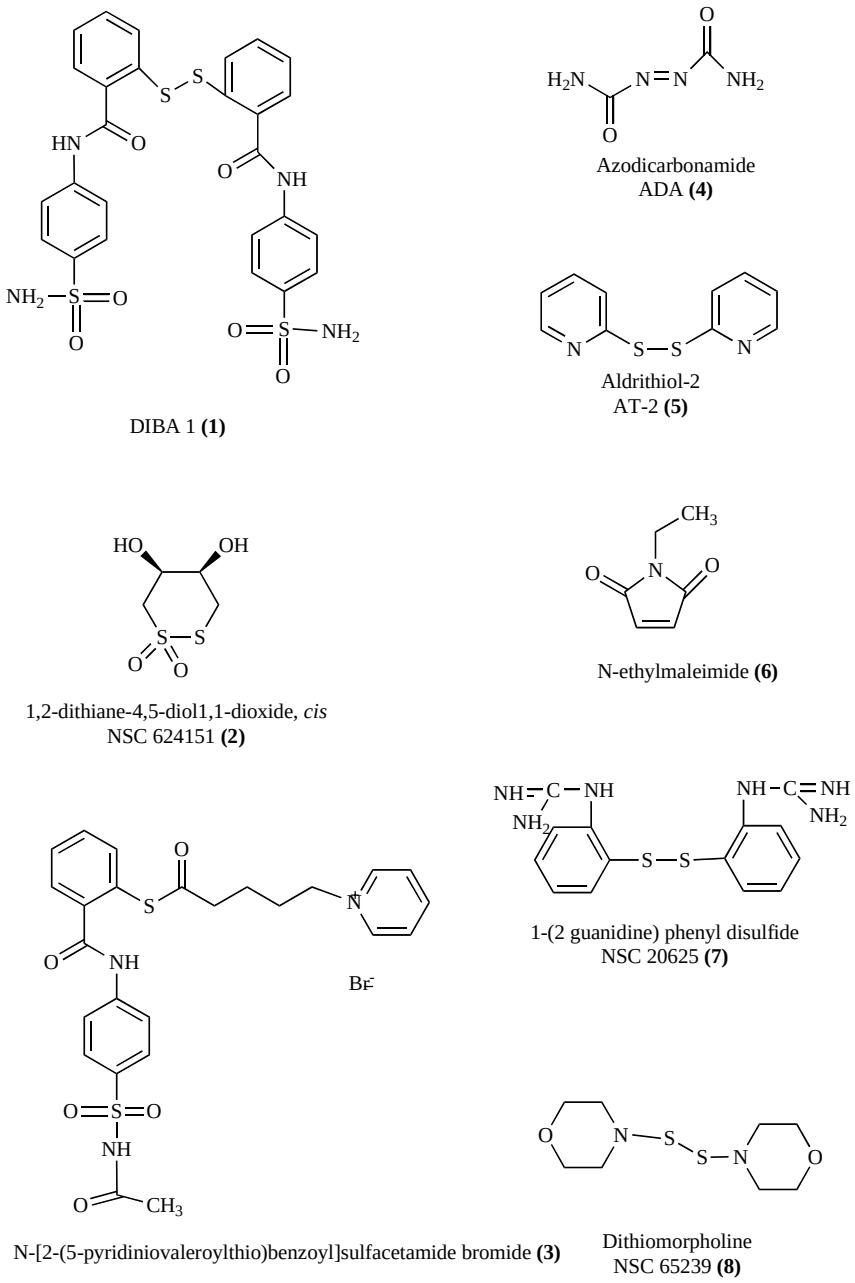

Fig. (3). Chemical structures of zinc finger disrupting compounds with antiviral activity.

Interestingly, computational and experimental studies have shown that exist differences in reactivity even between the two fingers in the HIV-1 NC protein. Modeling analysis of the docking configurations with DIBA-1, dithiane, NOBA and ADA, as well as chemical determinations with disulfide benzamides, dithiopyridine derivatives and pyridinioalkanoyl thioesters showed that the C 49 residue in the carboxy-terminal finger is more susceptible to electrophilic attack than the C in the amino-terminal finger [54, 66, 69, 71]. Given these variations among the reactivities of sulfur atoms within the same protein NC, it is reasonable to expect that the reactivity of zinc fingers of different proteins will also exhibit significant differences.

In conclusion, it is now clear that different zinc fingers provide different functions and nucleic acid recognition patterns that can produce biases in their chemical reactivities with ligands. Then, it is theorically feasible to design a therapeutic agent selectively targeted to the particular zinc finger motif present in a virus, but there is still a long way of research to be performed in order to get this goal. This crucial point is clearly evidenced by the very few studies about *in vivo* testing of zinc finger inhibitors. Ott *et al.* [72] reported small scale experiments to determine the inhibitory activity of several disulfides with proved *in vitro* efficacy in a retroviral murine model. Of the 14 compounds tested, only the 2, 2'-dithiopyridine (aldrithiol-2, AT-2) (**5**) (Fig. **3**) significantly delayed the onset of murine leukemia virus-induced Friend disease and decreased viral load [72], indicating the differential *in vitro* and *in vivo* effectiveness for this type of compounds. More recently, ADA was advanced to a phase I clinical trial in patients with advanced AIDS and documented virological failure. The compound showed moderate activity decreasing plasma viral RNA load, but some patients developed ADA-related cytotoxicity [73].

Since the systemic use of zinc finger reactive compounds in therapy is currently a distant objective, the scope of usefulness of these agents was expanded based on their viral inactivation properties. The loss of detectable infectivity after exposure of HIV-1 virions to AT-2 or *N*-ethylmaleimide (NEM) (**6**) (Fig. **3**) has been achieved with preservation of conformational and functional integrity of virion surface proteins [62, 74]. Virus inactivation by this type of compounds is time-dependent and appears to follow simple one-hit kinetics [63]. In contrast to virions inactivated by other conventional methods such as heat or formalin, viral envelope glycoproteins in these inactivated virions remained intact and functional, as detected by immunoprecipitation, high-performance liquid chromatography, fusion studies and dendritic cell capture. It is noticeably that safety studies involving direct intravenous administration of large amounts of AT-2 inactivated virus to nonhuman primates indicate that there is no detectable residual infectivity after treatment [75]. Retroviral inactivation by electrophilic compounds is of interest in the medical settings for the treatment of laboratory and biological samples, as well as in the prophylactic development of vaccines based in whoke killed particles.

Finally, other possibility taken into account for the NC zinc finger inhibitors was the evaluation of their potential as topical microbicide candidates. The ability found in lead thioesters to prevent cell-to-cell transmission of virus linked to their virucidal properties that destroy virion infectivity [68] are indicative of the promising perspectives for the utilization of zinc finger inhibitors as microbicidal agents in prevention of HIV transmission.

ORTHOMYXOVIRUSES: INFLUENZA

Influenza viruses cause global acute respiratory infections in humans with a diverse degree of severity. Currently available vaccines are not completely protective against influenza virus infection. Furthermore, the emergence of new viruses with high pathogenicity and virulence for humans has occurred in recent years [76, 77], with an increasing chance to produce a worldwide devastating pandemia. Thus, it is of great interest the development of antivirals for therapy or better vaccines for prevention of influenza infection.

Influenza virus is a negative-strand RNA virus with a segmented genome. The matrix protein M1 is the most abundant protein in influenza virus particles and plays critical functions in early and late steps of virus cycle, including virus entry to the cell and uncoating, regulation of viral RNA transcription and replication, assembly and budding of virus particles, and also as determinant of viral pathogenesis. M1 protein has a zinc finger motif with the sequence $CX_2CX_7HX_2H$ (CCHH) which is evolutionarily conserved among influenza viruses (Fig. **1**). However, the actual significance of the CCHH motif in influenza virus infection is still an enigma. It has been shown that only a small percentage (less than 10%) of influenza A M1 protein binds zinc, and its zinc content does not influence the *in vitro* RNA binding property of M1 [78, 79]. When small synthetic peptides corresponding to the CCHH motif of M1 were evaluated for their antiviral activity *in vitro* against influenza A virus controversial results were reported. A 19 amino acid peptide, designated peptide 6, spanning amino acids 148 to 166 of the M1 zinc finger region of influenza virus strain A/PR/8/34 (subtype H1N1) was inhibitory of the cytopathic effects induced by several influenza A and B strains in Madin-Darby canine kidney (MDCK) cells in culture [80] whereas other synthetic peptides corresponding to the CCHH motif of M1 protein of influenza virus strain A/WSN/33 (subtype H1N1) had no inhibitory effect on virus growth in MDCK cells [81]. In addition, the function of the CCHH motif in the influenza virus life cycle was also investigated by site-directed mutagenesis: mutant viruses containing an alanine replacement of cysteine and histidine residues in the CCHH motif, either individually or in combination, exhibited growth ability and cytopathogenic properties similar to wild type virus [81], suggesting that the motif does not provide a critical function in the influenza virus life cycle in cell cultures.

In contrast to the abovementioned results, conclusive evidences have shown that CCHH plays a central role in influenza virulence in mice. First, the intranasal treatment of influenza virus-infected mice with the M1 zinc finger peptide 6 was effective in preventing animal death and inhibiting virus pathogenic manifestations [82]. Second, some CCHH mutant viruses were highly attenuated in mice and, additionally, immunization with these attenuated viruses provided strong protection against lethal influenza virus challenge [83]. Presently it is unclear how CCHH mutations would lead to reduced virulence, but they provide an interesting alternative for the development of new master strains for live influenza virus vaccines.

ARENAVIRUSES: VIRAL HEMORRHAGIC FEVERS

Arenaviruses include several human pathogens able to cause severe hemorrhagic fevers such as Lassa virus, the agent of Lassa fever in Africa [84], and Junin virus (JUNV), the agent of Argentine hemorrhagic fever, an endemo-epidemic disease affecting the population of the most fertile zone in Argentina [85]. These two viruses generate periodic outbreaks of hemorrhagic fever with high mortality rate. Although several compounds were found to be selective inhibitors of the *in vitro* replication of arenaviruses [86] no reliable drug therapy is presently available for the treatment of infected patients. Ribavirin is the only compound that has shown partial efficacy against Lassa fever but with undesirable secondary reactions [87]. The only treatment for Argentine hemorrhagic fever consists of the early administration of standardized doses of convalescent plasma; however, this therapy is not efficient when it is initiated after 8 days of illness and a late neurological syndrome is observed in 10% of treated patients [88].

Among the five proteins expressed by arenavirus genome, a small 11-kDa protein named Z, due to its ability to bind zinc, contains a conserved CCCHCCCC RING-finger motif with the sequence $CX_2CX_9CX_2HX_2CX_2CX_{10}CX_2C$ (Fig. **1**) [89, 90, 91, 92]. Although the precise role of Z in the arenavirus life cycle is not totally elucidated, different investigators have proposed regulatory as well as structural functions for this protein during virus infection. Early studies based on *in vitro* transcription combined with immunodepletion of Z from infected cells suggested that Z was required for both mRNA synthesis and genome replication [93]. However, more recent studies performed with reverse genetics systems have shown that Z was not required for the RNA synthesis mediated by the viral polymerase, but rather Z exerted a dose-dependent inhibitory effect on both viral transcription and RNA replication [94, 95, 96]. Through this inhibitory activity, Z might contribute to the known restricted replicative ability and noncytopathic properties of many arenaviruses.

Since Z is a structural component of the virion closely associated to the viral nucleocapsid protein NP, Z has been proposed as the arenavirus counterpart of the matrix protein, found in most enveloped RNA negative strand viruses [97, 98, 99, 100]. The matrix proteins interact with membranes and are involved in the organization of viral components during assembly and budding, providing a link between the cytoplasmic tail of the glycoproteins and the nucleocapsid that contains the RNA genome [101]. Further evidence supporting the idea that Z functions as a matrix protein during arenavirus budding was provided by studies showing that arenavirus Z protein is strongly membrane-associated and is sufficient, in the absence of all other viral proteins, to release enveloped virus-like particles [102, 103]. In addition, the myristoylation of Z protein at the N-terminal domain appears to be essential for its membrane association and the consequent virus budding [104]. Thus, Eichler *et al.* [100] have assumed that Z is responsible to drive arenavirus budding through the recruitment of NP, complexed in the ribonucleoprotein, to the patches in the

cellular membranes enriched in envelope glycoproteins where virus assembly takes place.

In addition, the analysis of the amino acid sequence of Z protein in arenaviruses has shown that the RING domain is flanked by a C-terminal portion containing proline-rich motifs found in the so called late (L) domains that were identified in matrix proteins of enveloped viruses [105]. These late domains mediate protein-protein interactions and play a critical role in the virus budding process. It was found that the integrity of the late motifs and the RING finger domain is necessary for the Z-mediated regulatory and structural functions [102, 103, 106] turning this protein a very promising target for arenavirus chemotherapy.

Another outstanding property of Z is the ability of this protein to interact with several cellular proteins like the oncoprotein promyelocytic leukemia protein (PML) [107], the ribosomal protein P0 [108] and the eukaryotic translation initiation factor eIF4E [109]. These cellular interactions of Z may provide mechanisms to elucidate a viral strategy for the establishment of chronic infections, a typical property of arenaviruses.

In the search of agents reactive with the Z protein, several disulfides and azoic compounds from the National Cancer Institute repository were evaluated and found to be very effective inhibitors of arenaviruses [110]. According to their mode of action against the arenaviruses Junin, Tacaribe, Pichinde and lymphocytic chorio-meningitis virus, these compounds could be classified in three categories: i) virucidal agents able to inactivate cell-free virions, ii) antiviral agents that blocked the intracellular viral multiplication cycle, and iii) agents with both virucidal and antiviral properties.

The most effective inactivating agents included intermolecular aromatic disulfides, dithianes, and ADA, compounds that quickly inactivate arenaviruses in a concentration and time dependent manner, with inactivating concentration 50% (IC_{50}) values in the range 0.6-5.0 μM [111, 112]. Inactivated virions retained the biological functions of the viral envelope glycoproteins in virus binding and uptake, but were unable to perform viral RNA replication [111, 112, 113]. Furthermore, the electrophoretic pattern of the external glycoproteins as well as the nucleocapsid protein NP was unaffected. By contrast, the treatment of a recombinant Z protein with the virucidal compounds induced unfolding and oligomerization of Z to high-molecular-mass aggregates, confirming that this protein is the main target [113].

Accordingly with the proposed role of Z as analogue to a matrix protein, some compounds included in the second class of arenavirus inhibitors with antiviral properties appeared to block the virus multiplicaction cycle by interference with the process of intra-cellular virus assembly [112]. In addition, the Z-targeted inhibitors of arenavirus infection were able to block the interaction of Z with the cellular protein PML, as determined by confocal immuno-fluorescence, without affecting the RING motif of PML [113], and confirming that these compounds selectively disrupt the zinc binding residues in the viral RING finger domain.

Those compounds exhibiting both potent virucidal and antiviral activities against arenaviruses, such as the aromatic disulfides 1-(2-guanidine)phenyldisulfide (NSC20625) (**7**) (Fig. **3**) and AT-2, are the more promising type of agents to be used to affect Z function during therapeutic treatments. For several biothreat agents, including arenaviruses and filoviruses, person-to person trans-mission is not efficient and virus spread occurs primarily by contact with the reservoir or in hospital settings. Thus, compounds with combined inactivating and antiviral activities deserve further study to assess their perspectives as a barrier to minimize human exposure and prevent virus dissemination.

PAPILLOMAVIRUSES

Among the DNA viruses causing disease with significant mortality and morbidity, human papillomaviruses (HPV) comprise more than 100 different genotypes classified as high-risk and low-risk viruses according to the severity of the mucocutaneous disease. The high risk-viruses, including HPV-16, HPV-18, HPV-31 and HPV-45, are able to promote cervical cancer whereas the low-risk viruses are found in common and benign plantar, palmar and genital warts. HPV-16, the most common HPV type in malignant neoplasia, was found to be associated with 50% of all cervical cancers, a serious form of cancer affecting approximately a half million women worldwide with high mortality rate. There is no specific treatment for HPV-induced diseases and therapy for warts and advanced cases of cervical dysplasia include surgery or cytotoxic agents to remove the infected tissue as well as immune modulators like interferon [114, 115].

The transforming properties of HPV were ascribed to three oncoproteins encoded by the early genes E5, E6 and E7. E6 and probably E7 seem to play the central role in HPV oncogenesis development since pleiotropic effects were reported for these proteins with consequences on several biochemical and cellular processes such as transmembrane signaling, regulation of the cell cycle, differentiation, transformation and immortalization of cell lines.

HPV E6 protein contains about 150 amino acid residues and two $CX_2CX_{29}CX_2C$ zinc finger domains which chelate the metal ion by the four –SH moieties of the cysteine residues (Fig. **1**). This motif is unique among all other known zinc-binding proteins and is highly conserved among distantly related HPV and many animal papillomaviruses, suggesting that the structure and function of E6 are strongly dependent on these zinc fingers. In fact, mutations affecting the E6 zinc fingers affect HPV16 induced cellular transformation as well as the interactions of E6 with three cellular proteins, the cell cycle-regulator p53, the E6-associated protein (E6AP) and the E6-binding protein (E6BP), which are involved in p53 degradation and alterations in intracellular signaling respon-sible of the oncoproperties of E6 [116, 117, 118].

Thus, on a similar basis of the strategy assayed against retro-viruses and arenaviruses, the chemical alteration of the thiolates of the cysteine residues that coordinate zinc in the two E6 zinc fingers was intended using thiolate-reactive compounds. Three major groups of compounds were tested: disulfides, azoics and nitroso aromatics. By measuring the release of zinc ions after chemical alteration of the cysteine groups in the E6 zinc fingers, the interaction of E6 with the above mentioned cellular proteins E6AP and E6BP, and the viability of tumor cell lines that require the continuous expression of HPV E6, several disulfide derivatives were found reactive to E6 [119, 120] (Beerheide *et al*., 1999, 2000). The most active compound of this type, the 4,4'-dithiodimorpholine (**8**) (Fig. **3**) exhibited selective cytotoxicity toward the HPV-infected cells and was identified as a potential *in vitro* lead compound to develop anti-HPV inhibitors targeted to E6 zinc fingers.

OTHER HUMAN PATHOGENIC VIRUSES

Aside of the above mentioned pathogens, there are other viruses with severe pathogenicity in humans containing diverse zinc finger motifs in structural or nonstructural proteins. In these cases, like occurs with herpes simplex virus and the ICP0 protein, Ebola virus with the transactivator VP30, and the NSP10 protein of human coronavirus, studies about the roles of the corresponding zinc finger proteins in virus life cycle have been reported [121, 122, 123, 124]. But, currently no therapeutic applications centered on those proteins have been developed against these viruses.

CONCLUSION

The zinc finger motifs are undoubtedly a key factor for the structure, stability and functionality of the diverse viral proteins which play a central role in virus life cycle. The antiviral strategies leading to virus inhibition and/or inactivation by disruption of the

zinc finger motif have started about one decade ago. Currently the results are not successful, mainly due to the lack of selectivity of the chemical agents to attack the viral target without affecting cellular zinc finger proteins. However, the battle is not still lost.

The more precise knowledge of the structure and nature of the active site in a biomolecule, in this case the zinc finger protein, will allow the chemical modification to improve the quality and performance of the ideal compound, as described here for HIV.

Furthermore, the inactivating activity as well as the virus spread-inhibitory effect found in several zinc finger inhibitors open new perspectives of application for these agents, supporting their possible usage not only for the therapeutic treatment of human infections but also for the prevention of virus dissemination.

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ABBREVIATIONS

ADA	=	Azodicarbonamide
AIDS	=	Acquired immunodeficiency syndrome
AT-2	=	Aldrithiol-2
C	=	Cysteine
DIBAs	=	Disulfide-substituted benzamides
E6AP	=	E6-associated protein
E6BP	=	E6-binding protein
EC_{50}	=	Effective concentration 50%
eIF4E	=	Eukaryotic translation initiation factor
G	=	Glycine
H	=	Histidine
HAART	=	Highly active antiretroviral therapy
HIV	=	Human immunodeficiency virus
HPV	=	Human papillomaviruses
IC_{50}	=	Inactivating concentration 50%
JUNV	=	Junín virus
MDCK	=	Madin-Darby canine kidney
NC	=	Nucleocapsid protein
NEM	=	N-ethylmaleimide
NOBA	=	Nitrosobenzamide
P	=	Proline
PATE	=	Pyridinioalkanoyl thioesters
PML	=	Promyelocytic leukemia protein
TFIIIA	=	Transcription factor IIIA

REFERENCES

[1] Berg, J. M.; Shi, Y. *Science*, **1996**, *271*, 1081.
[2] Margoshes, M.; Vallee, B. L. *J. Am. Chem. Soc.*, **1957**, *79*, 4813.
[3] Maret W. *Biochemistry*, **2004**, *43*, 3301.
[4] Vallee, B. L.; Auld, D. S. *Biochemistry*, **1990**, *29*, 5647.
[5] Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.*, **1993**, *26*, 543.
[6] Hanas, J. S.; Hazuda, D.; Bogenhagen, D. F.; Wu, F. Y.-H.; Wu, C. W. *J. Biol. Chem.*, **1983**, *258*, 14120.
[7] Miller, J.; McLachlan, A. D.; Klug, A. *EMBO J.*, **1985**, *4*, 1609.
[8] Maret, W.; Vallee, B. L. *Methods Enzymol*., **1993**, *226*, 52.
[9] Andreini C.; Banci L.; Bertini I.; Rosato A. *J Proteome Res*., **2006**, *5*, 196.
[10] Berg, J. M.; Merkle, D. L. *J. Am. Chem. Soc*., **1989**, *111*, 3779.
[11] Laity, J. H.; Lee, B. M.; Wright, P. E. *Curr. Opin. Struct. Biol.*, **2001**, *11*, 39.
[12] Wolfe S.A.; Grant, R.A.; Elrod-Erickson, M.; Pabo, C.O. *Structure (Camb.)*, **2001**, *9*, 717.
[13] Leon O.; Roth, M. *Biol. Res.*, **2000**, *33*, 21.
[14] Klug A.; Schwabe, J.W. *FASEB J*., **1995**, *9*, 597.
[15] Iuchi S. *Cell. Mol. Life Sci.*, **2001**, *58*, 625.
[16] Miller, J.; Mc Lachlan, A.D.; Klug, A. *EMBO J*., **1985**, *4*, 1609.
[17] Frankel, A. D.; Berg, J. M.; Pabo, C. O. *Proc. Natl. Acad. Sci. U.S.A.*, **1987**, *84*, 4841.
[18] Alberts I.L.; Nadassy, K.; Wodak, S.J. *Protein Sci.*, **1998**, *7*, 1700.
[19] Karlin S.; Zhu, Z.Y. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 14231.
[20] Karlin S.; Zhu, Z.Y.; Karlin, K.D. *Proc. Natl Acad. Sci. USA*, **1997**, *94*, 14225.
[21] Ravasi T.; Huber T.; Zavolan M.; Forrest A.; Gaasterland T.; Grimmond S.; RIKEN GER Group; GSL Members; Hume D.A. *Genome Res.*, **2003**, *13*, 1430.
[22] Baron M.; Norman D.G.; Campbell ID. *Trends Biochem Sci.* **1991**, *16*, 13.
[23] Berg JM.: *Annu Rev Biophys Biophys Chem*., **1990**, *19*, 405.
[24] Mackay, J.P; Crossley, M. *Trends Biochem. Sci.*, **1998**, *265*, 1.
[25] Schwabe, J.W.R; Klug, A. *Nat. Struct. Biol*., **1994**, *1*, 345.
[26] Krishna SS.; Majumdar I; Grishin NV. *Nucleic Acids Res*., **2003**, *31*, 532.
[27] Grishin N.V. *Nucleic Acids Res.*, **2001**, *29*, 1703.
[28] Wang B.; Jones, D.N.; Kaine, B.P.; Weiss, M.A. *Structure*, **1998**, *6*, 555.
[29] Kutateladze T.G.; Ogburn K. D.; Watson W.T.; de Beer T.; Emr S.D.; Burd C. G.; Overduin M. *Mol Cell*., **1999**, *3*, 805.
[30] Bach, I. *Mech. Dev.*, **2000**, *91***,** 5.
[31] Freemont, P. S. *Ann. N Y Acad. Sci.*, **1993**, *684*, 174.
[32] Borden, K. L. *J. Mol. Biol*., **2000**, *295*, 1103.
[33] Joazeiro, C. A.; Weissman, A. M. *Cell*, **2000**, *102*, 549.
[34] Zhu W.; Zeng, Q.; Colangelo, C.M.; Lewis, M.; Summers, M.F.; Scott, R.A. *Nature Struct. Biol.*, **1996**, *3*, 122.
[35] M. Ghosh; L. M. Elsby; T. K. Mal; J. M. Gooding; S. G. E. Roberts; M. Ikura. *Biochem. J*., **2004**, 378, 317.
[36] Qian X.; Gozani, S.N.; Yoon, H.; Jeon, C.J.; Agarwal, K.; Weiss, M.A. *Biochemistry*, **1993**, *32*, 9944.
[37] B. K. Sharpe; J. M. Matthews; A. H.Y. Kwan; A. Newton; D. A. Gell; M. Crossley; J. P. Mackay. *Structure*, **2002**, *10*, 639.
[38] Bess, J.W. Jr; Powell, P.J.; Issaq, H.J.; Schumack, L.J.; Grimes, M.K.; Henderson, L.E.; Arthur, L.O. *J. Virol.*, **1992**, *66*, 840.
[39] Aldovini, A.; Young, R.A. *J. Virol*., **1990**, *64*, 1920.
[40] Dupraz, P.; Oertke, S.; Meric, C.; Damay, P.; Spahr, P.-F. *J. Virol*., **1990**, *64*, 4978.
[41] Gorelick, R.J.; Henderson, L.E.; Hanser, J.P.; Rein A. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 8420.
[42] Gorelick, R.J.; Nigida, S.M.Jr ; Bess, J.W.Jr; Arthur, L.O.; Henderson, L.E.; Rein, A. *J. Virol*., **1990**, *64*, 3207.
[43] Gorelick, R.J.; Chabot, D.J.; Rein, A.; Henderson, L.E.; Arthur, L.O. *J. Virol*., **1993**, *67*, 4027.
[44] Méric, C.; Gouilloud, E.; Spahr, P.-F. *J. Virol*., **1988**, *62*, 3328.
[45] Sakalian, M.; Wills, J.W.; Vogt, V.M. *J. Virol*., **1994**, *68*, 5969.
[46] Yu, Q; Darlis, J.L. *J. Virol.*, **1996**, *70*, 5791.
[47] Buckman, J.S., Bosche, W.J., Gorelick, R.J. J. Virol., 2003, 77, 1469.
[48] Levin, J.G.; Guo, J.; Rouzina, I.; Musier-Forsyth, K. Prog. *Nucleic Acid Res. Mol. Biol*., **2005**, *80*, 217.
[49] Thomas, J.A.; Gagliardi, T.D.; Alvord, W.G.; Lubomirski, M.; Bosche, W.J.; Gorelick, R.J. *Virology*, **2006**, *353*, 41.
[50] Topol, I.A.; McGrath, C.; Chertova, E.; Dasenbrock, C.; Lacourse, W.R.; Eissenstat, M.A.; Burt, S.K.; Henderson, L.E.; Casas-Finet, J.R. *Protein Science*, **2001**, *10*, 1434.
[51] Rice, W.G.; Supko, J.G.; Malspeis, L.; Buckheit, R.W.Jr; Clanton, D.; Bu, M.; Graham, L.; Scaheffer, C.A.; Turpin, J.A.; Domagala, J.; Gogliotti, R.; Bader, J.P.; Halliday, S.M.; Coren, L.; Sowder II, R.C.; Arthur, L.O.; Henderson, L.E. *Science*, **1995**, *270*, 1194.
[52] Rice, W.G.; Turpin, J.A.; Schaeffer, C.A.; Graham, L.; Clanton, D.; Buckheit, R.W.Jr ; Zaharevitz, D.; Summers, M.F.; Wallquist, A.; Covell, D.G. *J. Med. Chem.*, **1996**, *19*, 3606.
[53] Turpin, J.A.; Terpening, S.J.; Schaeffer, C.A.; Yu, G.; Glover, C.J.; Felsted, R.L.; Sausville, E.A.; Rice, W.G. *J. Virol*., **1996**, *70*, 6180.
[54] Tummino, P.J.; Scholten, J.D.; Harvey, P.J.; Holler, T.P.; Maloney, L.; Gogliotti, R.; Domagala, J.; Hupe, D. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 969.
[55] Witvrouw, M.; Balzarini, J.; Pannecouque, C.; Jhaumeer-Laulloo, S. ; Esté, J.A.; Schols, D.; Cherepanov, P.; Schmit, J.C.; Debyser, Z.; Vandamme, A.M.; Desmyter, J.; Ramadas, S.R.; De Clercq, E. *Antimicrob. Agents Chemother*. **1997**, *41*, 262.
[56] Berthoux, L.; Péchoux, C.; Darlix, J-L. *J. Virol.*, **1999**, *73*, 10000.

[57] Rice, W.G. ; Baker, D.C.; Schaeffer, C.A.; Graham, L.; Bu, M.; Terpening, S.; Clanton, D.; Schultz, R.; Bader, J.P.; Buckheit, R.W.Jr ; Field, L. ; Singh, P.K. ; Turpin, J.A. *Antimicrob. Agents Chemother*., **1997a**, *41*, 419.

[58] Rice, W.G. ; Schaeffer, C.A. ; Harten, B. ; Villinger, F.; South, T.L.; Summers, M.F.; Henderson, L.O.; Bess, J.W. Jr; Arthur, L.O.; McDougall, J.S.; Orloff, S.L.; Mendeleyev, J. ; Kun, E. *Nature*, **1993**, *361*, 473.

[59] Vandevelde, M.; Witvrouw, M.; Schmit, J.C.; Sprecher, S.; De Clercq, E.; Tassignon, J.P. *AIDS Res. Hum. Retroviruses* **1996**, *12*, 567.

[60] Rice, W.G.; Turpin, J.A.; Huang, M.; Clanton, D.; Buckheit, R.W.Jr; Covell, D.G.; Wallquist, A.; McDonnell, N.B.; DeGuzman, R.N.; Summers, M.F.; Zalkow, L.; Bader, J.P.; Haugwitz, R.D.; Sausville, E.A. *Nature Medicine*, **1997**, *3*, 341.

[61] Chertova, E.N.; Kane, B.P.; McGrath, C.; Johnson, D.G.; Sowder II, R.C.; Arthur, L.O.; Henderson, L.E. *Biochemistry*, **1998**, *37*, 17890.

[62] Morcock, D.R. ; Thomas, J.A. ; Gagliardi, T.D. ; Gorelick, R.J. ; Roser, J.D. ; Chertova, E.N. ; Bess Jr., J.W. ; Ott, D.E. ; Sattentau, Q.J. ; Frank, I. ; Pope, M. ; Lifson, J.D. ; Henderson, L.E. ; Crise, B.J. *J. Virol*., **2005**, *79*, 1533.

[63] Rein, A. ; Ott, D.E. ; Mirro, J. ; Arthur, L.O.; Rice, W. Henderson, L.E. *J. Virol*., **1996**, *70*, 4966.

[64] Turpin, J.A.; Schaeffer, C.A.; Terpening, S.J.; Graham, L.; Bu, M.; Rice, W.G. *Antiviral Chem. Chemother*. **1997**, *8*, 60.

[65] Turpin, J.A.; Song, Y.; Inman, J.K. ; Huang, M. ; Wallquist, A.; Maynard, A.; Covell, D.G.; Rice, W.G.; Appella, E. *J. Med. Chem.*, **1999**, *42*, 67.

[66] Basrur, V.; Song, Y.; Mazur, S.J.; Higashimoto, Y.; Turpin, J.A.; Rice, W.G.; Inman, J.K.; Appella, E. *J. Biol. Chem*., **2000**, *275*, 14890.

[67] Song, Y.; Goel, A.; Basrur, V.; Roberts, P.E.A.; Mikovits, J.A.; Inman, J.K.; Turpin, J.A.; Rice, W.G.; Appella, E. *Bioorg. Med. Chem*., **2002**, *10*, 1263.

[68] Srivastava, P.; Schito, M.; Fattah, R.J.; Hara, T.; Hartman, T.; Buckheit Jr., R.W.; Turpin, J.A.; Inman, J.K.; Appella, E. *Bioorg. Med. Chem.*, **2004**, *12*, 6237.

[69] Huang, M.; Maynard, A.; Turpin, J.A.; Graham, L.; Janini, G.M.; Covell, D.G.; Rice, W.G. *J. Med. Chem*.**1998**, *41*, 1371.

[70] Jenkins, L.M.; Durell, S.R.; Maynard, A.T.; Stahl, S.J.; Inman, J.K.; Appella, E.; Legault, P.; Omichinski, J.G. *J. Am. Chem. Soc*., **2006**, *128*, 11964.

[71] Halthout, Y.; Fabris, D.; Han, M.S.; Sowder, R.C.; Henderson, L.E.; Fenselau, C. *Drug Metab. Dispos*., **1996**, *24*, 1395.

[72] Ott, D.E. ; Hewes, S.M. ; Alvord, W.G.; Henderson, L.E.; Arthur, L.O. *Virology*, **1998**, *243*, 283.

[73] Goebel, F.D.; Hemmer, R.; Schmit, J.C.; Bogner, J.R.; De Clercq, E.; Witvrouw, M., Pannecouque, C.; Valeyev, R.; Vandevelde, M.; Margery, H.; Tassignon, J.P. *AIDS*, **2001**, *15*, 33.

[74] Rossio, J.L.; Esser, M.T.; Suryanarayana, K.; Schneider, D.K. ; Bess Jr., J.W. ; Vazquez, G.M.; Wiltrout, T.A.; Chertova, E.; Grimes, M.K.; Sattentau, Q.; Arthur, L.O.; Henderson, L.E.; Lifson, J.D. *J. Virol*., **1998**, *72*, 7992.

[75] Lifson, J.D.; Piatak, M.; Rossio, J.L.; Bess Jr., J.; Chertova, E.; Schneider, D.; Kiser, R.; Coalter, V.; Poore, B.; Iming, R.; Desrosiers, R.C.; Henderson, L.E.; Arthur, L.O. *J. Med. Primatol*., **2002**, *31*, 205.

[76] Lipatov, A.S.; Govorkova, E.A.; Webby, R.J.; Ozaki, H.; Peiris, M.; Guan, Y.; Poon, L.; Webster, R.G. *J. Virol.*, **2004**, *78*, 8951.

[77] Gillim-Ross, L.; Subbarao, K. *Clin. Microbiol. Rev*., **2006**, *19*, 614.

[78] Elster, C.; Fourest, E.; Baudin, F.; Larsen, K.; Cusack, S.; Ruigrock, R.W.H. *J. Gen. Virol*., **1994**, *75*, 37.

[79] Wakefield, L.; Brownlee, G.G. *Nucleic Acids Res.*, **1989**, *17*, 8569.

[80] Nasser, E.H. ; Judd, A.K. ; Sanchez, A. ; Anastasiou, D. ; Bucher, D.J. *J. Virol*., **1996**, *70*, 8639.

[81] Hui, E.K.-W.; Ralston, K.; Judd, A.K. ; Nayak, D.P. *J. Gen. Virol.*, **2003**, *84*, 3105.

[82] Judd, A.K.; Sanchez, A.; Bucher, D.J.; Huffman, J.H.; Bailey, K.; Sidwell, R.W. *Antimicrob. Agents Chemother*., **1997**, *41*, 687.

[83] Hui, E.K.-W.; Smee, D.F.; Wong, M.-H.; Nayak, D.P. *J. Virol.*, **2006**, *80*, 5697.

[84] McCormick, J.B.; Fisher-Hoch, S.P. *Curr. Top. Microbiol. Immunol.*, **2002**, *262*, 75.

[85] Peters, C.J. *Curr. Top. Microbiol. Immunol.*, **2002**, *262*, 65.

[86] Damonte, E.B.; Coto, C.E. *Adv. Virus Res.*, **2002**, *58*, 125.

[87] McCormick, J.B.; King, I.J.; Webb, P.A.; Scribner, C.L.; Craven, R.B.; Johnson, K.M.; Elliot, L.H.; Belmont-Williams, R. *N. Engl. J. Med*., **1986**, *314*, 20.

[88] Enria, D.A.; Maiztegui, J.I. *Antiviral Res.*, **1994**, *23*, 23.

[89] Salvato, M.S.; Shimomaye, E.M. *Virology*, **1989**, *173*, 1.

[90] Iapalucci, S.; López, N.; Rey, O.; Zakin, M.; Cohen, G.N.; Franze-Fernández, M.T. *Virology*, **1989**, *173*, 357.

[91] Djavani, M.; Lukashevich, I.S.; Sánchez, A.; Nichol, S.T.; Salvato, M.S.; *Virology*, **1997**, *235*, 414.

[92] Gibadulinova, A.; Zelnik, V.; Resiserova, L.; Zavodska, E.; Zatovicova, M.; Ciampor, F.; Pastorekova, S.; Pastorek, J. *Acta Virol.*, **1998**, *42*, 369.

[93] Garcin, D.; Rochat, S.; Kolakofsky, D. *J. Virol*. **1993**, *67*, 807 Garcin, D.; Rochat, S.; Kolakofsky, D. *J. Virol*. **1993**, *67*, 807.

[94] Lee, K. J.; Novella, I. S.; Teng, M. N.; Oldstone, M. B. A.; de la Torre, J. C. *J. Virol*., **2000**, *74*, 3470.

[95] Cornu, T. I.; de la Torre, J. C. *J. Virol*., **2001**, *75*, 9415.

[96] López, N.; Jácamo, R.; Franze-Fernández, M. T. *J. Virol.*, **2001**, *75*, 12241.

[97] Salvato, M.S.; Schweighofer, K.J.; Burns, J.; Shimomaye, E.M. *Virus Res*., **1992**, *22*, 185.

[98] Compans, R. W. In *The Arenaviridae*; Salvato, M. S., Ed.; Plenum Press: New York, **1993**; pp 3-16.

[99] Salvato, M. S. In *The Arenaviridae*; Salvato, M. S., Ed.; Plenum Press: New York, **1993**; pp 133-156.

[100] Eichler, R.; Strecker, T.; Kolesnikova, L.; ter Meulen, J.; Weissenhorn, W.; Becker, S.; Klenk, H. D.; Garten, W.; Lenz, O. *Virus Res*., **2004**, *100*, 249.

[101] Garoff, H.; Hewson, R.; Opstelten, D. J.. *Microbiol. Mol. Biol. Rev*., **1998**, *62*, 1171.

[102] Strecker, T.; Eichler, R.; ter Meulen, J.; Weissenhorn, W.; Klenk, H. D.; Garten, W.; Lenz, O. *J. Virol.*, **2003**, *77*, 10700.

[103] Perez M, Craven, R. C.; de la Torre, J. C. *Proc. Natl. Acad. Sci. U.S.A.*, **2003**, *100*, 12978.

[104] Perez, M.; Greenwald, D.L.; de la Torres, J.C. *J. Virol*., **2004**, *78*, 11443.

[105] Freed, E.O. *J. Virol*., **2002**, *76*, 4679.

[106] Cornu, T. I.; de la Torre, J. C. *J. Virol.*, **2002**, *76*, 6678.

[107] Borden, K. L.; Campbell-Dwyer, E. J.; Salvato, M. S. *J. Virol.*, **1998a**, *72*, 758.

[108] Borden, K. L. B.; Campbell-Dwyer, E. J.; Carlile, G. W.; Djavani, M.; Salvato, M. S. *J. Virol.*, **1998b**, *72*, 3819.

[109] Campbell Dwyer, E. J.; Lai, H.; MacDonald, R. C.; Salvato, M. S.; Borden, K. L. B. *J. Virol*., **2000**, *74*, 3293.

[110] García, C.C.; Candurra, N.A.; Damonte, E.B. *Antiviral Chem. Chemother*., **2000**, *11*, 231.

[111] García, C.C.; Candurra, N.A.; Damonte, E.B. *Antiviral Res.*, **2002**, *55*, 437.

[112] García, C.C.; Candurra, N.A.; Damonte, E.B. *Int. J. Antimicrob. Agents*, **2003**, *21*, 319.

[113] García, C.C.; Djavani, M.; Topisirovic, I. ; Borden, K.L.B. ; Salvato, M.S. ; Damonte, E.B. *J. Gen. Virol.*, **2006**, *87*, 1217.

[114] McGlennen, R.C. *Clin. Lab. Med*., **2000**, *20*, 383.

[115] Beutner, K.R.; Ferenczy, A. *Am. J. Med.*, **1997**, *102*, 28.

[116] Chen, J.J.; Reid, C.E.; Band, V.; Androphy, E.J. *Science*, **1995**, *269*, 529.

[117] Dalal, S.; Gao, Q.; Androphy, E.J.; Band, V. *J. Virol*., **1996**, *70*, 683.

[118] Nakagawa, S.; Watanabe, S.; Yoshikawa, H.; Taketani, Y.; Yoshiike, K. ; Kanda, T. *Virology*, **1995**, *212*, 535.

[119] Beerheide, W.; Bernard, H.U.; Tan, Y.J; Ganesan, A.; Rice, W.G.; Ting, A.E. *J. Natl. Cancer Inst*., **1999**, *91*, 1211.

[120] Beerheide, W.; Sim, M.M.; Tan, Y.J.; Bernard, H.U.; Ting, A.E. *Bioorg. Med. Chem*., **2000**, *8*, 2549.

[121] O'Rourke, D. ; Elliot, G. ; Papworth, M. ; Everett, R.; O'Hare, P. *J. Gen. Virol.*, **1998**, *79*, 537.

[122] Everett, R.D.; Rechter, S.; Papior, P.; Tavalai, N.; Stamminger, T.; Orr, A. *J. Virol.*, **2006**, *80*, 7995.

[123] Modrof, J. ; Becker, S. ; Mühlberger, E. *J. Virol*., **2003**, *77*, 3334.

[124] Joseph, J.S.; Saikatendu, K.S.; Subramanian, V.; Neuman, B.W.; Brooun, A.; Griffitj, M.; Moy, K.; Yadav, M.K.; Velasquez, J.; Buchmeier, M.J.; Stevens, R.C.; Kuhn, P. *J. Virol*., **2006**, *80*, 7894.