



Natural Alkaloids as Potential Anti-Coronavirus Compounds

Potansiyel Anti-Koronavirus Bileşikler Olarak Doğal Alkaloidler

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ABSTRACT

Coronaviruses (CoVs) are causative agents of the last three epidemics/pandemic; severe acute respiratory syndrome CoV (SARS-CoV), Middle East respiratory syndrome-CoV (MERS-CoV) and the last one SARS-CoV-2. Although meta-analysis of treatment studies against these three CoVs found no clear benefit of any specific regimen, currently, remdesivir and favipiravir are promising potential therapies for SARS-CoV-2. On the other hand, since natural products have always played a crucial role in drug discovery and development process against various diseases, many groups in the world, are now trying to find new or repurposed natural or naturally originated drugs against viruses and CoVs. Secondary metabolites of the plants, particularly alkaloids and terpenoids have been exhibited strong antimicrobial and anticancer activities besides synthetic drugs and other natural compounds (nucleosides and nucleotides and bacterial and fungi originated ones). The first isolated secondary metabolites have been converted into important drugs since 1800's such as morphine, codeine, cocaine, and quinine have alkaloid skeleton as well as some of the recent anticancer drugs vinblastine, vincristine, taxol, etc. This review includes the last two decades of publications about natural alkaloids rather than their plant extracts which showed some promising results against CoVs. Marine organisms are also another rich source to discover new lead drugs, however they were excluded in the present review article.

Keywords: Antiviral, alkaloids, coronaviruses, SARS-CoV-2, COVID-19, natural products

ÖZ

Şiddetli akut solunum sendromu koronavirüs (SARS-CoV), Orta Doğu solunum sendromu-CoV (MERS-CoV) ve en son SARS-CoV-2 olmak üzere CoV'ler son üç epidemi/pandeminin nedeni olan ajanlardır. Fakat, henüz bu üç CoV'ye karşı spesifik bir tedavi edici ajan mevcut değildir. Doğal ürünler, çeşitli hastalıklara karşı ilaç keşfi ve geliştirilmesi sürecinde her zaman önemli bir rol oynadıkları için şu anda dünyada pek çok grup virüsler ve özellikle koronavirüslere karşı doğal veya doğal orijinli ilaç etkin maddesi bulmak için çalışmaktadır. Bitkilerin sekonder metabolitleri, özellikle alkaloidler ve terpenoidler güçlü antimikrobiyal ve antikanser aktiviteler göstermektedirler, tabii ki sentetik ve diğer doğal ilaçların yanısıra (nükleozidler, nükleotidler ve bakteriyel ve mantar orijinli bileşikler). 1800'lerden itibaren morfin, kodein, kokain ve kinin gibi ilk izole edilmiş bileşikler ve 21. yy'da vinblastin, vinkristin, taksol gibi ilaca dönüşmüş bileşiklerin hepsi alkaloid yapısına sahiptir. Bu derleme, koronavirüslere karşı umut verici bazı sonuçlar göstermiş olan alkaloidleri içeren son 20 yıldaki yayınları kapsamaktadır. Deniz organizmaları yeni aday ilaçları keşfetmek için zengin bir kaynak oluşturmasına rağmen bu derlemede hariç tutulmuşlardır.

Anahtar Sözcükler: Antiviral, alkaloidler, koronavirüsler, SARS-CoV-2, COVID-19, doğal bileşikler

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Introduction

Viral infections are known as one of the major causes of deaths worldwide, especially serious outbreaks during the last 50 years. Coronaviruses (CoVs) belonging to the Family Coronaviridae, subfamily Coronavirinae are large (genome size 26-32 kb) enveloped, positive-sense single-stranded RNA viruses which can infect both animals and humans (1-6). The sizes of pleomorphic particles of the CoVs ranged between 80-150 nm in diameter (7) and their entire replication cycle takes place in the cytoplasm. CoVs can be subdivided into four genera: alpha-, beta-, gamma-, and delta-CoVs (2, 3, 8-11), and may cause a number of diseases and even death in animals and humans, and α - and β -CoVs infect only mammals. Until now, seven CoVs have been identified as human susceptible viruses. Among them, the HCoV-229E and HCoV-NL63 are human alpha-CoVs while H-CoVOC43 and HCoV-HKU1 beta-CoVs. The two of them, HCoV-229E and HCoV-OC43 have been identified in 1960 which caused mild-serious cold symptoms. The HCoV-HKU1 was determined in a clinical sample from an adult with severe pneumonia in Hong Kong as a mice-originated β -coronavirus in 2005. In fact, the above mentioned four strains showed low pathogenicity causing mild respiratory symptoms. The novel virus, due to highly similar properties to the SARS-CoV which appeared with a fatal acute respiratory syndrome in 2003 (12), was named SARS-CoV-2 by World Health Organisation on February 11, 2020. The three CoVs SARS-CoV, MERS-CoV, and SARS-CoV-2 are β -CoVs which led to severe and potentially fatal respiratory tract infections. In addition, enteric, hepatic and neurological problems and lethal pneumonia were observed in COVID-19 patients (13).

The new virus (SARS-CoV-2) with a single-stranded positive-sense RNA genome is 74.5%-99% identical to that of SARS-CoV. SARS-CoV-2, as a recombinant virus is originated from bats and is transmitted to humans, possibly using an intermediate host, such as the pangolin. The SARS-CoV-2 spike protein directly binds with the host cell ACE-2 receptor facilitating virus entry and replication. It is known that viruses, without an independent enzyme system, can mutate easily exhibiting some changes in virulence, antigens, and resistance (13-15).

The physicians couldn't use a standardized treatment in the beginning due to the observation of the varied symptoms of each patient of COVID-19, and they have mainly provided supportive care. As observed previously in SARS and MERS patients, a cytokine storm has occurred in some COVID-19 patients, even more strongly as a result of an overreaction of the immune system which could be linked a redox imbalance and oxidative stress. Therefore, due to lack of enough experience, the physicians have tried to treat patients with some antiviral and anti-inflammatory drugs in general, and immunosuppressive agents, if necessary.

In 2014, an Food and Drug Administration (FDA)-approved compounds library identified four small molecules against MERS-CoV includes chloroquine (Figure 7), chlorpromazine (Figure 1), loperamide (Figure 2), and lopinavir (Figure 3)

inhibiting of MERS-CoV replication in the micromolecular range (15).

However, a strategy and approaches to drug discovery and therapeutic options for CoVs specifically regarding on the key genomic elements of SARS and MERS (4,16,17) were published in 2016 included antivirals ribavirin, or additionally lopinavir-ritonavir (Figure 3), and according to the severity of

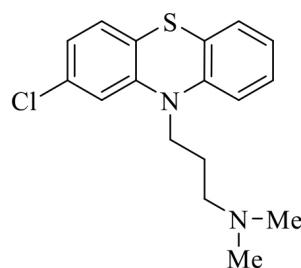


Figure 1. Chlorpromazine

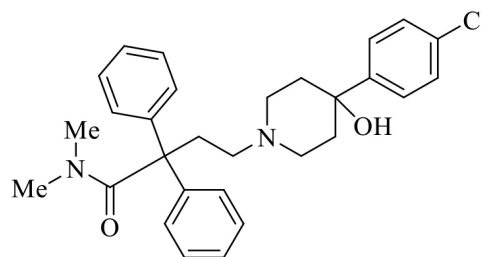


Figure 2. Loperamide

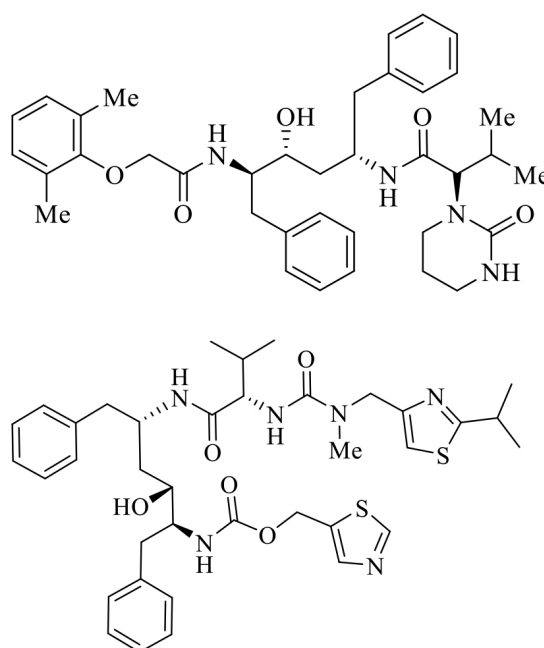


Figure 3. Lopinavir and ritonavir

the case, use of interferon combination with corticosteroids, and the convalescent plasma were used, if necessary. Any drugs or biologics have not been yet approved by the FDA for the treatment of COVID-19. However, remdesivir (Figure 4) was obtained emergency use authorization by the FDA on May 1st, 2020, based on preliminary results to the recovery of hospitalized patients with severe diseases in a shorter time. Favipiravir (Figure 5) and a few other repurposing drugs were used in China and Japan first, and they are now going to be experienced by the other countries' physicians to treat COVID-19.

The first applied drugs for the treatment of viral infections were interferons $-\alpha$, $-\beta$, $-\gamma$, and ribavirin (Figure 5) in cyclophilin inhibitors as indicated by Zumla et al. (4) who reported clinical features and treatment strategies of SARS and MERS besides the epidemiologic and virologic properties. Today, the drug discovery approach is based on mainly screening of the chemical libraries. This approach is being also used to find new anti-viral drugs by high-throughput screening (HTS) of many compounds which should be evaluated by detail antiviral assays. Various classes of drugs have been discovered using drug repurposing programs. In viral outbreaks, selection of candidate drugs should be made as either virus-based or host-based treatment options. The virus-based anti-CoV treatments consist of viral nucleosides, nucleotides and nucleic acids (4).

Today, there is an urgent need to rapidly identify and develop antiviral agents. Therefore, molecular docking and other

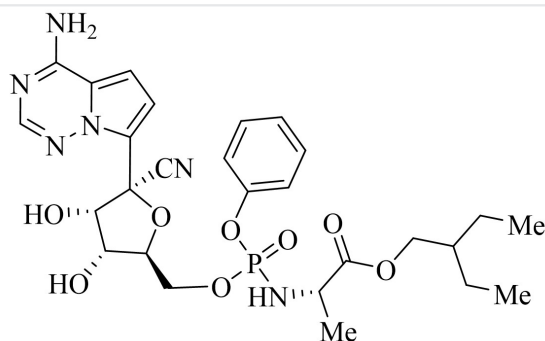


Figure 4. Remdesivir

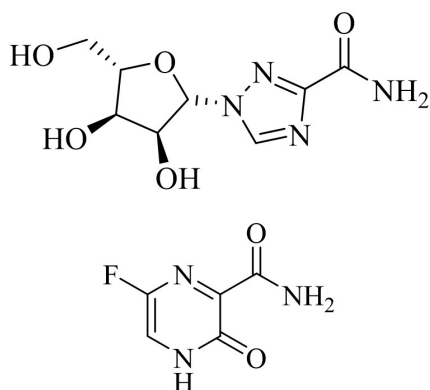


Figure 5. Ribavirin and favipiravir

computational studies may help and direct us by screening thousands of molecules within a short time to present valuable information about small molecules and natural products that inhibit important target proteins. In a very recent study, as indicated by Gyebi et al. (18) new potential antiviral drugs should be discovered and/or developed, based on the available knowledge about ligand-protein interaction of the SARS-CoV-2 and related CoVs.

The recent six months reports (Jan-July 2020) on virtual screening of antiviral drugs, a cumulative knowledge (19) has formed based on several databases and a number of studies (20, 21), covering small molecules (4,20,22,23) and natural agents (24-26). The major targets of SARS-CoV-2 are mainly viral spike proteins (27), 3-chymotrypsin-like protease (3CL^{pro}), 3 CL hydrolase (27), papain like protease (PL^{pro}), RNA-dependent RNA polymerase (28), envelop proteins (29), 2'-O-ribose methyltransferase (21, 30), and nonstructural protein-3 (Nsp3) (23) and nucleocapsid protein (31).

Some secondary metabolites and their derivatives possessing anti-inflammatory and anti-viral effects exhibited high binding affinity to 3CL^{pro} (18). Possible inhibitory role of some natural compounds including alkaloids and phenolics against the 3CL^{pro} of SARS-CoV-2 should be more rapidly investigated to find new natural lead compounds (32). There is an urgent need to search other sources and evaluate to obtain new compounds with antiviral activity. Because, no any drug exactly treats COVID-19, and some of the drugs used to cause several problems, namely viral resistance (33).

History of Alkaloids Isolated From Plants as Potential Drugs

Plants and their secondary metabolites have a pivotal role in the discovery of the novel drugs and might be a good solution in finding new antiviral drugs. Alkaloids as one the bioactive secondary metabolites are well known pharmacologically, over 10,000 alkaloids being isolated from plants or bacteria/fungi and/or marine organisms. However, the number of studies carried out on their antiviral and immunomodulatory properties. Since 19th century, many secondary metabolites have been isolated from plant extracts, most of them having alkaloid skeleton, such as morphine, quinine (Figure 6), codeine, striknin, brucine, veratrine, cocaine which were converted into important drugs to treat many disorders or diseases. Among them, quinine (Figure 6) was isolated in 1818 by two French pharmacists Pelletier and

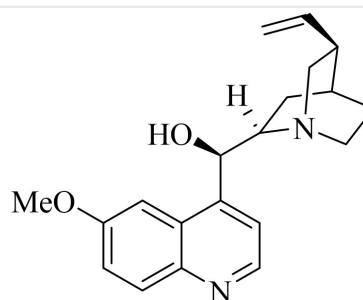


Figure 6. Quinine

Caventou and they ushered to the Scientific French Academia its isolation from *Cinchona* tree bark in 1820, and they have then presented extraction method to the drug industry without patenting to overcome malaria immediately, and then quinine (Figure 6) has been used to treat malaria until today. Its derivatives chloroquine (Figure 7) and hydroxychloroquine (Figure 8) are now used in the treatment of SARS-2 at the initial level of the disease. Therefore, alkaloids always took place as major potential compounds in the drug discovery, especially as antimicrobial, anti-inflammatory, and cytotoxic/anticancer agents. A literature survey indicates that (18,22,34-36) alkaloids may be potential antiviral agents with nitrogen-containing structures rather than other secondary metabolites. A study on the investigation of 54 medicinal plants against different viruses exhibited their antiviral potential (35).

Recent 20 Year Studies on Alkaloids as Potential Coronaviruses' Inhibitors

In the present review, we aimed to evaluate the last 20 years of studies carried out on the alkaloids as potent anti-coronaviral properties. For this purpose, first, we have searched the literature considering alkaloids isolated from the terrestrial plants which shown antiviral activity, particularly against CoVs, excluding studies on marine organisms as well as antiviral activities of the plant or animal extracts rather than their pure isolates.

A Brassicaceae family plant *Isatis indigotica* is a medicinal plant and used as a folkloric medicine. Among isolated 12 compounds from *I. indigotica* roots, three alkaloids indirubin, indican (Figure 12), indigo (Figure 10), and a glucosinolate sinigrin (Figure 9) and β -sitosterol were tested for anti-SARS-CoV 3CL^{pro} effects, of these compounds indigo, sinigrin, β -sitosterol inhibited cleavage activity of the 3CL^{pro} in cell-free and cell-based assay. Sinigrin

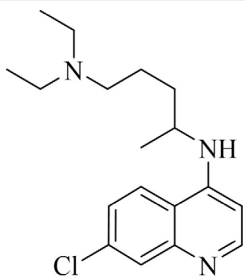


Figure 7. Chloroquine

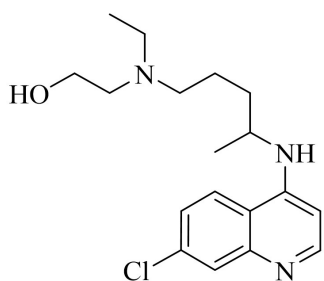


Figure 8. Hydroxychloroquine

showed a good correlation between the effects on cell-free and cell-based cleavage of the SARS-CoV 3CL^{pro}, and sinigrin and indigo were not found to be toxic to Vero Cells (34).

I. indigotica also used in the clinical treatment of several viral diseases. Isolated compounds from *I. indigotica* (isatin, indican, indigotin and indirubin) (Figures 11 and Figure 12), exhibited immunomodulatory and antiviral effects. Another study of Chang et al. (37) on *I. indigotica* extracts and the alkaloids; indigo (Figure 10) and indirubin (Figure 12) inhibited Japanese encephalitis virus replication *in vitro*.

Binding affinities of 20 alkaloids as well as some terpenoids isolated from some African plants consist of 8 indol alkaloids, 5 naphthoisoquinoline alkaloids, 5 cryptolepine alkaloids, a furoquinoline and a diterpene alkaloid were investigated against the 3-chymotrypsin-like protease (3CL^{pro}) (18). As a result of the study, the two natural alkaloids 10-hydroxyusambarensine (Figure 13) and cryptoquindoline (Figure 14), were found to be potent of inhibiting both SARS-CoV-2 and SARS-CoV. Therefore, they should be subjected to advance experimental studies against SARS-CoV-2 3CL^{pro} for the prevention and treatment of COVID-19.

In another comprehensive study, over 200 Chinese medicinal plant extracts were screened for antiviral activity against SARS-CoV, among them, the four extracts showed moderate to potent

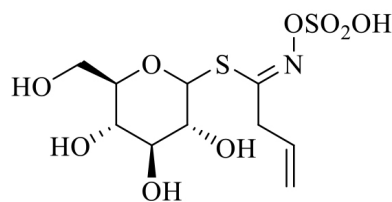


Figure 9. Sinigrin

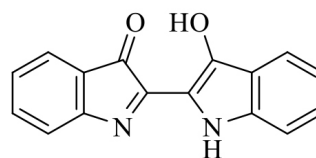


Figure 10. Indigo

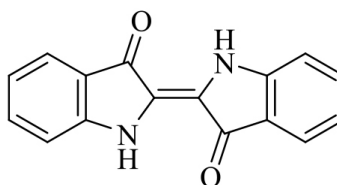


Figure 11. Indigotin

antiviral activities. From the tested four plant extracts (*Lycoris radiata*, *Artemisia annuna*, *Pyrrhosia lingua*, *Lindera aggregata*), *L. radiata* was the most potent extract, and a pure compound lycorine (Figure 15) was identified anti-SARS-CoV alkaloid with an EC_{50} value 15.7 ± 1.2 nM (38). In a recent study, lycorine was also investigated against several virus strains (HCoV-OC43, HCoV-NL63, MERS-CoV, and MHV-A59) inhibiting cell division with the IC_{50} values 0.15, 0.47, 1.63, and 0.31 μ M, respectively (22,38,39). However, the toxic effect of lycorine at low dosage (~ 1 mg/kg in dogs) should be considered (28).

Another study was performed in 2005 on a Chinese medicinal plant focusing to determine the anti-SARS-CoV activity of cepharanthine (Figure 16) *in vitro* (40). *Stephania tetrandra* and other related species of Menispermaceae family afforded bis-benzyloquinoline alkaloids including cepharanthine (Figure 16), fangchinoline (Figure 17), and tetrandrine (Figure 18). Although anticancer and anti-inflammatory activities of these alkaloids were previously studied, their antiviral activity studies are still ongoing. In 2019, a study reported on the antiviral activity of bis-benzyloquinoline alkaloids; cepharanthine, tetrandrine and fangchinoline showing

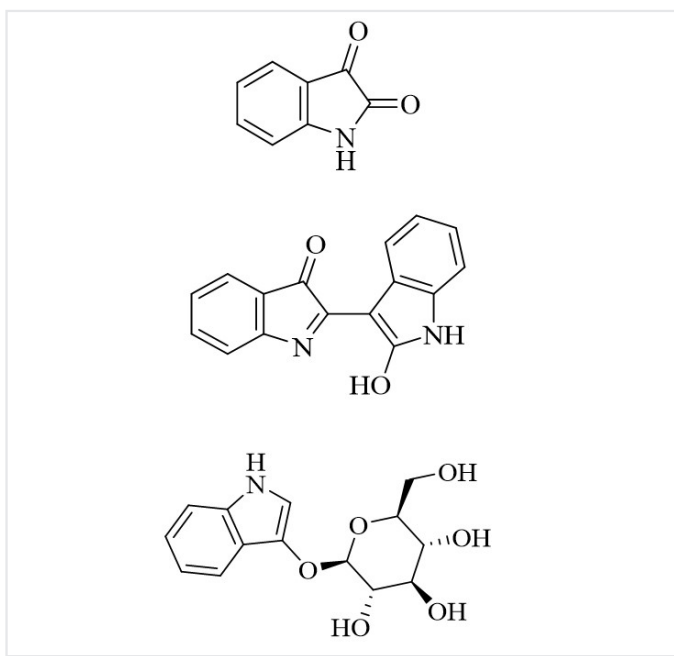


Figure 12. Isolated three alkaloids from *Isatis indigotica*

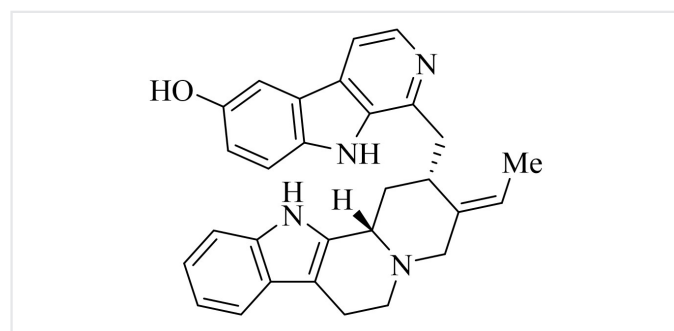


Figure 13. 10-hydroxyusambarensine

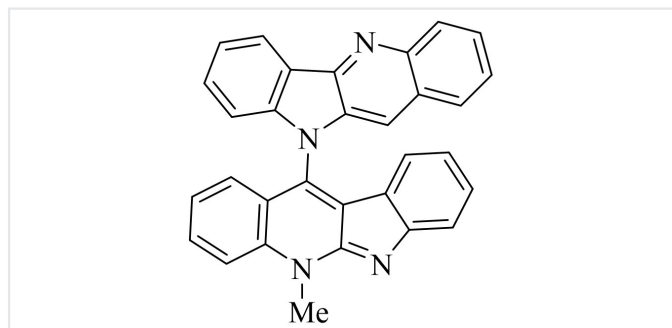


Figure 14. Cryptoquindoline

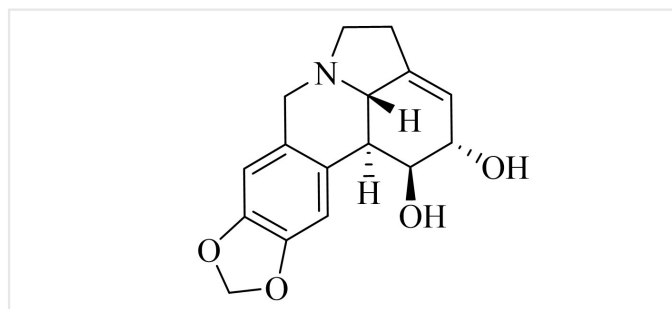


Figure 15. Lycorine

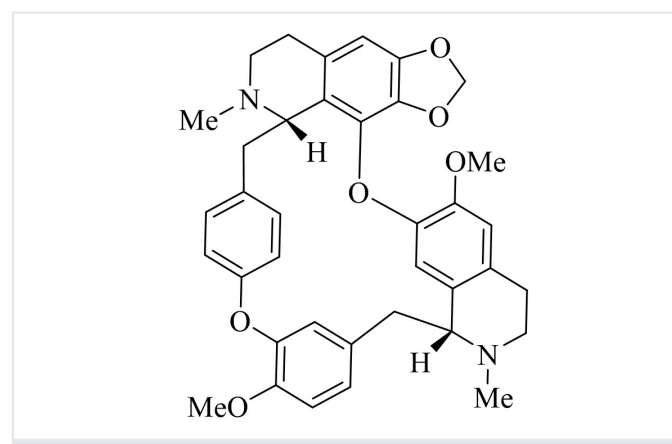


Figure 16. Cepharanthine

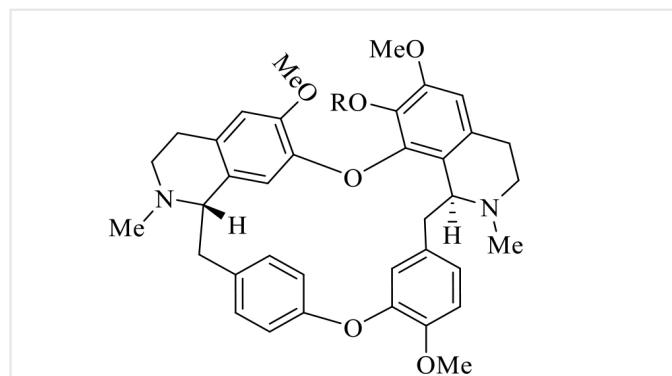


Figure 17. Fangchinoline
Figure 18. Tetrandrine

significant inhibition on virus human coronavirus HCoV-OC43-infected MRC-5 human lung cells. They subsequently suppressed its replication and inhibition of viral S and N protein expressions (41).

In a previous study, 17 alkaloids out of 49 alkaloids isolated from the methanol extract of another *Stephania* species *S. cepharantha* have shown anti herpes-simplex-virus activity (42).

Some natural and synthetic phenanthroindolizidines and phenanthroquinolizidines alkaloids were found to be potential in vitro inhibitors against enteropathogenic coronavirus transmissible gastroenteritis virus (TGEV). They also decreased cytopathic effect in Vero 76 cells infected by SARS CoV. These alkaloids, named “tylophorine compounds” (Figure 19) can be potent anti-coronavirus agents in the future to treat TGEV or SARS-CoV infections (43).

In a recent study, among 290 agents screened against the SARS and MERS CoVs, an alkaloid emetine (Figure 20) was found to be the most promising agent showing the lowest half-maximal effect. The most notable result was the observation of emetine in the human blood 300 times higher in the lungs. In fact, emetine was reported earlier as a MERS-CoV replication inhibitor and approved in 2015 by the FDA drug register list (44,45).

In a very recent study, some plant extracts and their isolates were searched against different human CoVs including the strains of SARS-CoV-2, SARS-CoV, MERS-CoV, and HCoV. Among the potent antiviral compounds, several alkaloids or alkaloid-like compounds were found to be promising agents besides some phenolics. Their action mechanisms are given

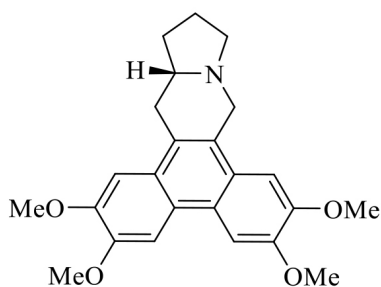


Figure 19. Tylophorine

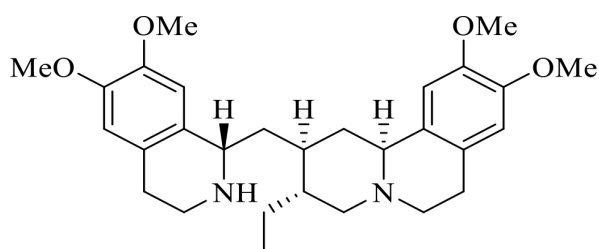


Figure 20. Emetine

below in parenthesis for each; including desmethoxyreserpine (by replication, 3CL^{pro} & entry) (Figure 21) and moupinamide (by PL^{pro}) (Figure 22) against SARS-CoV-2; sabadinine (by inhibition of CoV protease) (Figure 23), aurantiamide acetate (by inhibition of active pocket of CoV protease) (Figure 24), sinigrin (by inhibition of 3CL^{pro}) (Figure 9) and indigo (by inhibition of 3CL^{pro}) (Figure 10) against SARS-CoV 3CL^{pro} through inhibition of CoV-protease, tryptanthrin (Figure 25) and indigodole B (Figure 26) (by blocking viral RNA genome synthesis and PL^{pro} 2 activity against viral strain HCoV-NL63) (28,34).

Conclusion

In the 21st century, the COVID-19 pandemic poses a major challenge to mankind following 2002-2003 SARS and 2012

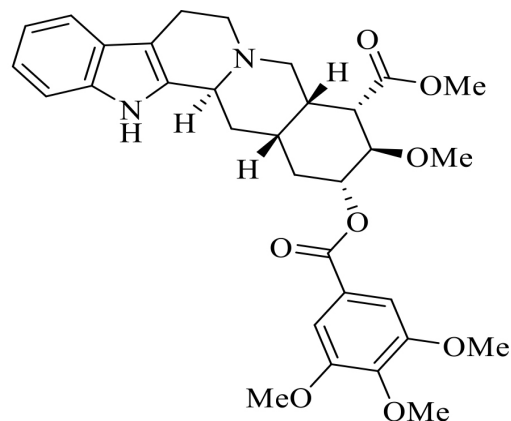


Figure 21. Desmethoxyreserpine

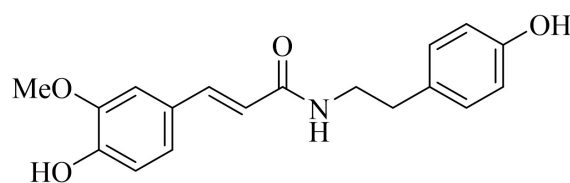


Figure 22. Moupinamide

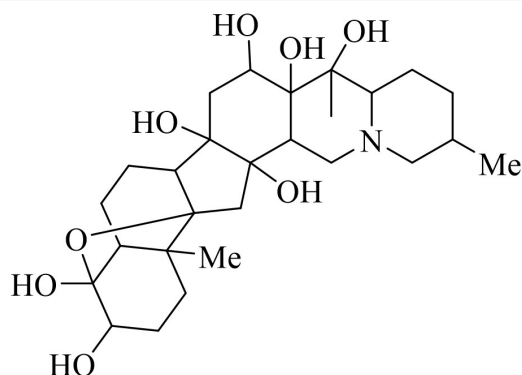


Figure 23. Sabadinine

MERS epidemics. Although a vaccine would be available in the near future, a variety of mutant forms of the CoVs would occur, particularly of the SARS-CoV-2. Therefore, we have to find new sources and new strategies to create new drugs from all possible sources. For this purpose, the nature is an unbelievable treasure with rich biodiversity of terrestrial plants and animals, as well as marine organisms which most of them have never studied yet. As a result of over the last 20 years in search of finding anti-viral drugs, some promising natural products were determined having alkaloid or terpenoid or phenolic structures. Among natural compounds tested for antiviral activity, about 100 alkaloids were found to be potent anti-viral agents, at least. This number will be increased by searching marine organisms. Microbes, especially bacteria and fungi are other resources to produce new drugs as well as nucleosides, nucleotides, and nucleic acids. Arbidol which is a small indol derivative which has been previously tried to use in the treatment of a bench of viral diseases including coronaviral ones and even now in COVID-19, but still more clinical trials are needed. Several indol alkaloids mentioned above in this review are also promising antiviral drugs. In this

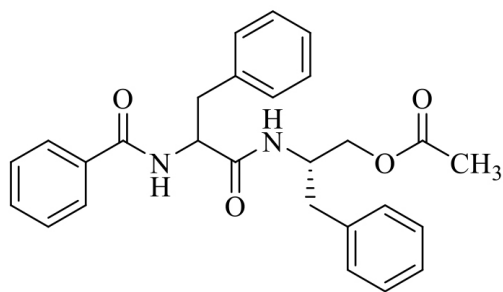


Figure 24. Aurantiamide acetate

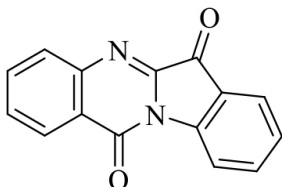


Figure 25. Tryptanthrin

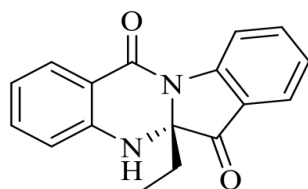


Figure 26. Indigodole B

alarming period, as emergency agents favipravir and remdesivir (Figure 4) have been used by some countries as known antiviral compounds and they are still subjected to a large number of clinical trials. Considering literature studies and presently used and developed drugs, most of the treatment mechanisms of the viral infections require the drugs which work as protein/enzyme inhibitors, in general. In the case of COVID-19, the SARS-CoV-2 uses ACE-2 as receptor, via binding of spike glycoprotein (S protein) to ACE-2, therefore soluble ACE-2 might be a most potent candidate for COVID-19 treatment. Beside many synthetic compounds, particularly natural compounds have been shown strong inhibition on main protease (3CL^{pro}) of the SARS-CoV-2, which can be lead drugs. The other protease (PL^{pro}) and some other enzyme inhibitors (such as neuraminidase inhibitors) in addition to RNA-dependent RNA polymerase inhibitors are another potential drugs. Thus, *in silico* virtual screening studies should be the first step in discovering new and/or developing repurposing drugs from both synthetic and natural sources, then *in vitro*, *in vivo* and clinical studies should be carried out immediately.

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Concept: , Design: , Data Collection or Processing: , Analysis or Interpretation: , Literature Search: , Writing:

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