

Recent Patents on Treatment of Severe Acute Respiratory Syndrome (SARS)

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Received: September 11, 2006; Accepted: October 3, 2006; Revised: November 6, 2006

Abstract: Severe acute respiratory syndrome (SARS) is an epidemic that spread worldwide in early 2003. The aetiological agent was originally defined as a novel coronavirus and later designated as the SARS coronavirus (SARS-CoV), which appears similar to other coronaviruses in both virion structure and genome organization with a single-stranded, plus-sense RNA. However, the epidemiology and pathogenesis of SARS remain poorly understood and there is currently no effective treatment. To date, considerable research has been done on detection, prevention and treatment of SARS. In this review, we mainly focus on the recent patents and research work on detecting, preventing and treating SARS.

Keywords: SARS, coronavirus, inhibitor, vaccine.

INTRODUCTION

Severe acute respiratory syndrome (SARS) is a life-threatening form of atypical pneumonia [1] caused by a novel human coronavirus, SARS-CoV [2-8], which resulted in over 8,000 cases and 774 deaths in 30 countries [9]. SARS-CoV is a member of the coronaviridae family. The SARS genome was first sequenced at BCCANCER agency and became available on the Internet at <http://www.ncbi.nlm.nih.gov/>. Preliminary analysis of a conserved region of the genome indicates that this strain constitutes a new group within the coronaviridae family, not closely related to any previously identified strain of the virus [7,8]. Rapid identification of the causal agent as a novel coronavirus (SARS-CoV) represents an extraordinary achievement in the history of global health. However, at present, no efficacious therapy for SARS is available, so to establish sensitive and efficient methods for diagnosis and treatments is of great importance. So far, investigations on detection, prevention and treatment of SARS have been extensively reported. The present article reviews recent patents on detecting, preventing and treating SARS.

SARS-COV GENOME STRUCTURE AND ITS REPLICATION

The coronaviruses are enveloped, positive-stranded RNA viruses with the largest single-stranded RNA genome (approximately 27-31 Kb in length) among known RNA viruses (Fig. 1A). The RNAs are polyadenylated and 5' capped, and are translated into large polyproteins following their entry into the host cell. Then the polyproteins are proteolytically cleaved by viral proteinases to yield the viral gene products. The SARS-CoV genome encodes the RNA-dependent RNA polymerase (Pol) and four structural proteins common to all coronaviruses, including the spike

glycoprotein (S), envelope (E), membrane (M) and nucleocapsid (N) proteins in the order Pol-S-E-M-N. The spike protein (S) is the major antigenic determinant for coronaviruses and is thought to be involved in receptor binding. E protein plays a role in viral assembly. The M glycoprotein, the most abundant transmembrane envelope glycoprotein in the virus particle, is important for virus budding and N protein is associated with viral RNA packaging (Fig. 1B) [10,11].

RNA INTERFERENCE PRINCIPLE

RNAi (RNA interference) has been demonstrated to be a powerful method for gene silencing. The idea of using RNAi for therapeutic purposes has extensively been exploited in treating various diseases such as cancer and dominantly inherited genetic disorders [12]. Development of therapeutic agents for SARS viral infection using short interfering RNA (siRNA) inhibitors exemplifies a powerful new means to combat emerging infectious diseases [13].

Wang *et al.* [12] exploited the possibility of using RNA interference as a therapeutic approach to fight the disease and they provided evidence that SARS-CoV replication could be efficiently inhibited by vector-derived siRNA-mediated RNAi in *vero* cells, so that siRNA has a potential to be developed into anti-SARS drugs. Tao *et al.* synthesized short hairpin RNA (shRNA) that specifically targeted the N gene sequence of SARS-CoV and assessed the inhibitory effect of this shRNA on SARS-CoV N antigen expression [14]. The results demonstrate that RNAi mediated silencing of SARS-CoV gene could effectively inhibit expression of SARS-CoV antigen, hence RNAi based strategy should be further explored as a more efficacious antiviral therapy of SARS-CoV infection.

Furthermore, the RNAi molecules targeting the replicase region of the hSARS virus, or combinations of different sites of hSARS virus genes also exhibited obvious effects on modulating the expression of SARS virus RNA [15]. Besides those mentioned above, RNAi has also been described in some other patents [16-18].

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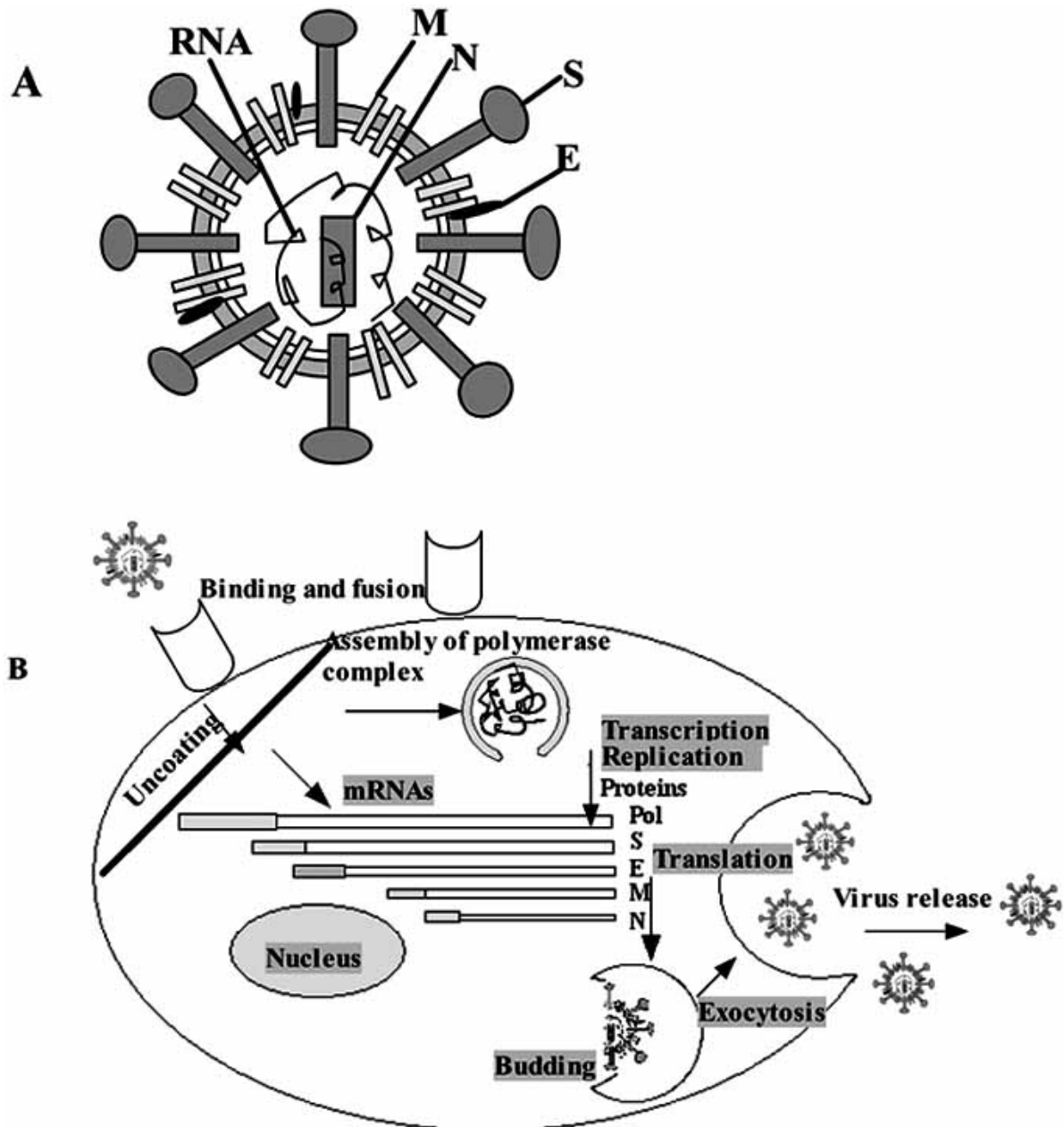


Fig. (1). The structure of SARS-CoV and its replication cycle (according to Holmes, 2003 [10]; Shigeta and Yamase, 2005 [11]).

(A). The structure of SARS-CoV. S: spike protein; M: membrane protein; E: envelope protein; N: envelope protein. (B) The replication cycle of SARS-CoV. SARS-CoV binds to cellular receptor (ACE2) using S1 domain of spike protein and induces fusion of virus envelope and cellular membrane. The naked genomic RNA that appears after penetration and uncoating of nucleocapsid is transcribed to mRNAs. The mRNA is translated into a polyprotein which is cleaved to RdRP, 3Clpro, helicase and other factors and four structural proteins known as S, M, E and N. M is required for virus budding; S has receptor binding and membrane fusion activities; E plays a role in coronavirus assembly; and N associates with viral RNA inside the virion.

SARS VACCINES

Regarding the serious infection of SARS-CoV, safe, effective, economical and easily administered vaccines are urgently needed.

Currently, strategies for SARS vaccines development include a whole inactivated SARS-CoV, full-length S protein, an attenuated virus or weak form of the virus, recombinant SARS proteins or DNA vaccines [19,20]. Both

the inactivated virus and recombinant S protein were targeted at inducing neutralizing antibodies, whereas an attenuated virus or weak form of the virus was targeted at inducing both cellular immunity and neutralizing antibodies [21].

Among those strategies for SARS vaccines development, the production of recombinant subunit vaccines has become a valuable modern strategy for the prevention of infectious diseases. Development of recombinant vaccines relies on the

identification of the best antigen and the choice of expression system. Currently, the SARS-CoV S glycoprotein and its truncated versions are considered the best candidates for the generation of a recombinant vaccine against this disease [22-28].

Besides the cell substrate for vaccine production, the plant system has been used for the production and convenient delivery of the subunit vaccines recently [29-31]. Several groups have reported the development of S protein recombinant plant-based vaccines against different CoVs for oral delivery [32-34].

For the SARS vaccines to be used in human, vaccine efficacy and safety must be evaluated. The most obvious question is how to evaluate the human efficacy of SARS vaccines without an outbreak of SARS. Then animal models for SARS were developed. At present SARS Accelerated Vaccine Initiative SAVI vaccines are first tested in ferrets and mice and then in non-human primates or other small animals for safety and immuno-genicity [21]. Other researchers evaluated recombinant viruses and virus-like particles or DNA vaccines for efficacy in hamsters [25], African green monkeys [35], Balb/c mice [23], ferrets [36], rhesus macaques [37] and C57BL/6 mice models [38].

Despite the usefulness of these animal models, however, no single animal species has been shown to reproduce all of the clinical signs and lethality that is observed in humans, who are infected with SARS-CoV [21]. Development of an animal model that mimics human disease will be the single most important advance in the development of a SARS vaccine [39].

INHIBITORS OF SARS 3C-LIKE PROTEINASE

Coronavirus replication and transcription function are encoded by the so-called "replicase" gene [40], which consists of two overlapping polyproteins, namely pp1a (~450 kDa) and pp1b (~750 kDa). Extensive proteolytic processing of these nonstructural polyproteins is required to provide the functional proteins for viral propagation. The cleavage process of the SARS-CoV polyproteins is mediated primarily by the main protease (Mpro), which is also known as dimeric chymotrypsin-like protease (3CLpro) [41-43]. Because of the functional importance of SARS 3C-like proteinase (3CLpro) in the viral life cycle, it has been recognized as a key target for structural-based drug design against SARS [41-45]. Inhibition of 3C proteases is believed to block proteolytic cleavage of the polyprotein, which in turn can retard the maturation and replication of the viruses by interfering with viral particle production. Currently, several groups have developed compounds targeting either the spike protein [46] to inhibit viral entry or 3CLpro to inhibit polyprotein processing [42-48].

Significant catalytic and structural similarities between rhinovirus 3C protease and coronavirus "3C-like" main protease suggest that selected inhibitors of rhinovirus 3C protease are useful against coronavirus main protease. A method of interfering with or preventing SARS viral replication activity by contacting a SARS-related coronavirus 3C-like protease with a therapeutically effective

amount of a rhinovirus 3C protease inhibitor was provided [49,50] (Table 1). Lai *et al.* [51] prepared imidazolium compounds (Table 1), analogs of calmidazolium chloride, as SARS coronavirus (3CL protease) inhibitors for the treatment of SARS. Ghosh *et al.* [52] described the design, synthesis, and biological evaluation of peptidomimetics (Table 1) as SARS chymotrypsin-like protease (SARS-3CLpro) inhibitors. These inhibitors exhibited antiviral activity against SARS-CoV in infected cells in the micromolar range.

The coronavirus main protease, Mpro, is considered to be a major target for drugs suitable for combating coronavirus infections including severe acute respiratory syndrome (SARS) [53]. An HPLC-based screening of electrophilic compounds to identify potential Mpro inhibitors revealed etacrynic acid tert-butylamide (Table 1) as an effective nonpeptidic inhibitor. Docking studies suggested a binding mode in which the Ph ring acts as a spacer bridging the inhibitor's activated double bond and its hydrophobic tert-Bu moiety. The latter is supposed to fit into the S4 pocket of the target protease. Furthermore, these studies revealed etacrynic acid amide (Table 1) as a promising lead for nonpeptidic active-site-directed Mpro inhibitors. In a fluorimetric enzyme assay using a novel fluorescence resonance energy transfer (FRET) pair labeled substrate, compound II showed a K_i value of 35.3 μM .

Shie *et al.* [54] described the inhibition of the SARS 3CL protease by peptidomimetic α,β -unsaturated esters. The most potent inhibitor (I) (Table 1) with an inhibition constant of 0.52 μM was obtained by condensation of the Phe-Phe dipeptide α,β -unsaturated ester with 4-(dimethylamino) cinnamic acid. The cell-based assays also indicate that I was a nontoxic anti-SARS agent with an EC_{50} value of 0.18 μM . In addition, they discovered anilide inhibitors against the SARS 3CL Protease [55]. Chen *et al.* [56] prepared N-substituted isatin derivations from the reaction of isatin and various bromides via two steps. Bioactivity assay results (*in vitro* tests) demonstrated that some of these compounds were potent and selective inhibitors against SARS coronavirus 3CL protease (Table 1). Jain *et al.* [57] synthesized a series of keto-glutamine analogs with a phthalhydrazido group at the α -position and tested them as reversible inhibitors against SARS 3CLpro. Attachment of tripeptide (Ac-Val-Thr-Leu) to these glutamine-based "warheads" generated significantly better inhibitors with IC_{50} values ranging from 0.60 to 70 μM (Table 1). Table 1 provides examples of inhibitor compounds that were useful as SARS-related 3C protease inhibitors.

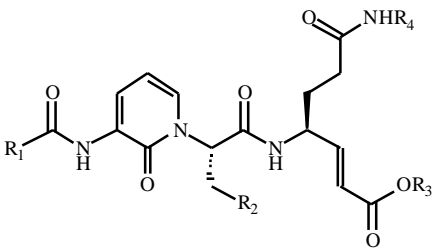
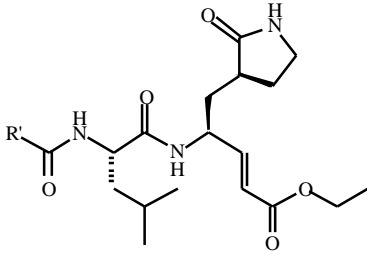
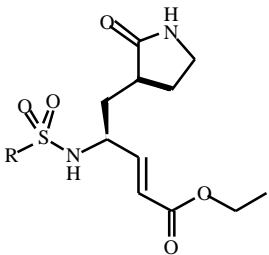
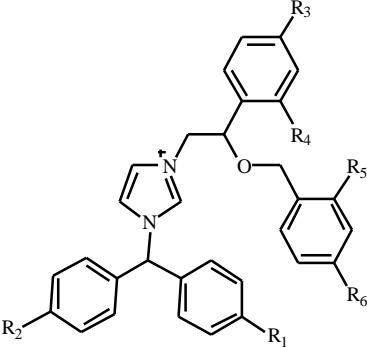
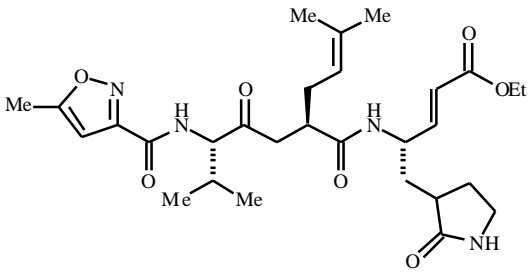
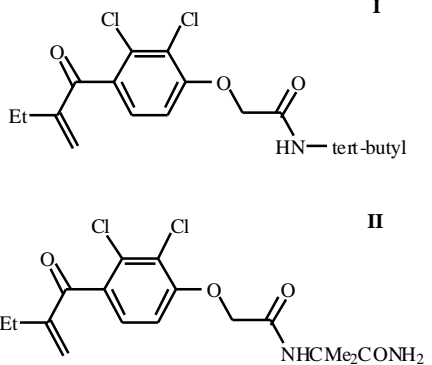
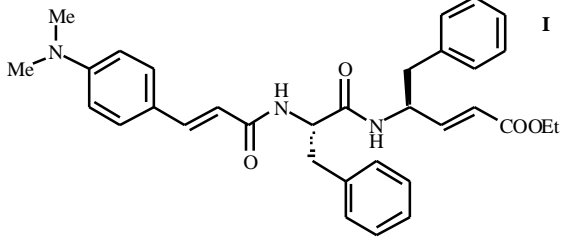
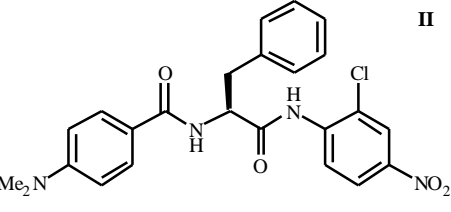
SARS-COV MAIN PROTEASE INHIBITORS

A group of dicyclic or multi-cyclic compounds effectively inhibit the activity of severe acute respiratory syndrome (SARS) coronavirus (CoV) main protease and hepatitis C virus (HCV) NS3 proteinase, a structural analog of SARS-CoV main protease [58] (Table 2).

OTHER INHIBITORS

In addition, certain nucleoside compounds and derivatives have been identified as potent inhibitors of the

Table 1. SARS-Related 3C Protease Inhibitors

	
US20040235952A1 [49]	US20060014821A1 [50]
	
US20060014821A1 [50]	CN1569841A [51]
	
(Ghosh et al., 2005) [52]	(Kaepler et al., 2005) [53]
	
(Shie et al., 2005a) [54]	(Shie et al., 2005b) [55]

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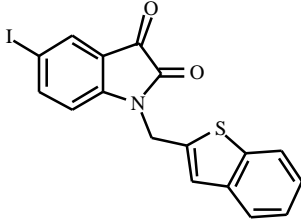
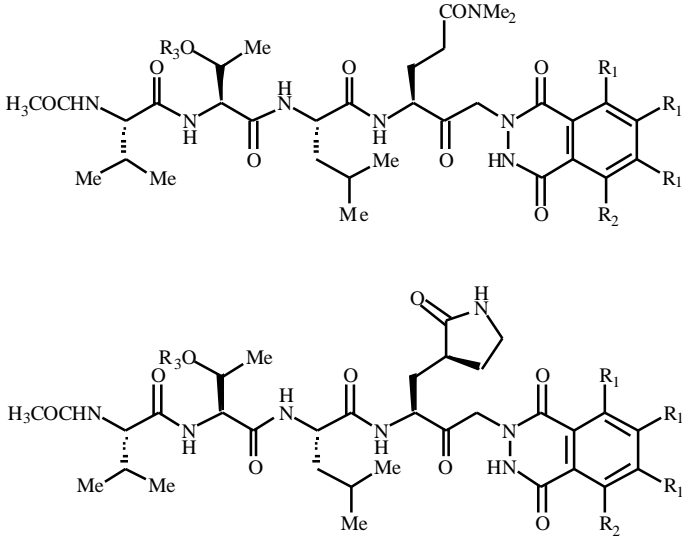
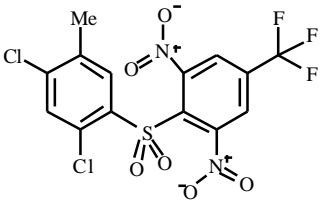
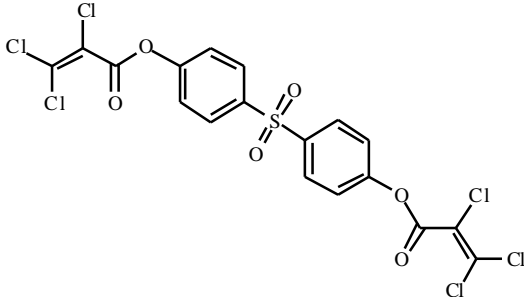
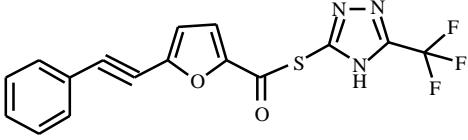
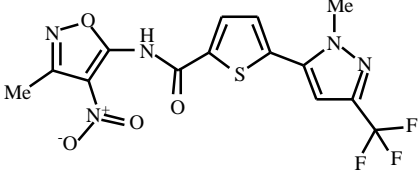
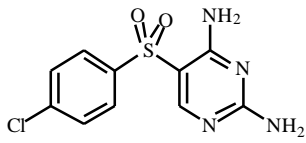
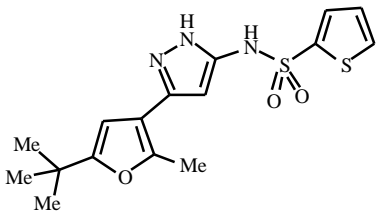
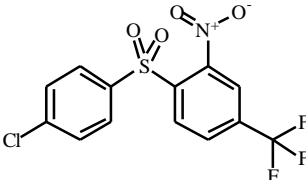
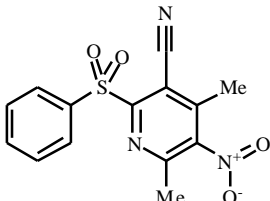
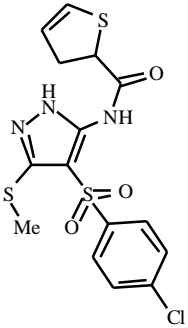
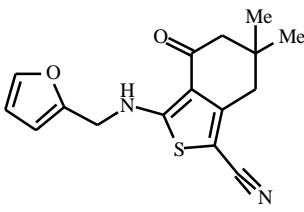
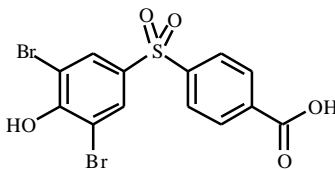
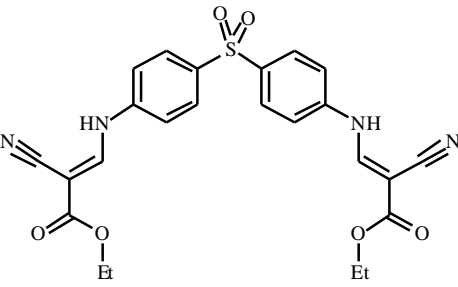
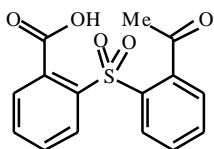
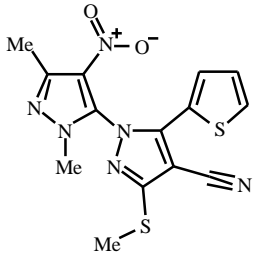

(Chen <i>et al.</i> , 2005) [56]

(Jain <i>et al.</i> , 2004) [57]

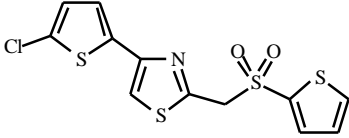
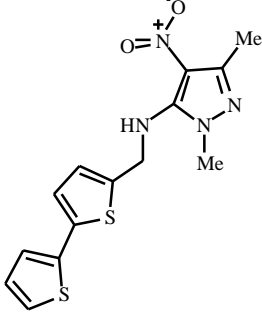
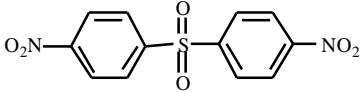
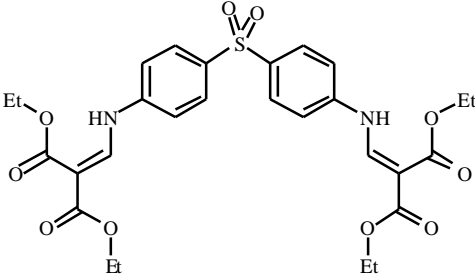
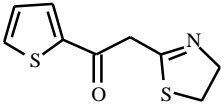
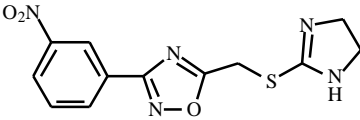
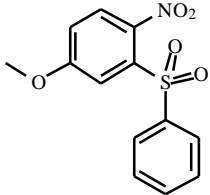
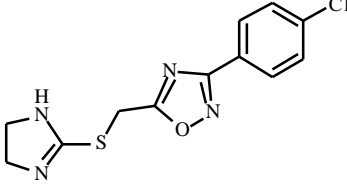
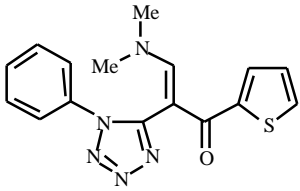
Table 2. SARS-CoV main Protease Inhibitors

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 <chem>Nc1nc(N)nc(S(=O)(=O)c2ccc(Cl)cc2)c1</chem>	 <chem>Cc1c(C)c(C)oc1-c1cc(C)nn1NS(=O)(=O)c2ccsc2</chem>
 <chem>C(F)(F)Fc1cc(C(=O)Nc2cc(Cl)cc2S(=O)(=O)c3ccccc3)nc1[N+](=O)[O-]</chem>	 <chem>Cc1c(C)nc(C#N)c([N+](=O)[O-])c1NS(=O)(=O)c2ccccc2</chem>
 <chem>Cs1c(C2=CC=CS2)nn1S(=O)(=O)c3ccc(Cl)cc3</chem>	 <chem>Cc1c(C#N)sc(C2=CC=CO2)N1C(=O)CC(C)C1#N</chem>
 <chem>O=C(O)c1ccc(S(=O)(=O)c2cc(Br)c(O)c(Br)c2)cc1</chem>	 <chem>CCOC(=O)C#Nc1ccc(S(=O)(=O)c2ccc(NC#N)cc2)cc1</chem>
 <chem>Cc1ccc(S(=O)(=O)c2ccccc2C(=O)O)cc1</chem>	 <chem>Cc1c(C#N)nn(C)c1S(=O)(=O)c2cc(C)nn2[N+](=O)[O-]</chem>

(Table 2) Contd....

	
	
	
	
	
<p style="text-align: center;">US20060019967A1(25 inhibitors) [58]</p>	

replication of coronaviruses, thus being useful in the treatment and prophylaxis of infection or illness due to coronaviruses. The inhibitors include two compounds (I or II) (Table 3), or their pharmaceutically acceptable salts. The compound is administered in combination with an inhibition effective amount of another agent active against the SARS virus, which includes interferon, ribavirin, levovirin or viramidine, an angiotensin II receptor blocker (e.g., losartan), 2'-C-methylcytidine or a pharmaceutically acceptable salt [59].

The release of inflammatory cytokines by leukocytes is a means by which the immune system combats pathogenic invasions, including infections. Cytokines are believed to be involved in cell-to-cell communications, acting as enhancing mediators for immune responses through interaction with specific cell-surface receptors on leukocytes. They include interleukins, lymphokines, interferons and tumor necrosis factor (TNF) [60]. Inhibitors of SARS-associated inflammatory cytokines are provided for use in treating SARS. They include a soluble recombinant SARS-associated inflammatory cytokine receptor, an antibody to a SARS-

associated inflammatory cytokine, a small molecule, a SARS-associated antisense oligonucleotide or a combination.

The administration of an effective amount of a suitable antibiotic agent, antifungal agent or antiviral agent in conjunction with one compound [61] (Table 3) to a patient is a therapeutic method for treating biological diseases. The compound can be used alone to reduce inflammation, as may occur during infection with antibiotic resistant bacteria, or certain viruses such as those that cause SARS or Ebola.

Glycopeptide antibiotics and their semisynthetic derivatives were used to treat or prevent viral infections and to manufacture a medicine to treat or prevent viral infections by SARS virus [62] (Table 3). Glycopeptide antibiotics from natural resources, their semisynthetic analogs and derivatives possess a broad anti-viral activity inhibiting the replication of BVDV, HIV, HSV, CMV, VZV, FCV and the SARS virus.

OTHER AGENTS OR METHODS FOR PREVENTING AND TREATING SARS

Alpha-interferons, interferon-beta, double-stranded ribonucleic acid (dsRNA) or the combinations were provided for treatment and/or prevention of SARS [63,64]. Administering of theaflavin and its derivatives in combination with a carrier was developed. Different carriers and different percentages of theaflavin and its derivatives in the composition were provided [65].

Aarhus *et al.* used subunits and oligomers of collections and/or ficolins, such as mannan-binding lectin in prophylactic and/or curative treatment of SARS, particularly, in individuals having a normal to low mannose-binding lectin (MBL) serum level. Furthermore, a method for treating SARS including determining the MBL serum level in an individual and administering MBL to the individual if relevant was described [66].

Triptolide compounds are effective in inhibiting cytokine production and thereby reduce symptoms, particularly in the immune hyperactive phase of SARS [67]. Baicain, a naturally occurring compound, extracted and purified from the chinese medicinal plant *Scutellaria baicalensis* Georgi (Chinese name: Huang Qin) exhibits potent antiviral activity against members of the order Nidovirales of the family *Coronaviridae* that infects humans and other animals, in particular SARS virus in human [68]. Extracts of *Scutellaria* comprising botanical drug substances or botanical ingredients were reported to be effective in preventing and treating SARS-CoV infection [69].

CURRENT & FUTURE DEVELOPMENTS

Substantial progress in detecting, preventing and treating SARS has been made in a short period of time after the outbreak of SARS in 2003. These advances present new opportunities to discover and develop novel and effective treatments for SARS in preparation for future outbreaks. Recently, other infective diseases such as avian flu and foot and mouth disease, have caused great economic losses and human disaster. From the experience in dealing with SARS and inventions in SARS research, we should be able to better deal with other future outbreaks.

ACKNOWLEDGEMENTS

We thank Dr. Qiu Zhang for assistance and Hongyu Zhou for drawing compound structures during the preparation of this review.

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