Natural molecules having anti-SARS-CoV activity – cannot they be effective against SARS-CoV-2?

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In the current scenario, developing treatments, identifying cures and formulating intervention strategies to fight against the COVID-19 outbreak, have become the major concern for researchers globally. Several studies have confirmed the molecular pathway of COVID-19 virulence that involves activation of proteins like angiotensin-converting enzyme 2 (ACE2), angiotensin (AT) receptor which is mainly AT1, and transmembrane protease serine 2 (TMPRSS2). Since the virus needs the involvement of these proteins, over and above its spike protein, for activation and infecting the host, it is obvious that targeting or blocking the activation of these molecules may play a critical role in the development of therapeutics for the cure and management of COVID-19. Many studies have been reported and several are ongoing to find a cure using these molecules as a target. While initially COVID-19 was thought to be affecting the human respiratory system, recent shreds of evidence indicate that this infection can reach beyond the lungs. It can invade and rampage almost all the organs in the body, and 'cytokine storm' is considered to be responsible for this. The reason for disease severity is not what matters anymore now, everyone is hoping for a cure soon enough. Therefore, there is an urgent need to search for some novel drugs/chemicals. Towards this end, natural products can contribute immensely since they have a long history of usage for the cure and management of various ailments, including viral infections. Various natural products (mainly from plants) have structural similarities to the molecules which by AT has been shown to interact with targets used by COVID-19 during its infection. This is indeed a positive indication for the development of natural products-based therapeutics. This aspect, therefore, warrants serious consideration for the cure and management of the COVID-19 pandemic.

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Keywords: Coronavirus, cure and management, herbal medicine, natural products, pandemic.

CORONAVIRUS (CoV) is a single-stranded RNA (ssRNA) virus of the Coronaviridae family which is comprised of several pathogenic species that are mainly responsible for upper respiratory and gastrointestinal tract infections in humans and other mammals[1]. Particularly, the human coronavirus (HCoV), such as 229E, OC43, NL63 and HKU1, may cause severe acute respiratory syndrome (SARS) and hence is called SARS-CoV; it is spreading world over among the human population[2]. SARS-CoV-1 and Middle East Respiratory Syndrome (MERS)-CoV in 2003 and 2012 respectively, were introduced as a global threat with a large number of casualties[3,4].

The recently introduced novel coronavirus (SARS-CoV-2 or 2019-nCoV) from Wuhan, China, caused the COVID-19 outbreak which was declared as a global pandemic by World Health Organization (WHO). It has spread to 200 countries and caused more than 477,000 deaths across the globe as on 24 June 2020. Similar to the earlier infections caused by SARS-CoV, MERS-CoV and Ebola, COVID-19 is incurable during its outbreak and thus, the scientific and medical fraternity has succumbed to this disease. The only procedure being adopted is to treat the infection symptomatically and to stop the spread of this virus through social distancing, lockdown or curfew. Hence, the discovery of a new drug/vaccine for 2019-nCoV is an urgent need of the hour.

The present work aims to review the selected anti-SARS-CoV molecules from natural origin and their possible role against SARS-CoV-2. Among 53 natural anti-CoV molecules chosen using different search engines like Scopus and PubMed, a total of 24 most effective molecules were selected for this study. Studies based on molecular docking without *in vitro*/*in vivo* experiments are rarely included here.

Structural similarity between SARS-CoV and SARS-CoV-2

CoV has seven strains, of which three are highly pathogenic, viz. SARS-CoV-1, MERS-CoV, and SARS-CoV-2, and caused severe CoV pandemic disease[5]. These have clinically important structural proteins such as spike (S), membrane (M), envelope (E) and nucleocapsid (N) (Figure 1). Some of the viruses, including SARS-CoV also have hemagglutinin esterase (HE) glycoprotein[6]. These are RNA viruses and their genome codes for seven genes that are conserved in the order of *ORF1a*, *ORF1b*, *S*, *OEF3*, *E*, *M* and *N* in 5′–3′ direction. The gene *ORF1a/b* produces two viral replicase proteins that are polyproteins (PP1a and PP1ab)[7]. Sixteen other mature nonstructural proteins (NSPs) arise from further processing of these two polyproteins. The NSPs take part in different viral functions, including formation of the replicase transcriptase complex. The remaining genome part of the virus encodes the mRNA which produces structural proteins, i.e. S, E, M, and N and other accessory proteins[7]. Another important envelope-associated protein which is expressed by only some strains of CoV is the HE protein[8]. The RNA genome of CoV is packed in the nucleocapsid protein and further covered with an envelope.

The SARS-CoV-2 has approximately 79% genome sequence identity with SARS-CoV and 50% with MERS-CoV (ref. 9). A study using sequence-based comparison confirmed that M, N and E proteins of SARS-CoV-2 and SARS-CoV have over 90% genetic similarity, while S protein of SARS-CoV and SARS-Cov-2 has 76% similarity[10]. Besides, homology modelling showed that SARS-CoV-2 also uses ACE2 receptor in humans for infection, similar to SARS-CoV (ref. 11). Recently, it has been demonstrated that human recombinant soluble ACE2 can significantly block early stages of SARS-CoV-2 infections[12]. Despite the name and genetic similarities, SARS-CoV-2 shows genetic and clinical differences with SARS-CoV. The S-protein found in SARS-CoV-2 is longer than that in SARS-CoV, and the receptor-binding regions of these viruses are completely different. Moreover, SARS-CoV nucleocapsid (N) protein can neutralize the immune response of the host, acting as an antagonist to the action of interferon (IFN)-γ, and it is still not known if the SARS-CoV-2 N-protein has the same ability. If not, this would partly explain why SARS-CoV has a higher mortality rate than SARS-CoV-2 (ref. 13). Similarly, the R0 (R through) of SARS-CoV-2 is two- to three-fold higher than that of SARS-CoV. Usually, a high R0 is associated with highly diffusible infections, which at the same time have a long incubation period, and characterized by mild to moderate symptoms, or latent infection associated with a low mortality rate[14]. SARS-CoV-2 receptor binding motif (RBM) contains structural changes in the hACE2-binding ridge, largely caused by a four-residue motif (residues 482–485: Gly–Val–Glu–Gly). This structural change allows the ridge to become more compact and form better contact with the N-terminal helix of hACE2 compared to SARS-CoV. Phe486 from the SARS-CoV-2 RBM can insert into a hydrophobic pocket. The corresponding residue in the SARS-CoV RBM is leucine, which likely forms a weaker contact with hACE2 due to its smaller side chain[15]. These hotspots on hACE2 are critical for coronavirus binding, because they involve two lysine residues that need to be accommodated properly in hydrophobic environments. Neutralizing the charges of the lysine residues is key to the binding of coronavirus receptor-binding domain (RBD) to hACE2. SARS-CoV-2 RBM has evolved strategies to stabilize the two hotspots; Gln493 and Leu455 stabilize hotspot-31, whereas Asn501 stabilizes hotspot-353. Therefore, biochemical data confirm that SARS-CoV-2 RBD has significantly higher hACE2-binding affinity than that of SARS-CoV, and that the above structural features of SARS-CoV-2 RBM contribute to the high hACE2-binding affinity of SARS-CoV2. Thus, both structural and biochemical data reveal that SARS-CoV-2 RBD recognizes hACE2 better than SARS-CoV RBD.

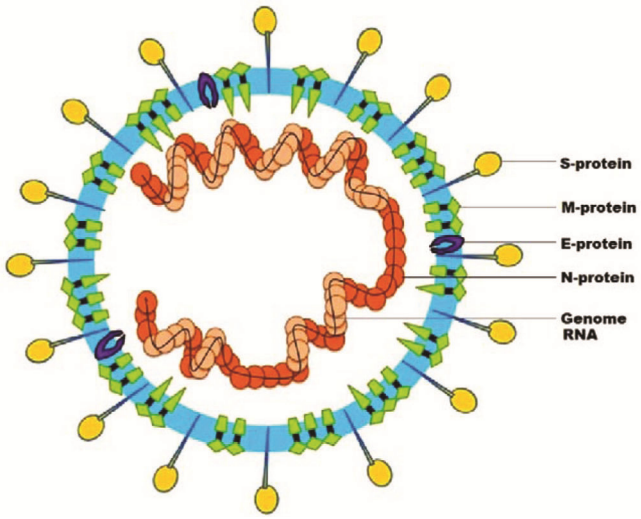

Figure 1. Structure of SARS-CoV (reproduced with permission from Li *et al.*[13]).

Important targets for drug development against SARS-CoV-2

The majority of medicinal plants contain many different compounds and most of them are quite complex. Plants contain a variety of materials having medicinal properties. Majority of them includes polysaccharides, polyphenols, tannins, saponins and various other complex materials that modulate and modify the effects of any ‘active principles’. It has been shown that the extract of whole plant does not mimic the isolated and purified constituents of the herbs. According to the literature, it is considered that the whole plant consists of various parts in most cases of

higher efficacy compared to individual components[16]. Therefore, a thorough screening of natural products-based drug-like molecules may lead to the development of therapeutics which could be used in the cure and management of COVID-19.

In the past decades, mainly after the SARS-CoV outbreak in 2003, scientists the worldover studied various targets to develop new antiviral drugs, which include the inhibition of essential proteases, helicase and viral proteins[17]. These enzymes are critical for the synthesis of RNA and protein of the virus. Amongst them, one of the important groups is non-structural proteins (NSPs), which are involved in transcription, translation, protein synthesis, processing and post-translational modification, virus replication and infection of the host. Similarly, helicase (NSP13), a multi-functional protein which includes the N-terminal metal-binding domain and helicase domain, is a necessary component for the replication of CoV. NSP13 can unravel double-stranded (ds) DNA and RNA along the 5′–3′ direction in an NTP-dependent manner[18]. Other groups of proteins are structural proteins of the virus, responsible for binding to human cell receptors.

Spike is the main structural protein that interacts with the host by binding to host cell receptors in order to mediate virus invasion and determine viral tissue or host tropism[19]. Spike is cleaved into S1 and S2 by the host cell protease like type-II transmembrane serine protease. The S1 subunit binds with the host cell surface receptors and the S2 subunit mediates virus–cell and cell–cell membrane fusion. There are some other proteins such as NSP1, NSP3c and ORF7a, called virulence factors which interfere with the innate immunity of the host. NSP1 interacts with host 40S ribosomal subunit that induces specifically host mRNA degradation and also inhibits type-I interferon production[20]. Similarly, NSP3c binds to ADP-ribose of the host to help CoV in resisting host innate immunity[21]. Besides, ORF7a protein binds to bone marrow matrix antigen 2 and inhibits its activity by blocking its glycosylation process[22].

Among others, 3C-like protease (3CLpro), a chymotrypsin-like cysteine protease, was found to be the most useful due to its role in the mutation of SARS-CoV by inducing autolytic cleavage of polyproteins pp1a/1ab (ref. 23). Also, 3CLpro was found to cleave the polyprotein at 11 sites with conserved Gln at the P1 position, which was found to be a required condition for efficient cleavage[24]. Papain-like protease (PLpro) was also found as an important target since it plays a vital role in the replication of CoV. In addition to its role in the processing of viral polyprotein, it strips ubiquitin and interferon-stimulated gene-15 from host cell proteins to aid CoV in its evasion of the host innate immune response. Hence, this target has an additional advantage over 3CLpro to inhibit dysregulation of signalling cascades in infected cells which might be responsible for the death of healthy cells nearby[25].

CoV helicase, a non-structural protein, is also considered to be a key target for drug development in this category, particularly against viral replication. This protein is a cleavage product of pp1ab, which binds to the 5′ overhang and moves in the 5′→3′ polarity to bring about negative supercoiling of dsRNA or dsDNA[26]. Apart from the above line of treatments, vaccination represents the preferred line of defence against viral infections due to its higher specificity compared to chemotherapeutic drugs. Immunogenic viral antigens such as the S protein in combination with potent adjuvants such as 3-deacylated monophosphoryl lipid-A or QS21 saponins from *Quillaja saponaria* may induce either humoral- or cell-mediated immunity by stimulating B-cells as well as $CD8^+$ T-cells[17]. In mice, a DNA vaccine encoding the spike (S) glycoprotein of SARS-CoV induced T-cell immunity and neutralizing antibodies, suggesting DNA vaccination as a powerful strategy against SARS-CoV[27]. Moreover, since patients recovering from SARS developed neutralizing antibodies effective in preventing infection, immunotherapy with neutralizing monoclonal antibodies represents a promising approach[17]. Besides these virus proteins, host proteins can also be potential targets, as these provide an attachment site for a virus to enter into the host cells. Such a critical enzyme is the angiotensin-converting enzyme 2, which has been proved by many studies to be the specific receptor for the spike RBD of SARS-CoV-2. Hence, potential CoV therapeutic drugs can be developed by inhibiting key proteins involved in different pathways.

Natural compounds with anti-CoV activity

Among other treatments, numerous molecules have been screened against SARS-CoV, MERS-CoV and other CoVs; many of them showed strong activity even at very low concentration[28]. Till date, hundreds of purified natural molecules together with their source extracts have been studied particularly for their anti-SARS-CoV activity[29]. A number of compounds, including scutellarein[30], tentrandrin[31], cepharanthine[31], and betulonic acid[32] were found to exhibit anti-CoV effects at very low IC_{50} values even with <1 μM concentration. Earlier studies suggested that natural molecules or their analogues were stronger than synthetic ones. Various mechanism-based studies showed that there is no certain pattern in the structures exhibiting anti-CoV activity because there is a diversity in the active molecules which include alkaloids, flavonoids, terpenoids, anthraquinones, saponins, polypeptides, steroids, tannins and lignans, among others. However, some studies proved that specific structure skeleton and site of substitution were responsible for the activity. In this direction, many coumarin derivatives found in *Saposhnikovia divaricata* showed activity against porcine epidemic diarrhoea virus, an enveloped single-stranded RNA coronavirus. Structure–activity relationship study

Table 1. Natural molecules having anti-SARS-CoV activity

Class of compounds	Compound	Natural source
Alkaloids	Berbamine	*Berberis amurensis*
	Cepharanthine	*Stephania cepharantha*
	Emetine	*Carapichea ipecacuanha*
	Fangchinoline	*Stephania tetrandra*
	Lycorine	*Lycoris radiate*
	Papaverine	*Papaver somniferum*
	Reserpine	*Rauvolfia serpentine*
	Tetrandrine	*Stephania tetrandra*
	Tylophorine	*Tylophora indica*
	7-Methoxycryptopleurine	*Tylophora indica*
Flavonoids	Amentoflavone	*Torreya nucifera*
	Apigenin	*Chamaemelum nobile*
	Bavachinin	*Psoralea corylifolia*
	Broussochalcone A	*Broussonetia papyrifera*
	Chrysin	*Passiflora caerulea*
	Corylifol A	*Psoralea corylifolia*
	Diplacone	*Paulownia tomentosa*
	Gallocatechin gallate	*Camellia sinensis*
	Hesperetin	*Isatis indigotica*
	Isobavachalcone	*Psoralea corylifolia*
	Juglanin	*Polygonum aviculare*
	Luteolin	*Reseda luteola*
	Mimulone	*Paulownia tomentosa*
	Myricetin	*Myrica nagi*
	Neobavaisoflavone	*Psoralea corylifolia*
	Papyriflavonol A	*Broussonetia papyrifera*
	Procyanidins A2 and B1	*Cinnamomum verum*
	Quercetin	*Quercus robur*
	Scutellarein	*Scutellaria lateriflora*
	Theaflavin-3,3′-digallate	*Camellia sinensis*
	Tomentins A–E	*Paulownia tomentosa*
	(–)-Catechin gallate	*Camellia sinensis*
	3′-*O*-Methyldiplacol	*Paulownia tomentosa*
	3-Isotheaflavin-3-gallate	*Camellia sinensis*
	4′-*O*-methylbavachalcone	*Psoralea corylifolia*
Terpenoids	Betulinic acid	*Betula pubescens*
	Betulonic acid	*Eucalyptus globules*
	Ferruginol	*Sequoia sempervirens*
	Miltirone	*Rosmarinus officinalis*
	Saikosaponins A, B_2, C and D	*Bupleurum chinense*
	Tanshinone I	*Salvia miltiorrhiza*
Anthraquinones	Aloe-emodin	*Aloe ferox*
	Emodin	*Rheum emodi*
	Rhein	*Rheum rhabarbarum*
Saponins	Aescin	*Aesculus hippocastanum*
	Glycyrrhizin	*Glycyrrhiza glabra*
Polypeptides	Hitachimycin	*Streptomyces scabriporus*
	Valinomycin	*Streptomyces* spp.
Steroids	Ginsenoside-Rb1	*Panax ginseng*
	β-Sitosterol	*Vitis vinifera*
Tannins	Cinnamtannin B1	*Cinnamomum verum*
	Tannic acid	*Quercus infectoria*
	Tetra-*O*-galloyl-*β*-D-glucose	*Phyllanthus emblica*
Lignans	*Hinokinin*	*Chamaecyparis obtuse*
	Savinin	*Pterocarpus santalinus*

(*Contd*)

Table 1. (*Contd*)

Class of compounds	Compound	Natural source
Miscellaneous	Chaetochromin	*Chaetomium* spp.
	Cinanserin	*Houttuynia cordata*
	Curcumin	*Curcuma longa*
	Indigo	*Indigofera tinctoria*
	Kazinol F	*Broussonetia papyrifera*
	Lutein	*Tropaeolum majus*
	Psoralidin	*Psoralea corylifolia*
	Sinefungin	*Streptomyces* spp.
	Sinigrin	*Isatis indigotica*
	Stictic acid	*Usnea articulate*

revealed that substitution at C-8 position could be responsible for the antiviral activity[33].

These molecules showed anti-CoV activity via multiple mechanisms such as inhibition of 3CLpro, PLpro, interaction of SARS-CoV S protein and ACE2, viral polymerase, virus replication/cell division, viral attachment and penetration stages[29]. Besides, these were found to block virus to host cell binding, 3a channel and viral entry to host cells. Table 1 provides a summary of selected molecules with anti-SARS-CoV activity.

Table 1 reveals that flavonoids are the most active compounds, perhaps due to hydroxyl substitution in their structure. The alkaloids are the second most active class, followed by terpenoids and anthraquinones. Although all the above molecules were found active against SARS-CoV, a few of them have been reported to be promising with a very low concentration[30–32]. Their mechanisms of action were also established in various *in vitro* studies[29]. Many of them are already approved drugs for other uses. Hence, a few most active molecules belonging to different chemical classes with their effective concentrations and the possible mechanisms are discussed here. Figure 2 shows their chemical structures.

Stictic acid

Stictic acid (**1**) is a bioactive compound found in many lichens, including *Usnea articulate* and *Lobaria pulmonaria*. This compound showed antiviral activity in virus-infected CRFK cells by inhibiting 3CLpro of feline infectious peritonitis virus (FIPV), a mutated form of the parental enteric form of feline enteric coronavirus, at the concentration <50 μM. It also showed activity to inhibit penetration of virus to the host cell. The activity was found more effective than that of ribavirin (<50 μM), which was used as a positive control. Stictic acid also inhibited FIPV replication in CRFK cells after viral infection. The EC_{50} value was measured at 21.83 μM, while the SI value was calculated to be 17.04 (ref. 34). Besides, at an IC_{50} value of <50 μM, compound **1** was found to produce potential effect against HIV-1 integrase[35].

Wassman *et al.*[36] found that **1** has the potential to react with Cys141 within the cysteine triad of tumour suppressor protein p53. As cysteine is also present in 3CLpro of SARS-CoV as the nucleophilic active site in the catalytic dyad (Cys144/His41), hence there is a possibility that **1** can also inhibit 3CLpro of coronavirus.

Lycorine

Lycorine (**2**), an alkaloid isolated from the stem cortex of *Lycoris radiata* (L′Hér.) Herb., showed potent *in vitro* activity against SARS-CoV with an EC_{50} value of 15.7 nM. Its 50% cytotoxic concentration (CC_{50}) was calculated to be 14.98 and 18.81 μM against the Vero E6 and HepG2 cell lines respectively, with a selective index of >900. Interferon-α was used as a standard, which showed inhibitory effects on virus-induced cytopathic effect with EC_{50} of 660.3 IU/ml and CC_{50} of >10,000 IU/ml (ref. 37). This compound was also used against normal Vero E6 and human HepG2 cell lines to evaluate its toxicity. The results showed that it was safe for further use as it showed a CC_{50} value of 14.98 μM (ref. 37). However, the mechanism of action was not elucidated as the study did not evaluate the interaction of lycorine with proteins and antigens of the virus. It inhibited the replication of HCoV-OC43, HCoV-NL63 and MERS-CoV in BHK-21 cells with EC_{50} values of 0.15, 0.47 and 1.63 μM respectively, whereas the CC_{50} values were recorded at 4.37, 3.81 and 3.14 μM respectively[38]. The compound also showed activity against mouse hepatitis virus (MHV)-A59, a β-CoV, with EC_{50} and CC_{50} values of 0.31 and 3.51 μM respectively. Besides, it inhibited the human enterovirus-71 (EV71) infection in mice. The results showed that it reduced mortality, clinical scores and pathological changes in the muscles of experimental animals by inhibiting viral replication at a dose of 0.4 mg/kg (ref. 39).

Myricetin

Myricetin (**3**), a common flavonoid distributed in many families, including Myricaceae and Anacardiaceae, has been found active against Rauscher leukaemia virus (RLV), HIV and SARS-CoV[40]. It displayed activity

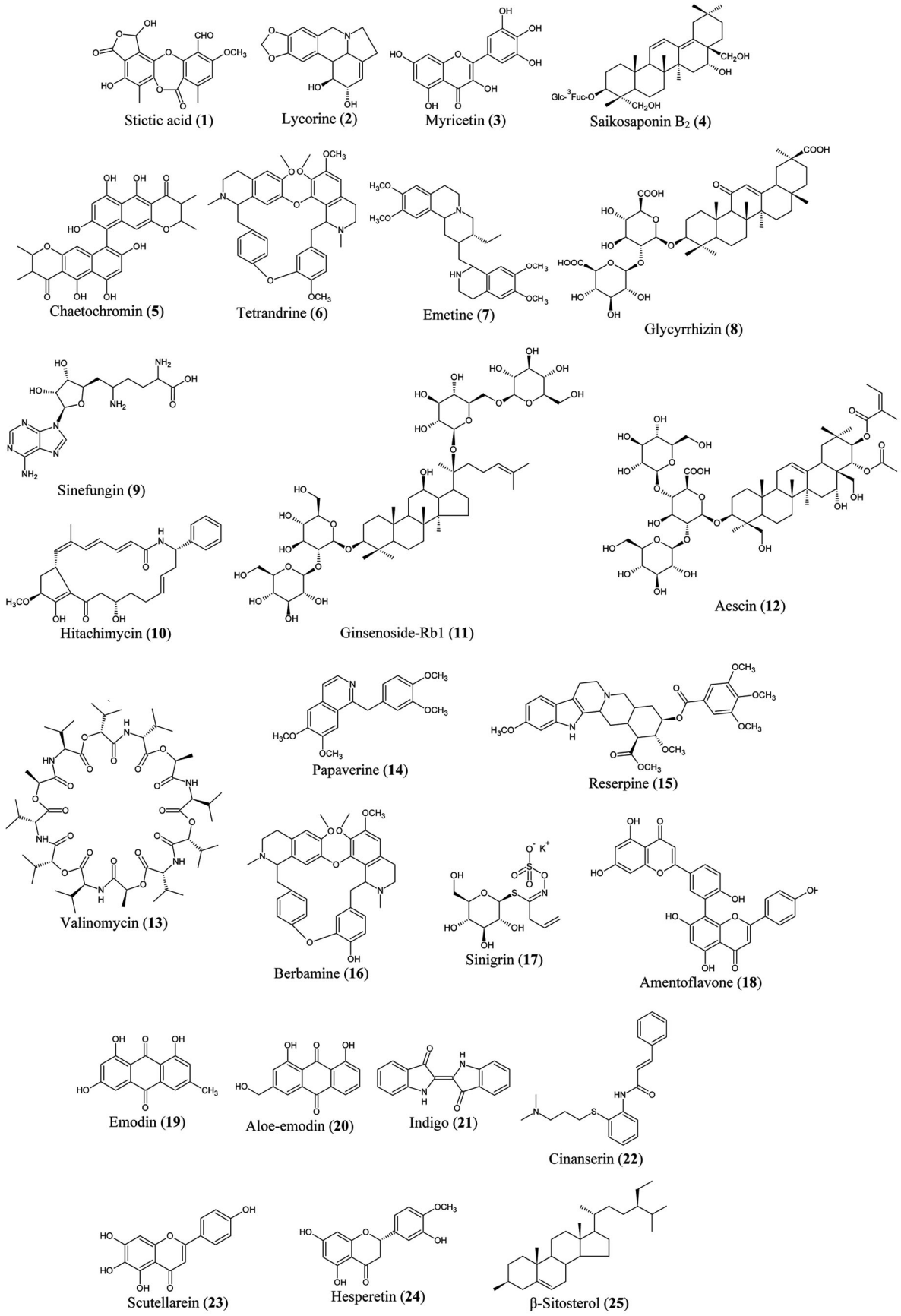

Figure 2. Chemical structures of important anti-SARS-CoV molecules from natural origin.

against SARS-CoV and inhibited its helicase protein by affecting the ATPase activity, with an IC_{50} value of 2.71 μM. The helicase activity was, however, not affected by the treatment of myricetin. Compound **3** was found to be safe for further use as it did not show cytotoxicity in MCF10A cells at 2 μM (ref. 30). The study also suggested that **3** can directly interact with ATP/ADP-binding pocket of coronavirus helicase protein[30]. Besides, myricetin-3-*O*-*β*-D-glucopyranoside obtained from *Camellia sinensis* was found as a possible molecule to inhibit 3CLpro of SARS-CoV-2 via a docking study[41].

Saikosaponin B_2

Triterpenoid glycosides, saikosaponins A, B_2, C and D, found in many plant species, including *Bupleurum chinense* DC, *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl., and *Scrophularia scorodonia* L. have been found to have antiviral effects against a number of pathogens such as human immunodeficiency virus, measles, influenza virus, herpes simplex virus (HSV), varicella-zoster virus and HCoV-229E (ref. 42). At concentrations of 25 μmol/l, saikosaponins A, B_2, C and D showed HCoV-229E inhibition activity by 67%, 100%, 58% and 62% respectively, whereas the EC_{50} values were recorded at 8.6, 1.7, 19.9 and 13.2 μmol/l respectively. Actinomycin D, a positive control showed activity with EC_{50} value of 0.02 μmol/l. Saikosaponin B_2 (**4**) was found to better inhibit HCoV-229E when used before the adsorption of the virus. Besides, the compound at 6 μmol/l also effectively prevented the attachment and penetration of the virus into host cells[42].

It is interesting to note that all tested saikosaponins were found nontoxic for normal lung MRC-5 cells at a concentration of 2.5 μmol/l, as no change in morphology and density was noticed in the cells. Furthermore, cell viability was recorded >60% when the concentration was increased to 25 μmol/l. No visible changes in cell morphology or cell density were observed at the concentrations tested[42].

Chaetochromin

Chaetochromin (**5**), an antidiabetic drug, first isolated from a fungal species of *Chaetomium*, showed inhibitory effect on recombinant FIPV 3CLpro with EC_{50} values of 1.19 and 4.86 μM during pre- and post-viral entry respectively. The cytotoxicity showed by the compound against CRFK cells was measured with a CC_{50} value of 10.53 μM. The results were found more effective than those of lopinavir, a potent protease inhibitor[34]. Chemically, this compound has two stereoisomers namely chaetochromin A and B, and their comparison of bioactivity is not known. Hence, this drug is given as a mixture rather than an isolated isomer.

Tetrandrine

Tetrandrine (**6**), isolated from *Stephania tetrandra* S. Moore, was earlier tested for its activity against Ebola virus and found to inhibit penetration of virus to the host cells in an *in vitro* study. Moreover, it inhibited Ebola virus-induced infection of human macrophages *in vivo*[43]. This compound produced inhibitory effects against the multiplication of HCoV-OC43, HCoV-NL63, MERS-CoV, and MHV-A59 with EC_{50} values of 0.29, 2.05, 12.68, and 4.81 μM respectively, in BHK-21 cells. Also, the CC_{50} values were measured to be >20 μM against all tested viruses[38]. It inhibited the death of HCoV-OC43-infected MRC-5 human lung cells at the early stage of infection. The suppression of virus replication and inhibition of S- and N-protein expression were also recorded during the study at IC_{50} value of 295.6 nM and SI value >40. It is interesting to note that **6** did not show cytotoxicity up to the concentration of 10 μM (ref. 31).

In view of its effect against SARS-CoV, Henan Provincial People's Hospital (China) has started clinical trials on this drug against COVID-19, and has already found encouraging results up to phase III trials; and phase IV trials are in progress[44]. This study targeted to enrol at least 60 COVID-19 patients with mild to severe neocoronary pneumonia. Tetrandrine tablets were given to them together with standard treatment regimens to reduce the clinical progress, improve prognosis and reduce the incidence of pulmonary fibrosis.

Emetine

Emetine (**7**), a drug obtained from the roots of *Carapichea ipecacuanha*, was found effective in inhibiting the replication and penetration of four different CoVs, viz. HCoV-OC43, HCoV-NL63, MERS-CoV and MHV-A59 in BHK-21 cells. The EC_{50} values for these viruses were 0.30, 1.43, 0.34, and 0.12 μM while the CC_{50} values were 2.69, 3.63, 3.08, and 3.51 μM respectively, in BHK-21 cells[38]. The study also confirmed that emetine can inhibit the replication of both DNA and RNA viruses.

Glycyrrhizin

Glycyrrhizin (**8**), purified from the roots of *Glycyrrhiza glabra* L., was evaluated for its anti-SARS-CoV activity *in vitro*, and found that it inhibited virus infection with an EC_{50} value of 300 μg/ml. The compound inhibited replication in Vero cells with a selectivity index of 67. During the virus adsorption to Vero cells EC_{50} was calculated to be 2400 μg/ml, while after virus adsorption the EC_{50} value was found to be 600 μg/ml, which showed that the compound is comparably more effective when added after virus adsorption. The CC_{50} value was measured to >20,000 μg/ml for all experimental conditions[45].

The compound inhibited both adsorption and penetration of CoV, which is usually an initial phase of the replication cycle of a virus. The expression of virus antigens was found considerably lower in the cells treated with 1000 μg/ml concentration, while replication was completely blocked at a concentration of 4000 μg/ml. As far as the mechanism is concerned, it induced NOS in Vero cells and virus replication was inhibited after adding β-NONOate, a nitrous oxide donor to the culture medium[45]. Although its antiviral mechanism is not clear enough, its role against PKC, CK2, AP1 and NFκB has been well-established.

Previously, compound **8** was also tested for its activity against the replication of flaviviruses at different concentrations. The compound was found effective against viruses with EC_{50} values ranging from 316 to 625 μg/ml (ref. 46). Glycyrrhizin was found non-cytotoxic up to 2500 μg/ml, as no inhibition in cell growth was observed at this concentration. Hoever *et al.*[47] reported that after introducing 2-acetamido-β-D-glucopyranosylamine into the glycosidic chain of **8**, its anti-CoV activity was found to increase by 10-fold than that of compound **8** itself. On the other hand, the activity was increased up to 70-fold after forming its amides and conjugates with two amino acids and one COOH group at C-30. These structures were, however, found to be more toxic than that of pure glycyrrhizin.

Pharmacokinetics studies of **8** showed that it hydrolyses to 18β-glycyrrhetinic acid when taken via the oral route. The hydrolysed product, after getting absorbed in the gut, metabolises to 3β-monoglucuronyl-18β-glycyrrhetinic acid in the liver, and finally reaches the blood. The metabolite then circulates in the bloodstream. A clinical study with six participants showed that 0.31–0.67% metabolite was excreted by urine when a dose of 600 mg of **8** was given orally[48]. Higher doses at 354 and 430 per day were clinically proven to be effective against 37 patients of SARS-CoV. The compound could treat all associated-symptoms like dry cough, chest distress and dyspnea[49].

Sinefungin

Sinefungin (**9**), a nucleoside found in *Streptomyces*, inhibited the activities of N7-MTase and 2′O-MTases of Vaccinia virus with IC_{50} values of 12.0 and 39.5 nM respectively[50]. At IC_{50} value of 55 nM, it also inhibited fungal N7-MTase in an *in vivo* model[51]. Its activity against the SARS-CoV N7-MTase was evaluated using MT–yeast, MT–human and MT–SARS, and found that the compound repressed the growth of these yeast strains at a concentration of 100 μM. The strongest activity was shown against MT–yeast, and the study found that it is a broad-spectrum inhibitor against N7-MTases of yeasts, humans and CoVs[52].

Hitachimycin

Hitachimycin (**10**), a well-known bioactive compound found in *Streptomyces scabriporus*, exhibited FIPV 3CLpro inhibitory activity in CRFK cells. The FIPV 3CLpro has been found responsible for poly-protein processing in CoV during its replication. The EC_{50} values for pre-viral entry, post-viral entry and prophylactic antiviral activity were found at 3.83, 4 and 16.22 μM respectively. This activity was found more effective than that of ribavirin which was used as a reference drug. The compound was found safe as it showed cytotoxicity with a CC_{50} value of 56.87 μM (ref. 34).

Ginsenoside-Rb1

Ginsenoside-Rb1 (**11**) isolated from *Panax ginseng*, was found to have anti-SARS-CoV activity in Vero cells at the concentration of 100 μM (ref. 53). This molecule showed no toxicity in NIH-3T3 cells even at 100 μM. Cell viability was not affected during and after the experiment[54]. Its antiviral activity was also evaluated against H1N1 virus and found that its administration at 1 and 2 mg/ml prior to the infection in mice reduced weight loss and complete protection over lethal infection[55]. Besides, it has the ability to inhibit replication of the virus. The overall results suggested that compound **11** may be further used as a lead molecule for antiviral drug discovery against SARS-CoV-2 after obtaining its toxicity and bioavailability profile.

Aescin

Aescin (**12**), a bioactive found in *Aesculus hippocastanum* L., produced antiviral effect against SARS-CoV in Vero E6 cells with EC_{50} value of 6.0 μM and CC_{50} value of 15 μM. Based on the results, the selectivity index (SI) was calculated to be 2.5 (ref. 53). This molecule has been least studied for its antiviral activity in comparison to other molecules considered here. Since compound **12** showed remarkable activity with lesser concentration, its further use in higher models may produce better results.

Valinomycin

Valinomycin (**13**), a fungus-derived dodecadepsipeptide found in *Streptomyces* species, showed inhibitory activity against SARS-CoV with an EC_{50} value of 0.85 μM and CC_{50} value of 68 μM (SI = 80). SARS virus-infected Vero E6 cells were treated with valinomycin and found that it inhibited the multiplication and penetration of the virus[53]. Its activity against HCoV-OC43, HCoV-NL63, MERS-CoV and MHV-A59 has been established with EC_{50} values of 4.43, 1.89, 6.07, and 6.78 μM and CC_{50}

values of 6.15, 4.12, 5.88, and 5.11 μM respectively in BHK-21 cells[38].

Papaverine

Papaverine (**14**), naturally occurring in the capsules of *Papaver somniferum*, is an established antispasmodic drug. This alkaloid showed anti-SARS-CoV activity against the replication of HCoV-OC43, HCoV-NL63, MERS-CoV and MHV-A59 with EC_{50} values of 1.61, 7.32, 9.45 and 11.46 μM respectively, in BHK-21 cells. The respective CC_{50} values were recorded at 12.11, 11.71, 11.98 and 12.44 μM (ref. 38).

Reserpine

Reserpine (**15**), the well-known alkaloidal drug naturally found in *Rauvolfia serpentina* (L.) Benth. ex Kurz, was shown to have anti-SARS-CoV activity. The SI calculated for this compound was 7.3 with EC_{50} and CC_{50} values of 3.4 μM and 25 μM respectively. Compound **15** was also found to inhibit virus penetration into Vero E6 cells[53]. As this drug is already in clinical use, its trial against COVID-19 can be conducted by following earlier trials mainly for dose determination.

Berbamine

Berbamine (**16**), a natural alkaloid found in *Berberis amurensis* and other species of the Berberidaceae family, was screened for its antiviral efficacy. This compound was found effective against the replication of HCoV-OC43, HCoV-NL63, MERS-CoV and MHV-A59 with EC_{50} values of 1.48, 9.46, 13.14 and 10.91 μM respectively. A CC_{50} value of >20 μM against all the above viruses was also measured in an *in vitro* study in BHK-21 cells[38]. The molecule was only studied for its preliminary antiviral activity, the mechanism of action was not evaluated. Moreover, toxicity studies are lacking; hence, such studies are required before using it on higher models.

Sinigrin

Sinigrin (**17**) is a glucosinolate which is found in many species of family Brassicaceae, including *Isatis indigotica* Fort. It showed activity against 3CLpro which is responsible for mediating proteolytic processing of replicase polypeptides (pp1-a and pp1-ab) into functional proteins of the virus. The IC_{50} value for cell-based cleavage activity of this compound was measured at 217 μM, with CC_{50} value for Vero cell death at >10,000 μM. It effectively blocked the cleavage process of 3CLpro. In addition, it was found non-toxic to Vero cells as it showed a CC_{50} value of >10,000 μM (ref. 56).

Amentoflavone

Amentoflavone (**18**), isolated from the leaves of *Torreya nucifera*, has been found to inhibit SARS-CoV 3CLpro with an IC_{50} value of 8.3 μM. The structure–activity relationship study showed that an apigenin moiety at position C-3′ is responsible for the inhibitory activity of 3CLpro (ref. 57). Apigenin, luteolin and quercetin were used to understand the structure–activity relationship and found that apigenin showed 3CLpro inhibitory activity by 40% at 200 μM, while luteolin and quercetin showed activity with IC_{50} values of 20.2 and 23.8 μM respectively. This is interesting because these compounds are also AhR ligands. Thus, it might be useful to test other AhR ligands as well.

Emodin

Emodin (**19**), one of the active compounds of *Rheum emodi*, furnished antiviral activity against most of the pathogenic viruses like hepatitis-B, -C, HSV and CoV. Particularly against hepatitis B, it showed activity by inhibiting the DNA replication and HBsAg secretion of the virus in G2.2.15 cells with an IC_{50} value of 21 μg/ml (ref. 58). This activity was also confirmed by Dang *et al.*[59] using mice models at a dose of 287.95 mg/kg/day for 21 days. Emodin also inhibited influenza A virus (IAV) replication, reduced expressions of TLR2/3/4/7, MyD88 and TRAF6, decreased phosphorylations of p38/JNK MAPK and nuclear translocation of NF-κB p65 induced by IAV (ref. 60).

Interestingly, its activity against SARS-CoV is highly effective as it was found to block the interaction of CoV spike protein with ACE-2 and to inhibit the infectivity of coronavirus spike proteinpseudo-typed retrovirus in Vero E6 cells. It showed inhibition of the interaction between S protein and ACE2 by 60% at a concentration of 400 μM. The IC_{50} value for this activity was measured at 200 μM (ref. 61). Emodin inhibited the **3a** ion channel of HCoV-OC43 with a $K_{1/2}$ value of 20 μM. The virus-induced cytopathic effect was found to reduce with a concentration of 8 μM, moreover, it decreased the number and size of plaques in the RD cells after virus infection[62].

Aloe-emodin

Aloe-emodin (**20**), an anthraquinone found in many plant species, including *Aloe vera*, *Cassia angustifolia* and *Rheum emodi*, was shown to inhibit the cleavage activity of SARS-CoV 3CLpro in cell-free and cell-based assays

with IC_{50} values of 132 and 366 μM respectively. The CC_{50} value of cell death was measured at 11,592 μM (ref. 56).

Indigo

Indigo (**21**), more popularly known for its dyeing properties, is a natural compound found in *Indigofera tinctoria*, *Isatis indigotica*, etc. This compound showed inhibitory effect on cell-free and cell-based cleavage activity of SARS-CoV 3CLpro with IC_{50} values of 300 and 752 μM respectively. Indigo was found non-toxic to Vero cells with a CC_{50} value of 7375 μM (ref. 56).

Cinanserin

Cinanserin (**22**), found in *Houttuynia cordata*, is a well-known serotonin antagonists that was also found effective against murine CoV. At an IC_{50} value of 5 μM, this was found to inhibit the catalytic activity of 3CLpro of SARS-CoV and HCoV-229E. This molecule has already undergone clinical trials during 1960 and showed encouraging results; hence, it has been considered for further use[63]. As cinanserin has been already evaluated for its efficacy and safely in humans, it can be tested against SARS-CoV-2 without considering toxicity issues.

Scutellarein

Scutellarein (**23**), a flavone occurs in *Scutellaria lateriflora*, was found to inhibit SARS-CoV helicase protein by affecting the ATPase activity of nsP13 by more than 90% at a concentration of 10 μM. The IC_{50} value of scutellarein measured for this activity was 0.86 μM. The molecule was found to be non-toxic against MCF10A cells up to a concentration of 2 μM. The study also suggested that the molecule can directly interact with ATP/ADP-binding pocket of the SARS-CoV helicase protein[30].

Hesperetin

Hesperetin (**24**), a flavonoid found in many citrus fruits and other plants such as *Isatis indigotica*, inhibited the cleavage activity of SARS-CoV 3CLpro in cell-free assay with IC_{50} value of 60 μM, whereas CC_{50} value was recorded at 2718 μM (ref. 56). It also showed inhibitory effects on Sindbis virus with an IC_{50} value of 68 μM, using the plaque assay[64]. Although limited studies have been done with this interesting molecule, including some docking studies, it needs further exploration with advanced research using higher models. As the molecule has not yet been studied against SARS-CoV-2, its effect against COVID-19 can be evaluated.

β-Sitosterol

β-Sitosterol (**25**) is a well-known phytosterol found in most plant species showing diverse pharmacological activities. It blocked the cleavage process of SARS-CoV 3CLpro with an IC_{50} value of 1210 μM in Vero cells. It was found non-toxic to Vero cells with a CC_{50} value of 1475 μM (ref. 56).

Toxicity studies

An extensive survey of the literature revealed that most of the molecules discussed here are non-toxic at lower doses. Interestingly, few of them are already in use as medicines for other purposes. Many of these molecules such as glycyrrhizin, aescin and reserpine have been found safe under an optimum concentration and used clinically for several years in original or modified forms[53]. Although used frequently, glycyrrhizin in higher doses and/or for a long duration may cause severe adverse effects, including pseudoaldosteronism, which is mainly due to its metabolite, viz. 3*β*-monoglucuronyl-18*β*-glycyrrhetinic acid[48]. Its use is recommended only under medical supervision. Similarly, reserpine, a US Food and Drug Administration-approved drug often prescribed in combination with hydrochlorothiazide for hypertension, is safe and can be taken under medical supervision[65]. Emodin, at lower doses of 0.6 and 2.5 mg/ml, showed no toxicity; however, at a higher dose of 6 mg/ml, it caused reduction in the average foetal body weight per litter in mice. Emodin also caused toxic effects in rats and mice when used at a dose of 60 mg/ml or higher[66]. The toxicity of myricetin has also been evaluated using both *in vitro* and *in vivo* experiments. Most of these studies suggested that this molecule is safe for clinical research. An *in vivo* study by Yang *et al.*[67] did not reveal any toxic symptoms when a dose of 1000 mg/kg was intraperitoneally administered to mice.

Among the several toxicity studies on *β*-sitosterol[68], an important study by Paniagua-Pérez *et al.*[69] found that this molecule is neither cytotoxic nor genotoxic to mice up to LD_{50} of 1000 mg/kg. Malini and Vanithakumari[70] reported that its subcutaneous treatment to rats at 10 mg/kg for two months was safe, as no toxic signs appeared either externally nor internally, which was confirmed by microscopic studies of liver and kidney. Besides the functions of liver and kidney, all other blood parameters, including haemoglobin, glucose, bilirubin, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were also found normal. However, the study found a drastic fall in the blood protein levels at 10 mg/kg, albeit only in males, whereas a dose-dependent decrease in the blood cholesterol levels was recorded after treatment with *β*-sitosterol[70]. This phytosterol was also used clinically to treat conditions of prostate enlargement, enrolling 519

males in a study. Although it did not significantly reduce prostate size in comparison to the control group, its role in improving urinary symptoms and flow measures was encouraging[71]. A randomized clinical trial of plant sterols containing β-sitosterol found that the dose of 1.6 g per day to 26 subjects for one month significantly reduced hypercholesterolemia[72]. Similarly, hesperetin was shown clinically to inhibit the activity of acetyl-CoA acetyltransferase (ACAT)-1 and ACAT-2 genes, reduce the activity of microsomal triglyceride transfer protein and upregulate the low-density lipoproteins receptor. It also reduced cholesterol level in human subjects[73]. Hence, based on the preclinical and clinical studies, these molecules can be considered safe and evaluated against SARS-CoV using higher experimental models.

Beyond the dyeing properties, indigo showed diverse biological activities, including *in vitro* anti-SARS-CoV. This molecule has been evaluated for its toxicity in animals and found that it was safe in lower doses, but it had low oral toxicity at an LD_{50} of 5 g/kg (ref. 74). On the other hand, amentoflavone was tested for its toxicity in human umbilical vein endothelial cells and recorded an LD_{50} of 290 μM (ref. 75). Hence, based on its toxicity profile, it has been used in rat models at 40 mg/kg (orally) or 10 ml/kg (i.v.) to evaluate antitumour activity.

Thus, earlier studies have revealed that most of the above-mentioned natural molecules are safe under certain doses/concentrations. These molecules can be evaluated for their anti-SARS-CoV activity both in animals and humans, as there is no toxicity concern with any of these natural entities. On the other hand, a few molecules considered in this study have limited toxicity data. These compounds have either not been evaluated for their toxicity or only *in vitro* studies were performed to measure their toxic profile. Hence, it is necessary to evaluate the toxicity of such molecules before using them in further studies.

Opportunities and challenges

Plant natural products have long been used as a valuable source of molecules with diverse therapeutic potential. Plant-derived natural products represent an important basket for the identification of novel drug candidates. The biggest advantage of drug discovery from plant natural products is that sometimes well-recognized ethnopharmacological information about the traditional medicinal use of whole plants or their extracts is available, which can provide us with the knowledge of the type of natural product effective in therapeutics[76]. When compared to synthetic and combinatorial compounds, plant-derived natural products have unique structural characteristics, high chemical diversity, biochemical specificity and other molecular properties that make them attractive targets as lead structures for drug discovery in new disease targets. In the recent past, many reports have been published on drug discovery from natural products with key challenges and possible solutions, as they have been found important scaffolds in searching new drugs, including flavonoids, terpenoids, polyketides, phenylpropanoids and alkaloids[77–79]. Despite the great potential of plant natural products to be used as new drug candidates, there are many challenges, including their complex isolation and characterization methods, and poor yield in most of the cases[80].

Previous studies suggested that natural molecules are non-toxic in nature when consumed in lower amounts because most of them are already part of our daily diet, e.g. tea polyphenols. The main challenges in using molecules such as emodin and scutellarin as drugs are their poor solubility and bioavailability. The shorter gastrointestinal transit time and poor membrane permeability affect the absorption of a drug into the GIT. Hence, it is an important point to be considered for the sustainable release of an oral drug in GIT for its maximum utilization[81]. There are several studies associated with improvement in the bioavailability of natural molecules. In this direction, Yang *et al.*[82] prepared a nanosuspension to increase the bioavailability and dissolution rate of scutellarin to improve its drug-like properties. Hence, using advanced tools like nanotechnology can help improve the drug-like properties of molecules with poor bioavailability.

Combination therapy using more than one agent or treatment procedure, has been well-accepted globally mainly against infectious diseases caused by microorganisms[83]. It has been noticed that there are many compounds which are either inactive or poorly active against infection when used alone, but in combination with other molecules, they show stronger activity against the same infection. A common example of this is the treatment of tuberculosis with a combination of ethambutol, isoniazid, pyrazinamide and rifampin[84]. Hence, a combination of more than one natural compound is a good choice, which needs profound research. In addition, the route/delivery of a drug plays a key role in the line of treatment against a particular pathological condition. There are several drugs which show different efficacies when administered through different routes; for example, diclofenac which is used as oral, topical or injection[85]. Selected molecules having poor absorption or poor membrane permeability through oral route can be tested via the intravenous or intramuscular route. These routes are also important if a patient is not in a position to take medicines through the mouth. In some cases, other routes like nasal, ear or eye are also helpful, in which sometimes there is need of a carrier to target the drug to a particular site[86].

Apart from the above-mentioned points, another key challenge in drug development is mimicking the results of lower to higher experimental models. In many cases, molecules showing activity in docking studies are inactive

in *in vitro* or *ex vivo* models, and similarly, the active molecules of an *in vitro*/*ex vivo* model show no activity in the *in vivo* model. Unlike the *in vitro*/*ex vivo* system, this happens because of the complex physiology of animals or humans, which involves multiple mechanisms. Furthermore, the physiology of humans is different from that of animals, and hence, a molecule exhibiting activity in an animal model (*in vivo*) may be inactive in humans (clinical trials). In the present study, most of the molecules were tested for their anti-SARS-CoV activity using *in vitro* models. The activity on higher models has either not been studied or may be under progress. Therefore, by considering the above challenges, research on higher models, including clinical trials should be conducted on active molecules using advanced tools and techniques. Studies for improving the drug-like properties, including pharmacokinetics, of the most active molecules should be conducted to develop a drug against SARS-CoV.

Conclusion

The COVID-19 pandemic has pushed the world to a state never witnessed before, as thousands of people have died, and many more are under critical care. As no effective treatment is yet available for CoV, scientists from all over the world are looking for possible solutions against this pandemic. Studies confirmed that SARS-CoV-2 has similarity to the earlier known SARS-CoV in several ways, and hence, a few anti-SARS-CoV drugs have also been considered for the prophylaxis and management of COVID-19 pneumonia. Previous research revealed that these molecules, including myricetin, tetrandrine, glycyrrhizin, aescin, reserpine, emodin and scutellarein have the potential to inhibit the essential proteases, helicase and viral proteins of SARS-CoV at very low concentrations. These molecules also have the capability to inhibit penetration of the virus to the host cell. Hence, there is tremendous scope for developing new drug leads from anti-SARS-CoV against anti-SARS-CoV-2 because, besides similarity in most of the proteins, SARS-CoV-2 has almost 80% identical genome sequence to SARS-CoV.

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Received 12 May 2020; revised accepted 6 July 2020

doi: 10.18520/cs/v119/i5/757-770