Antiviral Strategies Against Human Coronaviruses

K. Pyrc*, B. Berkhout and L. van der Hoek

Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

Abstract: Since the mid 60's the human coronaviruses (HCoV), represented by HCoV-OC43 and HCoV-229E, were generally considered relatively harmless viruses. This status changed dramatically with the emergence of SARS-CoV in 2002/2003. The SARS-CoV pandemic took 774 lives around the globe and infected more than 8000 people in 29 countries. SARS-CoV is believed to be of zoonotic origin, transmitted from its natural reservoir in bats through several animal species (e.g., civet cats, raccoon dogs sold for human consumption in markets in southern China). The epidemic was halted in 2003 by a highly effective global public health response, and SARS-CoV is currently not circulating in humans. The outbreak of SARS-CoV and the danger of its re-introduction into the human population, as well as the danger of the emergence of other zoonotic coronaviral infections triggered an intense survey for an efficient treatment that resulted in the evaluation of several anticoronaviral compounds.

HCoV-NL63 and HCoV-HKU1 were identified shortly after the SARS-CoV outbreak. The 4 human coronaviruses HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 cause mild respiratory illnesses when compared to SARS, but these infections are involved in 10 – 20 % of hospitalizations of young children and immunocompromised adults with respiratory tract illness. Therefore, there is an urgent need for a successful therapy to prevent disease induction or a vaccine to prevent new infections. This review summarizes the current status of anticoronaviral strategies.

Keywords: Human coronavirus, antiviral, therapy, inhibition.

CORONAVIRUSES

Coronaviruses, a genus of the *Coronaviridae* family, are enveloped viruses with a plus-strand RNA genome. The genomic RNA is 27−32 kb in size, capped and polyadenylated. Coronaviruses have been identified in bats, mice, rats, chickens, turkeys, swine, dogs, cats, rabbits, horses, cattle and humans and cause highly prevalent diseases such as respiratory, enteric, cardiovascular and neurological disorders. Originally, coronaviruses were classified on the basis of antigenic cross-reactivity, and three antigenic groups were recognized. When coronavirus genome sequences began to accumulate, the original antigenic groups were converted into genetic groups based on similarity of the nucleotide sequences [1,2].

Clinical Manifestations

Coronaviruses received relatively little attention as human pathogens, as they were considered to be common cold viruses. Inoculation of HCoV-229E and HCoV-OC43 in healthy volunteers revealed that infection with these viruses causes common cold, but more severe lower respiratory tract infections (LRTI) were observed in infants and immunocompromized persons [3-19]. Coryza occurs more often in patient with HCoV-229E infection, while HCoV-OC43 positive patients frequently have sore throat manifestations [18]. The SARS epidemic started in the Guandong province of China in 2003. At first it was suggested that known viruses were involved, but soon thereafter SARS-CoV was identified as the responsible pathogen [20-22]. SARS-CoV probably originated from a wild animal reservoir, most likely bats, and was transmitted in a zoonotic event to humans via civet cats that are traded as food in China [23]. The short but wide-spread epidemic was terminated at the end of 2003 and has not returned thereafter [24]. The last two viruses: HCoV-NL63 and HCoV-HKU1 were identified in 2004 and 2005, respectively [25,26]. Infection by these viruses probably displays a spectrum of diseases similar to HCoV-229E and HCoV-OC43 infection. A clear link between HCoV-NL63 and respiratory diseases was established in a prospective population-based study (PRI.DE) on LRTI in children less than three years of age in Germany [27,28]. Of the children with HCoV-NL63 infection, 45% had laryngotracheitis (croup) compared to only 6% in the control group. Multivariate analysis demonstrated that the chance of croup is 6.6 times higher in HCoV-NL63-positive children than in HCoV-NL63-negative children [9]. The respiratory symptoms accompanying an HCoV-HKU1 infection are usually rhinorrhoea, fever, cough, febrile seizure and wheezing, and disease manifestations like bronchiolitis and pneumonia [14]. It has been suggested that HCoV-HKU1 might also trigger gastrointestinal disease [16].

*Address correspondence to this author at the Laboratory of Experimental Virology, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands; Tel: +31 20 566 75 10; Fax: +31 20 691 65 31; E-mail: k.a.pyrc@gmail.com

Genome Structure

The coronaviral genome encompasses 27 - 32 kb and contains at least seven open reading frames (ORF) and untranslated regions at the 5' and 3' ends. The two large 5' terminal ORFs 1a and 1ab encode non-structural proteins that are required for viral replication. The remaining ORF's in the 3' part of the genome encode the four structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N) for group I coronaviruses and an additional haemaglutinin esterase (HE) protein for group II coronaviruses. Between the structural protein genes several accessory ORF's are located. The position and order of these ORF's varies between species [25]. Recent studies have shown that some of the accessory proteins of SARS-CoV are structural proteins that are incorporated in the virion [29-31].

Replication Cycle

Coronavirus infection starts with the recognition of a specific receptor on the host cell surface by the coronaviral S protein, followed by internalization of the virion core either by direct fusion of the viral membrane with the plasma membrane or via endocytosis [32,33]. Viral RNA is released into the cytoplasm and "cap-dependent" initiation of translation results in the immediate expression of viral proteins and subsequent RNA replication [34]. Full-length genomic RNA and a nested set of subgenomic mRNAs (sg mRNA) are generated in the membrane-associated replication centers (double membrane vesicles, DMV's) [35]. These sg mRNAs are functional templates for translation of structural proteins. Full-length viral RNA which is bound to the N protein is encapsidated in newly assembled virion particles that are released from the host cell via exocytosis or apoptosis.

1871-5265/07 $50.00+.00

ANTIVIRAL AGENTS TARGETING DIFFERENT STEPS OF THE VIRAL REPLICATION CYCLE

Cell Entry

The coronavirus S protein is a 180 – 200 kDa type I membrane glycoprotein, incorporated in a virion as a surface protein. The S protein is composed of a short C-terminal tail inside the virion, a single transmembrane domain, and a rod - like domain (S2) with a globular "head" (S1). The main function of the S protein is binding to the cellular receptor by the receptor binding site (RBS) on the S1 domain, resulting in fusion with the cellular membrane. Group II coronavirus S proteins contain a furin cleavage site between S1 and S2, and cleavage of the S protein is critical to reach an active conformation [36,37]. The receptor specificity varies between species, especially for group II viruses: MHV uses CEACAM-1 [38], HCoV-OC43 and BCoV use O-acetylated sialic acid [39] and SARS-CoV uses angiotensin converting enzyme 2 (ACE2) [40]. Before the discovery of HCoV-NL63, it was generally thought that all group I coronaviruses use CD13 (also known as aminopeptidase N) as receptor, because all representatives (HCoV-229E, porcine, feline and canine coronaviruses) engage this molecule for cell entry [41-44]. However, HCoV-NL63 does not use CD13, but ACE2 as receptor [45].

After binding of S to the receptor enveloped viruses employ two different strategies to enter their target cells. One involves fusion of viral / cellular membrances on the cell surface while the second strategy uses endocytosis and subsequent acidification of the environment, which is needed directly or indirectly (by pH dependent activation of endosomal proteinases) for virion / cell fusion.

Inhibition of Cell Attachment

The S protein of coronaviruses is highly glycosylated. This carbohydrate shield may be used as a target for compounds specifically binding to sugar moieties, e.g. lectins, thus coating the protein and blocking the interaction with the receptor. Plant lectins are known to inhibit the infection of viruses that contain a glycosylated envelope such as human immunodeficiency virus type 1, cytomegalovirus and human T-cell leukemia virus type I [46-48]. The mannose-specific plant lectins derived from *Galanthus nivalis* (Common Snowdrop), *Hippeastrum hybrid* (Amaryllis) and *Allium porrum* (leek) inhibit replication of SARS-CoV and feline coronavirus [49,50]. These lectins indeed block the S - receptor interaction as inhibition is observed at a very early stage of replication [49].

A more specific inhibitory approach is provided by specific monoclonal or polyclonal antibodies directed to the RBS of the S protein or the domain of the cellular receptor that is recognized by the S protein. Several reports describe RBS - specific antibodies that efficiently inhibit receptor binding [51-55]. These antibodies can be generated by immunization of laboratory animals with proteins [51-53,56] or complete viruses [54,57] and subsequent purification of specific antibodies. Alternatively, *in vitro* generation of human antibodies is possible based on antibody phage display library screening [58-60]. The CR304 human monoclonal antibody (mAb) and 80R mAb immunoglobulin IgG1 are potent SARS-CoV-RBS binders that block the S – ACE2 interaction [51,60]. These mAbs can limit SARS-CoV infection below the detection levels in an *in vivo* mouse and ferret model [60-62]. A broader protection is provided by polyclonal antibodies, as the possible selection of viral escape mutants is less likely [63,64].

Only few studies have tested monoclonal and polyclonal antibodies against the other human coronaviruses. Inhibition of HCoV-229E infection can be achieved *in vitro* by polyclonal antibodies directed to complete virus or the RBS, and also a monoclonal antibody directed to the HCoV-229E receptor, aminopeptidase N, inhibits HCoV-229E replication [65]. HCoV-NL63 can be neutralized by pooled immunoglobulins (IVIG) [63].

Small-molecular-weight inhibitors interacting with the viral receptor provide another therapeutic option, e.g. N-(2-aminoethyl)-1 aziridine-ethanamine (NAAE), which binds to the ACE2 protein [66].

Another approach to influence coronavirus entry is to induce downregulation of the receptor on the cell surface. Treatment of Vero E6 cells with interleukin 4 and interferon γ decreases the expression of ACE2 at the transcriptional level, thus limiting the susceptibility of the cells to SARS-CoV infection [67]. Although these results were presented only for SARS-CoV, it might be expected that HCoV-NL63 infection will also be restricted since this virus uses the same receptor. The clinical consequences of such a treatment are currently not known. ACE2 together with ACE are involved in regulation of the renin–angiotensin system and have an important role in maintaining blood pressure homeostasis, as well as fluid and salt balance [68-70]. Moreover, ACE2 is involved in protection against lung damage [71,72], and decreasing ACE2 levels or inactivating ACE2 as part of a SARS treatment can therefore have serious adverse effects.

Inhibition of Virus – Cell Fusion

A second step of coronavirus cell entry is the virion fusion with the host cell membrane and release of the virion core with the RNA genome into the cytoplasm. Depending on the virus species, the fusion can occur directly on the cell surface or may require internalization into the endosome [37]. Treatment with inhibitors of vacuolar acidification like ammonium chloride, chloroquine (an anti malaric drug, approved for use in humans) or bafilomycin A, demonstrated that HCoV-229E and SARS-CoV require the endosomal pathway for entry [33,73]. HCoV-NL63 may engage a different route for entry since vacuolar acidification inhibitors have only limited influence [73,74]. Thus, inhibition of the fusion process with chloroquine has limitations and may be applied only for some coronaviruses [33,63,73].

The S proteins of coronaviruses exhibit a characteristic membrane fusion mechanism that is driven by conformational changes in the S protein, involving rearrangement and interaction of heptad repeat regions (HR1 and HR2) located in the S2 domain. Association of the HR1 and HR2 domains into a 6-helix bundle triggers membrane fusion. Characteristic for group I viruses is the insertion of 14 additional amino acid in both HR regions, when compared to group II viruses [75]. Several reports showed that treatment with synthetic peptides corresponding to the HR1 and HR2 regions results in profound inhibition of SARS-CoV replication [76-78]. For non-SARS human coronaviruses such a study was performed only for HCoV-NL63 and a clear inhibition was observed *in vitro* with a synthetic HR2 peptide, illustrating that group I coronaviruses use the same fusion mechanism as group II viruses [63]. Such an antiviral approach is not a novelty, as it was presented for several other viruses, including the T20 peptide that has been approved as anti – HIV-1 drug [79]. Besides inhibition by HR – derived peptides, the association between HR1 and HR2 can also be blocked by HR - specific antibodies [80-83].

Furthermore, Sainz *et al.* developed peptide fusion inhibitors based on non-HR regions of S2. Five regions within the SARS-CoV S2 subunit with a high propensity to interact with the lipid interface of membranes were identified and peptides analogous to these regions are effective inhibitors of SARS-CoV [84].

Coronaviral Transcription

Coronavirus replication is a complex, not yet fully understood process [85,86]. The 5' end of coronavirus genomic RNA contains the untranslated leader sequence that ends with the Transcription Regulation Sequence (TRS) element. These sequence elements are also present upstream of each ORF (body TRSs). During trans-

cription the RNA-dependent RNA-polymerase (RdRp) has been proposed to pause once one of the body TRS is copied during minus strand synthesis. Subsequent strand transfer to the leader TRS adds a common leader sequence to each minus strand sg mRNA [87]. This discontinuous transcription mechanism is based on base-pairing of the nascent minus strand copy RNA with the plus strand leader TRS [88-91].

Polymerase Inhibitors

All coronaviruses encode their own RdRp. The RdRp gene is located partially in the 1a ORF and mostly in the 1b ORF [92]. The enzymatic activity of the RdRp protein and membrane-associated RdRp activity was demonstrated [93,94]. The polymerase is the most conserved protein among coronaviral species, providing a convenient target for broad anti-coronaviral drugs. Unfortunately, no RdRp specific inhibitors have been developed according to our knowledge.

In silico molecular docking studies to identify potential inhibitors have been performed for the aurintricarboxylic acid (ATA) and RdRp protein, showing a possible interaction [95]. *In vitro* experiments with ATA indeed showed inhibition of SARS-CoV and HCoV-NL63, but *in vitro* studies with the purified enzyme have not been performed [63,96]. Nucleoside analogues that might be considered as RdRp inhibitors are described below.

Nucleoside Analogues

As mentioned above, the coronaviral positive-strand RNA is copied by the viral RdRp via a negative-strand intermediate. Nucleoside analogues can inhibit virus replication by interference with various processes, e.g. they can be incorporated in the new strand and cause chain termination [97] or decrease the processivity and fidelity of transcription, resulting in an "error catastrophy" [98]. Furthermore, several pyrimidine ribonucleoside analogs can also act as antimetabolites, inhibiting UMP synthase and thereby interfering with the UTP metabolism [99]. Nucleoside analogues are known for their inhibition of several types of viruses, including HIV, pestivirus, hepacivirus, flaviviruses, hepatitis C virus and West Nile virus [100-105]. Only a few nucleoside inhibitors can in fact inhibit the replication of coronaviruses. The most promising drug candidates are β-D-N4-hydroxycytidine [106] and 6-azauridine [63,107] that can inhibit SARS-CoV and HCoV-NL63 replication *in vitro*.

Ribavirin, a purine analogue, was incorporated in clinical practice during the SARS-CoV outbreak because of its broad antiviral activity against numerous RNA viruses. However, the anti-coronaviral activity of this compound is rather poor [98,108,109]. *In vitro* inhibition of SARS-CoV indicated that the IC_{50} value is relatively high, whereas no inhibitory action was observed on HCoV-NL63 [63,107,110,111]. Also experimental therapy of SARS patients with this compound showed no positive effect [109]. Another widely known compound, the imidazole nucleoside analogue mizoribine [112-118], exhibits only marginal anti - coronavirus activity [111].

Helicase Inhibitors

The coronaviral helicase domain is enzymatically active and efficiently unwinds ds DNA and ds RNA [119,120]. The helicase domain is encoded in ORF1b by the nsp13 gene, which is located downstream of the RdRp gene. Coronaviral helicase shares sequence similarity with the superfamily 1 helicases. This enzyme family is common among viruses, being encoded by e.g. alphaviruses, rubiviruses, hepatitis E viruses and arteriviruses [121,122]. Biochemical and genetic data suggest a role of these proteins in diverse aspects of viral replication: transcription, RNA stability, cell-to-cell movement and virus biogenesis [123-129]. Adamantane-derived bananins are potent inhibitors of SARS-CoV helicase activity and replication. Six bananin derivatives have been tested in an ATPase assay: bananin, iodobananin, adeninobananin, vanillinbananin, eubananin, and ansabananin. Iodobananin and vanillinbananin appeared to be the most effective SARS-CoV helicase inhibitors. Unfortunately, only bananin was tested against SARS-CoV replication *in vitro* and exhibited a reasonable inhibitory potency in the micromolar range [130].

Small Interfering RNA and Antisense Phosphorodiamidate Morpholino Oligomers

RNA interference (RNAi) is a mechanism for sequence-specific mRNA degradation by dsRNA molecules. Long dsRNA molecules are recognized and cleaved into short 21-24 nt fragments by an endogenous DICER enzyme with RNase III activity. The resulting small interfering (si) RNA molecules are incorporated into the RNA-induced silencing complex (RISC), which degrades mRNA molecules with perfect sequence complementarity to the siRNA. Antiviral RNAi can be induced by synthetic siRNA that is incorporated directly into RISC [131]. Several groups developed effective siRNA's, against different regions of a coronavirus genome, including the 5' leader genome sequence [132,133], TRS region [133], nsp1 gene [134], nsp12 gene [135], RdRp gene [136,137], S gene [63,135,138-140], E [140,141], M [140,141], N gene [140-143] and 3' UTR [133]. The effectiveness of siRNAs against respiratory tract diseases in a therapeutic setting was demonstrated recently by the intranasal administration of siRNA targeting respiratory syncytial virus, parainfluenzavirus, and SARS-CoV, with or without transfection reagents, in mouse and monkey models (9, 39, 59). An inhaled siRNA coctail in low doses may offer a fast, potent, and easily administered antiviral tool to control coronaviral infection in humans. Although the field is moving fast, to date no RNAi therapy is approved for use in humans [144-146].

A similar approach, targeting directly the viral RNA, employs antisense phosphorodiamidate morpholino oligomers (PMO). While RNAi is based on degradation of the viral RNA, PMO's bind to complementary sequences and form a steric block [147]. PMO's that efficiently inhibit SARS-CoV replication *in vitro* target the ORF 1a initiation codon, ORF 1a/1b pseudoknot structure, TRS, S2M motif, 3'-UTR pseudoknot structure, and 3' UTR [148].

Agents Targeting Translation and Protein Processing

During replication of coronaviruses, several structural and non-structural proteins are produced. Translation of coronaviral proteins is a rather difficult target for therapy, as it employs a cellular mechanism [149]. In contrast, a therapeutic window is presented by the post-translational processing of the 1a / 1ab polyprotein, which is performed exclusively by viral proteinases. This proteolytic processing is a key regulatory mechanism in the expression of the replicative proteins. Three proteinases are encoded by the N-terminal part of the 1a/1ab polyprotein. Two papain-like proteinase domains ($PL1^{pro}$ and $PL2^{pro}$) are encoded by the nsp3 gene and process 3 cleavage sites, including an autoproteolytic release [150]. The second proteinase of HCoV-NL63 is named chymotrypsin-like proteinase or "main proteinase" (M^{pro}) to stress its dominant function. M^{pro} is encoded by nsp5 and recognizes 11 cleavage sites in the 1ab polyprotein.

Main Proteinase Inhibitors

The coronaviral M^{pro} displays very low amino acid variability between coronavirus species and is therefore a convenient target for broad range inhibitors. Several studies have described potent inhibition of the M^{pro} enzyme by different compounds [151-177]. As the crystal structure is solved for SARS-CoV M^{pro} [178], the most popular method for *de novo* identification of inhibitors is computer-based docking of existing drugs and compounds, followed by an *in vitro* M^{pro} activity assay [151-154]. Furthermore, there are several computational methods that allow an efficient in silico structure – based screening. Despite the power of these methods, only a few agents identified this way were able to inhibit SARS-CoV M^{pro} in the micromolar range [151,155,179].

Agents inhibiting M^{pro} are either peptide-based inhibitors or small non-peptide compounds, both binding (reversibly or irreversibly) to the catalytic site of M^{pro} and thus inhibiting its activity. Unfortunately, most studies have focused exclusively on SARS-CoV, while these proteinase inhibitors may have the capacity to inhibit all coronaviruses. As an exception, a broad approach was presented by Yang *et al*, who described several M^{pro} inhibitors targeting all group I and II human coronaviruses [156].

Papain – Like Proteinase Inhibitors

The papain-like proteinase (PL^{pro}) consists of two domains: $PL1^{pro}$ and $PL2^{pro}$, of which in most cases both have catalytic activity except for SARS-CoV and IBV, which lack the $PL1^{pro}$ [119,180-187]. PL^{pro}s cut between two small amino acids with short uncharged side chains [188-191].

Coronaviral PL^{pro} is a multi-functional protein with catalytic domains that mediate different enzymatic activities. The PL^{pro} protein is a more difficult target for antiviral agents because of its high variability. One study describes that zinc ion and its conjugates can act as inhibitors of the SARS-CoV Cys-His catalytic dyad activity. No such assays have been performed for other coronaviruses [192].

ANTIVIRAL AGENTS WITH UNSPECIFIED MODE OF ACTION

Steroids

Proinflammatory cytokines released by stimulated macrophages in the alveoli play a prominent role in SARS pathogenesis, resulting in cytokine dysregulation [193,194]. During the SARS-CoV outbreak in 2003 several patients received anti-inflammatory corticosteroids that may reduce the damaging effect of the local inflammatory response [195]. Steroids inhibit cytokine, chemokine and adhesion molecule production and antagonize the action of proinflammatory cytokines by interfering with the Jak-STAT intracellular signalling pathways. In several studies early use of high-dose steroids (methylprednisolone) resulted in improvement of clinical symptoms, decreased incidence of acute respiratory distress syndrome and mortality [196-198]. However, other studies show lack of direct effect of steroid treatment in SARS patients [194,199,200]. It is also worth mentioning that immunosupression caused by steroid treatment may increase the danger of secondary infections, in fact occasionally increasing the mortality in SARS patients [201].

Interferons

Interferons (IFN) play a key role in the antiviral defense by activation of the innate immune system in response to the presence of dsRNA. There are several hundreds of genes transcriptionally regulated by IFNs in response to viral invasion. Additionally, type I IFNs (α and β) upregulate surface expression of MHC class I and II molecules, enhancing the presentation of viral antigens [202-204].

The employment of exogenous IFN as an antiviral agent has been described for animal coronaviruses [205-208] and the first study on human coronaviruses appeared already in 1983 [209]. An antiviral effect of recombinant human leukocyte IFN-α was observed in healthy volunteers challenged with HCoV-229E. The percentage of individuals that developed respiratory illness was significantly lower for the group treated with IFN (5.7%) compared to the control group (37.0%). Another study presented the inhibitory effect of IFN-α delivered intranasally. The majority of the placebo recipients (73%) developed a cold, whereas only 41% of interferon recipients developed disease [210]. The same effect of type I IFNs has been observed for SARS-CoV both *in vitro* and *in vivo* [110,211-214], but type II IFN (IFN-γ) is a poor inhibitor of SARS-CoV replication [215]. Although IFN-γ by itself appeared to have little inhibitory effect, it acted synergistically in combination with IFN β [216,217]. A similar synergistic effect was observed during treatment with IFN-β and ribavirin [218,219].

Still, the *in vivo* data is limited. Prophylactic treatment of SARS-CoV infected macaques with pegylated IFN-α significantly reduced viral replication, excretion and pulmonary damage, but post-infection treatment yielded only intermediate protection. Moreover, treatment with IFN-α during the SARS-CoV outbreak showed no benefit [196].

Nitric Oxide

Nitric oxide (NO) is an important cell-to-cell signalling molecule and is involved in a wide range of biological processes [220]. It also plays a key role in the host defence against bacteria, protozoa, and tumour cells. Antiviral activity of NO has been described for several viruses [221-224], but the antiviral mechanism is not known. Several NO donors (DETA NONOate, SNAP) can inhibit SARS-CoV, but not HCoV-NL63 [63,107,225]. It has been suggested that IFN-γ activity against SARS-CoV is partially due to its stimulatory effects on inducible NO synthase (iNOS) expression [226]. Clinical trials in Beijing during the SARS-CoV epidemic suggested that inhalation of NO gas may improve the patient's outcome [227].

Calpain Inhibitors

Calpains are mammalian intracellular calcium-dependent cysteine proteinases [228]. Calpain plays a regulatory role in membrane and cytoskeletal remodeling, including mitosis and apoptosis regulation [229]. It was demonstrated that two commercially available calpain inhibitors (calpain inhibitor III and VI) inhibit SARS-CoV replication in cell culture [106], but they failed to inhibit HCoV-NL63 replication [63].

Herbs

Several compounds of herbal origin have strong anti-coronaviral activity. Glycyrrhizin is a bioactive compound isolated from the liqorice root [230]. It exhibits anti-tumoural, anti-inflammatory and antiviral properties [231-236]. The mechanism of glycyrrhizin's activity against SARS-CoV [107,219] is unclear as the compound affects many cellular signalling pathways [237-240]. One possible mode of action includes stimulation of iNOS expression and subsequent increase of the NO concentration in treated cells [241]. The advantage of glycyrrhizin treatment is that it has been approved as an intravenous drug and it is commercially available as SNMC (Stronger Neo-Minophagen C). [242]. Glycyrrhizin is inactive against HCoV-NL63 [63].

There are several other examples of plant-derived compounds that can inhibit coronavirus replication. Baicalin is a flavonoid compound derived from *Scutellaria baicalensis* that inhibits SARS-CoV *in vitro* [219]. Escin, active against SARS-CoV but not HCoV-NL63, is an approved drug derived from the horse chestnut tree [63,157]. Reserpine, a naturally occurring alkaloid produced by several members of the *Rauwolfia* genus, also inhibits SARS-CoV replication [157]. Several other plant compounds have been mentioned in other sections e.g. quercetine, isatin and mannose binding lectins, illustrating the potency of this natural reservoir.

Antibiotics

Glycopeptide antibiotics are commonly used for treatment of bacterial infections. They alter the cell wall characteristics and interfere with DNA and RNA synthesis. But these compounds can also inhibit several animal and human viruses by inhibiting virus replication or interfering with proper glycosylation of viral proteins, e.g. bleomycin for Rauscher murine leukemia virus [243], tunicamycin for herpex simplex virus [244] and teicoplanin, DA40 and DMDA40, vancomycin, eremomycin and their derivatives for HIV-1 and HIV-2 [245,246]. Two compounds – eremomycin and vancomycin - also display anti SARS-CoV activity [247].

Another antibiotic, actinomycin D, the first antibiotic shown to have anti-cancer activity, can inhibit replication of HCoV-229 [248]. However, the high cytotoxicity of the compound limits its usability as an anticoronaviral drug. Valinomycin, a cyclododecadepsipeptide antibiotic that is a natural product of Streptomyces [249], possesses antifungal, insecticidal-nematocidal, and antibacterial activities. It was recently demonstrated to exhibit *in vitro* anti SARS-CoV activity [157,250]. No inhibitory effect of actinomycin D or valinomycin was observed for HCoV-NL63 [63].

6. FUTURE DIRECTIONS

The outbreak of SARS-CoV in 2003 brought the coronaviruses back in the spotlight. The sudden appearance of SARS-CoV and the subsequent SARS epidemic met us unprepared. The current status of the anticoronaviral drugs has improved, but the majority of studies focus exclusively on compounds that target SARS-CoV. Realizing that many undiscovered coronaviruses may reside in animal reservoirs, this approach seems too restricted, leaving us unprepared for a future strike by another coronavirus species. Broadly active anticoronaviral compounds should be designed to quickly respond to new zoonotic epidemics of pathogenic coronaviruses. The antivirals should be potent, but some toxicity may be allowed.

Future studies on anticoronavirals should also focus on a preventive therapy for known coronaviral pathogens and quick-response drugs with specificity and broad activity. The major requirement for these drugs is lack of toxicity or side effects, considering the fact that these infections are relatively mild and very common. Of special interest are the natural compounds used for several years in folk medicine (e.g. leek, lucretia, garlic extracts, Chinese herbs). Unfortunately, there is no coherent study on activity of these compounds against different coronaviruses. Another option is the development of a broad siRNA cocktail against variety of respiratory viruses delivered by inhalation.

ABBREVIATIONS

ACE2	=	Angiotensin converting enzyme 2
ATA	=	Aurintricarboxylic acid
DMV	=	Double membrane vesicle
E	=	Envelope protein/gene
HE	=	Haemaglutinin esterase protein/gene
HCoV	=	Human coronavirus
HR	=	Heptad repeat
IFN	=	Interferon
iNOS	=	Inducible NO synthase
IVIG	=	Intravenous immunoglobulins
LRTI	=	Lower respiratory tract infection
M	=	Membrane protein/gene
mAb	=	Monoclonal antibody
M^{pro}	=	Main proteinase
N	=	Nucleocapsid protein/gene
NAAE	=	N-(2-aminoethyl)-1 aziridine-ethanamine
NO	=	Nitric oxide
ORF	=	Open reading frame
PL_{pro}	=	Papain-like proteinase domains
PMO	=	Phosphorodiamidate morpholino oligomers
RBS	=	Receptor binding site
RdRp	=	RNA-dependent RNA-polymerase
RISC	=	RNA-induced silencing complex
RNAi	=	RNA interference
S	=	Spike protein/gene
SARS-CoV	=	Severe acute respiratory syndrome coronavirus
sg mRNA	=	Subgenomic mRNA
siRNA	=	Small interfering RNA
TRS	=	Transcription Regulation Sequence

REFERENCES

[1] Gonzalez, J.M.; Gomez-Puertas, P.; Cavanagh, D.; Gorbalenya, A.E.; Enjuanes, L. *Arch. Virol.*, **2003**, *148*, 2207.
[2] Holmes, K.V.; Lai, M.M.C. In *Fields Virology* Fields, Knipe, Howley, Eds.; Lippincott-Raven Publishers: Philadelphia, **1996**; pp. 1075-1093.
[3] Baker, S.C. *Pediatr. Infect. Dis. J.*, **2004**, *23*, 1049.
[4] McIntosh, K. *J. Infect. Dis.*, **2005**, *191*, 489.
[5] Ebihara, T.; Endo, R.; Ma, X.; Ishiguro, N.; Kikuta, H. *J. Med. Virol.*, **2005**, *75*, 463.
[6] Arden, K.E.; Nissen, M.D.; Sloots, T.P.; Mackay, I.M. *J. Med. Virol.*, **2005**, *75*, 455.
[7] Bastien, N.; Robinson, J.L.; Tse, A.; Lee, B.E.; Hart, L.; Li, Y. *J. Clin. Microbiol.*, **2005**, *43*, 4567.
[8] Chiu, S.S.; Chan, K.H.; Chu, K.W.; Kwan, S.W.; Guan, Y.; Poon, L.L.; Peiris, J.S. *Clin. Infect. Dis.*, **2005**, *40*, 1721.
[9] van der Hoek, L.; Sure, K.; Ihorst, G.; Stang, A.; Pyrc, K.; Jebbink, M.F.; Petersen, G.; Forster, J.; Berkhout, B.; Uberla, K. *PLoS. Med.*, **2005**, *2*, e240.
[10] Kaiser, L.; Regamey, N.; Roiha, H.; Deffernez, C.; Frey, U. *Pediatr. Infect. Dis. J.*, **2005**, *24*, 1015.
[11] Lau, S.K.; Woo, P.C.; Yip, C.C.; Tse, H.; Tsoi, H.W.; Cheng, V.C.; Lee, P.; Tang, B.S.; Cheung, C.H.; Lee, R.A.; So, L.Y.; Lau, Y.L.; Chan, K.H.; Yuen, K.Y. *J. Clin. Microbiol.*, **2006**, *44*, 2063.
[12] Choi, E.H.; Lee, H.J.; Kim, S.J.; Eun, B.W.; Kim, N.H.; Lee, J.A.; Lee, J.H.; Song, E.K.; Kim, S.H.; Park, J.Y.; Sung, J.Y. *Clin. Infect. Dis.*, **2006**, *43*, 585.
[13] Garbino, J.; Crespo, S.; Aubert, J.D.; Rochat, T.; Ninet, B.; Deffernez, C.; Wunderli, W.; Pache, J.C.; Soccal, P.M.; Kaiser, L. *Clin. Infect. Dis.*, **2006**, *43*, 1009.
[14] Sloots, T.P.; McErlean, P.; Speicher, D.J.; Arden, K.E.; Nissen, M.D.; Mackay, I.M. *J. Clin. Virol.*, **2006**, *35*, 99.
[15] Woo, P.C.; Lau, S.K.; Tsoi, H.W.; Huang, Y.; Poon, R.W.; Chu, C.M.; Lee, R.A.; Luk, W.K.; Wong, G.K.; Wong, B.H.; Cheng, V.C.; Tang, B.S.; Wu, A.K.; Yung, R.W.; Chen, H.; Guan, Y.; Chan, K.H.; Yuen, K.Y. *J. Infect. Dis.*, **2005**, *192*, 1898.
[16] Vabret, A.; Dina, J.; Gouarin, S.; Petitjean, J.; Corbet, S.; Freymuth, F. *Clin. Infect. Dis.*, **2006**, *42*, 634.
[17] Tyrrell, D.A.; Bynoe, M.L. *Br. Med. J.*, **1965**, *5448*, 1467.
[18] Reed, S.E. *J. Med. Virol.*, **1984**, *13*, 179.
[19] McIntosh, K.; Chao, R.K.; Krause, H.E.; Wasil, R.; Mocega, H.E.; Mufson, M.A. *J. Infect. Dis.*, **1974**, *130*, 502.
[20] Drosten, C.; Gunther, S.; Preiser, W.; van der Werf, S.; Brodt, H.R.; Becker, S.; Rabenau, H.; Panning, M.; Kolesnikova, L.; Fouchier, R.A.; Berger, A.; Burguiere, A.M.; Cinatl, J.; Eickmann, M.; Escriou, N.; Grywna, K.; Kramme, S.; Manuguerra, J.C.; Muller, S.; Rickerts, V.; Sturmer, M.; Vieth, S.; Klenk, H.D.; Osterhaus, A.D.; Schmitz, H.; Doerr, H.W. *N. Engl. J. Med.*, **2003**, *348*, 1967.
[21] Ksiazek, T.G.; Erdman, D.; Goldsmith, C.S.; Zaki, S.R.; Peret, T.; Emery, S.; Tong, S.; Urbani, C.; Comer, J.A.; Lim, W.; Rollin, P.E.; Dowell, S.F.; Ling, A.E.; Humphrey, C.D.; Shieh, W.J.; Guarner, J.; Paddock, C.D.; Rota, P.; Fields, B.; DeRisi, J.; Yang, J.Y.; Cox, N.; Hughes, J.M.; LeDuc, J.W.; Bellini, W.J.; Anderson, L.J. *N. Engl. J. Med.*, **2003**, *348*, 1953.
[22] Peiris, J.S.; Lai, S.T.; Poon, L.L.; Guan, Y.; Yam, L.Y.; Lim, W.; Nicholls, J.; Yee, W.K.; Yan, W.W.; Cheung, M.T.; Cheng, V.C.; Chan, K.H.; Tsang, D.N.; Yung, R.W.; Ng, T.K.; Yuen, K.Y. *Lancet*, **2003**, *361*, 1319.
[23] Lau, S.K.; Woo, P.C.; Li, K.S.; Huang, Y.; Tsoi, H.W.; Wong, B.H.; Wong, S.S.; Leung, S.Y.; Chan, K.H.; Yuen, K.Y. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 14040.
[24] Peiris, J.S.; Guan, Y.; Yuen, K.Y. *Nat. Med.*, **2004**, *10*, S88.
[25] van der Hoek, L.; Pyrc, K.; Jebbink, M.F.; Vermeulen-Oost, W.; Berkhout, R.J.; Wolthers, K.C.; Wertheim-van Dillen, P.M.; Kaandorp, J.; Spaargaren, J.; Berkhout, B. *Nat. Med.*, **2004**, *10*, 368.
[26] Woo, P.C.; Lau, S.K.; Chu, C.M.; Chan, K.H.; Tsoi, H.W.; Huang, Y.; Wong, B.H.; Poon, R.W.; Cai, J.J.; Luk, W.K.; Poon, L.L.; Wong, S.S.; Guan, Y.; Peiris, J.S.; Yuen, K.Y. *J. Virol.*, **2005**, *79*, 884.
[27] Forster, J.; Ihorst, G.; Rieger, C.H.; Stephan, V.; Frank, H.D.; Gurth, H.; Berner, R.; Rohwedder, A.; Werchau, H.; Schumacher, M.; Tsai, T.; Petersen, G. *Eur. J. Pediatr.*, **2004**, *163*, 709.
[28] Konig, B.; Konig, W.; Arnold, R.; Werchau, H.; Ihorst, G.; Forster, J. *J. Clin. Microbiol.*, **2004**, *42*, 4632.
[29] Schaecher, S.R.; Mackenzie, J.M.; Pekosz, A. *J. Virol.*, **2007**, *81*, 718.
[30] Huang, C.; Ito, N.; Tseng, C.T.; Makino, S. *J. Virol.*, **2006**, *80*, 7287.
[31] Huang, C.; Narayanan, K.; Ito, N.; Peters, C.J.; Makino, S. *J. Virol.*, **2006**, *80*, 210.
[32] Kooi, C.; Cervin, M.; Anderson, R. *Virology*, **1991**, *180*, 108.
[33] Blau, D.M.; Holmes, K.V. *Adv. Exp. Med. Biol.*, **2001**, *494*, 193.

[34] Lai, M.M.; Cavanagh, D. *Adv. Virus Res.*, **1997**, *48*, 1.
[35] Brockway, S.M.; Clay, C.T.; Lu, X.T.; Denison, M.R. *J. Virol.*, **2003**, *77*, 10515.
[36] de Haan, C.A.; Stadler, K.; Godeke, G.J.; Bosch, B.J.; Rottier, P.J. *J. Virol.*, **2004**, *78*, 6048.
[37] Simmons, G.; Gosalia, D.N.; Rennekamp, A.J.; Reeves, J.D.; Diamond, S.L.; Bates, P. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 11876.
[38] Williams, R.K.; Jiang, G.S.; Holmes, K.V. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 5533.
[39] Vlasak, R.; Luytjes, W.; Spaan, W.; Palese, P. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 4526.
[40] Li, W.; Moore, M.J.; Vasilieva, N.; Sui, J.; Wong, S.K.; Berne, M.A.; Somasundaran, M.; Sullivan, J.L.; Luzuriaga, K.; Greenough, T.C.; Choe, H.; Farzan, M. *Nature*, **2003**, *426*, 450.
[41] Yeager, C.L.; Ashmun, R.A.; Williams, R.K.; Cardellichio, C.B.; Shapiro, L.H.; Look, A.T.; Holmes, K.V. *Nature*, **1992**, *357*, 420.
[42] Oh, J.S.; Song, D.S.; Park, B.K. *J. Vet. Sci.*, **2003**, *4*, 269.
[43] Tresnan, D.B.; Levis, R.; Holmes, K.V. *J. Virol.*, **1996**, *70*, 8669.
[44] Delmas, B.; Gelfi, J.; L'Haridon, R.; Vogel, L.K.; Sjostrom, H.; Noren, O.; Laude, H. *Nature*, **1992**, *357*, 417.
[45] Hofmann, H.; Pyrc, K.; van der Hoek, L.; Geier, M.; Berkhout, B.; Pohlmann, S. *Proc. Natl. Acad. Sci. U. S. A*, **2005**, *102*, 7988.
[46] Spear, G.T.; Zariffard, M.R.; Xin, J.; Saifuddin, M. *Immunology*, **2003**, *110*, 80.
[47] Balzarini, J.; Schols, D.; Neyts, J.; Van Damme, E.; Peumans, W.; De Clercq, E. *Antimicrob. Agents Chemother.*, **1991**, *35*, 410.
[48] Yang, D.; Haraguchi, Y.; Iwai, H.; Handa, A.; Shimizu, N.; Hoshino, H. *Int. J. Cancer*, **1994**, *56*, 100.
[49] Balzarini, J.; Vijgen, L.; Keyaerts, E.; Van Damme, E.; Peumans, W.; De Clercq, E.; Egberink, H.; Van Ranst, M. In *Abstracts of the 17th International Conference on Antiviral Research.* Antiviral Research: Tucson, USA, 2004; pp. A27-90.
[50] Vijgen, L.; Keyaerts, B.; Van Damme, E.; Peumans, W.; De Clercq, E.; Balzarini, J.; Van Ranst, M. *Antiviral Res.*, **2004**, *123*, A76.
[51] Sui, J.; Li, W.; Murakami, A.; Tamin, A.; Matthews, L.J.; Wong, S.K.; Moore, M.J.; Tallarico, A.S.; Olurinde, M.; Choe, H.; Anderson, L.J.; Bellini, W.J.; Farzan, M.; Marasco, W.A. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 2536.
[52] Greenough, T.C.; Babcock, G.J.; Roberts, A.; Hernandez, H.J.; Thomas, W.D., Jr.; Coccia, J.A.; Graziano, R.F.; Srinivasan, M.; Lowy, I.; Finberg, R.W.; Subbarao, K.; Vogel, L.; Somasundaran, M.; Luzuriaga, K.; Sullivan, J.L.; Ambrosino, D.M. *J. Infect. Dis.*, **2005**, *191*, 507.
[53] He, Y.; Lu, H.; Siddiqui, P.; Zhou, Y.; Jiang, S. *J. Immunol.*, **2005**, *174*, 4908.
[54] Tripp, R.A.; Haynes, L.M.; Moore, D.; Anderson, B.; Tamin, A.; Harcourt, B.H.; Jones, L.P.; Yilla, M.; Babcock, G.J.; Greenough, T.; Ambrosino, D.M.; Alvarez, R.; Callaway, J.; Cavitt, S.; Kamrud, K.; Alterson, H.; Smith, J.; Harcourt, J.L.; Miao, C.; Razdan, R.; Comer, J.A.; Rollin, P.E.; Ksiazek, T.G.; Sanchez, A.; Rota, P.A.; Bellini, W.J.; Anderson, L.J. *J. Virol. Methods*, **2005**, *128*, 21.
[55] Hwang, W.C.; Lin, Y.; Santelli, E.; Sui, J.; Jaroszewski, L.; Stec, B.; Farzan, M.; Marasco, W.A.; Liddington, R.C. *J. Biol. Chem.*, **2006**, *281*, 34610.
[56] He, Y.; Zhou, Y.; Liu, S.; Kou, Z.; Li, W.; Farzan, M.; Jiang, S. *Biochem. Biophys. Res. Commun.*, **2004**, *324*, 773.
[57] Chou, T.H.; Wang, S.; Sakhatskyy, P.V.; Mboudjeka, I.; Lawrence, J.M.; Huang, S.; Coley, S.; Yang, B.; Li, J.; Zhu, Q.; Lu, S. *Virology*, **2005**, *334*, 134.
[58] Liang, M.F.; Du, R.L.; Liu, J.Z.; Li, C.; Zhang, Q.F.; Han, L.L.; Yu, J.S.; Duan, S.M.; Wang, X.F.; Wu, K.X.; Xiong, Z.H.; Jin, Q.; Li, D.X. *Biomed. Environ. Sci.*, **2005**, *18*, 363.
[59] Duan, J.; Ji, X.; Feng, J.; Han, W.; Zhang, P.; Cao, W.; Guo, X.; Qi, C.; Yang, D.; Jin, G.; Gao, G.; Yan, X. *Antivir. Ther.*, **2006**, *11*, 117.
[60] Ter Meulen, J.; Bakker, A.B.; van den Brink, E.N.; Weverling, G.J.; Martina, B.E.; Haagmans, B.L.; Kuiken, T.; de Kruif, J.; Preiser, W.; Spaan, W.; Gelderblom, H.R.; Goudsmit, J.; Osterhaus, A.D. *Lancet*, **2004**, *363*, 2139.
[61] Sui, J.; Li, W.; Roberts, A.; Matthews, L.J.; Murakami, A.; Vogel, L.; Wong, S.K.; Subbarao, K.; Farzan, M.; Marasco, W.A. *J. Virol.*, **2005**, *79*, 5900.
[62] Subbarao, K.; McAuliffe, J.; Vogel, L.; Fahle, G.; Fischer, S.; Tatti, K.; Packard, M.; Shieh, W.J.; Zaki, S.; Murphy, B. *J. Virol.*, **2004**, *78*, 3572.
[63] Pyrc, K.; Bosch, B.J.; Berkhout, B.; Jebbink, M.F.; Dijkman, R.; Rottier, P.; van der Hoek, L. *Antimicrob. Agents Chemother.*, **2006**, *50*, 2000.
[64] Ter Meulen, J.; van den Brink, E.N.; Poon, L.L.; Marissen, W.E.; Leung, C.S.; Cox, F.; Cheung, C.Y.; Bakker, A.Q.; Bogaards, J.A.; van Deventer, E.; Preiser, W.; Doerr, H.W.; Chow, V.T.; de Kruif, J.; Peiris, J.S.; Goudsmit, J. *PLoS. Med.*, **2006**, *3*, e237.
[65] Bonavia, A.; Zelus, B.D.; Wentworth, D.E.; Talbot, P.J.; Holmes, K.V. *J. Virol.*, **2003**, *77*, 2530.
[66] Towler, P.; Staker, B.; Prasad, S.G.; Menon, S.; Tang, J.; Parsons, T.; Ryan, D.; Fisher, M.; Williams, D.; Dales, N.A.; Patane, M.A.; Pantoliano, M.W. *J. Biol. Chem.*, **2004**, *279*, 17996.
[67] de Lang, A.; Osterhaus, A.D.; Haagmans, B.L. *Virology*, **2006**,
[68] Crackower, M.A.; Sarao, R.; Oudit, G.Y.; Yagil, C.; Kozieradzki, I.; Scanga, S.E.; Oliveira-dos-Santos, A.J.; da Costa, J.; Zhang, L.; Pei, Y.; Scholey, J.; Ferrario, C.M.; Manoukian, A.S.; Chappell, M.C.; Backx, P.H.; Yagil, Y.; Penninger, J.M. *Nature*, **2002**, *417*, 822.
[69] Skeggs, L.T.; Dorer, F.E.; Levine, M.; Lentz, K.E.; Kahn, J.R. *Adv. Exp. Med. Biol.*, **1980**, *130*, 1.
[70] Boehm, M.; Nabel, E.G. *N. Engl. J. Med.*, **2002**, *347*, 1795.
[71] Imai, Y.; Kuba, K.; Rao, S.; Huan, Y.; Guo, F.; Guan, B.; Yang, P.; Sarao, R.; Wada, T.; Leong-Poi, H.; Crackower, M.A.; Fukamizu, A.; Hui, C.C.; Hein, L.; Uhlig, S.; Slutsky, A.S.; Jiang, C.; Penninger, J.M. *Nature*, **2005**, *436*, 112.
[72] Kuba, K.; Imai, Y.; Rao, S.; Gao, H.; Guo, F.; Guan, B.; Huan, Y.; Yang, P.; Zhang, Y.; Deng, W.; Bao, L.; Zhang, B.; Liu, G.; Wang, Z.; Chappell, M.; Liu, Y.; Zheng, D.; Leibbrandt, A.; Wada, T.; Slutsky, A.S.; Liu, D.; Qin, C.; Jiang, C.; Penninger, J.M. *Nat. Med.*, **2005**, *11*, 875.
[73] Huang, I.C.; Bosch, B.J.; Li, F.; Li, W.; Lee, K.H.; Ghiran, S.; Vasilieva, N.; Dermody, T.S.; Harrison, S.C.; Dormitzer, P.R.; Farzan, M.; Rottier, P.J.; Choe, H. *J. Biol. Chem.*, **2006**, *281*, 3198.
[74] Hofmann, H.; Simmons, G.; Rennekamp, A.J.; Chaipan, C.; Gramberg, T.; Heck, E.; Geier, M.; Wegele, A.; Marzi, A.; Bates, P.; Pohlmann, S. *J. Virol.*, **2006**, *80*, 8639.
[75] Bosch, B.J.; Van Der Zee, R.; de Haan, C.A.; Rottier, P.J. *J. Virol.*, **2003**, *77*, 8801.
[76] Bosch, B.J.; Martina, B.E.; Van Der Zee, R.; Lepault, J.; Haijema, B.J.; Versluis, C.; Heck, A.J.; De Groot, R.; Osterhaus, A.D.; Rottier, P.J. *Proc. Natl. Acad. Sci. U. S. A*, **2004**, *101*, 8455.
[77] Zhu, J.; Xiao, G.; Xu, Y.; Yuan, F.; Zheng, C.; Liu, Y.; Yan, H.; Cole, D.K.; Bell, J.I.; Rao, Z.; Tien, P.; Gao, G.F. *Biochem. Biophys. Res. Commun.*, **2004**, *319*, 283.
[78] Yuan, K.; Yi, L.; Chen, J.; Qu, X.; Qing, T.; Rao, X.; Jiang, P.; Hu, J.; Xiong, Z.; Nie, Y.; Shi, X.; Wang, W.; Ling, C.; Yin, X.; Fan, K.; Lai, L.; Ding, M.; Deng, H. *Biochem. Biophys. Res. Commun.*, **2004**, *319*, 746.
[79] Lambert, D.M.; Barney, S.; Lambert, A.L.; Guthrie, K.; Medinas, R.; Davis, D.E.; Bucy, T.; Erickson, J.; Merutka, G.; Petteway, S.R., Jr. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 2186.
[80] Keng, C.T.; Zhang, A.; Shen, S.; Lip, K.M.; Fielding, B.C.; Tan, T.H.; Chou, C.F.; Loh, C.B.; Wang, S.; Fu, J.; Yang, X.; Lim, S.G.; Hong, W.; Tan, Y.J. *J. Virol.*, **2005**, *79*, 3289.
[81] Lai, S.C.; Chong, P.C.; Yeh, C.T.; Liu, L.S.; Jan, J.T.; Chi, H.Y.; Liu, H.W.; Chen, A.; Wang, Y.C. *J. Biomed. Sci.*, **2005**, *12*, 711.
[82] Lip, K.M.; Shen, S.; Yang, X.; Keng, C.T.; Zhang, A.; Oh, H.L.; Li, Z.H.; Hwang, L.A.; Chou, C.F.; Fielding, B.C.; Tan, T.H.; Mayrhofer, J.; Falkner, F.G.; Fu, J.; Lim, S.G.; Hong, W.; Tan, Y.J. *J. Virol.*, **2006**, *80*, 941.
[83] Tripet, B.; Kao, D.J.; Jeffers, S.A.; Holmes, K.V.; Hodges, R.S. *J. Struct. Biol.*, **2006**, *155*, 176.
[84] Sainz, B., Jr.; Mossel, E.C.; Gallaher, W.R.; Wimley, W.C.; Peters, C.J.; Wilson, R.B.; Garry, R.F. *Virus Res.*, **2006**, *120*, 146.
[85] Sawicki, S.G.; Sawicki, D.L. *J. Virol.*, **1990**, *64*, 1050.
[86] Sawicki, S.G.; Sawicki, D.L. *Adv. Exp. Med. Biol.*, **1998**, *440*, 215.
[87] Spaan, W.; Delius, H.; Skinner, M.; Armstrong, J.; Rottier, P.; Smeekens, S.; van der Zeijst, B.A.; Siddell, S.G. *EMBO J.*, **1983**, *2*, 1839.
[88] Hofmann, M.A.; Chang, R.Y.; Ku, S.; Brian, D.A. *Virology*, **1993**, *196*, 163.
[89] Konings, D.A.; Bredenbeek, P.J.; Noten, J.F.; Hogeweg, P.; Spaan, W.J. *Nucleic Acids Res.*, **1988**, *16*, 10849.
[90] Sethna, P.B.; Hung, S.L.; Brian, D.A. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 5626.
[91] de Haan, C.A.; van Genne, L.; Stoop, J.N.; Volders, H.; Rottier, P.J. *J. Virol.*, **2003**, *77*, 11312.
[92] Lee, H.J.; Shieh, C.K.; Gorbalenya, A.E.; Koonin, E.V.; La Monica, N.; Tuler, J.; Bagdzhadzhyan, A.; Lai, M.M. *Virology*, **1991**, *180*, 567.
[93] Cheng, A.; Zhang, W.; Xie, Y.; Jiang, W.; Arnold, E.; Sarafianos, S.G.; Ding, J. *Virology*, **2005**, *335*, 165.
[94] Gosert, R.; Kanjanahaluethai, A.; Egger, D.; Bienz, K.; Baker, S.C. *J. Virol.*, **2002**, *76*, 3697.
[95] Yap, Y.; Zhang, X.; Andonov, A.; He, R. *Comput. Biol. Chem.*, **2005**, *29*, 212.
[96] He, R.; Adonov, A.; Traykova-Adonova, M.; Cao, J.; Cutts, T.; Grudesky, E.; Deschambaul, Y.; Berry, J.; Drebot, M.; Li, X. *Biochem. Biophys. Res. Commun.*, **2004**, *320*, 1199.
[97] Lai, C.L.; Yuen, M.F. *J. Med. Virol.*, **2000**, *61*, 367.
[98] Crotty, S.; Cameron, C.E.; Andino, R. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 6895.
[99] Cihak, A.; Rada, B. *Neoplasma*, **1976**, *23*, 233.
[100] Barreiro, P.; Garcia-Benayas, T.; Rendon, A.; Rodriguez-Novoa, S.; Soriano, V. *AIDS Rev.*, **2004**, *6*, 234.
[101] Hollecker, L.; Choo, H.; Chong, Y.; Chu, C.K.; Lostia, S.; McBrayer, T.R.; Stuyver, L.J.; Mason, J.C.; Du, J.; Rachakonda, S.; Shi, J.; Schinazi, R.F.; Watanabe, K.A. *Antivir. Chem. Chemother.*, **2004**, *15*, 43.
[102] Morrey, J.D.; Smee, D.F.; Sidwell, R.W.; Tseng, C. *Antiviral Res.*, **2002**, *55*, 107.
[103] Schinazi, R.F.; Mellors, J.; Bazmi, H.; Diamond, S.; Garber, S.; Gallagher, K.; Geleziunas, R.; Klabe, R.; Pierce, M.; Rayner, M.; Wu, J.T.; Zhang, H.; Hammond, J.; Bacheler, L.; Manion, D.J.; Otto, M.J.; Stuyver, L.; Trainor, G.; Liotta, D.C.; Erickson-Viitanen, S. *Antimicrob. Agents Chemother.*, **2002**, *46*, 1394.
[104] Stuyver, L.J.; Lostia, S.; Adams, M.; Mathew, J.S.; Pai, B.S.; Grier, J.; Tharnish, P.M.; Choi, Y.; Chong, Y.; Choo, H.; Chu, C.K.; Otto, M.J.; Schinazi, R.F. *Antimicrob. Agents Chemother.*, **2002**, *46*, 3854.

[105] Stuyver, L.J.; Whitaker, T.; McBrayer, T.R.; Hernandez-Santiago, B.I.; Lostia, S.; Tharnish, P.M.; Ramesh, M.; Chu, C.K.; Jordan, R.; Shi, J.; Rachakonda, S.; Watanabe, K.A.; Otto, M.J.; Schinazi, R.F. *Antimicrob. Agents Chemother.*, **2003**, *47*, 244.

[106] Barnard, D.L.; Hubbard, V.D.; Burton, J.; Smee, D.F.; Morrey, J.D.; Otto, M.J.; Sidwell, R.W. *Antivir. Chem. Chemother.*, **2004**, *15*, 15.

[107] Cinatl, J.; Morgenstern, B.; Bauer, G.; Chandra, P.; Rabenau, H.; Doerr, H.W. *Lancet*, **2003**, *361*, 2045.

[108] Anderson, J.P.; Daifuku, R.; Loeb, L.A. *Annu. Rev. Microbiol.*, **2004**, *58*, 183.

[109] Chiou, H.E.; Liu, C.L.; Buttrey, M.J.; Kuo, H.P.; Liu, H.W.; Kuo, H.T.; Lu, Y.T. *Chest*, **2005**, *128*, 263.

[110] Stroher, U.; DiCaro, A.; Li, Y.; Strong, J.E.; Aoki, F.; Plummer, F.; Jones, S.M.; Feldmann, H. *J. Infect. Dis.*, **2004**, *189*, 1164.

[111] Saijo, M.; Morikawa, S.; Fukushi, S.; Mizutani, T.; Hasegawa, H.; Nagata, N.; Iwata, N.; Kurane, I. *Antiviral Res.*, **2005**, *66*, 159.

[112] Hosoya, M.; Shigeta, S.; Ishii, T.; Suzuki, H.; De Clercq, E. *J. Infect. Dis.*, **1993**, *168*, 641.

[113] Nakagawa, M.; Nakahara, K.; Maruyama, Y.; Kawabata, M.; Higuchi, I.; Kubota, H.; Izumo, S.; Arimura, K.; Osame, M. *J. Neurovirol.*, **1996**, *2*, 345.

[114] Ichikawa, Y.; Kyo, M.; Hanafusa, T.; Kohro, T.; Kishikawa, H.; Fukunishi, T.; Nagano, S.; Shinji, Y. *Transplantation*, **1998**, *65*, 134.

[115] Pancheva, S.; Dundarova, D.; Remichkova, M. *Z. Naturforsch. [C]*, **2002**, *57*, 902.

[116] Yanagida, K.; Baba, C.; Baba, M. *Antiviral Res.*, **2004**, *64*, 195.

[117] Naka, K.; Ikeda, M.; Abe, K.; Dansako, H.; Kato, N. *Biochem. Biophys. Res. Commun.*, **2005**, *330*, 871.

[118] Hamaguchi, M.; Kawahito, Y.; Tsubouchi, Y.; Kohno, M.; Yamamoto, A.; Ishino, H.; Wada, M.; Yoshikawa, T. *Clin. Rheumatol.*, **2006**,

[119] Thiel, V.; Ivanov, K.A.; Putics, A.; Hertzig, T.; Schelle, B.; Bayer, S.; Weissbrich, B.; Snijder, E.J.; Rabenau, H.; Doerr, H.W.; Gorbalenya, A.E.; Ziebuhr, J. *J. Gen. Virol.*, **2003**, *84*, 2305.

[120] Ivanov, K.A.; Thiel, V.; Dobbe, J.C.; van der Meer, Y.; Snijder, E.J.; Ziebuhr, J. *J. Virol.*, **2004**, *78*, 5619.

[121] Gorbalenya, A.E.; Koonin, E.V.; Donchenko, A.P.; Blinov, V.M. *Nucl. Acids Res.*, **1989**, *17*, 4847.

[122] Gorbalenya, A.E.; Koonin, E.V.; Donchenko, A.P.; Blinov, V.M. *FEBS Lett.*, **1988**, *235*, 16.

[123] De, I.; Sawicki, S.G.; Sawicki, D.L. *J. Virol.*, **1996**, *70*, 2706.

[124] Janda, M.; Ahlquist, P. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 2227.

[125] O'Reilly, E.K.; Tang, N.; Ahlquist, P.; Kao, C.C. *Virology*, **1995**, *214*, 59.

[126] O'Reilly, E.K.; Wang, Z.; French, R.; Kao, C.C. *J. Virol.*, **1998**, *72*, 7160.

[127] Osman, T.A.; Buck, K.W. *J. Virol.*, **1996**, *70*, 6227.

[128] Petty, I.T.; French, R.; Jones, R.W.; Jackson, A.O. *EMBO J.*, **1990**, *9*, 3453.

[129] Rouleau, M.; Smith, R.J.; Bancroft, J.B.; Mackie, G.A. *Virology*, **1994**, *204*, 254.

[130] Tanner, J.A.; Zheng, B.J.; Zhou, J.; Watt, R.M.; Jiang, J.Q.; Wong, K.L.; Lin, Y.P.; Lu, L.Y.; He, M.L.; Kung, H.F.; Kesel, A.J.; Huang, J.D. *Chem. Biol.*, **2005**, *12*, 303.

[131] Stevenson, M. *N. Engl. J. Med.*, **2004**, *351*, 1772.

[132] Li, T.; Zhang, Y.; Fu, L.; Yu, C.; Li, X.; Li, Y.; Zhang, X.; Rong, Z.; Wang, Y.; Ning, H.; Liang, R.; Chen, W.; Babiuk, L.A.; Chang, Z. *Gene Ther.*, **2005**, *12*, 751.

[133] Wu, C.J.; Huang, H.W.; Liu, C.Y.; Hong, C.F.; Chan, Y.L. *Antiviral Res.*, **2005**, *65*, 45.

[134] Ni, B.; Shi, X.; Li, Y.; Gao, W.; Wang, X.; Wu, Y. *Antivir. Ther.*, **2005**, *10*, 527.

[135] Li, B.J.; Tang, Q.; Cheng, D.; Qin, C.; Xie, F.Y.; Wei, Q.; Xu, J.; Liu, Y.; Zheng, B.J.; Woodle, M.C.; Zhong, N.; Lu, P.Y. *Nat. Med.*, **2005**, *11*, 944.

[136] Lu, A.; Zhang, H.; Zhang, X.; Wang, H.; Hu, Q.; Shen, L.; Schaffhausen, B.S.; Hou, W.; Li, L. *Virology*, **2004**, *324*, 84.

[137] Wang, Z.; Ren, L.; Zhao, X.; Hung, T.; Meng, A.; Wang, J.; Chen, Y.G. *J. Virol.*, **2004**, *78*, 7523.

[138] Zhang, Y.; Li, T.; Fu, L.; Yu, C.; Li, Y.; Xu, X.; Wang, Y.; Ning, H.; Zhang, S.; Chen, W.; Babiuk, L.A.; Chang, Z. *FEBS Lett.*, **2004**, *560*, 141.

[139] Qin, Z.L.; Zhao, P.; Zhang, X.L.; Yu, J.G.; Cao, M.M.; Zhao, L.J.; Luan, J.; Qi, Z.T. *Biochem. Biophys. Res. Commun.*, **2004**, *324*, 1186.

[140] He, M.L.; Zheng, B.J.; Chen, Y.; Wong, K.L.; Huang, J.D.; Lin, M.C.; Peng, Y.; Yuen, K.Y.; Sung, J.J.; Kung, H.F. *FEBS Lett.*, **2006**, *580*, 2414.

[141] Shi, Y.; Yang, D.H.; Xiong, J.; Jia, J.; Huang, B.; Jin, Y.X. *Cell Res.*, **2005**, *15*, 193.

[142] Zhao, P.; Qin, Z.L.; Ke, J.S.; Lu, Y.; Liu, M.; Pan, W.; Zhao, L.J.; Cao, J.; Qi, Z.T. *FEBS Lett.*, **2005**, *579*, 2404.

[143] Tao, P.; Zhang, J.; Tang, N.; Zhang, B.Q.; He, T.C.; Huang, A.L. *Chin Med. J. (Engl.)*, **2005**, *118*, 714.

[144] Jia, F.; Zhang, Y.Z.; Liu, C.M. *J. Biotechnol.*, **2006**,

[145] Konstantinova, P.; de Vries, W.; Haasnoot, J.; Ter Brake, O.; de Haan, P.; Berkhout, B. *Gene Ther.*, **2006**, *13*, 1403.

[146] Stram, Y.; Kuzntzova, L. *Virus Genes*, **2006**, *32*, 299.

[147] Summerton, J. *Biochim. Biophys. Acta*, **1999**, *1489*, 141.

[148] Neuman, B.W.; Stein, D.A.; Kroeker, A.D.; Churchill, M.J.; Kim, A.M.; Kuhn, P.; Dawson, P.; Moulton, H.M.; Bestwick, R.K.; Iversen, P.L.; Buchmeier, M.J. *J. Virol.*, **2005**, *79*, 9665.

[149] Masters, P.S. *Adv. Virus Res.*, **2006**, *66*, 193.

[150] Gao, F.; Ou, H.Y.; Chen, L.L.; Zheng, W.X.; Zhang, C.T. *FEBS Lett.*, **2003**, *553*, 451.

[151] Chen, L.; Gui, C.; Luo, X.; Yang, Q.; Gunther, S.; Scandella, E.; Drosten, C.; Bai, D.; He, X.; Ludewig, B.; Chen, J.; Luo, H.; Yang, Y.; Yang, Y.; Zou, J.; Thiel, V.; Chen, K.; Shen, J.; Shen, X.; Jiang, H. *J. Virol.*, **2005**, *79*, 7095.

[152] Kaeppler, U.; Stiefl, N.; Schiller, M.; Vicik, R.; Breuning, A.; Schmitz, W.; Rupprecht, D.; Schmuck, C.; Baumann, K.; Ziebuhr, J.; Schirmeister, T. *J. Med. Chem.*, **2005**, *48*, 6832.

[153] Lu, I.L.; Mahindroo, N.; Liang, P.H.; Peng, Y.H.; Kuo, C.J.; Tsai, K.C.; Hsieh, H.P.; Chao, Y.S.; Wu, S.Y. *J. Med. Chem.*, **2006**, *49*, 5154.

[154] Chen, L.; Li, J.; Luo, C.; Liu, H.; Xu, W.; Chen, G.; Liew, O.W.; Zhu, W.; Puah, C.M.; Shen, X.; Jiang, H. *Bioorg. Med. Chem.*, **2006**,

[155] Gan, Y.R.; Huang, H.; Huang, Y.D.; Rao, C.M.; Zhao, Y.; Liu, J.S.; Wu, L.; Wei, D.Q. *Peptides*, **2006**, *27*, 622.

[156] Yang, H.; Xie, W.; Xue, X.; Yang, K.; Ma, J.; Liang, W.; Zhao, Q.; Zhou, Z.; Pei, D.; Ziebuhr, J.; Hilgenfeld, R.; Yuen, K.Y.; Wong, L.; Gao, G.; Chen, S.; Chen, Z.; Ma, D.; Bartlam, M.; Rao, Z. *PLoS. Biol.*, **2005**, *3*, e324.

[157] Wu, C.Y.; Jan, J.T.; Ma, S.H.; Kuo, C.J.; Juan, H.F.; Cheng, Y.S.; Hsu, H.H.; Huang, H.C.; Wu, D.; Brik, A.; Liang, F.S.; Liu, R.S.; Fang, J.M.; Chen, S.T.; Liang, P.H.; Wong, C.H. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 10012.

[158] Al Gharabli, S.I.; Shah, S.T.; Weik, S.; Schmidt, M.F.; Mesters, J.R.; Kuhn, D.; Klebe, G.; Hilgenfeld, R.; Rademann, J. *Chembiochem.*, **2006**, *7*, 1048.

[159] Chen, L.; Chen, S.; Gui, C.; Shen, J.; Shen, X.; Jiang, H. *J Biomol. Screen.*, **2006**,

[160] Dooley, A.J.; Shindo, N.; Taggart, B.; Park, J.G.; Pang, Y.P. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 830.

[161] Tsai, K.C.; Chen, S.Y.; Liang, P.H.; Lu, I.L.; Mahindroo, N.; Hsieh, H.P.; Chao, Y.S.; Liu, L.; Liu, D.; Lien, W.; Lin, T.H.; Wu, S.Y. *J. Med. Chem.*, **2006**, *49*, 3485.

[162] Yang, S.; Chen, S.J.; Hsu, M.F.; Wu, J.D.; Tseng, C.T.; Liu, Y.F.; Chen, H.C.; Kuo, C.W.; Wu, C.S.; Chang, L.W.; Chen, W.C.; Liao, S.Y.; Chang, T.Y.; Hung, H.H.; Shr, H.L.; Liu, C.Y.; Huang, Y.A.; Chang, L.Y.; Hsu, J.C.; Peters, C.J.; Wang, A.H.; Hsu, M.C. *J. Med. Chem.*, **2006**, *49*, 4971.

[163] Wu, C.Y.; King, K.Y.; Kuo, C.J.; Fang, J.M.; Wu, Y.T.; Ho, M.Y.; Liao, C.L.; Shie, J.J.; Liang, P.H.; Wong, C.H. *Chem. Biol.*, **2006**, *13*, 261.

[164] Du, Q.; Wang, S.; Wei, D.; Sirois, S.; Chou, K.C. *Anal. Biochem.*, **2005**, *337*, 262.

[165] Du, Q.S.; Wang, S.Q.; Zhu, Y.; Wei, D.Q.; Guo, H.; Sirois, S.; Chou, K.C. *Peptides*, **2004**, *25*, 1857.

[166] Chen, L.R.; Wang, Y.C.; Lin, Y.W.; Chou, S.Y.; Chen, S.F.; Liu, L.T.; Wu, Y.T.; Kuo, C.J.; Chen, T.S.; Juang, S.H. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 3058.

[167] Lee, T.W.; Cherney, M.M.; Huitema, C.; Liu, J.; James, K.E.; Powers, J.C.; Eltis, L.D.; James, M.N. *J. Mol. Biol.*, **2005**, *353*, 1137.

[168] Lin, C.W.; Tsai, F.J.; Tsai, C.H.; Lai, C.C.; Wan, L.; Ho, T.Y.; Hsieh, C.C.; Chao, P.D. *Antiviral Res.*, **2005**, *68*, 36.

[169] Liu, Z.; Huang, C.; Fan, K.; Wei, P.; Chen, H.; Liu, S.; Pei, J.; Shi, L.; Li, B.; Yang, K.; Liu, Y.; Lai, L. *J. Chem. Inf. Model.*, **2005**, *45*, 10.

[170] Liu, Y.C.; Huang, V.; Chao, T.C.; Hsiao, C.D.; Lin, A.; Chang, M.F.; Chow, L.P. *Biochem. Biophys. Res. Commun.*, **2005**, *333*, 194.

[171] Martina, E.; Stiefl, N.; Degel, B.; Schulz, F.; Breuning, A.; Schiller, M.; Vicik, R.; Baumann, K.; Ziebuhr, J.; Schirmeister, T. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 5365.

[172] Shie, J.J.; Fang, J.M.; Kuo, C.J.; Kuo, T.H.; Liang, P.H.; Huang, H.J.; Yang, W.B.; Lin, C.H.; Chen, J.L.; Wu, Y.T.; Wong, C.H. *J. Med. Chem.*, **2005**, *48*, 4469.

[173] Bacha, U.; Barrila, J.; Velazquez-Campoy, A.; Leavitt, S.A.; Freire, E. *Biochemistry*, **2004**, *43*, 4906.

[174] Blanchard, J.E.; Elowe, N.H.; Huitema, C.; Fortin, P.D.; Cechetto, J.D.; Eltis, L.D.; Brown, E.D. *Chem. Biol.*, **2004**, *11*, 1445.

[175] Hsu, J.T.; Kuo, C.J.; Hsieh, H.P.; Wang, Y.C.; Huang, K.K.; Lin, C.P.; Huang, P.F.; Chen, X.; Liang, P.H. *FEBS Lett.*, **2004**, *574*, 116.

[176] Kao, R.Y.; Tsui, W.H.; Lee, T.S.; Tanner, J.A.; Watt, R.M.; Huang, J.D.; Hu, L.; Chen, G.; Chen, Z.; Zhang, L.; He, T.; Chan, K.H.; Tse, H.; To, A.P.; Ng, L.W.; Wong, B.C.; Tsoi, H.W.; Yang, D.; Ho, D.D.; Yuen, K.Y. *Chem. Biol.*, **2004**, *11*, 1293.

[177] Kao, R.Y.; To, A.P.; Ng, L.W.; Tsui, W.H.; Lee, T.S.; Tsoi, H.W.; Yuen, K.Y. *FEBS Lett.*, **2004**, *576*, 325.

[178] Anand, K.; Ziebuhr, J.; Wadhwani, P.; Mesters, J.R.; Hilgenfeld, R. *Science*, **2003**, *300*, 1763.

[179] Shie, J.J.; Fang, J.M.; Kuo, T.H.; Kuo, C.J.; Liang, P.H.; Huang, H.J.; Wu, Y.T.; Jan, J.T.; Cheng, Y.S.; Wong, C.H. *Bioorg. Med. Chem.*, **2005**, *13*, 5240.

[180] Liu, D.X.; Tibbles, K.W.; Cavanagh, D.; Brown, T.D.; Brierley, I. *Adv. Exp. Med. Biol.*, **1995**, *380*, 413.

[181] Lim, K.P.; Ng, L.F.; Liu, D.X. *J. Virol.*, **2000**, *74*, 1674.

[182] Gorbalenya, A.E.; Koonin, E.V.; Lai, M.M. *FEBS Lett.*, **1991**, *288*, 201.

[183] Bonilla, P.J.; Hughes, S.A.; Weiss, S.R. *J. Virol.*, **1997**, *71*, 900.

[184] Baker, S.C.; Shieh, C.K.; Soe, L.H.; Chang, M.F.; Vannier, D.M.; Lai, M.M. *J. Virol.*, **1989**, *63*, 3693.

[185] Kanjanahaluethai, A.; Baker, S.C. *J. Virol.*, **2000**, *74*, 7911.

[186] Ziebuhr, J.; Thiel, V.; Gorbalenya, A.E. *J. Biol. Chem.*, **2001**, *276*, 33220.

[187] Herold, J.; Gorbalenya, A.E.; Thiel, V.; Schelle, B.; Siddell, S.G. *J. Virol.*, **1998**, *72*, 910.

[188] Ziebuhr, J.; Heusipp, G.; Seybert, A.; Siddell, S.G. *Adv. Exp. Med. Biol.*, **1998**, *440*, 115.
[189] Ziebuhr, J.; Siddell, S.G. *J. Virol.*, **1999**, *73*, 177.
[190] Ziebuhr, J.; Snijder, E.J.; Gorbalenya, A.E. *J. Gen. Virol.*, **2000**, *81*, 853.
[191] Lim, K.P.; Liu, D.X. *Virology*, **1998**, *245*, 303.
[192] Han, Y.S.; Chang, G.G.; Juo, C.G.; Lee, H.J.; Yeh, S.H.; Hsu, J.T.; Chen, X. *Biochemistry*, **2005**, *44*, 10349.
[193] Nicholls, J.M.; Poon, L.L.; Lee, K.C.; Ng, W.F.; Lai, S.T.; Leung, C.Y.; Chu, C.M.; Hui, P.K.; Mak, K.L.; Lim, W.; Yan, K.W.; Chan, K.H.; Tsang, N.C.; Guan, Y.; Yuen, K.Y.; Peiris, J.S. *Lancet*, **2003**, *361*, 1773.
[194] Jones, B.M.; Ma, E.S.; Peiris, J.S.; Wong, P.C.; Ho, J.C.; Lam, B.; Lai, K.N.; Tsang, K.W. *Clin. Exp. Immunol.*, **2004**, *135*, 467.
[195] Bianchi, M.; Meng, C.; Ivashkiv, L.B. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 9573.
[196] Zhao, Z.; Zhang, F.; Xu, M.; Huang, K.; Zhong, W.; Cai, W.; Yin, Z.; Huang, S.; Deng, Z.; Wei, M.; Xiong, J.; Hawkey, P.M. *J. Med. Microbiol.*, **2003**, *52*, 715.
[197] So, L.K.; Lau, A.C.; Yam, L.Y.; Cheung, T.M.; Poon, E.; Yung, R.W.; Yuen, K.Y. *Lancet*, **2003**, *361*, 1615.
[198] Sung, J.J.; Wu, A.; Joynt, G.M.; Yuen, K.Y.; Lee, N.; Chan, P.K.; Cockram, C.S.; Ahuja, A.T.; Yu, L.M.; Wong, V.W.; Hui, D.S. *Thorax*, **2004**, *59*, 414.
[199] Booth, C.M.; Matukas, L.M.; Tomlinson, G.A.; Rachlis, A.R.; Rose, D.B.; Dwosh, H.A.; Walmsley, S.L.; Mazzulli, T.; Avendano, M.; Derkach, P.; Ephtimios, I.E.; Kitai, I.; Mederski, B.D.; Shadowitz, S.B.; Gold, W.L.; Hawryluck, L.A.; Rea, E.; Chenkin, J.S.; Cescon, D.W.; Poutanen, S.M.; Detsky, A.S. *JAMA*, **2003**, *289*, 2801.
[200] Cinatl, J., Jr.; Michaelis, M.; Morgenstern, B.; Doerr, H.W. *Int. J. Mol. Med.*, **2005**, *15*, 323.
[201] Wang, H.; Ding, Y.; Li, X.; Yang, L.; Zhang, W.; Kang, W. *N. Engl. J. Med.*, **2003**, *349*, 507.
[202] Pestka, S. *Biopolymers*, **2000**, *55*, 254.
[203] Faltynek, C.R.; Kung, H.F. *Biofactors*, **1988**, *1*, 227.
[204] Pestka, S.; Kotenko, S.V.; Muthukumaran, G.; Izotova, L.S.; Cook, J.R.; Garotta, G. *Cytokine Growth Factor Rev.*, **1997**, *8*, 189.
[205] Sperber, S.J.; Hayden, F.G. *J. Interferon Res.*, **1989**, *9*, 285.
[206] Fuchizaki, U.; Kaneko, S.; Nakamoto, Y.; Sugiyama, Y.; Imagawa, K.; Kikuchi, M.; Kobayashi, K. *J. Med. Virol.*, **2003**, *69*, 188.
[207] Pei, J.; Sekellick, M.J.; Marcus, P.I.; Choi, I.S.; Collisson, E.W. *J. Interferon Cytokine Res.*, **2001**, *21*, 1071.
[208] Jordan, L.T.; Derbyshire, J.B. *Vet. Microbiol.*, **1995**, *45*, 59.
[209] Higgins, P.G.; Phillpotts, R.J.; Scott, G.M.; Wallace, J.; Bernhardt, L.L.; Tyrrell, D.A. *Antimicrob. Agents Chemother.*, **1983**, *24*, 713.
[210] Turner, R.B.; Felton, A.; Kosak, K.; Kelsey, D.K.; Meschievitz, C.K. *J. Infect. Dis.*, **1986**, *154*, 443.
[211] Spiegel, M.; Pichlmair, A.; Muhlberger, E.; Haller, O.; Weber, F. *J. Clin. Virol.*, **2004**, *30*, 211.
[212] Hensley, L.E.; Fritz, L.E.; Jahrling, P.B.; Karp, C.L.; Huggins, J.W.; Geisbert, T.W. *Emerg. Infect. Dis.*, **2004**, *10*, 317.
[213] Dahl, H.; Linde, A.; Strannegard, O. *Scand. J. Infect. Dis.*, **2004**, *36*, 829.
[214] Tan, E.L.; Ooi, E.E.; Lin, C.Y.; Tan, H.C.; Ling, A.E.; Lim, B.; Stanton, L.W. *Emerg. Infect. Dis.*, **2004**, *10*, 581.
[215] Zheng, B.; He, M.L.; Wong, K.L.; Lum, C.T.; Poon, L.L.; Peng, Y.; Guan, Y.; Lin, M.C.; Kung, H.F. *J. Interferon Cytokine Res.*, **2004**, *24*, 388.
[216] Sainz, B., Jr.; Mossel, E.C.; Peters, C.J.; Garry, R.F. *Virology*, **2004**, *329*, 11.
[217] Scagnolari, C.; Vicenzi, E.; Bellomi, F.; Stillitano, M.G.; Pinna, D.; Poli, G.; Clementi, M.; Dianzani, F.; Antonelli, G. *Antivir. Ther.*, **2004**, *9*, 1003.
[218] Morgenstern, B.; Michaelis, M.; Baer, P.C.; Doerr, H.W.; Cinatl, J., Jr. *Biochem. Biophys. Res. Commun.*, **2005**, *326*, 905.
[219] Chen, F.; Chan, K.H.; Jiang, Y.; Kao, R.Y.; Lu, H.T.; Fan, K.W.; Cheng, V.C.; Tsui, W.H.; Hung, I.F.; Lee, T.S.; Guan, Y.; Peiris, J.S.; Yuen, K.Y. *J. Clin. Virol.*, **2004**, *31*, 69.
[220] Ignarro, L.J. *Biosci. Rep.*, **1999**, *19*, 51.
[221] Lane, T.E.; Paoletti, A.D.; Buchmeier, M.J. *J. Virol.*, **1997**, *71*, 2202.
[222] Pope, M.; Marsden, P.A.; Cole, E.; Sloan, S.; Fung, L.S.; Ning, Q.; Ding, J.W.; Leibowitz, J.L.; Phillips, M.J.; Levy, G.A. *J. Virol.*, **1998**, *72*, 7084.
[223] Reiss, C.S.; Komatsu, T. *J. Virol.*, **1998**, *72*, 4547.
[224] Torre, D.; Pugliese, A.; Speranza, F. *Lancet Infect. Dis.*, **2002**, *2*, 273.
[225] Keyaerts, E.; Vijgen, L.; Chen, L.; Maes, P.; Hedenstierna, G.; Van Ranst, M. *Int. J. Infect. Dis.*, **2004**, *8*, 223.
[226] Akerstrom, S.; Mousavi-Jazi, M.; Klingstrom, J.; Leijon, M.; Lundkvist, A.; Mirazimi, A. *J. Virol.*, **2005**, *79*, 1966.
[227] Chen, L.; Liu, P.; Gao, H.; Sun, B.; Chao, D.; Wang, F.; Zhu, Y.; Hedenstierna, G.; Wang, C.G. *Clin. Infect. Dis.*, **2004**, *39*, 1531.
[228] Perrin, B.J.; Huttenlocher, A. *Int. J. Biochem. Cell Biol.*, **2002**, *34*, 722.
[229] Neumar, R.W.; Xu, Y.A.; Gada, H.; Guttmann, R.P.; Siman, R. *J. Biol. Chem.*, **2003**, *278*, 14162.
[230] Baltina, L.A. *Curr. Med. Chem.*, **2003**, *10*, 155.
[231] Pompei, R.; Flore, O.; Marccialis, M.A.; Pani, A.; Loddo, B. *Nature*, **1979**, *281*, 689.
[232] Lin, J.C. *Antiviral Res.*, **2003**, *59*, 41.
[233] Lampi, G.; Deidda, D.; Pinza, M.; Pompei, R. *Antivir. Chem. Chemother.*, **2001**, *12*, 125.
[234] Crance, J.M.; Scaramozzino, N.; Jouan, A.; Garin, D. *Antiviral Res.*, **2003**, *58*, 73.
[235] Sasaki, H.; Takei, M.; Kobayashi, M.; Pollard, R.B.; Suzuki, F. *Pathobiology*, **2002**, *70*, 229.
[236] Shibata, S. *Yakugaku Zasshi*, **2000**, *120*, 849.
[237] O'Brian, C.A.; Ward, N.E.; Vogel, V.G. *Cancer Lett.*, **1990**, *49*, 9.
[238] Harada, S.; Karino, A.; Shimoyama, Y.; Shamsa, F.; Ohtsuki, K. *Biochem. Biophys. Res. Commun.*, **1996**, *227*, 102.
[239] Cherng, J.M.; Lin, H.J.; Hsu, Y.H.; Hung, M.S.; Lin, J.C. *Antiviral Res.*, **2004**, *62*, 27.
[240] Lee, J.; Jung, E.; Park, J.; Jung, K.; Park, E.; Kim, J.; Hong, S.; Park, J.; Park, S.; Lee, S.; Park, D. *J. Invest. Dermatol.*, **2005**, *124*, 405.
[241] Yi, H.; Nakashima, I.; Isobe, K. *Am. J. Chin Med.*, **1996**, *24*, 271.
[242] Miyake, K.; Tango, T.; Ota, Y.; Mitamura, K.; Yoshiba, M.; Kako, M.; Hayashi, S.; Ikeda, Y.; Hayashida, N.; Iwabuchi, S.; Sato, Y.; Tomi, T.; Funaki, N.; Hashimoto, N.; Umeda, T.; Miyazaki, J.; Tanaka, K.; Endo, Y.; Suzuki, H. *J. Gastroenterol. Hepatol.*, **2002**, *17*, 1198.
[243] Muller, W.E.; Yamazaki, Z.I.; Zahn, R.K. *Biochem. Biophys. Res. Commun.*, **1972**, *46*, 1667.
[244] Katz, E.; Margalith, E.; Duksin, D. *Antimicrob. Agents Chemother.*, **1980**, *17*, 1014.
[245] Balzarini, J.; Pannecouque, C.; De Clercq, E.; Pavlov, A.Y.; Printsevskaya, S.S.; Miroshnikova, O.V.; Reznikova, M.I.; Preobrazhenskaya, M.N. *J. Med. Chem.*, **2003**, *46*, 2755.
[246] Preobrazhenskaya, M.N.; Olsufyeva, E.N. *Antiviral Res.*, **2006**, *71*, 227.
[247] Balzarini, J.; Keyaerts, E.; Vijgen, L.; Egberink, H.; De Clercq, E.; Van Ranst, M.; Printsevskaya, S.S.; Olsufyeva, E.N.; Solovieva, S.E.; Preobrazhenskaya, M.N. *Antiviral Res.*, **2006**, *72*, 20.
[248] Kennedy, D.A.; Johnson-Lussenburg, C.M. *J. Virol.*, **1979**, *29*, 401.
[249] Perkins, J.B.; Guterman, S.K.; Howitt, C.L.; Williams, V.E.; Pero, J. *J. Bacteriol.*, **1990**, *172*, 3108.
[250] Cheng, Y.Q. *Chembiochem.*, **2006**, *7*, 471.

Received: December 18, 2006 Accepted: January 12, 2007