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Teaser Recent advances in the research and development of small-molecule anti-human coronavirus therapies.

Reviews • KEYNOTE REVIEW

Recent discovery and development of inhibitors targeting coronaviruses

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Human coronaviruses (CoVs) are enveloped viruses with a positive-sense single-stranded RNA genome. Currently, six human CoVs have been reported including human coronavirus 229E (HCoV-229E), OC43 (HCoV-OC43), NL63 (HCoV-NL63), HKU1 (HCoV-HKU1), severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), and MiddleEast respiratory syndrome (MERS) coronavirus (MERS-CoV). They cause moderate to severe respiratory and intestinal infections in humans. In this review, we focus on recent advances in the research and development of small-molecule anti-human coronavirus therapies targeting different stages of the CoV life cycle.

Introduction

Coronaviruses (CoVs) primarily cause multiple respiratory and intestinal infection in humans and animals [1]. Although the history of CoVs began in the 1940's [2,3], the identification of first human CoVs were reported in the 1960's, as causative agents for mild respiratory infections. Subsequently they were named as (i) human CoV 229E (HCoV-229E) and (ii) HCoV-OC43 [4–6]. In the 1970's, the discovery of new viruses, studies about the mechanism of action as well as the replication and pathogenesis of CoVs were active among virologists. This led to discovery of another four new human coronaviruses, namely (iii) HCoV- Hong Kong University 1 (HKU1) [7,8] (iv) HCoV-NL63, (v) severe acute respiratory syndrome (SARS)-CoV and (vi) Middle East respiratory syndrome (MERS)-CoV. The first four CoVs are universally circulated and contribute approximately one-third of common cold in humans [9]. However, in severe cases, they can cause life-threatening pneumonia and bronchiolitis in children and immunocompromised individuals [10–12] such as those undergoing chemotherapy and those with HIV-AIDS [13–15]. Besides that, these four coronaviruses have been associated with enteric and neurological diseases [16–20]. In 2003, SARS-CoV was identified as a causative agent during the global pandemic SARS. According to the World Health Organization (WHO), the emergence by SARS-CoV had affected 8422 cases in 32 countries, 916 of which died with the fatality rate of 10-15% [21]. Following this outbreak, ten years after, another highly pathogenic coronavirus MERS-CoV epidemic surfaced in Middle Eastern countries in 2013 [22]. However, the

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major outbreak was happened in the Republic of Korea in 2015 [23]. The virus infection was majorly observed in adults, although it can affect any age of people [24]. Within a short time, the virus affected a total number of 1401 individuals, 543 of which died with the mortality of rate of ~39% worldwide, while in Saudi Arabia alone it was 37.5%[25].In the last two decades, there have been extensive studies on human coronaviruses, especially on SARS- and MERS-CoVs that led not only to understand coronaviruses biology but has also driven the discovery of new therapeutics for in case if any future outbreaks. In this review, we focus on the recent development of inhibitors targeting coronaviruses.

Taxonomy, structure and replication of human coronaviruses

Coronaviruses are members of two subfamilies of *Coronavirinae* and *Torovirinae* in the family of *Coronaviridae*, which in turn comprise the order *Nidovirales* [26] (Fig. 1). The *Coronavirinae* subfamily is further classified into four main genera: α-coronavirus, β-coronavirus, γ-coronavirus and δ-coronavirus based on the International Committee for Taxonomy of Viruses (Fig. 1). HCoV-229E and HCoV-NL6 belong to α-coronavirus, HCoV-HKU1, SARS-CoV, MERS-CoV, and HCoV-OC43 are β-coronaviruses, and they both infect only mammals. γ-Coronavirus and δ-coronavirus infect birds, but some of them can also infect mammals [27]. Based on current sequence databases, it has been discovered that all human CoVs have animal origins; SARS-CoV, MERS-CoV, HCoV-NL63, and HCoV-229E are considered to have originated in bats; HCoV-OC43 and HKU1N are likely originated from rodents [28,29].

Under the electron microscope, coronaviruses are enveloped, single-stranded positive-sense RNA virus with the largest genome size, ranging approximately from 26-32-kilobases found to date [30]. The genomic RNA, which acts as a messenger RNA (mRNA), plays an important role in the initial RNA synthesis of the infectious cycle, template for replication and transcription and as a substrate for packaging into the progeny virus. In all CoVs, 5′ two-thirds of the genome encodes a replicase polyproteins, pp1ab, which comprises of two overlapping open reading frames (ORFs), ORF1a and ORF1b. These ORFs are then processed by viral proteases to cleave into 16 non-structural proteins that are involved in genome transcription and replication. The 3′ terminus encodes CoV canonical set of four structural proteins; including (i) the nucleocapsid (N) protein, a basic RNA-binding protein, (ii) a spike protein (S), a type of glycoprotein I, (ii) a membrane protein (M) that spans the membrane, and (iii) an envelope protein (E), a highly hydrophobic protein that covers the entire structure of the coronavirus [31] (Fig. 2). These accessory proteins are not only important for virion assembly but may also have an additional link that they suppress the host immune response to facilitate viral replication.

The replication of coronavirus begins with the binding of its spike protein (S) on the cell surface molecules of the host. This receptor recognition is important for initiating virus entry into the host cells, thereby playing a major role in the tissue and host species tropism of viruses. The receptors used by all human CoV are known (see Table 1): Aminopeptidase N by HCoV-229E [32], 9-O-acetylated sialic acid by HCoV-OC43 and HCoV-HKU1 [33,34], angiotensin-converting enzyme 2 (ACE2) by SARS-CoV [35] and HCoV-NL63 [36,37] and dipeptidyl peptidase 4 (DPP4) by MERS-CoV [38].

Apart from this, some CoVs may also enter into the cells with the help of proteases; for example, the role of cathepsin L has been linked with the SARS-and MERS-CoVs entry, transmembrane protease serine 2 (TMPRSS2) and airway trypsin-like protease TMPRSS11D could activate the S protein for virus entry at the cell membrane during HCoV-229E and SARS-CoV infection [39–41].

Upon the entry, the viral particle is uncoded and ready for translation ORF 1a and 1b into polyproteins pp1a (4382 amino

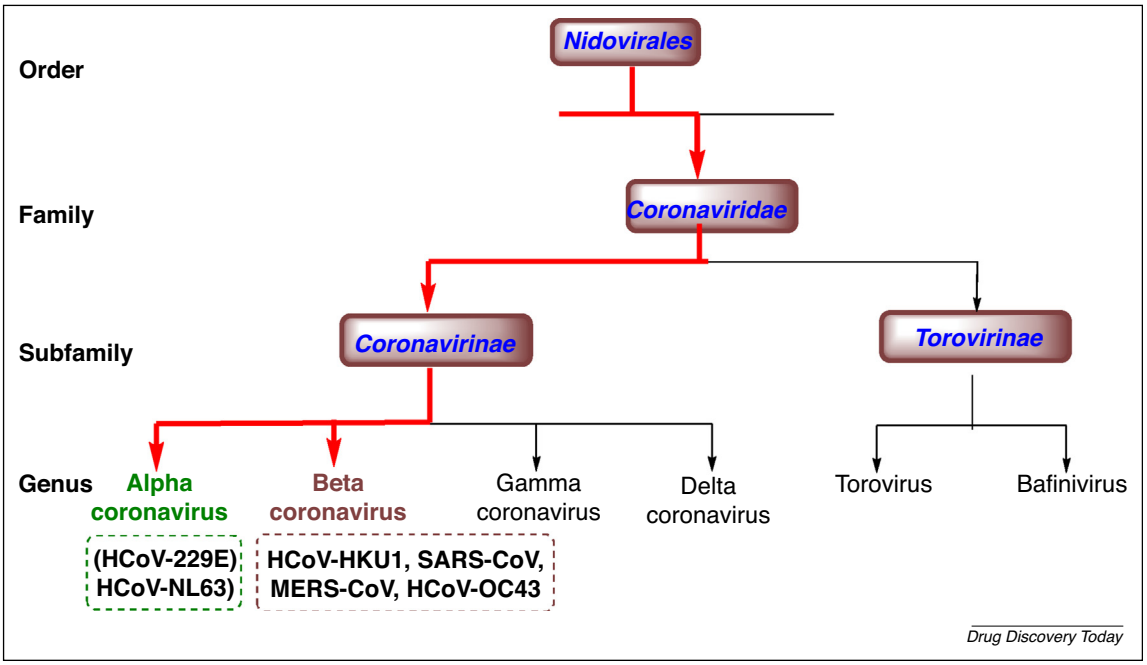

FIGURE 1

Schematic representation of the taxonomy of *Coronaviridae* (according to the International Committee on Taxonomy of Viruses). The six human coronaviruses belong to the Alpha- and Beta-coronaviruses genuses, respectively.

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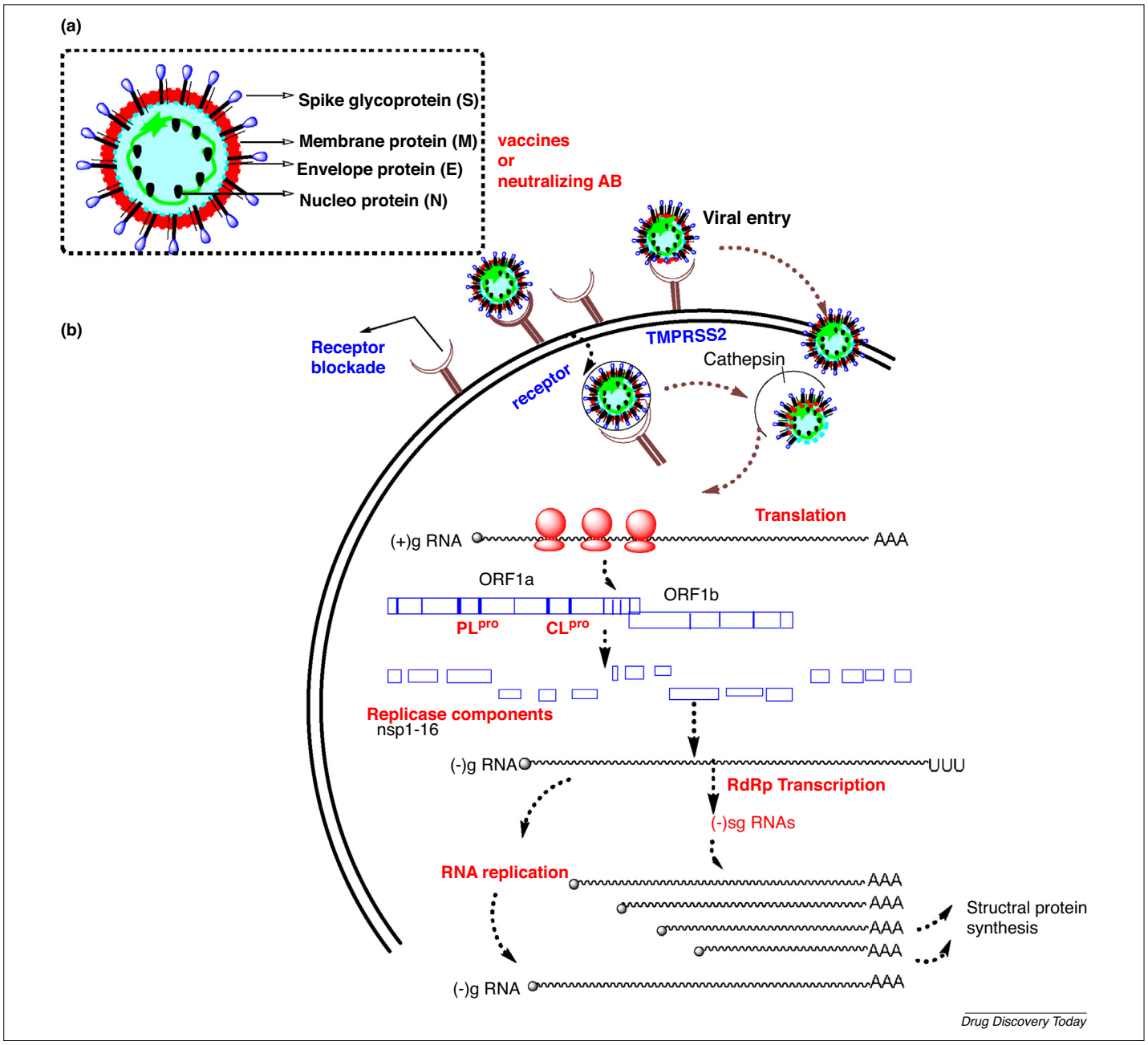

FIGURE 2

(A) Structure of coronavirus and (B) its replication: (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, ORF: open reading frame, ACE2: Angiotensin converting enzyme, CD13: human aminopeptidase N).Entry targets: (I) Spike protein (RBD, Fusion intermediates); (II) Receptors; a. DPP4/CD26 for MERS, b. ACE2 for SARS and HCoV-NL63, c. 9-O-Acetylated sialic acid for HCoV-OC43 and HCoV-HU1, d. CD13 for HCoV-229E (III) Surface proteases: TMPRSS2. (IV) Endosomal proteases. Polyprotein processing targets: a. Papain-like protease (PL^{pro}), b. 3C-like protease ($3CL^{pro}$). Replicase targets: a. ADP-ribose-1'-phosphatase (nsp3), b. RNA-dependent and RNA polymerase (nsp12), c. Helicase (nsp13), d.Exonuclease (nsp14), e. Endoribonuclease (nsp15), f. 2'-*O*-methyltransferase.

acids) and pp1ab (7073 amino acids) that are processed by proteases 3-C-like protease (3CLpro) and papain-like protease (PLpro). Subsequently, these polyproteins are cleaved into at least 15 non-structural proteins (nsp), which assembles and form the replication-transcription complex. With the aid of replicases, the full-length positive strand of genomic RNA is transcribed to form a full-length negative-strand template for the synthesis of new genomic RNAs. These mRNAs are then transcribed and translated to produce the structural and accessory proteins. Interrupting any replication processes would become a potential molecular target to develop therapeutics.

Development of anti-CoV therapeutics

Although all human CoVs are a real threat to human populations, numerous researches have mainly been focused on SARS- and MERS-CoVs infections. Because they were responsible for severe illness when compared to other CoVs. Numerous agents have been identified to inhibit the entry and/or replication of SARS- and

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TABLE 1

Classification, discovery, cellular response and natural host of the coronaviruses

hCoV genera	Coronaviruses	Discovery	Cellular receptor	Natural Host(s)
α-Coronaviruses	HCoV-229E	1966	Human aminopeptidase N (CD13)	Bats
	HCoV-NL63	2004	ACE2	Palm Civets, Bats
β-Coronaviruses	HCoV-OC43	1967	9-O-Acetylated sialic acid	Cattle
	HCoV-HKU1	2005	9-O-Acetylated sialic acid	Mice
	SARS-CoV	2003	ACE2	Palm Civets,
	MERS-CoV	2012	DPP4	Bats, Camels

MERS-CoVs in cell culture or animal models [41,42]. Due to the high morbidity and mortality rates of SARS and MERS, several antiviral drugs and immunomodulators have been used empirically or evaluated in uncontrolled trials [41,43–53] (see for representative examples 1–6 in Fig. 3).

Ribavirin (**1**) has been widely used for treating a variety of viral infections, but for SARS, the clinical outcome of the antiviral intervention had no significant effect on patients [41,44]. On the other hand, the patients who received ribavirin (**1**), lopinavir (**10**, see Fig. 4)- ritonavir (**2**) and a corticosteroid had lower 21-day acute respiratory distress syndrome (ARDS) and death rates than those who received ribavirin and a corticosteroid [54,55] combination therapy using interferon α-1 and corticosteroid was associated with improved oxygen saturation and more rapid resolution of radiographic lung opacities than systemic corticosteroid alone (uncontrolled study) [56]. The use of the corticosteroid, methylprednisolone (**3**, Fig. 3) as a therapeutic intervention for SARS patients was associated with an increased 30-day mortality rate (adjusted OR = 26.0, 95% CI = 4.4–154.8). However, disseminated fungal infection and avascular osteonecrosis occurred following the prolonged systemic corticosteroid therapy [57–59]. A randomized, placebo-controlled study showed that plasma SARS-CoV RNA levels in weeks 2-3 of the illness were higher in patients given hydrocortisone (n = 10) than those given normal saline (n = 7) in the early phase of the illness, suggesting that early use of pulsed methylprednisolone (**3**) might prolong viremia. In the case of patients with MERS, the combination of ritonavir (**2**) + interferon α2a or interferon α-2b resulted in no significant effect on clinical outcome; case-control study showed significantly improved survival (14 out of 20 and 7 out of 24 in the treated and control groups, respectively; P = 0.004) at 14 days, but not at 28 days [46–50].

Retrospective analyses showed that the combination of ritonavir (**2**) + interferon β-1a had no significant effect on clinical outcome [48]. In another study, the combination of ribavirin (**1**), ritonavir (**2**) + interferon α-2a resolved viremia within 2 days after commencement of treatment in a patient with severe MERS. Patients with severe MERS who were treated with methylprednisolone (**3**) with or without antivirals and interferons had no favorable response [48,49].

Approaches for the development of anti-viral drugs

Typically, the drug-discovery program to develop new potent antiviral agents and to obtain approval for clinical use takes more than 10 years. Until now, no effective vaccines or drugs are approved, while potent inhibitors are in clinical development to treat coronavirus infections.To speed up the discovery of potential

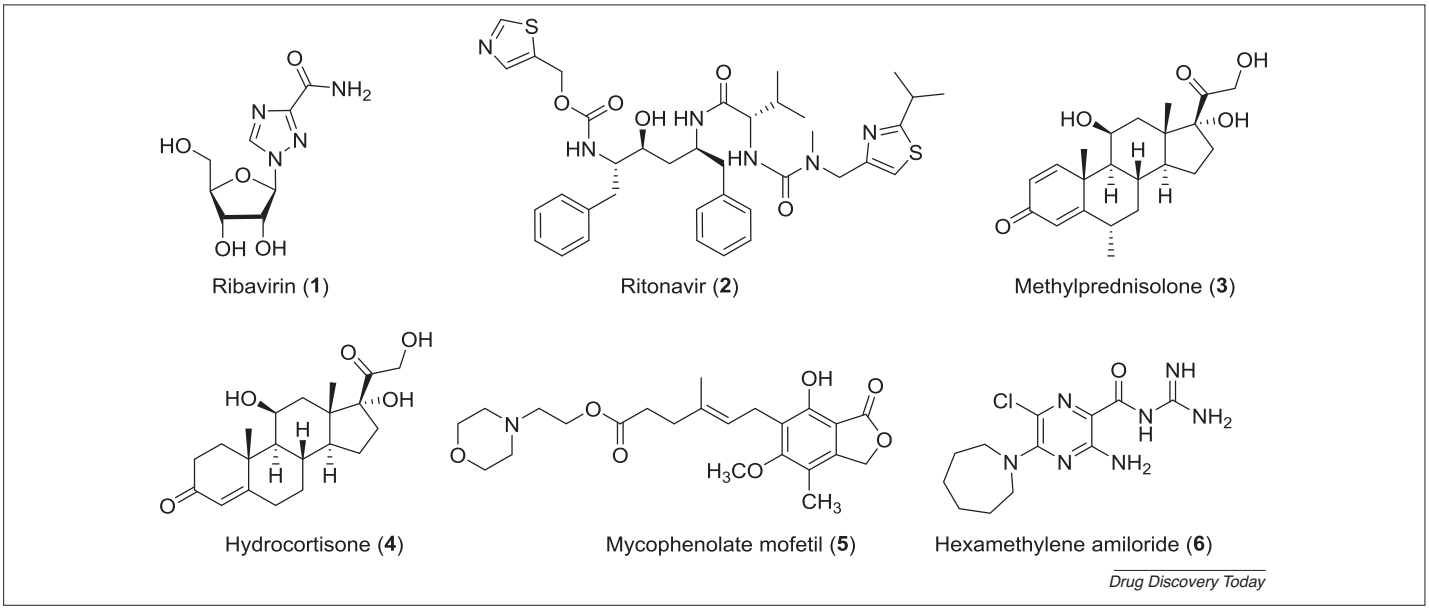

FIGURE 3

Potential anti-viral therapeutics used in patients with SARS and MERS infections.

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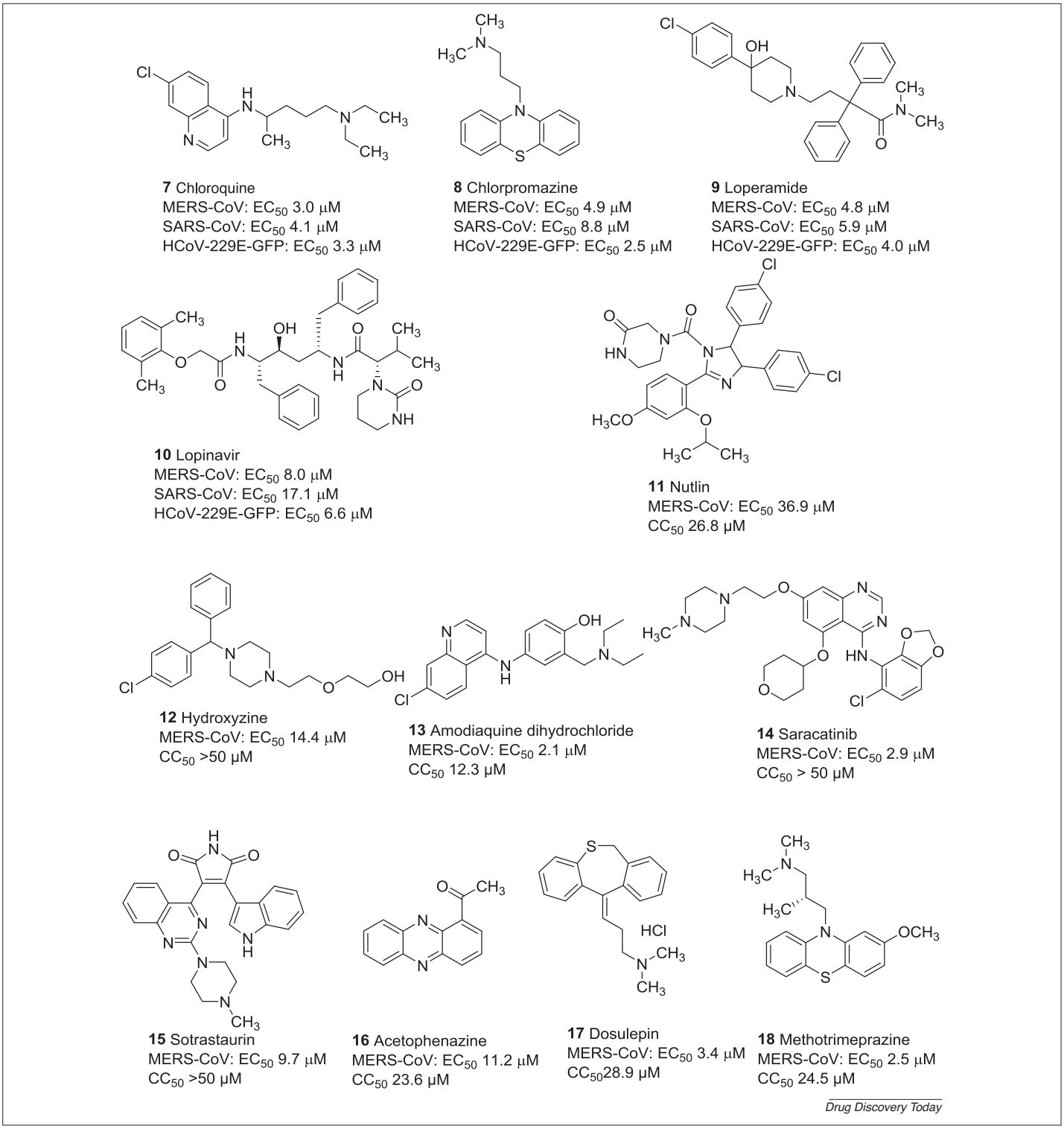

FIGURE 4

Drugs repurposed on coronaviruses infections.

treatment options for human pathogenic coronaviruses, two general approaches were employed, especially for SARS- and MERS-CoVs that are linked with more severe diseases than the other CoVs.

The first approach is repurposing of broadly acting antiviral drugs that have been used for other viral infections or other indications. These drugs have the obvious benefits of being already available with known pharmacokinetic and pharmacodynamic properties, solubility, metabolic stability, side effects, and dosing regimens. Drugs including interferon α, β, and γ, ribavirin (**1**) and inhibitors of cyclophilin [60–62] were discovered using this approach.

The combination therapy of interferon and ribavirin showed the best result in treating MERS-CoV infection. Also, different

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TABLE 2

Broad-spectrum [235_TD$DIFF]inhibitorsof human coronaviruses

Compound name	Bioactivity	HCoV-OC43 EC_{50} (CC_{50})	HCoV-NL63 EC_{50} (CC_{50})	MERS-CoV EC_{50} (CC_{50})	MHV-A59 EC_{50} (CC_{50})
Lycorine	Inhibits cell division, antineoplastic, antiviral	0.15 (4.37)	0.47 (3.81)	1.63 (3.14)	0.31 (3.51)
Emetine	Inhibits RNA, DNA, and protein synthesis	0.30 (2.69)	1.43 (3.63)	0.34 (3.08)	0.12 (3.51)
Mycophenolate mofetil	Immune suppressant, antineoplastic, antiviral	1.58 (3.43)	0.23 (3.01)	1.54 (3.17)	0.27 (3.33)
Phenazopyridine	Analgesic	1.90 (>20)	2.02 (>20)	1.93 (>20)	0.77 (>20)
Mycophenolic acid	Immune suppressant, antineoplastic, antiviral	1.95 (3.55)	0.18 (3.44)	1.95 (3.21)	0.17 (4.18)
Pyrvinium pamoate	Anthelmintic	3.21 (>20)	3.35 (>20)	1.84 (19.91)	4.12 (19.98)
Monensin sodium	Antibacterial	3.81 (20)	1.54 (>20)	3.27(>20)	0.18 (>20)

types of interferons including IFN-α-2b and IFN-β-1b have been employed in the management of patients with MERS-CoV infection [63]. Recently, several research groups reported the discovery of anti-viral drugs using the drug repurposingapproach.

Shen et al reported broad-spectrum inhibitors of human coronaviruses, see Table 2 [64]. They identified seven compounds (lycorine, emetine, monensin sodium, mycophenolate mofetil, mycophenolic acid, phenazopyridine, and pyrvinium pamoate) from HTS screening as broad-spectrum inhibitors according to their strong inhibition of replication by four CoVs *in vitro* at low-micromolar concentrations. These seven broad-spectrum inhibitors suppressed the replication of all CoVs in a dose-dependent manner and with low EC_{50} values. Although the cytotoxic concentration for some compound is as high as of their inhibitions, phenazopyridine, pyrvinium pamoate, and monensin sodium displayed less CC_{50} values. Further, *in vivo* studies showed lycorine protected mice against lethal HCoV-OC43 infection.

In a search of potential anti-viral agents against CoVs, de Wilde et al identified four drugs such as chloroquine (**7**), chlorpromazine (**8**), loperamide (**9**) and lopinavir (**10**) from the screening of FDA approved drugs library (Fig. 4) [65]. They all were able to inhibit the replication of MERS-CoV, SARS-CoV as well as HCoV-229E in the low micromolar range, which suggest that they could be used for the broad spectral anti-viral activity. As a mode of action, 7 inhibited the replication of MERS-CoV in a dose-dependent manner with an EC_{50} of 3.0 μM and the inhibition contributed to the blockade of the virus at a very early stage. Compound 7 was previously reported as an effective anti-viral agent against flavivirus, influenza virus, HIV [66], Ebola virus [67], and Nipha-Hendra virus [68]. Chlorpromazine (**8**) was another hit compound resulted from the screening and inhibited the replication of MERS-CoV with an EC_{50} of 4.9 μM. Chlorpromazine (**8**) is the first antipsychotic drug developed for the treatment of schizophrenia [69] and mechanistically it inhibited the clathrin-mediated endocytosis. It has also been reported to inhibit the replication of hepatic C virus [70] (HCV), alphavirus [71], mouse hepatitis virus (MHV-2) [72] and other coronavirus SARS-CoV [73]. The mechanistic study of chlorpromazine (**8**) on MERS-CoV indicated that it inhibited the virus at both an early and postentry stage, suggesting that an effect on clathrin-mediated endocytosis was not only the sole antiviral mechanism. Loperamide (**9**), an antidiarrheal opioid receptor agonist, which reduces intestinal motility [74], inhibited the replication of MERS-CoV. Additionally, it inhibited the other two coronaviruses in the low micromolar range (4 to 6 μM). Lopinavir (**10**) is an anti-HIV protease inhibitor, which also inhibited the replication of MERS-CoV with an EC_{50} of 8.0 μM. It was previously reported to inhibit the SARS-CoV main protease (Mpro) [75] and therefore, it was presumed that it might also target the M^{pro} of MERS-CoV.

Current anti-MERS-CoV agents have been primarily resulted from previous drugs used for the SARS-CoV infection. To identify potential antiviral agents against MERS-CoV, Shin *et al.* screened a library consisting of 2334 approved drugs and pharmaceutically active compounds [76]. This yielded a series of hit compounds, primarily categorized as anti-protozoal, anti-cancer, anti-psychotics (**11–18**, Fig. 4), with micromolar inhibitory activity ranging from 2.1 to 14.4 μM (Fig. 4). Among them, saracatinib (**14**) was particularly important as it showed an excellent anti-MERS-CoV activity with an EC_{50} of 2.9 μM and a CC_{50}>50 μM. Saracatinib (**14**) is an orally available small molecule drug used for the treatment of tumor malignancies through the Src-family of tyrosine kinases (SFKs) inhibition. It also inhibited other coronaviruses SARS-CoV (EC_{50} 2.4 μM) and HCoV-229E (EC_{50} 5.1 μM), and feline infectious peritonitis (FIPV, EC_{50} 7.0 μM) within a not-toxic range of concentration. An *in vitro* study of the anti-viral effect of saracatinib (**14**) [found to suppress the early stages of the MERS-CoV life cycle in Huh-7 cells through a possible suppression of the SFK signaling pathways. Interestingly, co-treatment of saracatinib (**14**) with gemcitabine, a deoxycytidine analog that is commonly used for the treatment of cancers [77,78] showed a synergistic antiviral effect with a minimal cytotoxic effect. This supports the hypothesis of using them in a combination therapy to treat CoV diseases.

In continuation, several classes of compounds that have been used for other indications identified as potent inhibitors of SARS- and MERS-CoVs. These drugs were classified into different therapeutic groups as neurotransmitter inhibitors, estrogen receptor antagonists, kinase signaling inhibitors, protein-processing inhibitors, inhibitors of lipid or sterol metabolism and inhibitors of DNA synthesis or pair (see, for representative examples, Fig. 5 and the activities in Table 3). Antidiarrheal agent loperamide (**9**), or anti-HIV-1 agent lopinavir (**10**) were able to inhibit both MERS- and SARS-CoVs infection in the low-micromolar range and was linear with the study by de Wilde *et al.* [79]. Antiparasitics, mefloquine (**20**), and amodiaquine dihydrochloride (**21**) or antibacterial, emetine dihydrochloride hydrate (**19**) in which that function was not linked to coronaviruses in general, showed antiviral activity against both CoVs. Cathepsins are important for the fusion step during virus entry of coronavirus [80]. Cathepsin inhibitor, E-64-D (**22**), blocked the MERS-CoV and SARS-CoV at the entry stage. The neurotransmitter inhibitor triflupromazine (**33**) inhibited both SARS-CoV and MERS-CoV. In addition to that,

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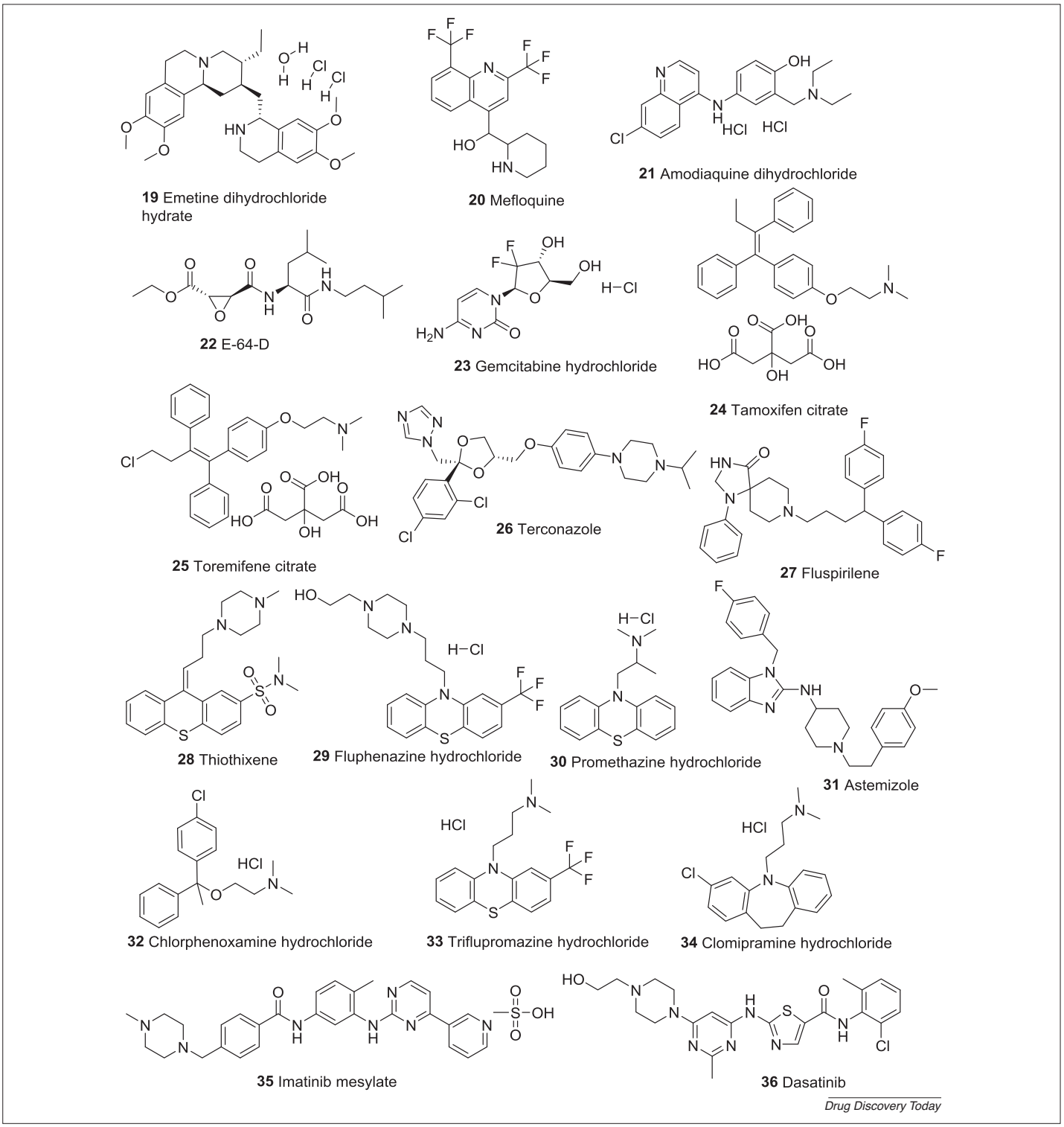

FIGURE 5

Repurposing of various classes of drugs on SARS-and MERS-CoVs.

other neurotransmitters fluphenazine (**29**) and promethazine (**30**) were reported to inhibit MERS-CoVs protein-mediated cell-cell fusion with IC_{50} values of about 20 and 29 μM, respectively [81]. Kinase signaling pathway inhibitors imatinib mesylate (**35**) and dasatinib (**36**) are known inhibitors of the Abelson murine leukemia viral oncogene homolog-1 pathway (ABL-1) and were 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 example, gemcitabine hydrochloride, **23**) those were active against at least one coronavirus, suggest that these drugs have potential as antiviral therapy against coronaviruses. Toremifene citrate (**25**) is an

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TABLE 3

Compounds with activity against MERS-CoV and SARS-CoV

Pharmaceutics	Class	MERS-CoV EC_{50} (μM)	SARS-CoV EC_{50} (μM)
Emetine dihydrochloride hydrate (**19**)	Antibacterial agent	0.014	0.051
Mefloquine (**20**)	Antiparasitic agent	7.41	15.55
Amodiaquinedihydrochloridedehydrate (**21**)	Antiparasitic agent	6.21	1.27
Loperamide (**9**)	Antidiarrheal agent	4.8	5.90
Lopinavir (**10**)	HIV-1 inhibitor	8.0	24.4
E-64-D (**22**)	Cathepsin inhibitor	1.27	0.76
Gemcitabine hydrochloride (**23**)	DNA metabolism inhibitor	1.21	4.95
Tamoxifen citrate (**24**)	Estrogen receptor inhibitor	10.11	92.88
Toremifene citrate (**25**)	Estrogen receptor inhibitor	12.91	11.96
Terconazole (**26**)	Sterol metabolism inhibitor	12.20	15.32
Fluspirilene (**27**)	Neurotransmitter inhibitor	7.47	5.96
Thiothixene (**28**)	Neurotransmitter inhibitor	9.29	5.31
Fluphenazine hydrochloride (**29**)	Neurotransmitter inhibitor	5.86	21.43
Promethazine hydrochloride (**30**)	Neurotransmitter inhibitor	11.80	7.54
Astemizole (**31**)	Neurotransmitter inhibitor	4.88	5.59
Chlorphenoxamine hydrochloride (**32**)	Neurotransmitter inhibitor	12.64	20.03
Triflupromazine hydrochloride (**33**)	Neurotransmitter inhibitor	5.75	6.39
Clomipramine hydrochloride (**34**)	Neurotransmitter inhibitor	9.33	13.23
Imatinibmesylate (**35**)	Kinase signaling inhibitor	17.68	9.82
Dasatinib (**36**)	Kinase signaling inhibitor	5.46	2.10

estrogen receptor 1 antagonist that inhibits both MERS-CoV and SARS-CoV with EC_{50} of 12.9 and 11.97 μM. respectively.

The second approach for anti-CoV drug discovery involves the *de novo* development of novel, specific agents based on the genomic and biophysical understanding of the individual coronavirus. Examples include siRNA molecules or inhibitors that target specific viral enzymes involved in the viral replication cycle, mAbs that target the host receptor, inhibitors of host cellular proteases, inhibitors of virus endocytosis by the host cell, human or humanized mAbs that target the S1 subunit RBD and antiviral peptides that target the S2 subunit.

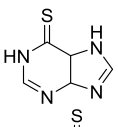
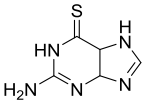
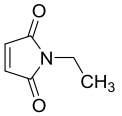
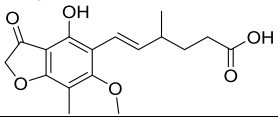
Virus-based anti-CoV therapeutics

Despite their high species diversity, CoVs share key genomic elements that are essential for the design of therapeutic agents. The large replicase polyprotein 1a (pp1a) and pp1ab are processed and cleaved by two viral proteases, PL^{pro}, and the $3CL^{pro}$, to produce nonstructural proteins (NSPs) such as RNA-dependent RNA polymerase (RdRp) and helicase, which are involved in the transcription and replication of the virus [82]. Numerous enzyme inhibitors targeting these proteins have shown anti-CoV activities *in vitro*.

Inhibitors that target nucleosides or nucleotides are building blocks of viral nucleic acids and they have broad-spectrum activity against a wide range of coronaviruses as well as other viruses, in general. Mycophenolate (**5**, Fig. 3), is an immunosuppressant drug used to prevent rejection in organ transplantation. It inhibits inosine monophosphate dehydrogenase, a key rate-limiting enzyme in the *de novo* purine synthesis pathway which converts inosine monophosphate to guanosine monophosphate [83]. The active molecule, mycophenolic acid (**46**, see for structure Table 4),

TABLE 4

Structure and IC_{50} of compounds against MERS-CoV PL protease

Compound	Chemical structure	IC_{50} (μM)	
		Peptide cleavage	DUB activity
6-Mercaptopurine (**43**)		26.9	25.8
6-Thioguanine (**44**)		24.4	12.4
N-Ethylmaleimide (**45**)		45.0	ND
Mycophenolic acid (**46**)		247.6	222.5

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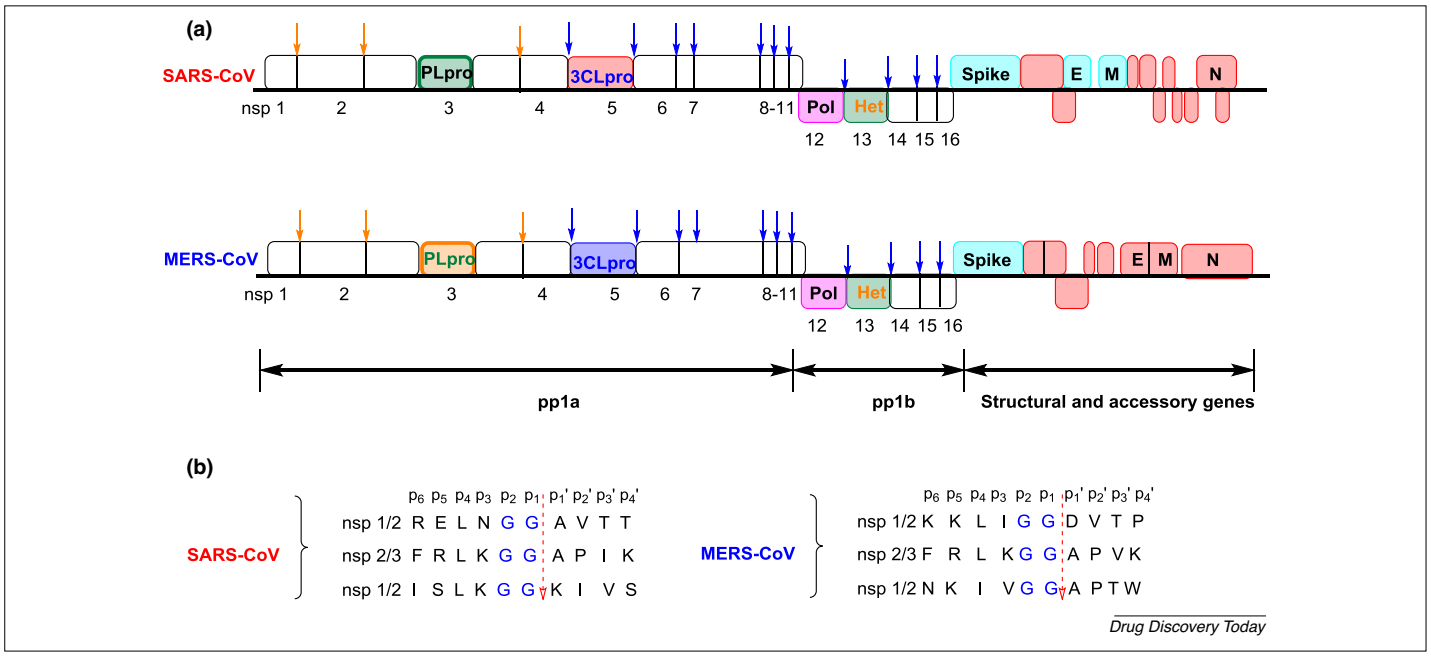

FIGURE 6

Overview of SARS and MERS-CoVs 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).

exhibits broad-spectrum *in vitro* anti-viral activity against various viruses, including hepatitis B virus (HBV), hepatitis C virus (HCV) and arboviruses [82]. Recently, mycophenolic acid was identified as a potential anti-MERS-CoV drug by a high-throughput screening approach and has a potent anti-MERS-CoV activity *in vitro* [83]. However, a subsequent study in a non-human primate model did not provide a positive result as the treatment of MERS-CoV infected common marmosets with mycophenolic acid had a worse outcome than untreated animals did [84].

Proteases are indispensable for the viral life cycle and they play an essential role in viral replication by mediating the maturation of viral replicases. Therefore, targeting proteases has become an attractive approach for developing potential antiviral drugs. Protease inhibitors block the replication of coronaviruses (CoVs), including the causative agents of MERS and SARS infection. The papain-like protease (PL^{pro}) and a 3C-like protease ($3CL^{pro}$ also known as the main protease) are important two proteases that mediate the process replicase polyproteins pp1a and pp1b. PL^{pro} is a cysteine protease that uses the thiol group of cysteine as a nucleophile to attack the carbonyl group of the scissile peptide bond for cleavage at first three positions of its polyprotein to produce three nonstructural 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 (Fig. 6).

Dehaen *et al.* reported a novel library of fused 1,2,3-triazole derivatives against coronavirus 229E in HEL cells [85]. Structure-activity relationship studies showed that some compounds displayed moderate inhibitory activities in micromolar range without alterations of the normal cell morphology in confluent HEL cell cultures at concentrations up to 100 μM. For example, compounds **37-41** (see Fig. 7). Although the authors claimed that these inhibitors could inhibit the 3CL protease of CoV-229E based on the molecular modeling of previously reported inhibitors, they could not confirm with the experiments.

Grünweller *et al.* reported the broad-spectrum activity of natural product silvestrol (**42**, Fig. 7), a specific inhibitor of the DEAD-box RNA helicase eIF4A, for MERS-CoV and HCoV-22E on viral translation using a dual luciferase assay and virus-infected primary cells [86]. Silvestrol was recently shown to have potent antiviral activity in Ebola virus-infected human macrophages. They found that silvestrol is also a potent and non-toxic inhibitor of cap-dependent viral mRNA translation in CoV-infected human embryonic lung fibroblast (MRC-5) cells. It was found to be highly effective against both infections with EC_{50} values of 1.3 nM and 3 nM, respectively. For MERS-CoV, the potent antiviral activities of silvestrol were also confirmed using peripheral blood mononuclear cells (PBMCs) as a second type of human primary cells. Mechanistically Silvestrol strongly inhibited the expression of CoV structural and nonstructural proteins (N, nsp8) and the formation of viral replication/transcription complexes. They also confirmed that silvestrol found to inhibit human rhinovirus (HRV) A1 and poliovirus 1 (PV), respectively.

Snijder et al reported alisporivir, a non-immunosuppressive cyclosporin A-analog (structure not shown), inhibited the replication of different human coronaviruses, including 229E, MERS- and SARS-coronavirus in low micromolar concentrations [87]. Theinvestigation suggest that alisporivir inhibits MERS- and SARS-CoV replication by cell-culture based screening assays relying on the rapid cytopathic effect (CPE) observed in coronavirus-infected cells.

MERS-CoV and SARS-CoV PL proteases inhibitors

In anti-viral therapy, P^{Lpro} is an important target as it is a multi-functional protein involved in proteolytic, deubiquitination,

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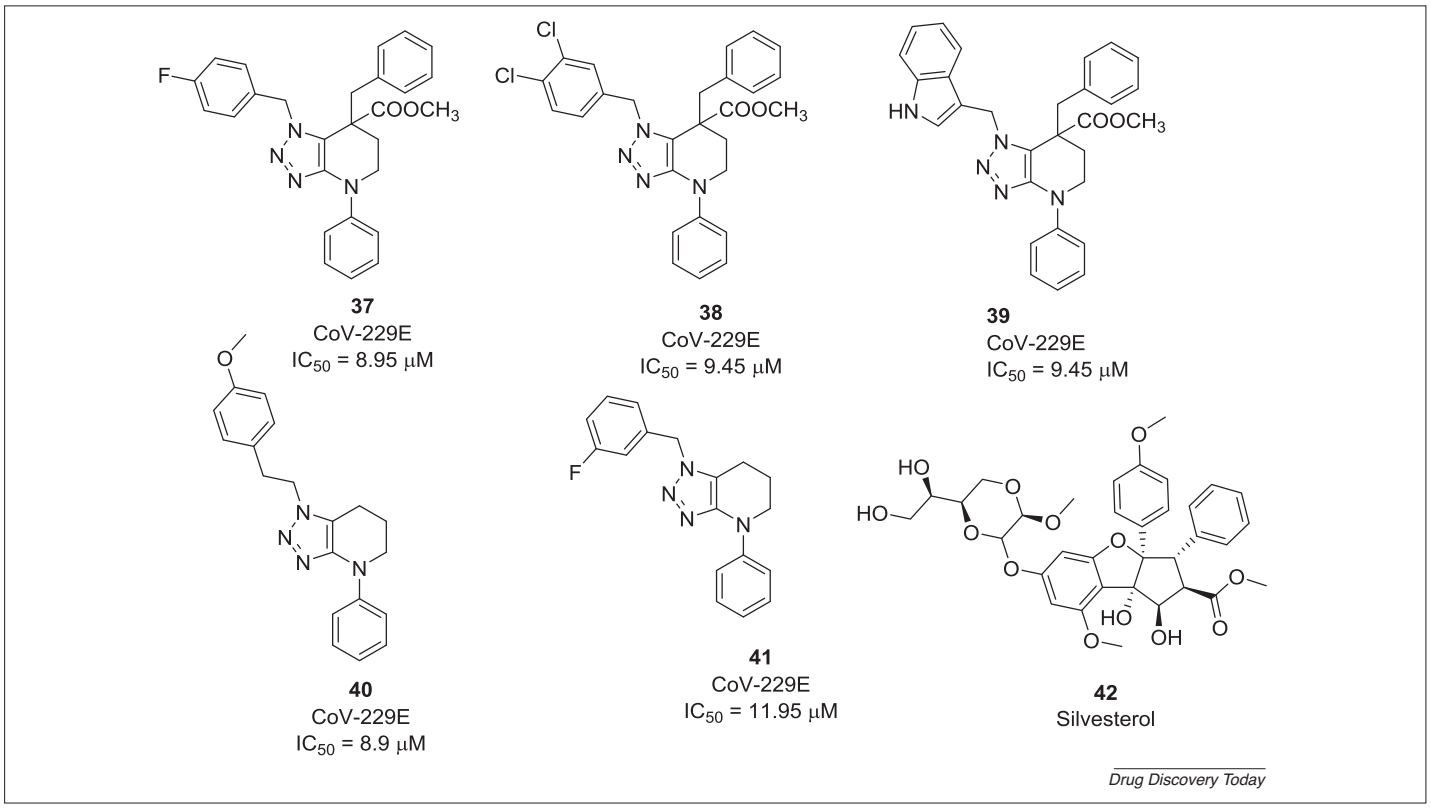

FIGURE 7

1,2,3-Triazole derivatives (37-42) against coronavirus-229E.

de-ISGylation (ISG = interferon-stimulated gene), and viral evasion of the innate immune response in addition to its proteolytic activity [88,89]. Several X-ray crystallography studies have facilitated the characterization of these PL^{pro} enzymes and the identification of PL^{pro} inhibitors [90]. Numerous SARS-CoV PL^{pro} inhibitors belonging to different classeshave been identified, including small-molecule inhibitors, thiopurine compounds, natural products, zinc ion, and zinc conjugate inhibitors and naphthalene inhibitors [91]. 6-Mercaptopurine (**43**), 6-thioguanine (**44)** and *N*-ethylmaleimide (**45**) as well as the immunosuppressive drug, mycophenolic acid (**46**), were all independently able to inhibit the proteolytic activity and deubiquitination of MERS-CoV PLpro (Table 4) [92]. Compared with *N*-ethylmaleimide (**45**), 6-mercaptopurine (**43**), 6-thioguanine (**44)** were more effective inhibitors, while mycophenolic acid (**46**) was a less effective inhibitor against the MERS-CoV PL^{pro}.

Disulfiram (**47**, Fig. 8) is an FDA drug and has been used in alcohol aversion therapy. This drug was reported to inhibit the activity of methyltransferase [93], kinase [94], and urease [94], all by reacting with cysteine residues, suggesting broad-spectrum characteristics [95]. Notably, disulfiram (**47**) has been reported as an allosteric inhibitor of MERS-CoV PL^{pro} [95]. It was suggested that the administration of **41** together with compound **44** and/or **45**, could synergistically inhibit MERS-CoV papain-like protease [95].

8-(Trifluoromethyl)-9*H*-purin-6-amine (**48**, F2124–0890, Fig. 8) was identified as a selective dual inhibitor of both PL^{pro} enzymes of MERS-CoV and SARS-CoV through a high-throughput screening of molecule library containing 25,000 chemical entities [96]. As a mode of action, this compound acts as a competitive inhibitor against MERS-CoV with an IC_{50} value of 6.0 μM, while acts as an allosteric inhibitor against SARS-CoV (IC_{50} 11 μM). Compound **48** was first synthesized in 1958 as a potential anticancer agent, and in the late 1980s and early 1990s, the compound was used as a reactant for designing arrhythmia and antiviral drugs as well as compounds set to regulate plant growth [97,98].

Naphthalene amides **49-52** (Fig. 8) [99–102] were reported to inhibit MERS-CoV PL^{pro} but were inactive against SARS-CoV PL^{pro}, suggesting the structural difference in the binding mode of both PL proteases. This was supported by the recent X-ray crystal structures of SARS-CoV PL^{pro} complex with the lead inhibitors **50** and **51**. These structures revealed that inhibitors did not bind to the catalytic site of SARS CoV PL^{pro} but to the BL2 loop, which appears to prevent the accessibility of substrate to the active site, and thereby inhibiting the enzymatic activity. Structural and sequence analysis at BL2 loop of SARS-CoV PLproand MERS CoV PL^{pro} suggested that they both have a difference in key amino acid residues that are responsible for inhibitor binding. For example, Y269 and Q270 are responsible for inhibitor binding in SARS-CoV PL^{pro}, whereas T274 and A275 in MERS CoV PL^{pro}. These findings suggest that making dual inhibitors targeting PL^{pro} of SARS-and MERS-CoVs is difficult.

Park *et al*. assessed the inhibitory activity of polyphenols derived from *B. papyrifera* against SARS- and MERS-CoVs [103]. The isolated polyphenols markedly inhibited 3CL and PL CoV proteases of both SARS and MERS. The IC_{50} values of these compounds, though higher than those of peptide-derived inhibitors, were still in the low micromolar range. In particular, the isolated compounds

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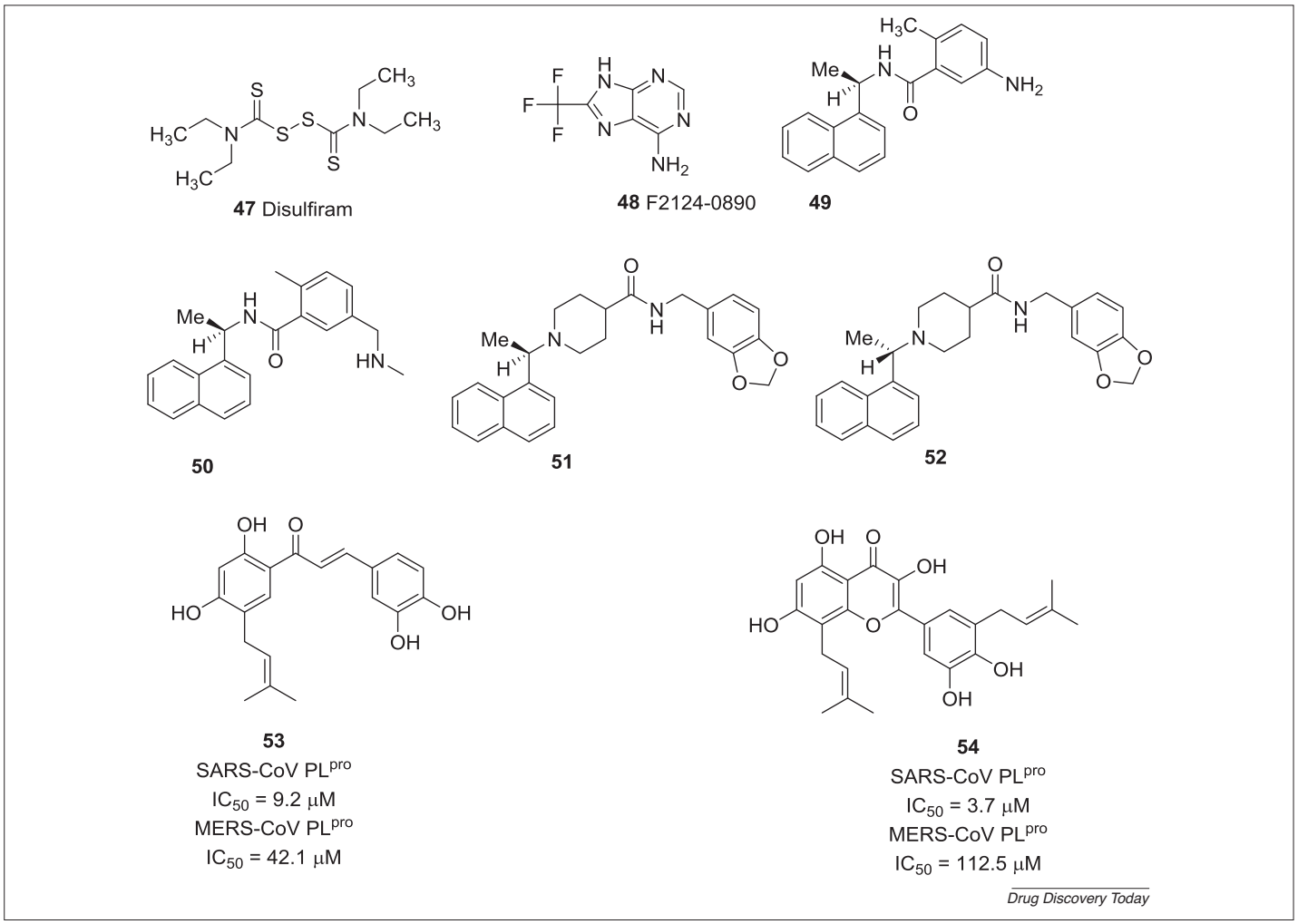

FIGURE 8

Representative examples of MERS- and/or SARS-CoV PL^{pro} inhibitors (47-54).

exerted significant SARS-CoV PL^{pro} inhibitory activity through noncompetitive inhibition. Compounds **53** and **54**, showed the most potent PL^{pro} inhibitory activity with IC_{50} values of 9.2 and 3.7, respectively. Additionally, they performed detailed protein-inhibitor SPR-binding analyses of compound **54** with SARS-CoV PL^{pro}. Compound **54** strongly inhibited the cleavage of both ubiquitin and ISG15 (IC_{50} values of 7.6 and 8.5 μM, respectively).

3CL^{pro} are cysteine proteases, which are analogs to the main picornavirus 3C protease, a family of viruses that also cause respiratory illness. A broad-spectrum anti-CoV inhibitor N3 (**55**, peptide derivative, Fig. 9) was identified to inhibit the proteolytic activity of MERS-CoV 3CL^{pro} with an IC_{50} of 0.28 μmol/L. The X-ray crystal structure of MERS-CoV 3CL^{pro} with inhibitor **55** confirms that inhibition of 3CL^{pro} was similar to the mechanism to other CoVs [104], as the inhibitor binds with the interface of domain I and II of MERS-CoV 3CL^{pro} with an EC_{50} of about 0.3 μM [104].

AG7088 (**56**, peptide derivative), a potent inhibitor of rhinovirus 3C^{pro} with Michael acceptor functionality, failed to inhibit SARS-CoV 3CL^{pro} [105]. However, a series of AG7088 analogs were reported to combat CoVs by targeting 3CL^{pro} [106]. The screening of peptidomimetics (**57–60**; see Fig. 9) which contains a Michael acceptor group, (i.e., α,β-unsaturated carbonyl) showed moderate anti-CoV activities [107–109]. Enterovirus inhibitors **61**, **62**, and **63**, were recently shown to inhibit MERS-CoV with EC_{50} values ranging from 1.7 to 4.7 μM [110]. These inhibitors provide an excellent starting point for the development of natural substrate mimicking (or peptidomimetics) compounds against SARS- and MERS-CoV 3CL^{pro}. Benzotriazole derivatives (**64**,**65**) that have an activated carbonyl functionality displayed inhibition against both SARS-CoV 3CL^{pro} and MERS-CoV 3CL^{pro} [111].

The 5-chloropyridyl esters GRL-001 (**66**) and **67** (Fig. 10) have been shown to block the replication of SARS- and MERS-CoV 3CL^{pro} [112,113] and could serve as potential leads for the future drug development for anti-coronavirus therapy. Pyrazolone based neuraminidase (NA) inhibitors **68–70** (Fig. 10) were reported to inhibit the MERS-CoV 3CL^{pro} with moderate potencies in the range of 5.8-7.5 μM. The pharmacophore moieties phenyl at R3 and carboxylate, either R1 or R4 were suggested to be essential for the antiviral activity [114]. A dipeptidyl transition state 3CL^{pro} inhibitor GC376 (**71**) inhibited the activity of MERS-CoV 3CL^{pro} with an EC_{50} of 1.6 μM by fluorescence resonance energy transfer

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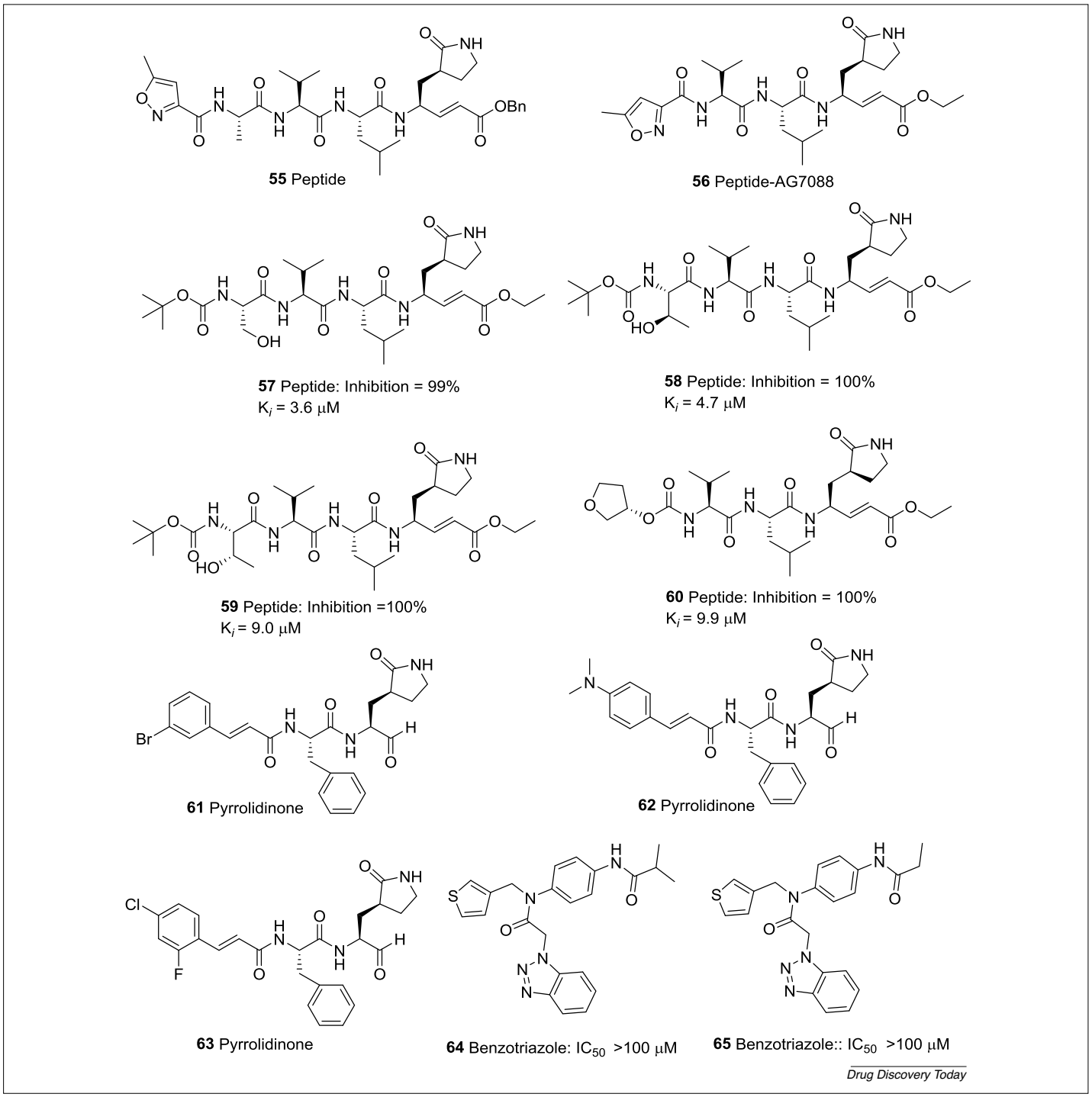

FIGURE 9

Structure of $3CL^{pro}$ inhibitors that contain Michael acceptor, aldehyde and activated carbonyl functional groups.

(FRET) assay [115]. Another pyrrolidinone based peptide GC813 (**72**) as well as its derivatives **73**-**74** displayed inhibition for MERS-CoV with EC50 values of 0.5 μM, 0.5 μM, and 0.8 μM in cell culture [116].

Kenichi et al reported new non-peptide SARS-CoV $3CL^{pro}$ inhibitors [117] by introducing decahydroisoquinoline at the S2 position by connecting the cyclohexyl group of the substrate-based inhibitor **75** (Fig. 11) [118]. The resulting compounds (**76**-**80,** Fig. 11) showed moderate but very promising inhibitory activities against SARS-CoV $3CL^{pro}$, which suggested that the decahydroisoquinoline scaffold could be a novel scaffold at the S2 position for SARS-CoV $3CL^{pro}$ inhibition.

The co-crystal structures of SARS-CoV $3CL^{pro}$ with decahydroisoquinoline inhibitors (**76**-**80**) revealed that P2-decahydroisoquinoline scaffold was inserted into a large S2 pocket and the P1-imidazole was occupied into the S1 pocket as

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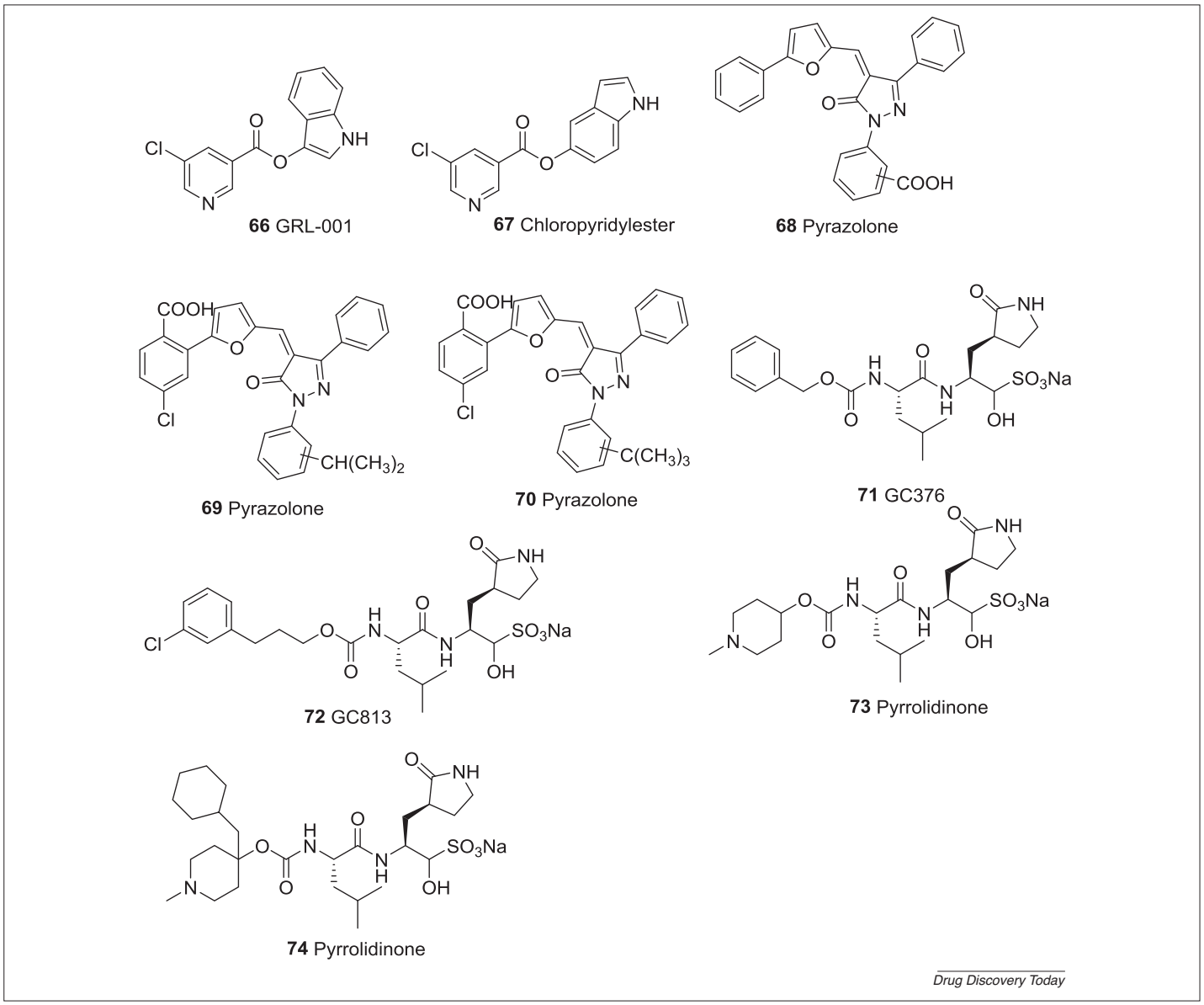

FIGURE 10

Structure of 3CL^{pro} inhibitors (66-74).

expected. The study was extended to find out the novel inhibitors that interact at S3 to S4 site [119]. For this reason, a non-prime site substituent and warheads combined with the decahydroisonoline was designed and evaluated against SARS-CoV 3CL^{pro}. The resulting analogs have Ac-Thr-Gly-OH (compound **81,** Fig. 11), instead of an original Ac-Thr-Val sequence in **75**, since an isopropyl side chain of the Val in **75** is directed to an outward of SARS 3CL^{pro} and no interactions with SARS 3CL^{pro} at the Val site is detected. Indeed the compound showed about 2.4 times potent inhibitory activities for SARS 3CL^{pro} when combined with a non-prime site substituent. This finding indicated not only the expected additional interactions with the SARS 3CL^{pro} but also the possibility of new inhibitors containing a fused-ring system as a hydrophobic scaffold and a new warhead such as thioacetal.

It was reported that mature SARS 3CL protease is subject to degradation at the188Arg/189Gln site [120]. Therefore, R188I mutant protease with high activity and stability was prepared. Kenichi et al were involved in developing inhibitors SARS 3CL R188I mutant protease. As a result, the compound **75** was developed with an IC_{50} value of 98 nM [118]. However, due to problems like enzymatic digestions of peptide chains and α-proton racemization, these compounds are not further taken forward. To solve this problem, they designed another derivative Sk23 (**82,** Fig. 11) [121] with serine backbone against the mutant protease. The compound Sk23 (Fig. 11) showed a weak inhibitory activity. In a way to develop small molecule inhibitors, they further investigated the structural modifications on the inhibitor SK23 and found isoserine backbone could be an alternative to the serine [122].One of the resulting analog SK40 (**83,** Fig. 11) showed an IC_{50} value 43 μM against the mutant protease. The compound further characterized for its microbial and cytotoxicity activities. However, it did not show inhibitory activities for any screened microbials and no cytotoxicity.

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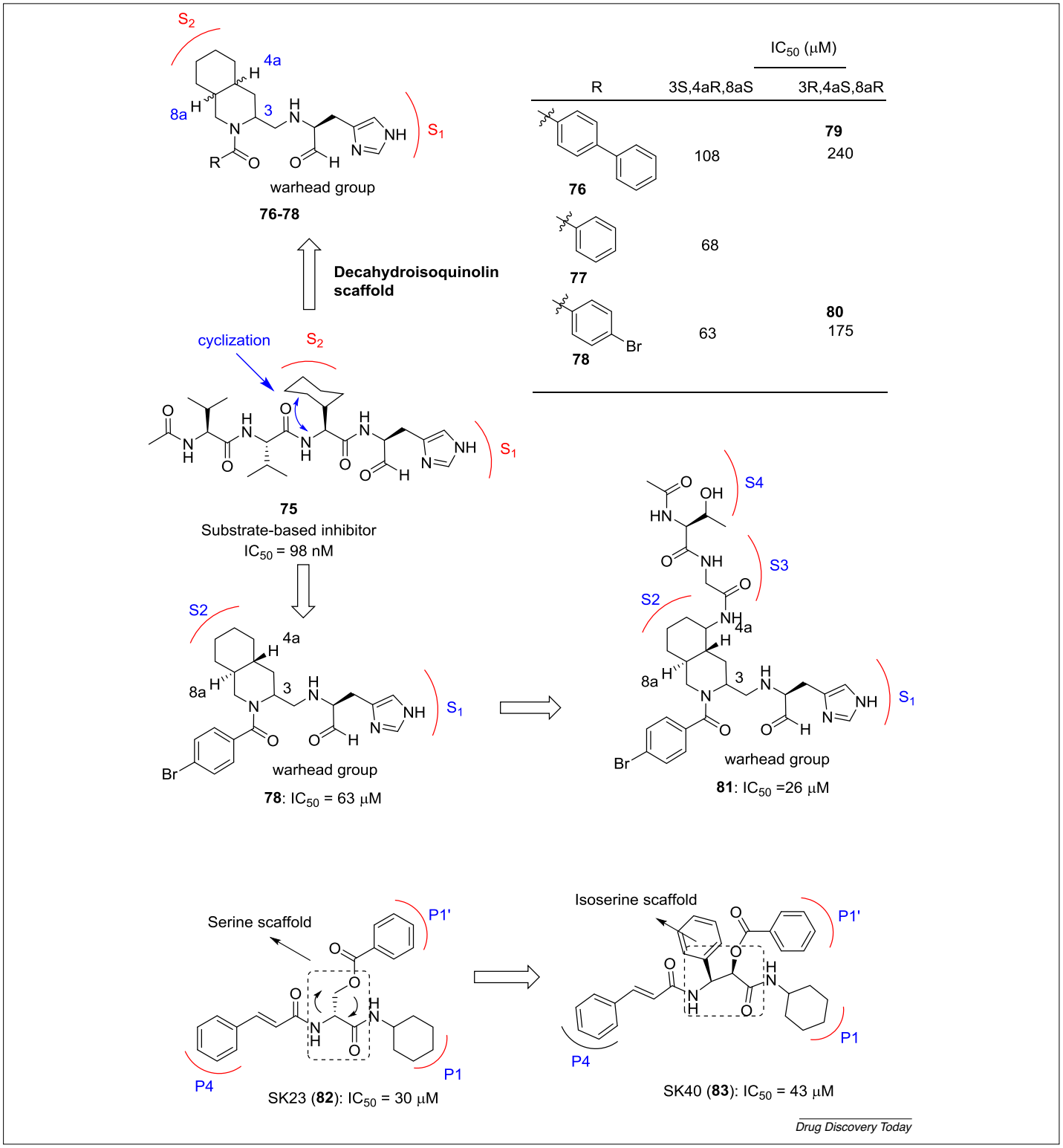

FIGURE 11

Non-peptide SARS-CoV 3CL^{pro} inhibitors contain P2-decahydroisoquinoline, serine and isoserine scaffolds.

Groutas *et al.* reported the novel class of peptidomimetic MERS-CoV3CL^{pro} inhibitors that embody a piperidine moiety [116]. These inhibitors were designed based on the dipeptidyl aldehyde bisulfite adduct inhibitor, designated GC376 (**84**, Fig. 12), which was clinically demonstrated for its efficacy. Attachment of the piperidine moiety to a dipeptidyl component permits the resultant hybrid inhibitor to engage in favorable binding interactions with the S3and S4 subsites of the enzyme. Some of these peptidomimetics showed excellent inhibition of MERS-CoV as well as the SARS-CoV infections (see, for example, compounds **85**,**86**,

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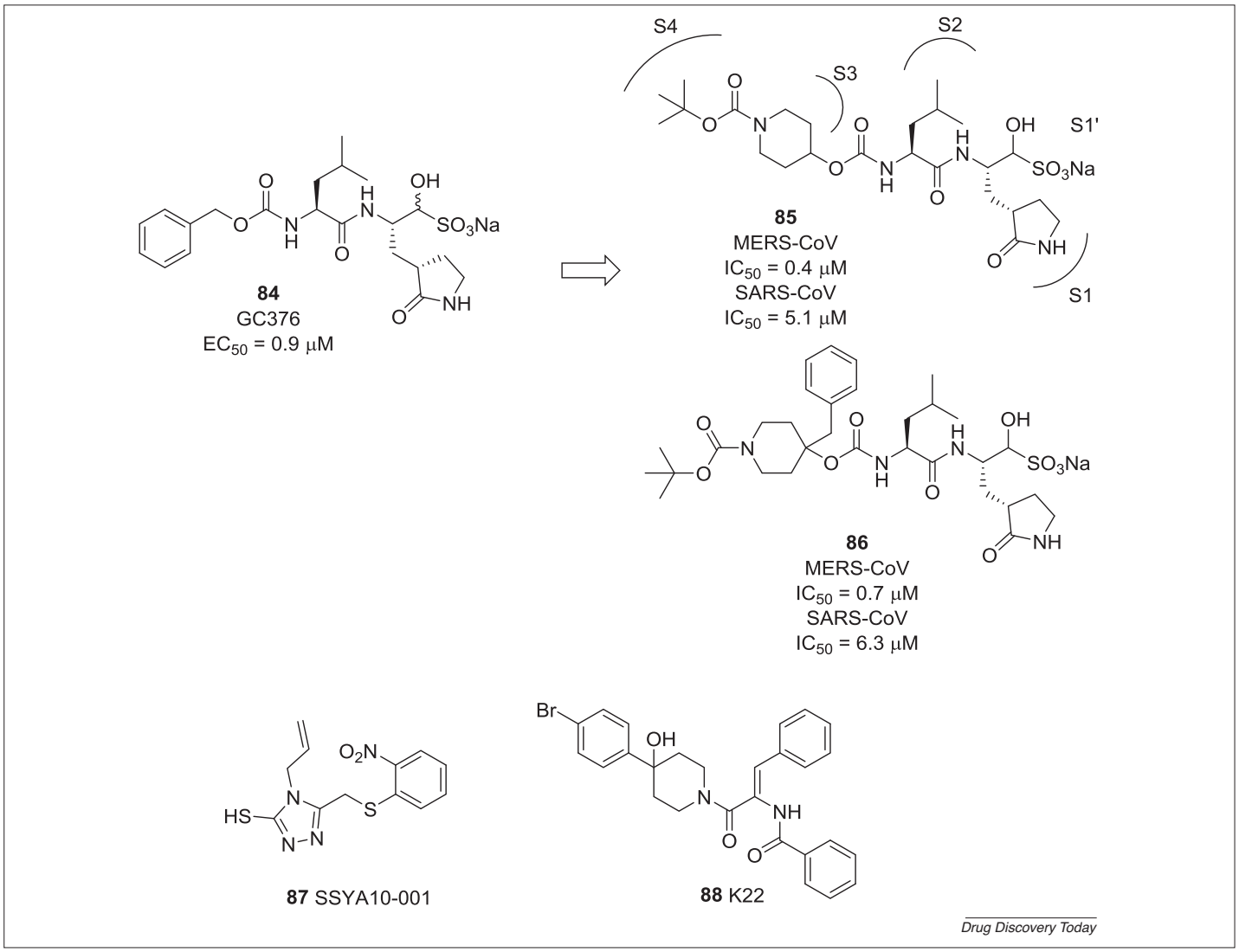

FIGURE 12

Dipeptidyl aldehyde bisulfite adduct inhibitors (84-86), replicase (87) and RNA synthesis inhibitors (88).

Fig. 12). Compounds **85** and **86** displayed potent inhibition toward MERS-CoV in both enzyme and cell-based systems, with low cytotoxicity (CC_{50}>100 μM).

Replicase inhibitors

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.

Recently, Adedeji *et al.* [123,124] reported a small 1,2,4-triazole derivative **87** (SSYA10-001, see Fig. 12) that inhibited the viral NTPase/helicase (known as nonstructural protein 13, nsp13) of both SARS-and MERS-CoVs. The antiviral activity of **87** inhibits MERS-CoV and SARS-CoV replication with EC_{50} values of 25 μM and 7 μM, respectively, and no significant cytotoxicity was observed up to the concentration of 500 μM. There have been, so far, no helicase inhibitors approved antiviral therapy and thus compound **87** could serve as a potential lead for the development of effective broad-spectrum anti-coronavirus drugs.

Membrane-bound viral RNA synthesis inhibitors

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. Antiviral agents that target membrane-bound coronaviral RNA synthesis is important for the replication and therefore, represent a novel and attractive target. Lundin A. *et al.* [125] discovered an inhibitor, designated K22 (**88**,Fig. 12) that targets membrane-bound coronaviral RNA synthesis and showed potent antiviral activity of MERS-CoV infection with remarkable efficacy [126].

Host-based anti-CoV treatment options

The host innate interferon response is crucial for the control of viral replication after infection [127]. Although CoVs can suppress the interferon response for immune evasion, they remain

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susceptible to interferon treatment *in vitro* [128,129]. The interferon response can be augmented by the administration of recombinant interferons or interferon inducers. Recombinant interferon-α and interferon- β inhibit the replication of both SARS-CoV and MERS-CoV *in vitro* and animal models [84,130–135]. Various combinations of interferon- α or-β with other antivirals such as ribavirin and/or lopinavir-ritonavir have been used to treat patients with SARS or MERS. Overall, combination treatments consisting of interferons and ribavirin did not consistently improve outcomes [47–49,136,137]. The apparent discrepancy between *in vitro* findings and *in vivo* outcomes may be related to the high EC_{50}/Cmax ratios of these drugs and the delay between symptom onset and drug administration [83,138].

This delay is especially relevant for MERS patients, as they have a much shorter median time interval between symptom onset and death than do SARS patients [139,42]. The use of recombinant interferon β-1b, which has the lowest EC_{50}/Cmaxratio against MERS-CoV among tested preparations of recombinant interferons, should be evaluated in combination with other effective antivirals in clinical trials at early stages of the infection [83,84].

Nitazoxanide (**89**, Fig. 13) is another potent type I interferon inducer that has been used in humans for parasitic infections [132]. A synthetic nitrothiazolyl–salicylamide derivative exhibits broad-spectrum antiviral activities against both RNA and DNA viruses including canine CoV, influenza viruses, HBV, HCV, HIV, rotavirus, norovirus, and flaviviruses [132]. It has been evaluated in Phase II and Phase III clinical trials for the treatment of HCV infection and influenza and has a good safety profile [132,140,141].

In addition to direct potentiation of the interferon response, other cell signaling pathways have been identified as potential anti-CoV treatment targets. Cyclophilins interact with SARS-CoV nsp1 to modulate the calcineurin pathway, which is important in the T cell-mediated adaptive immune response [142]. The calcineurin inhibitor cyclosporine (**90**, Fig. 13) inhibits a broad range of CoVs *in vitro* [142–144]. However, its immunosuppressive effects and high EC_{50}/Cmax ratio at standard therapeutic dosages limit its clinical application.

Trametinib (**91**), selumetinib (**92**), everolimus, rapamycin, dasatinib, and imatinib are examples of inhibitors of kinase signaling pathways, which are active against SARS-CoV and MERS-CoV (see, for representative example **91**, **92** in Fig. 13). Their mechanism of action is to block the ABL1, ERK-MAPK and/or PI3K-AKT-mTOR pathways, which may block early viral entry and/or postentry events. However, the major drawback of the inhibitors may be associated with immunopathology [145,146].

CoVs utilize specific host factors for virus entry and replication. Specific monoclonal or polyclonal antibodies, peptides or functional inhibitors can target the host receptor. For example, anti-DPP4 mAbs inhibit MERS-CoV cell entry *in vitro* [147]. For the

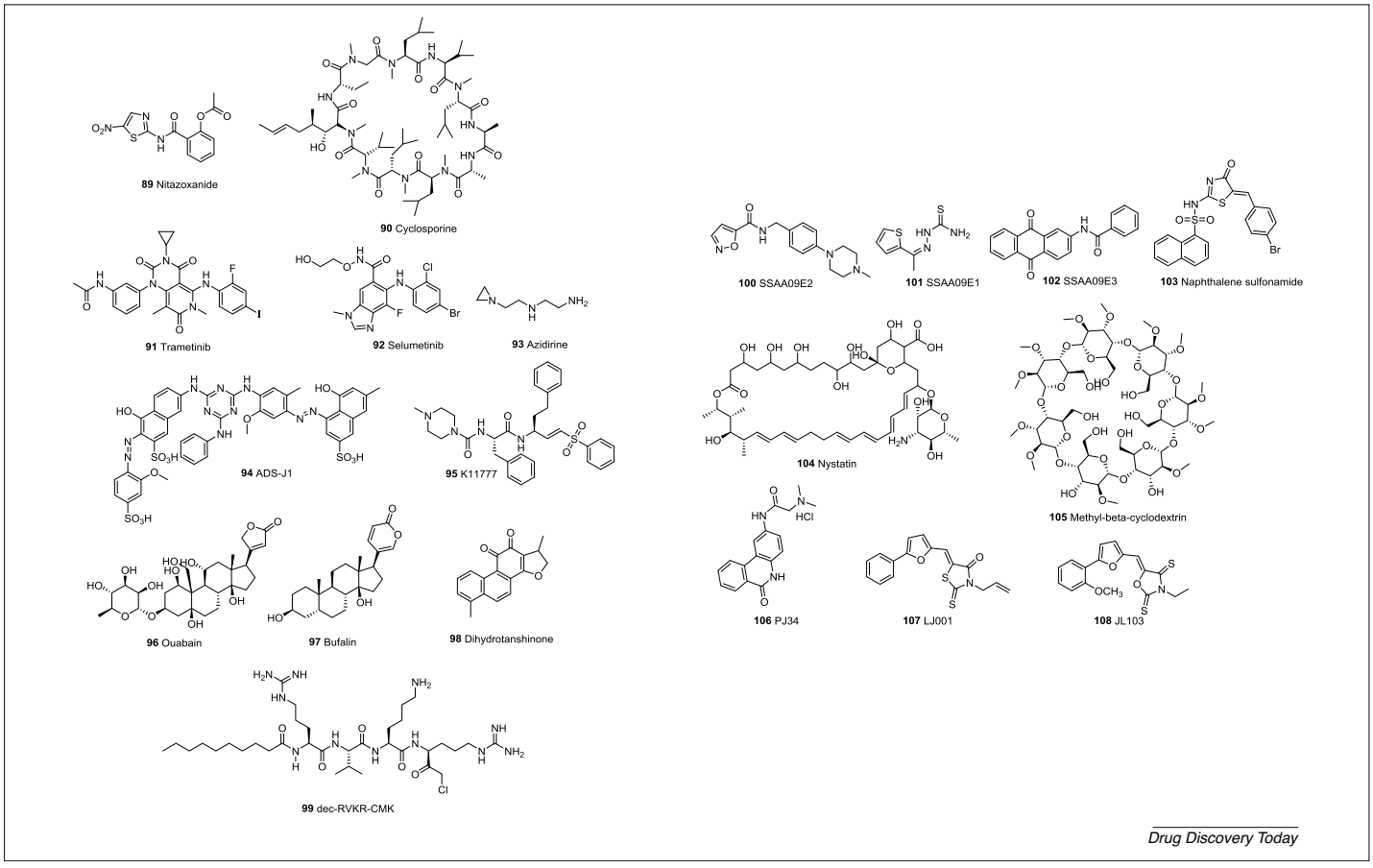

FIGURE 13

Representative host-based anti-Coviral drugs for CoV infections.

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treatment of SARS-CoV, small-molecule entry inhibitors such as *N*-(2-aminoethyl)-1-aziridineethanamine (93, NAAE) inhibit the catalytic activity of ACE2 and SARS-CoV S-mediated cell-cell fusion *in vitro* [148].

Neurotransmitter inhibitors including chlorpromazine (**8**), fluphenazine (**29**), and promethazine (**30**), were reported to inhibit cell-cell fusion in a moderate level with EC_{50} values of about 23, 15, and 17 μM, respectively [81]. Additionally, they disrupt clathrin-mediated endocytosis to inhibit MERS-CoV [81]. An HIV entry inhibitor targeting gp41 (**94**, ADS-J1, Fig. 13) could inhibit more than 90% of MERS-CoV pseudovirus infection in NBL-7 and Huh-7 cells by interrupting the entry of pseudotyped MERS-CoV with an EC_{50} of 0.6 μM in the DPP4-expressing cell line and with a CC_{50} of 26.9 μM in NBL-7 and Huh-7 cells by MTT assay [81].

The entry of CoVs into host cells via the endosomal and/or cell surface pathways is facilitated by host proteases that cleave and activate S protein. Cathepsins are cysteine proteases that are involved in the endosomal pathway and can be inhibited by cathepsin inhibitors such as K11777 (**95**) and its related vinyl sulfone analogs [149]. These compounds seem to be safe and effective against various parasitic infections in animal models and have broad-spectrum activities against enveloped RNA viruses such as CoVs (SARS-CoV, MERS-CoV, HCoV-229E, and HCoV-NL63), filoviruses (Ebola and Marburg viruses) and paramyxoviruses [149–152].

Ouabain (**96**) and bufalin (**97**) (Fig. 13) can inhibit MERS-CoV entry by blocking clathrin-mediated endocytosis [153]. Dihydrotanshinone (**98**, Fig. 13), a lipophilic compound, showed a decimal reduction at 0.5 μg/mL and excellent antiviral effects at ≥2 μg/mL with a reduction in titer from 6.5 Log to 1.8 Log $TCID_{50}$/mL by using a pseudovirus expressing MERS-CoV spike protein [154]. During the biosynthesis of MERS-CoV S protein, the furin inhibitor decanoyl-RVKR-chloromethylketone (dec-RVKR-CMK, **99**, Fig. 13) at 75 μM can lead to a decrease of the 85-kDa cleaved product in MERS-CoV S wt and S2′ mutant [155].

Recently some small molecules were discovered and characterized as inhibitors of SARS-CoV replication that block viral entry by three different mechanisms [156]. (i) compound **100** (SSAA09E2) acts through a novel mechanism of action, by blocking early interactions of SARS-S with the receptor for SARS-CoV, angiotensin-converting enzyme 2 (ACE2); (ii) Compound **101** (SSAA09E1) acts later, by blocking cathepsin L, a host protease required for processing of SARS-S during viral entry; and (iii) compound **102** (SSAA09E3) also acts later and does not affect interactions of SARS-S with ACE2 or the enzymatic functions of cathepsin L but prevents fusion of the viral membrane with the host cellular membrane. Naphthalene sulfonamide derivative **103** a selective clathrin inhibitor targeting its amino-terminal domain, and tetradecyltrimethylammonium bromide (MiTMAB), a dynamin I and II GTPase inhibitor were reported to inhibit the HCoV-NL63 replication by inhibiting the clathrin-mediated entry [157]. Importantly, no cytotoxic effect was observed for the tested inhibitors applied to LLC-Mk2 cells.

HCoV-OC43 remains incessantly one of the most important etiological factors for respiratory tract diseases in humans. Recently, it was found that HCoV-OC43 employs caveolin-1 dependent endocytosis for the entry [158]. Subsequently, cholesterol-binding or depleting agents such as nystatin (**104**, Fig. 13) or methyl-β-cyclodextrin (MβCD) (**105**, Fig. 13) were reported to inhibit the virus replication via an aveolin-1 caveolin-1 dependent endocytosis entry.

Another class of anti-CoV agents that target S to inhibit CoV entry is the carbohydrate-binding agents. Griffithsin is an antiviral protein originally isolated from the red alga *Griffithsia* spp. [159]. It binds specifically to oligosaccharides on viral surface glycoproteins such as S and HIV glycoprotein and inhibits a broad range of CoVs, including SARS-CoV, HCoV-229E, HCoV-OC43 and HCoV-NL63 *in vitro* and SARS-CoV-infected mice [159,160]. The optimal delivery modes and safety profiles of these agents in humans should be further evaluated.

Alternatively, an increasing number of agents that target specific binding sites or functions of these proteins are being generated through crystallography and functional assays. Examples include the viroporin inhibitor hexamethylene amiloride (**6**, Fig. 1), which reduces the ion channel activity of E in SARS-CoV and HCoV-229E, and compound **106** (PJ34, Fig. 13), which binds to a distinct ribonucleotide-binding pocket at the N-terminal domain of N in HCoV-OC43 [161–163]. However, these agents are likely to exhibit a narrow-spectrum as the binding sites and functions of these proteins are unique to individual CoVs. Novel lipophilic thiazolidine derivatives, such as **107** (LJ001, Fig. 13 and **108** (JL103, Fig. 13), are membrane-binding photosensitizers that produce singlet oxygen molecules to induce changes in the properties of lipid membranes and prevent fusion between viral and target cell membranes. They exhibit broad-spectrum activities against numerous enveloped viruses and may be active against CoVs [164–167]. In addition to the above-mentioned molecules, there are numerous peptide-based inhibitors of coronaviruses [168].

Several nucleic acid synthesis inhibitors have broad-spectrum activity against SARS-CoV and MERS-CoV viruses. Inosine monophosphate dehydrogenase (IMPDH) inhibitors such as ribavirin and mycophenolic acid inhibit an important step in *de novo* synthesis of nucleic acids (discussed earlier). Mizoribine, another IMPDH also proved to inhibit the synthesis of nucleic acids. Mizoribine (**109**, Fig. 14), an approved immunosuppressant in organ transplantation with limited adverse side effects, has shown *in vitro* activity against HCV and bovine viral diarrhea virus (BVDV) and was considered as an alternative to ribavirin/IFN combinations for treatment of HCV infections [169] Mizoribine exerts its activity through selective inhibition of inosine monophosphate synthetase and guanosine monophosphate synthetase, resulting in the complete inhibition of guanine nucleotide synthesis without incorporation into nucleotides. The chemotherapeutic gemcitabine has shown *in vitro* activity against MERS-CoV and SARS-CoV (discussed earlier). Remdesivir (development code GS-5734, or **110**, Fig. 12) is an antiviral drug, a novel nucleotide analog prodrug. It was developed by Gilead Sciences as a treatment for Ebola virus disease and Marburg virus infections, though it has subsequently also been found to show reasonable antiviral activity against more distantly related viruses such as respiratory syncytial virus, Junin virus, Lassa fever virus, and MERS-coronavirus [170]. Remdesivir was rapidly pushed through clinical trials due to the 2013–2016 West African Ebola virus epidemic crisis, eventually being used in at least one human patient despite its early development stage at the time. Preliminary results were promising and it was used in the emergency setting for the 2018 Kivu Ebola outbreak along with further clinical trials.

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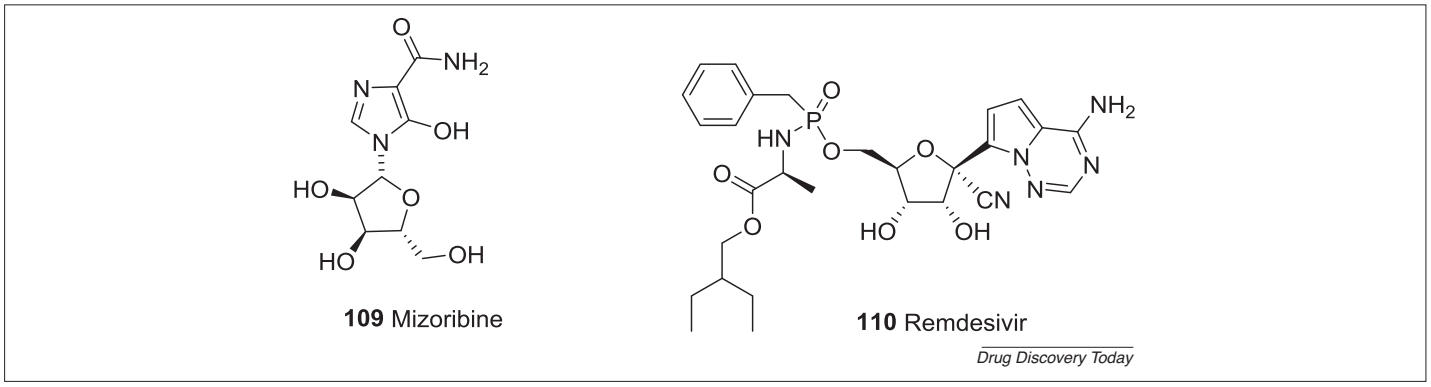

FIGURE 14
Nucleic acid synthesis inhibitors.

In vitro, remdesivir showed potent antiviral activity against both Malaysian and Bangladesh genotypes of Nipah virus and reduced replication of Nipah virus Malaysia in primary human lung microvascular endothelial cells by more than four orders of magnitude, warranting further testing of the efficacy of remdesivir against Nipah virus infection *in vivo*. In contrast, to control animals, which all succumbed to the infection, all remdesivir-treated animals survived the lethal challenge, indicating that remdesivir represents a promising antiviral treatment for Nipah virus infection also [171].

Conclusions

Human coronaviruses utilizes host cellular components to achieve various physiological processes, including viral entry, genomic replication, and the assembly and budding of virions, thereby resulting in pathological damage to the host. Therefore, interrupting any stages of the viral life cycle would become a potential therapeutic target for developing antiviral therapies. Although numerous anti-human coronaviral agents have been identified through various approaches, no specific treatment is currently available for HCoVs, to date. One of the main reasons for that is most of the identified agents were not properly evaluated for *in vitro* and *in vivo* studies.

Our increasing understanding of novel emerging coronaviruses will be accompanied by increasing opportunities for the reasonable design of therapeutics. Importantly, understanding this basic information about coronavirus protease targets will not only aid the public health against SARS-CoV and MERS-CoV but also help in advance to target new coronaviruses that may emerge in the future.

In spite of huge efforts taken by both academia and pharmaceutical industries, no coronavirus protease inhibitor has yet successfully been marketed.

Author contributions

M.M and S.M collected the data. T.P wrote the manuscript, which was revised by all.

Conflict of interest

The authors declare no competing financial interest.

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