Pillaiyar et al., Med chem 2015, 5:8
DOI: 10.4172/2161-0444.1000287

Medicinal chemistry

Review Article Open Access

Middle East Respiratory Syndrome-Coronavirus (MERS-CoV): An Updated Overview and Pharmacotherapeutics

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Abstract

In 2012, a novel human coronavirus (CoV) associated with severe respiratory tract infection, Middle East Respiratory Syndrome (MERS-CoV) was first recognized and since then 1401 patients were infected across the world (26 countries) with this virus, 543 (~39%) of which died. The diseases present severe respiratory infection often with shock, acute kidney injury and coagulopathy. Its human-to-human transmission through close contact has raised a global concern about its potential pandemic. This review describes the strategies used to develop effective pharmacotherapeutics for MERS-CoV, which are based on the experience gained from SARS-CoV outbreak in 2003.

Keywords: Coronavirus; Middle East respiratory syndrome; Severe acute respiratory syndrome; Respiratory tract infection; Positive-sense RNA; Inhibitors; Anti-viral agents; Dipeptidyl peptidase 4; Papain-like protease and 3C-like protease

Abbreviations: hCoV: Human Coronavirus; MERS: Middle East Respiratory Syndrome; SARS: Severe Respiratory Syndrome; RNA: Ribonucleic Acid; DPP4: Dipeptidyl Peptidase 4; PLpro: Papain-Like Protease; 3CL-Pro: 3C-like Protease; HCoV-OC43: Human Coronaviruses OC43; HCoV-229E: Human Coronaviruses 229E; HCoV-EMC: Human Coronavirus-Erasmus Medical Center; TMPRSS2: Transmembrane Protease, Serine 2; RdRp: RNA Dependent and RNA Polymerase; Nsp: Non-Structural Protein; ACE-2: Angiotensin Converting Enzyme-2; CEACAM: Carcinoembryonic Antigen-Related Adhesion Molecules; SP: Signal Peptide; FP: Fusion Peptide; HR: Heptad Repeat; TM: Transmembrane; CP: Cytoplasmic Domain; RBD: Binding Domain; S: Spike; ADA: Adenosine Deaminase; Mab: Monoclonal Antibody; CPE: Cytopathic Effect; 6-HB: Six-Helix Bundle; HIV: Human Immunodeficiency Virus; DNA: Deoxyribonucleic Acid; MHV-2: Mouse Hepatitis Virus-2; ABL-1: Homolog-1 Pathway; IFN: Interferon; IFNTM: Interferon Transmembrane; NEM: *N*-ethyl Maleimide; 6-TG: 6-Thioguanine; 6MP: 6-Mercaptopurine; 6-TG: 6-Thioguanine; CNS: Central Nervous System

Introduction

At first, human coronaviruses were identified in 1960 as the causative agents for the first mild respiratory infections and were subsequently named as human coronaviruses 229E (HCoV-229E) and human coronaviruses OC43 (HCoV-OC43) [1,2]. In 2003, the new human coronavirus was identified as an etiological agent of the first global pandemic of the 21st century, severe-acute respiratory syndrome (SARS) and the virus was named as SARS-CoV. SARS is an atypical form of pneumonia; affected more than 800 people across three continents with a mortality rate about 10% [3-6]. In the aftermath of SARS epidemic, two additional human coronaviruses such as HCoV-NL63 in 2004 [7] and HCoV-HKU1 in 2005 [8] as well as at least 60 novel bat associated CoVs, including some closely related to SARS-CoV, were identified [9].

Most recently, a novel human coronavirus called Middle East respiratory syndrome coronavirus (MERS-CoV); previously called human coronavirus-Erasmus Medical Center (HCoV-EMC) was discovered by Zaki et al. in Saudi Arabia in 2012 [10,11] and spread to 26 countries, including United Arab Emirates, Jordan, Qatar, Egypt, the United Arab Emirates, Kuwait, Turkey, Oman, Yemen, Lebanon, Algeria, Malaysia, Bangladesh, Indonesia (none were confirmed), Austria, [12] Tunisia, the United Kingdom, France, Germany, Greece, Netherlands, South Korea [13,14], the United States [15,16], China [17] Thailand [18], and the Philippines [19] (Table 1). As of July 2015, a total of 1401 patients were infected with this virus, of which 543 (~39%) died [20-22]. Even though the transmission rate is comparatively slow when compared to SARS-CoV, the MERS-CoV infection continues to grow.

Coronaviruses belong to one of the two subfamilies of Coronavirinae and Torovirinae in the family of Coronaviridae, which inturn comprise the order, Nidovirales (Figure 1) [23,24]. Coronaviruses are classified into four genera (α, β, γ, δ) and each genus can be further divided into lineage subgroups. SARS-CoV and MERS-CoV belong to lineage 'b' and 'c' of Betacoronavirus respectively. However, MERS-CoV constitutes a sister species in the group 'c' along with bat coronaviruses HKU4 and HKU5 [25,26]. The close relationship of MERS-CoV to HKU4 and HKU5 suggests a zoonotic origin bat coronaviruses. The reports strengthen that Camels and Egyptian cave bats are likely to be major intermediate hosts for MERS-CoV infection [27,28]. Human-to-human transmission has now alarmed the healthcare societies with a higher prevalence in immunocompromised patients or patients with underlying the diseases [29,30]. The MERS infection resembled SARS, as both are human CoVs and exhibit severe respiratory infection with extra-pulmonary involvements and high case of fatality-rate. However, the additional unique symptom of MERS-CoV infection is associated with renal failure. The recent study shows that central nervous system (CNS) could be another target of MERS infection as three cases involved with neurological symptoms [31].

Intervention of MERS-CoV Infection

The emergence of MERS-CoV and the retransmission of SARS-CoV from zoonotic reservoirs to humans [32-34] have enhanced the concern of possible repetition of the 2003 SARS episode. One of the

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Received July 27, 2015; **Accepted** August 18, 2015; **Published** August 24, 2015

Citation: Pillaiyar T, Manickam M, Jung SH (2015) Middle East Respiratory Syndrome-Coronavirus (MERS-CoV): An Updated Overview and Pharmacotherapeutics. Med chem 5: 361-372. doi: 10.4172/2161-0444.1000287

Reporting country	Cases				Total	Deaths
Middle East	**2012**	**2013**	**2014**	**2015**		
Saudi Arabia	5	136	679	237	1057	467
United Arab Emirates	0	12	57	11	81	11
Jordan	2	0	10	7	19	6
Qatar	0	7	2	4	13	5
Oman	0	1	1	4	6	3
Iran	0	0	5	1	6	2
Kuwait	0	2	1	0	3	2
Egypt	0	0	1	0	1	0
Lebanon	0	0	1	0	1	0
Yemen	0	0	1	0	1	1
Europe	**2012**	**2013**	**2014**	**2015**		
United Kingdom	1	3	0	0	4	3
Germany	1	1	1	0	3	2
France	0	2	0	0	2	1
Netherlands	0	0	2	0	2	0
Greece	0	0	1	0	1	1
Turkey	0	0	1	0	1	1
Austria	0	0	1	0	1	0
Italy	0	1	0	0	1	0
Asia	**2012**	**2013**	**2014**	**2015**		
China	0	0	0	1	1	0
Malaysia	0	0	1	0	1	1
Philippines	0	0	0	3	3	0
South Korea	0	0	0	185	185	36
Thailand	0	0	0	1	1	0
Rest of the world	**2012**	**2013**	**2014**	**2015**		
Algeria	0	0	0	2	2	1
Tunisia	0	3	0	0	3	1
United States of America	0	0	2	0	2	0
Total	9	168	769	455	1401	543

Table 1: Confirmed MERS cases and deaths by country of reporting (March 2012-July 2015) [21,22].

important challenges in the epidemic of MERS-CoV is that no effective therapeutics is currently available. Therefore, developing treatments is paramount important to save lives and stop MERS-CoV to spread.

Coronaviruses are enveloped, single-stranded positive-sense RNA virus, extraordinarily a large RNA genome ranging from 26-36-kilobases. Coronaviruses contain proteins that contribute the overall structure; spike (S), Envelop (E), membrane (M) and neucleocapsid (Figure 2).

The search for antiviral agents in the post coronavirus outbreak (SARS-CoV) resulted in the identification of several anti-viral targets for MERS-CoV. First, the antiviral agent that may target coronavirus entry and spread are concerned with targeting the coronavirus spike protein; virus replication begins after the entry to the host cells through its spike protein (S), and upon the entry, the virus particle is uncoated and ready for translation. Second, the antiviral agents those targeting the proteases; both $3CL^{pro}$ and PL^{pro} are essential for replication, making them attractive targets. Third one targets the replicases (helicases); required for virus replication in host cells, and thus may serve as a feasible target for anti-MERS therapy (Figure 2). Further inhibition of virus replication, interfering with the host immune response and a combination therapy also take part in the classification of antiviral agents. This review describes the strategies used to develop effective pharmacotherapeutics for MERS-CoV by comparing with its close associated coronaviruses, especially SARS-CoV; a highly pathogenic human coronavirus outbreak emerged in 2003. Since the clinical, epidemiological and virological features for MERS-CoV are very similar to SARS-CoV, we have compared SARS-CoV with MERS-CoV for the readers to understand and thus it would be helpful for the development of new therapeutics.

Viral Entry or Fusion Inhibitors

In order to better understand the biology of coronaviruses, timely identification of receptor could reveal important clues to its zoonotic transmission, and its pathogenicity and therefore important to design possible pharmacotherapies. Additionally, surface receptors play an

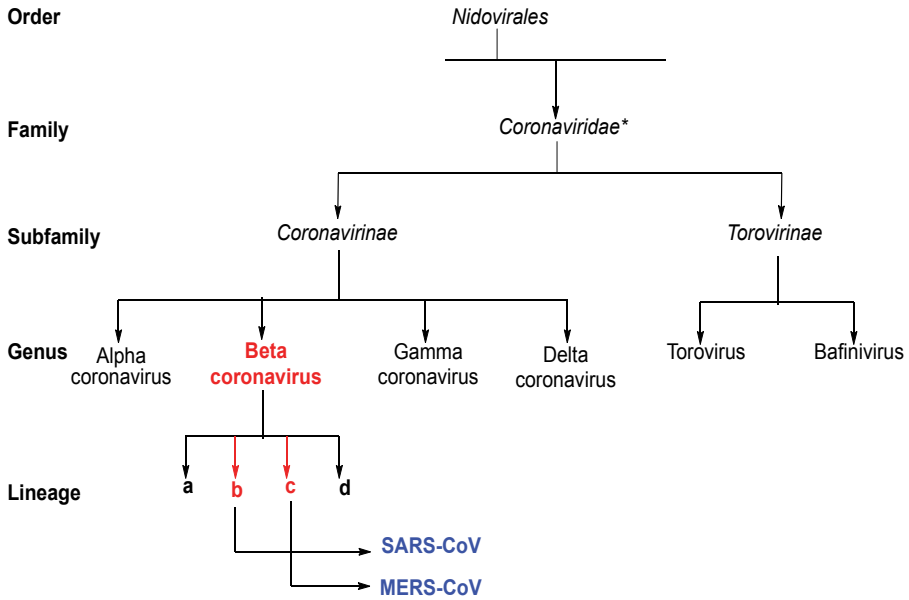


Figure 1: Schematic representation of taxonomy of *Coronaviridae* (according to the International Committee on Taxonomy of Viruses). Both SARS-CoV and MERS-CoV belong to the genus of Betacoronavirus but with different lineages. **Coronaviridae* is together with *Arteriviridae, Mesoniviridae*, and *Roniviridae* in the family.

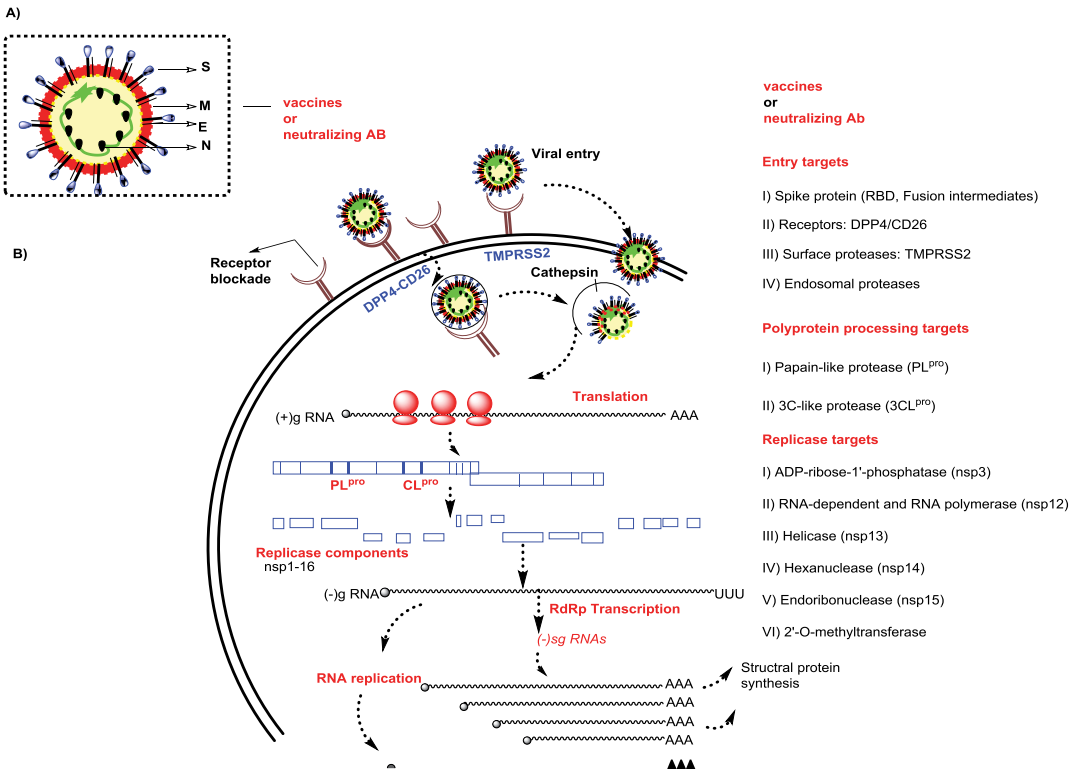


Figure 2: (A) Structure of Coronavirus and (B) anti-viral targets for MERS-CoV. (Ab: Antibody; DPP4: Dipeptidase Peptidyl 4; TMPRSS2: Transmembrane Protease, Serine 2; PL^{pro}: Papain-like Protease; $3CL^{pro}$: 3-C-like Protease; RdRp: RNA dependent and RNA polymerase; nsp: Non-Structural Protein).

important role in initiating virus entry into the host cells, thereby playing a major role in the tissue and host species tropism of viruses. Angiotensin converting enzyme-2 (ACE-2) was identified as a receptor for SARS-CoV, as it is attached to the defined specific receptor domain on S mediates the virus entry [35]. Some betacoronaviruses uses immunoglobulin-related carcinoembryonic antigen-related adhesion molecules (CEACAM) to enter cells, whereas for several alpha- and beta-coronaviruses [36], two peptidase have been recognized as cellular receptors [37,38]. Therefore inhibition of MERS-CoV binding to the cellular receptor of the host may be a promising approach for the treatment.

Like other coronavirus, MERS-CoV enters into the target cell either through endocytosis or plasma membrane fusion, while the latter is an important pathway. Similar to SARS-CoV, MERS-CoV binds to the host cells through interaction between the receptor binding domain (RBD) in its spike protein (s) and its receptor dipeptidyl peptidase-4 (DPP4) [39-41]. The recent study confirmed that MERS-CoV does not rely on the same receptor as SARS-CoV uses angiotensin converting enzyme-2 (ACE-2) for the cell entry [42].

This S protein of MERS-CoV is type I transmembrane glycoprotein which contains 1353 amino acids and can be cleaved into two subunit S1 and S2 (Figure 3A). The S1 subunit which contains RBD is responsible for binding to the target cellular receptor and S2 mediates the membrane fusion. Dipeptidyl peptidase-4 (DPP4, Figure 3B) also known as adenosine deaminase (ADA)-complexing protein-2 or CD26, was recently identified as a key functional receptor of the host cell for this virus [39], with the exception of mouse DPP4 [43]. MERS-CoV is the first that has been identified to use DPP-4 as a functional receptor for the entry into the cells [39]. DPP4 is an intrinsic 766-amino acid-long type II transmembrane gylcoproteins, expressed as a homodimer on the cell surface, which is involved in the cleavage of dipeptides [39,44]. It plays a major role in the glucose metabolism and is associated with various immunological functions, chemotaxis modulations, cell adhesions and apoptosis [39,44]. In human, the expression of DPP4 was found predominantly on the bronchial epithelial and alveolar cells in the lower parts of the lungs [44,45].

Viral entry inhibitors targeting RBD of S1 subunit in the S protein (cellular receptor dipeptidyl peptidase-4 (DPP4)

The binding motif of RBD in S1 subunit binds to the side surface of DPP4, in which the interaction is very similar to the interaction between ADA and DPP4 [39]. An *in vitro* study of MERS infection in ferret, known to be susceptible for many respiratory viruses, including SARS-CoV and influenza virus, revealed that adenosine deaminase, a DPP4 binding protein, competed for virus binding and acts as natural antagonist for MERS-CoV infection [39]. However, a screening of typical DPP4 inhibitors such as sitagliptin, vildagliptin and saxagliptin (see the structures in Figure 4) do not block the MERS-CoV infection [39]. This result has given a crucial point that the development of effective therapeutic and vaccines that target the binding interface between the S1 domain (RBD) of virus and receptor DPP4 may prove to be a promising approach for the effective treatment of MERS CoV infection.

Targeting RBD, two kinds of highly specific human monoclonal antibodies (MERS-4 and MERS-27) were identified using a non-immune yeast-displayed scFv library to screen against the recombinant MERS-CoV RBD. The most potent mAb, MERS-4 showed potent neutralizing activities against pseudotyped MERS-CoV infection in DPP4-expressing Huh-7 cells with the IC_{50} value of 0.056 μg/mL and

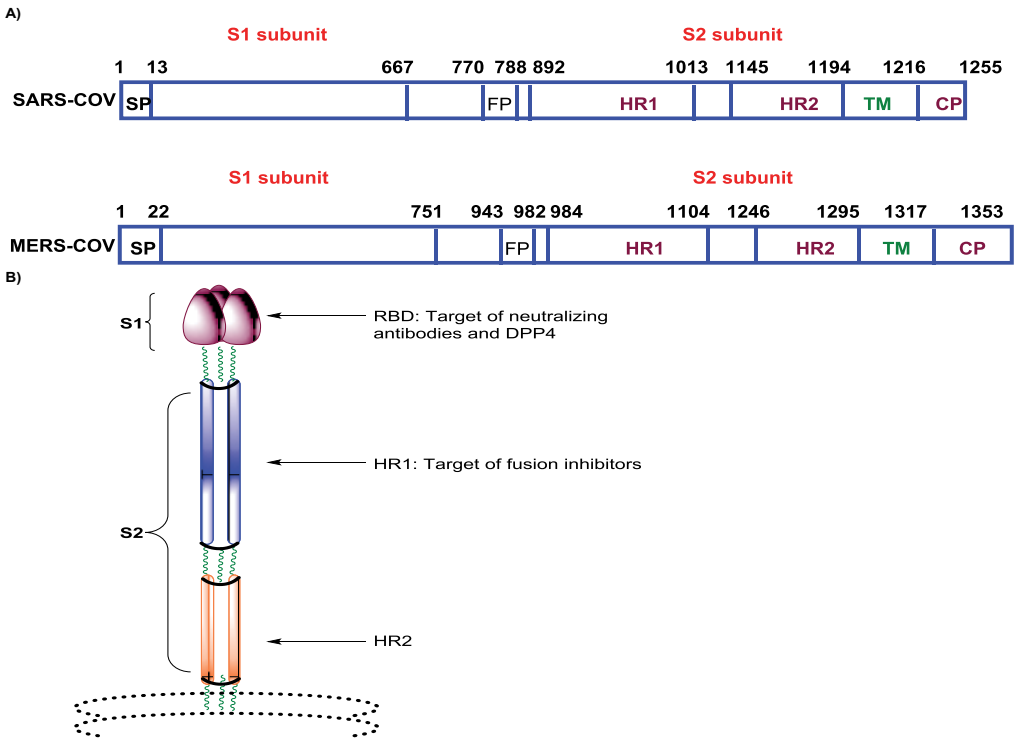


Figure 3: Structure of MERS-CoV S protein. (A): Structure of MERS-CoV S protein compared with SARS-CoV S Protein (B) SP: Signal Peptide; FP: Fusion Peptide; HR1: Heptad Repeat 1 Domain; HR2: Heptad Repeat 2 Domain; TM: Transmembrane; CP: Cytoplasmic Domain.

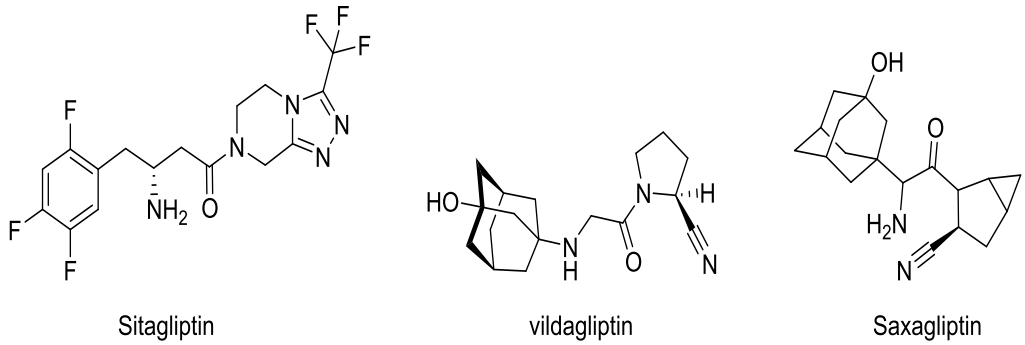


Figure 4: Structure of typical DPP4 inhibitors.

inhibited the formation of MERS-CoV-induced CPE during live MERS infection of permissive Vero E6 cells with an IC_{50} of 0.5 μg/mL [46].

In addition, the other human monoclonal antibodies (mAb), m336, m337 and m338 from a very large naïve-antibody library (containing ~10(11) antibodies) were tested against live MERS-CoV and found these are the first fully human mAbs to neutralize the pseudovirus and live virus with exceptionally high neutralizing activity for MERS-CoV [47]. Especially the most potent mAb, m336 inhibited >90% MERS-CoV pseudovirus infection (IC_{90}) in DPP4-expressing Huh-7 cells at a concentration of 0.039 μg/mL. The highest affinity of m336 showed the most potent live MERS-CoV neutralizing activity in inhibiting the formation of MERS-CoV-induced cytopathic (CPE) during live MERS infection of permissive Vero E6 cells with an IC_{50} value of 0.07 μg/mL.

Tang et al. discovered neutralizing mAbs by using a non-immune yeast-displayed scFv library [48]. The most potent antibody, 3B11, neutralized live MERS-CoV in the plaque reduction neutralization tests with an IC_{50} of 1.83 μg/mL.

Du et al. identified a recombinant protein containing a 212-amino acid fragment (residues 377-588) in the truncated RBD (residues 372-606) in the S1 subunit of MERS-CoV S protein fused with Fc receptor of human IgF (S377-588-Fc) [49]. This protein, denoted as S377-588-Fc, efficiently binds to the MERS-CoV receptor, DPP4, and potently inhibited MERS-CoV infection in DPP4 expressing cells. Particularly the truncated protein S377-588-Fc of MERS-CoV S protein induced strong MERS-CoV S-specific antibodies in vaccinated mice, blocking the binding of MERS-CoV to its cellular receptor DPP4 and effectively neutralizing MERS infection.

Viral entry inhibitors targeting RBD of S2 subunit in the S protein (cellular receptor dipeptidyl peptidase-4 (DPP-4)

S Protein of coronavirus plays indispensable roles in receptor recognition, membrane fusion and thereby initiating the infection. In this process, heptad repeats 1 and 2 (HR1 and HR2) of the S protein assemble into a complex called six-helix bundle (6-HB) fusion core structure, which represents a key membrane fusion architecture. The discovery of T20, an HR2 peptide was approved by the US FDA as the first HIV fusion/entry inhibitor, has opened a new avenue to identify and develop peptidic viral entry inhibitors against enveloped viruses with class 1 fusion proteins such as Nipahvirus, Hendravirus, Ebola virus and other paramyxoviruses, Newcastle disease virus, simian immunodifficiency virus, feline immunodeficiency virus and respiratory syncytial virus [50-53].

Cao et al. [54] and later Lu et al. [55] studied the structure and function of the heptad repeat domains HR1 and HR2 in the S protein S2 subunit of MERS-CoV (Figure 5), particularly six-helix bundle fusion core structure formed by the HR1 and HR2 domains, with the aim of designing a novel candidate as MERS-CoV fusion inhibitor. The HR sequences were variably truncated and then connected with a flexible amino acid linker. As a result, two heptad repeat peptides HR1P and HR2P, spanning amino acid residues in HR1 and HR2 domains, respectively, were identified as potent inhibitors of MERS-CoV replication, inhibiting MERS-CoV S protein mediated cell-cell fusion [55]. More specifically, HR2P was the most potent in inhibiting MERS-CoV S protein mediated cell-cell fusion and six-helix bundle fusion core formation. HR2P could effectively inhibit MERS-CoV replication in Vero cells in a dose-dependent manner (IC_{50} value of ~ 0.6 μM) with low or no *in vitro* toxic effect. (Selectivity index for HR2P is >1,667). Addition of hydrophilic residues into HR2P resulted in significant improvement of its stability, solubility and antiviral activity. It was interesting to note that MERS-CoV HR2P could not inhibit SARS-CoV pseudovirus infection in 293T/ACE2 cells, while the SARS-CoV HR2P peptide SC-1 was effective in inhibiting SARS-CoV infection, indicating that HR2P peptide is a MERS-Specific fusion inhibitor.

Repurposing of clinically developed drugs targeting viral entry

First and foremost need for the MERS epidemic is more countermeasures that can be used to control, at least, the early episode of an epidemic to provide an immediate treatment response while appropriate therapies are being developed. Given the time and cost associated with the intellectual right for developing the novel pharmaceutics, one feasible and rapid advancement in the drug discovery is repurposing of existing clinically approved drugs. This approach has several advantages; including availability, lower cost, and safety/tolerability.

Screening of a library of drugs either clinically developed or with a well-defined cellular pathway from different classes of therapeutics identified a series of compounds with activity against MERS-CoV, SARS-CoV and both together [56,57] (Table 2). These compounds were grouped into 16 different therapeutic classes based on their recognized mechanism of action. Drugs that inhibited both coronaviruses included neurotransmitter inhibitors, estrogen receptor antagonists, kinase signaling inhibitors, protein-processing inhibitors, inhibitors of lipid or sterol metabolism and inhibitors of DNA synthesis or pair. Antidiarrheal agent or HIV-1 protease inhibitor were identified to inhibit MERS-CoV infection in the low-micromolar range. Antiparasitics or antibacterials in which those function was not obviously linked to coronaviruses in general, showed antiviral activity against MERS-CoV. Cathepsin inhibitor, E-64-D, blocked the MERS-CoV and SARS-CoV: cathepsins are important for the fusion step during virus entry of coronavirus [58]. Two of the neurotransmitter inhibitors, including chloropromazine hydrochloride and triflupromizine inhibit the dopamine receptor that led to inhibit both SARS-CoV and MERS-CoV. The similarity of chlorpromazine hydrochloride and flupromizine in the chemical structure would suggest that the inhibition of these coronaviruses have the same mechanism of action. Chloropromazine hydrochloride, an inhibitor of clathrin-mediated endocytosis for virus entry, reported

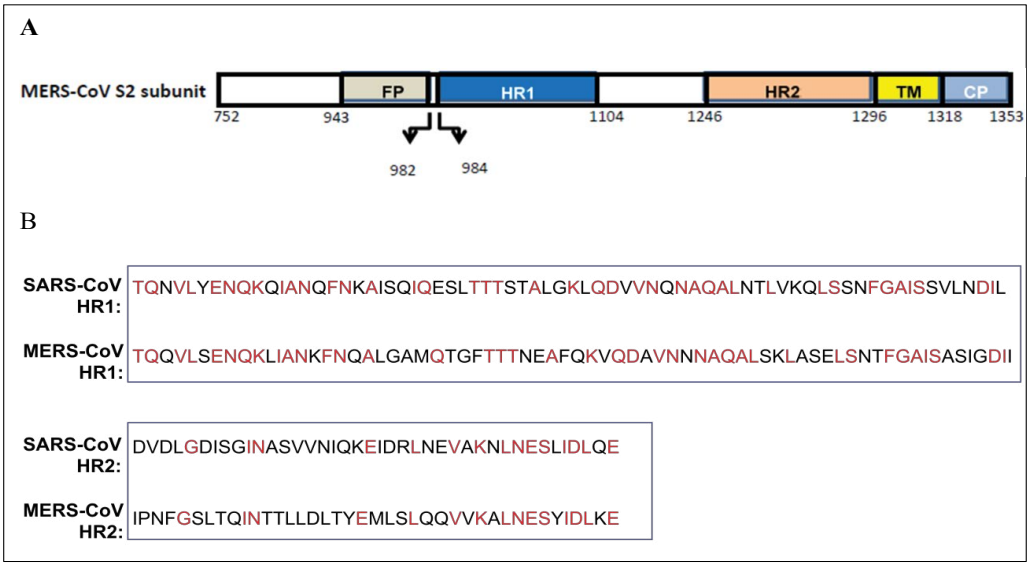


Figure 5: (A) Schematic representation of MERS-CoV S protein S2 subunit. FP: Fusion Peptide; HR1: Heptad Repeat 1 Domain; HR2: Heptad Repeat 2 Domain; TM: Transmembrane; CP: Cytoplasmic domain. (B) Sequence similarities between the H1R and H2R domains in S2 of SARS-CoV and H1R and H2R domains in S2 of MERS-CoV. (For MERS-CoV: HR1P, residues 986-1055 and HR2P, residues 1246-1285; for SARS-CoV: HR1P, residues 894-963 and HR2P, residues 1144-1183). Identical amino acids are highlighted in red color. The figure was simplified from the picture reported by Lu et al. [55].

Pharmaceutics	Class	MERS-CoV EC_{50} (μM)	SARS-CoV EC_{50} (μM)
Emetine dihydrochloride hydrate	Antibacterial agent	0.014	0.051
Chloroquine diphosphate	Antiparasitic agent	6.27	6.53
Hydroxychloroquine sulfate	Antiparasitic agent	8.27	7.96
Mefloquine	Antiparasitic agent	7.41	15.55
Amodiaquine dihydrochloride dihydrate	Antiparasitic agent	6.21	1.27
loperamide	Antidiarrheal agent	4.8	5.90
Lopinavir	HIV-1 inhibitor	8.0	24.4
E-64-D	Cathepsin inhibitor	1.27	0.76
Gemcitabine hydrochloride	DNA metabolism inhibitor	1.21	4.95
Tamoxifen citrate	Estrogen receptor inhibitor	10.11	92.88
Toremifene citrate	Estrogen receptor inhibitor	12.91	11.96
Terconazole	Sterol metabolism inhibitor	12.20	15.32
Triparanol	Sterol metabolism inhibitor	5.28	
Anisomycin	Protein-processing inhibitor	0.003	0.19
Cycloheximide	Protein-processing inhibitor	0.189	0.04
Homoharringtonine	Protein-processing inhibitor	0.071	
Benztropine mesylate	Neurotransmitter inhibitor	16.62	21.61
Fluspirilene	Neurotransmitter inhibitor	7.47	5.96
Thiothixene	Neurotransmitter inhibitor	9.29	5.31
Chlorpromazine hydrochloride	Neurotransmitter inhibitor	9.51	12.97
Fluphenazine hydrochloride	Neurotransmitter inhibitor	5.86	21.43
Promethazine hydrochloride	Neurotransmitter inhibitor	11.80	7.54
Astemizole	Neurotransmitter inhibitor	4.88	5.59
Chlorphenoxamine hydrochloride	Neurotransmitter inhibitor	12.64	20.03
Thiethylperazine maleate	Neurotransmitter inhibitor	7.86	
Triflupromazine hydrochloride	Neurotransmitter inhibitor	5.75	6.39
Clomipramine hydrochloride	Neurotransmitter inhibitor	9.33	13.23
Imatinib mesylate	Kinase signaling inhibitor	17.68	9.82
Dasatinib	Kinase signaling inhibitor	5.46	2.10

Table 2: Compounds with activity against MERS-CoV and SARS-CoV.

to inhibit the replication of alphaviruses (hCoV-229E), hepatits C virus, infectious bronchitis virus and mouse hepatitis virus-2 (MHV-2) [59-63]. These studies suggest that the drug chlorpromazine may act similarly on these viruses and have potential as a broad-spectrum coronavirus inhibitor. In addition to that, three neurotransmitter inhibitors (chloropromazine, promethazine, and fluphenazine) were reported to inhibit MERS-CoV S protein mediated cell-cell fusion with IC_{50} values of about 20, 20 and 29 μM, respectively [64].

Kinase signaling pathway inhibitors imatinib mesylate and dasatinib are known inhibitors of the Abelson murine leukemia viral oncogene homolog-1 pathway (ABL-1), and active against both MERS-CoV and SARS-CoV. The data suggest that the ABL-1 pathway may be important for the viral replication and inhibitors of this pathway may have the potential in the discovery of antiviral agents.

The identified DNA synthesis inhibitors (for instance, Gemcitabine hydrochloride) those were active against at least one coronavirus, suggesting that these drugs have potential as antiviral therapy coronaviruses. Toremifine citrate is an estrogen receptor 1 antagonist that inhibits both MERS-CoV and SARS-CoV with EC_{50} of 12.9 and 11.97 μM respectively.

The interferon (IFN) response is an integral component of innate immunity against viral infections and the IFN-induced transmembrane proteins (IFNTM) 1 to 3 inhibit infections of several enveloped viruses [65-67] including hCoV-229E [68] and SARS-CoV [69]. The inhibition usually occurs during the fusion of viral membrane with an endosomal membrane [65,70-72], and might be due to an IFN-induced accumulation of cholesterol in late endosomes. Recent report suggested that MERS-CoV is sensitive to inhibition by IFITM proteins [73]. In 293T cells, IFITM-mediated inhibition of SARS-CoV or MERS-CoV entry was less efficient than blockade of human coronaviruses 229E and NL63. However, the similar difference was not observed in A549 cells, suggesting that cellular context and/or IFITM protein expression levels can influence inhibition efficacy.

Interferon Therapy

MERS-CoV elicits attenuated innate immune responses with delayed proinflammatory cytokine induction in cell culture and *in vivo* [74,75]. It is inhibited by type 1 interferons IFN-α, IFN-β and IFN-λ more effectively than SARS-CoV. Especially, IFN-β has a significant *in vitro* antiviral effect on MERS-CoV than SARS-CoV, suggesting a potential therapeutic use for interferons. The Interferon-alfa therapy was reported be effective for patients with probable SARS, treated with corticosteroids or corticosteroids plus subcutaneous interferon alfa-consensus-1 [76].

Ribavirin was extensively used in SARS patients without any beneficial effect and was complicated by haemolytic anaemia and metabolic disturbances in many cases [77,78]. *In vitro* study of ribavirin combined with interferon exhibit anti-MERS-CoV activity [79] and it was observed that the activity of interferon was enhanced by adding of ribavirin [80]. A combination therapy using interferon and ribavirin was tried in 5 patients with MERS; the median time from admission to therapy was 19 days [81]. The treatment was given to severely ill patients and none of the cases were responded to the therapeutic intervention and all died of their illness. This may probably be the late administration of the combination therapy in the critical stage of the disease [81].

Protease Inhibitors

Protease plays an indispensable role during virus life cycle: it is essential for viral replication by mediating the maturation of viral replicases and thus becomes an attractive target of potential antiviral drugs. Protease inhibitors block the replication of coronaviruses (CoVs), including the causative agents of MERS and SARS infection providing a promising foundation for the development of new antiviral agents.

Both and MERS and SARS coronaviruses are enveloped, single-stranded positive-sense RNA virus, extraordinarily a large RNA genome ranging from 26-36-kilobases. Each of their genes encodes two replicase polyprotein pp1a and pp1b that are processed by viral proteases, the papain-like protease (PL^{pro}) and a 3C-like protease ($3CL^{pro}$ also known as the main protease). PL^{pro} is responsible for cleavage at first three position of its polyprotein to produce 3 non-structural proteins, while $3CL^{pro}$ cleaves the remaining 11 locations, releasing non-structural proteins from nsp4 to nsp16. As a result, sequence motifs recognized by MERS-CoV PL^{pro} and SARS-CoV PL^{pro} are (L/I)XGG↓(A/D)X and LXGG↓(A/K)X, respectively (Figure 6).

Papain-like protease (PL^{pro}) inhibitors

High-throughput screening of molecule library containing 25,000 chemical entities against both PLpro enzymes of MERS-CoV and SARS-CoV identified a novel covalent low molecular weight dual inhibitor (Figure 7A) [82]. This was the first MERS-CoV PL^{pro} inhibitor published to date. The mode of action suggested that this compound acts as a competitive inhibitor against MERS-CoV with an IC_{50} value of 6 μM, while the same acts as an allosteric inhibitor against SARS-CoV (IC_{50} 11 μM). It was interesting to note that the previously reported MERS-CoV PL^{pro} inhibitors (Figure 7B-7E) [83-86] were not active against SARS-CoV PL^{pro}, which infers the difference in the binding mode of both PL proteases. The difference was clearly explained in the recent study, two SARS-CoV PL^{pro} complex crystal structures with the lead inhibitors (C and D) revealed that inhibitors bind not to the catalytic site of SARS CoV PL^{pro} but to the BL2 loop, blocking the entrance of active site. The BL2 appears to prevent the accessibility of substrate to the active site, and thereby inhibiting of enzymatic activity. Structural and sequence analysis at BL2 loop of SARS-CoV PL^{pro} revealed that the two residues Y269 and Q270 responsible for inhibitor binding, are replaced by T274 and A275 in MERS CoV PL^{pro}, making difficult for SARS-CoV PL^{pro} inhibitors binding to MERS-CoV $3CL^{pro}$.

In anti-viral therapy, PL^{pro} has been shown to be an important target as it is a multifunctional protein involved in deubiquitination, de-ISGylation (ISG: Interferon-Stimulated Gene), and viral evasion of the innate immune response in addition to its proteolytic activity. 6-Thiopurine analogues and *N*-ethyl maleimide (NEM) as well as the immunosuppressive drug, mycophenolic acid, were all independently able to inhibit the proteolytic activity and deubiqutination of MERS-CoV PL^{pro} (Table 3) [87]. Compared with NEM, 6MP and 6-TG were more effective inhibitors, while mycophenolic acid was a less effective inhibitor against the MERS-CoV PL^{pro}.

3C-like protease ($3CL^{pro}$) inhibitors

Ren et al. found that the wide-spectrum anti-CoV inhibitor N3 (Figure 8) can inhibit the proteolytic activity of MERS-CoV $3CL^{pro}$ with an IC_{50} of 0.28 μmol/L and by solving the crystal structure of MERS-CoV $3CL^{pro}$ with inhibitor N3 confirms that inhibition of protease through a similar mechanism to other CoVs [88].

AG7088, a potent inhibitor of rhinovirus $3C^{pro}$ with Michael acceptor functionality, failed to inhibit SARS-CoV $3CL^{pro}$ [89]. Interestingly, a series of AG7088 analogues were reported to combat CoVs by targeting $3CL^{pro}$ [90]. The screening of SARS-CoV $3CL^{pro}$ peptidomimetics (M-1 to M-10; Figure 8) which contain a Michael acceptor, (i.e., α,β-unsaturated carbonyl) [91-93], displayed inhibition

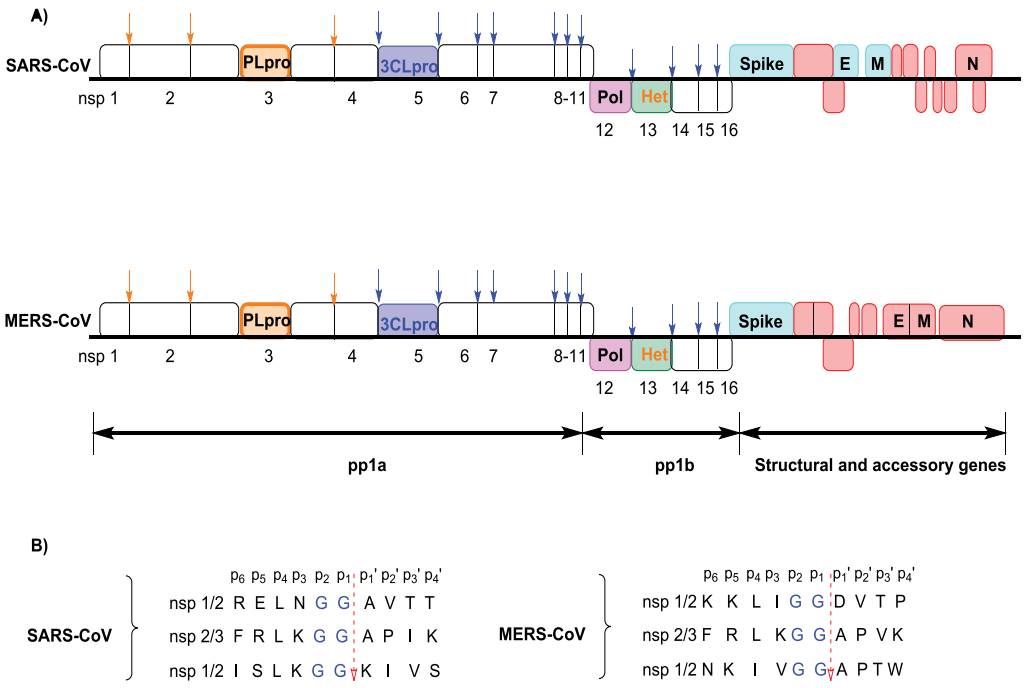


Figure 6: Outline of SARS and MERS-coronaviruses polyproteins. (A) Cleavage positions of PL^{pro} and $3CL^{pro}$ are shown by arrows (B) Cleavage site comparison between SARS and MERS PL^{pro} enzymes (For SARS-PL^{pro}: (L/I)XGG↓(A/D)X and for MERS-PL^{pro}: LXGG↓(A/K)X). This figure was inspired from Lee et al. [82].

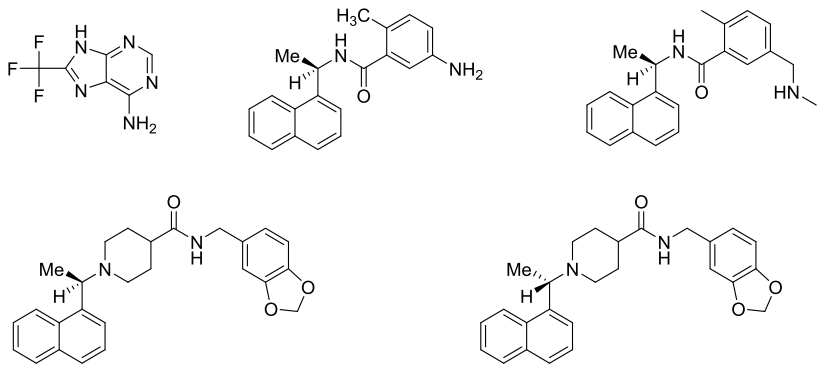


Figure 7: A hit compound (A) of MERS-CoV PL^{pro} obtained from HTS and SARS-CoV PL^{pro} lead inhibitors (B-E). In anti-viral therapy, PL^{pro} has been shown to be an important target as it is a multifunctional protein involved in deubiquitination, de-ISGylation (ISG: Interferon-Stimulated Gene) and viral evasion of the innate immune response in addition to its proteolytic activity. 6-Thiopurine analogues and *N*-ethyl maleimide (NEM) as well as the immunosuppressive drug, mycophenolic acid, were all independently able to inhibit the proteolytic activity and deubiqutination of MERS-CoV PL^{pro} (Table 3) [87]. Compared with NEM, 6MP and 6-TG were more effective inhibitors, while mycophenolic acid was a less effective inhibitor against the MERS-CoV PL^{pro}.

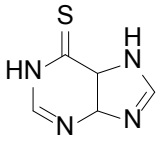
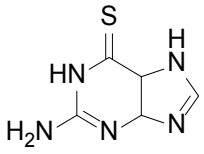
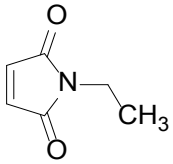
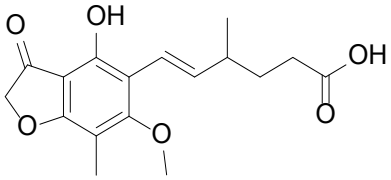
Compound	Chemical structure	IC_{50} (μM)	
		Peptide cleavage	DUB activity
6-Mercaptopurine (6MP)		26.9	25.8
6-Thioguanine (6-TG)		24.4	12.4
N-Ethylmaleimide (NEM)		45.0	ND
Mycophenolic acid		247.6	222.5

Table 3: Structure and IC_{50} of compounds against MERS-CoV PL protease.

against MERS-CoV 3CL^{pro}, especially compounds M-5 to M-8 showed comparatively good inhibitions with the Ki values in the micromolar range [94]. These compounds were basically reported for their inhibitory activity against SARS-CoV 3CL protease. Structure-activity relationship of these compounds show that the S2-subsite of MERS-CoV 3CLprotease is small and can only accommodate a smaller group P2-isobutyl but no bigger substitutions.

These inhibitors provide an excellent starting point for the development of natural substrate mimicking (or peptidomimetics) compounds against MERS CoV 3CL protease. GRL-001, a 5-chloropyridyl ester derived compound reported for the inhibition of SARS-CoV 3CL^{pro} activity [86,95], has shown to block the replication of MERS-CoV 3CL^{pro} [96]. This GRL-001 would serve as a potential lead for the future drug development for anticoronavirus therapy.

Recent kinetic studies revealed that MERS-CoV 3CL^{pro} is less efficient at processing a peptide substrate (Kd ~ 52 μM, but SARS-CoV 3CL^{pro}; IC_{50} <50 nM) being a weekly associated dimer [94]. kinetic studies of peptidomimetic inhibitors contain a Michael acceptor group, known for irreversible binding, demonstrated that MERS-CoV 3CL protease undergone a significant ligand-induced dimerization upon binding, the reaction of covalent bond formation with active site cysteine ensures the compete inhibition of enzyme at low molecular concentration. On the contrary, the non-covalent (or reversible) inhibitors act as activators at low concentration and the inhibition was

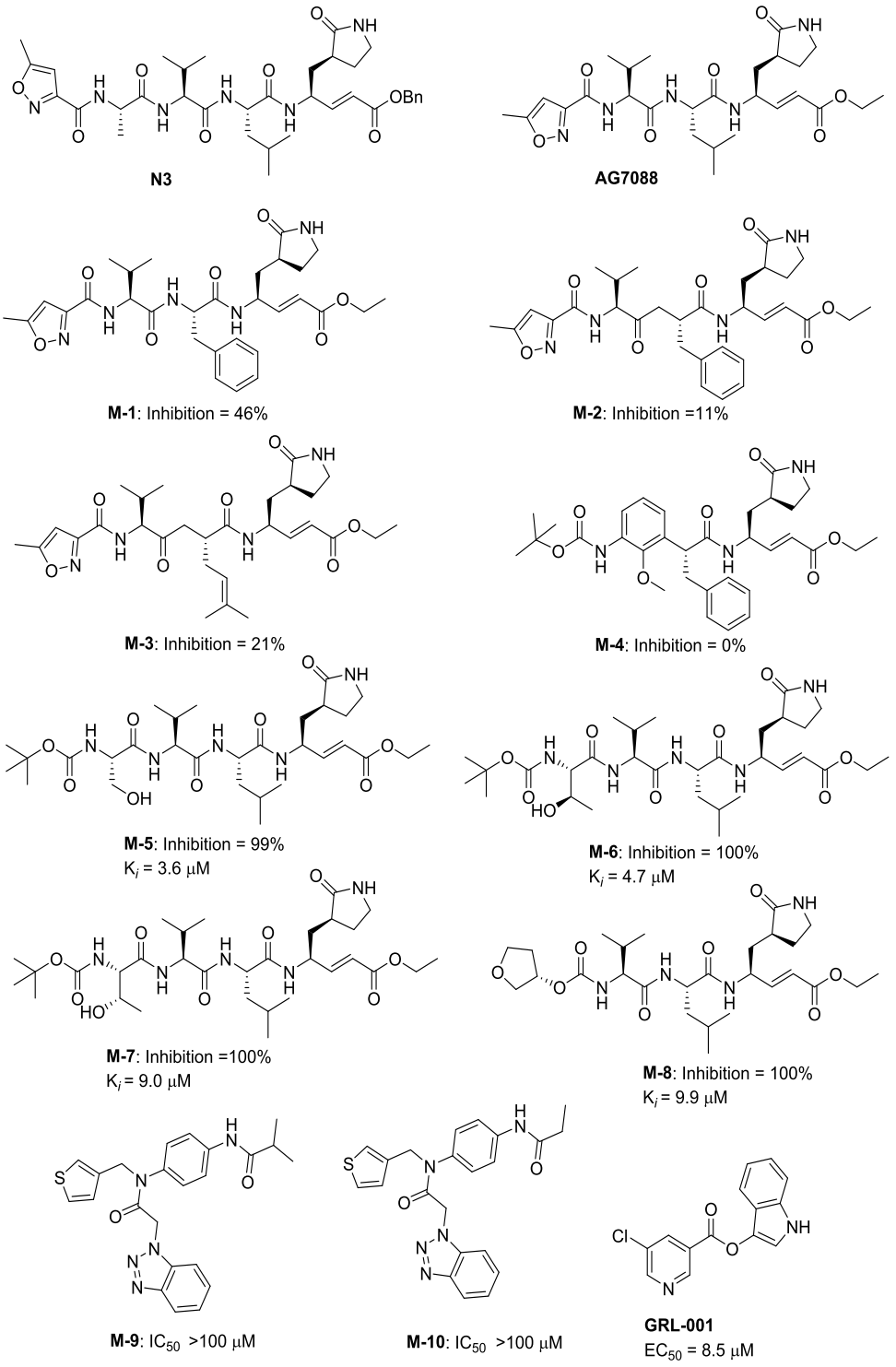


Figure 8: Structure of 3CL protease inhibitors contain Michael acceptor group (N3, AG7088, M-1 to M-8) and activated carbonyl functionality (M-9, M-10 and GRL-001).

achieved only at high concentration. Based on this observation, the compounds that inhibit irreversibly MERS-CoV $3CL^{pro}$ may serve as a starting point for the development of anti-MERS therapy.

Replicase Inhibitors

Targeting MERS-CoV helicase (nsp13)

Helicases are ubiquitous proteins that are required for a wide range of biological processes, such as genome replication, recombination, displacement of proteins bound to NAs and chromatin remodeling. Helicase (nsP13) protein is a critical component, required for virus replication in host cells, and thus may serve as a feasible target for anti-MERS and anti-SARS chemical therapies.

The recent approach was taken by Adedeji et al. [97,98], who recently reported a small 1,2,4-triazole derivative compound called

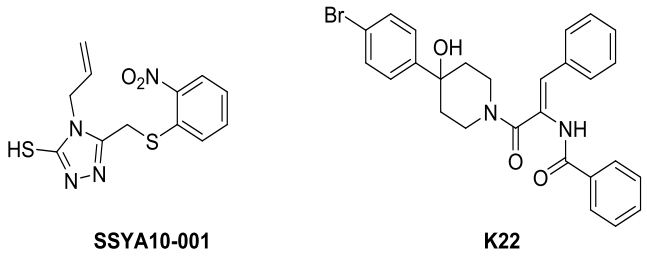


Figure 9: Structure of helicase inhibitor SYA10-001 and viral RNA synthesis inhibitor k22.

SSYA10-001 (Figure 9) that inhibited the viral NTPase/helicase (known as nonstructural protein 13, nsp13) of both SARS-CoV and MERS-CoV. The antiviral activity of SSYA10-001 inhibits MERS-CoV and SARS-CoV replication with EC_{50} values of 25 μM and 7 μM, respectively, and no significant cytotoxicity was observed even at 500 μM. There have been, so far, no helicase inhibitors approved antiviral therapy and thus compound SSYA10-001 could serve as potential lead for the development of effective broad spectrum anti-coronavirus drugs.

Targeting membrane-bound viral RNA synthesis

Like all RNA viruses, coronaviruses employ host cells membranes to assemble the viral replicase complex. This evolutionary conserved strategy provides a compartment for viral RNA synthesis, a crucial step in the coronavirus life cycle that is enriched in replicative viral and host cell-derived proteins and believed to protect from antiviral host cells defense mechanism. Antiviral agents that target membrane-bound coronaviral RNA synthesis, which is important for the replication represent a novel and attractive target. Lundin et al. [99] discovered an inhibitor, designated K22 that targets membrane-bound coronaviral RNA synthesis and showed potent antiviral activity of MERS-CoV infection with remarkable efficacy, illustrated by reduction of viral replication and substantial reduction of dsRNA in MERS CoV infected primary HEA cultures. MERS-CoV can readily replicate on primary HAE cells by infecting non-ciliated cells expressing the cellular receptor DPP4 [100].

Conclusion and Perspectives

The Middle East respiratory infection-coronavirus (MERS-CoV) was identified in 2012 in Saudi Arabia and was recognized as a sixth human coronavirus identified to the date. The disease has been associated with a high-mortality rate; until June 2015, 1167 patients were infected across the world with this virus and 479 (41%) of which died. Alarmingly, the human-human transmission of this deadly virus has raised a global concern about the potential for MERS pandemic.

A feasible and rapid advancement in the drug discovery for the development of effective chemotherapeutics against MERS-CoV can be achieved by repurposing the existing and clinically approved drugs.

Unlike the PL^{pro} inhibitors of SARS-CoV, the CL^{pro} inhibitors of SARS-CoV showed inhibitory activity against MERS-CoV $3CL^{pro}$ with almost similar level of potency. Thus, it should be highly considered that evaluating SARS-CoV $3CL^{pro}$ inhibitors against MERS-CoV $3CL^{pro}$ may provide promising leads for the development of new anti-MERS agents.

The discovery of monoclonal antibodies (mAbs), especially m336 with potent live MERS-CoV neutralizing activity and the HR2 peptide, a potent inhibitor of MERS-CoV S protein mediated cell-cell fusion may consider as advancements, and these should be considered taking into clinical studies for the treatment of MERS infection.

Establishment of effective animal for the evaluation of efficacy of candidate vaccine is of foremost important. Unlike SARS-CoV, it has been shown that MERS-CoV is unable to replicate in small animal models like hamsters, ferrets and mice [80,101-103] significantly restricting the efficacy evaluation of MERS vaccines.

The recent study shows that central nervous system (CNS) could be another target of MERS-CoV infection as three cases involved with neurological symptoms. Therefore patients with progressive or worse CNS findings are to be given special attentions.

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