A mini-review of the anti-SARS-CoV-2 potency of Amaryllidaceae alkaloids

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Abstract

Background

Nature has perennially served as an infinite reservoir of diverse chemicals with numerous applications benefiting humankind. In recent years, due to the emerging COVID-19 pandemic, there has been a surge in studies on repurposing natural products as anti-SARS-CoV-2 agents, including plant-derived substances. Among all types of natural products, alkaloids remain one of the most important groups with various known medicinal values. The current investigation focuses on Amaryllidaceae alkaloids (AAs) since AAs have drawn significant scientific attention as anti-SARS-CoV-2 agents over the past few years.

Purpose and Study design

This study serves as a mini-review, summarizing recent advances in the anti-SARS-CoV-2 potency of AAs covering two aspects: structure-activity relationship and mechanism of action (MOA).

Methods

The study covers the period from 2019 to 2023. The information in this review were retrieved from some databases including Web of Science, ScienceDirect, PubMed and Google scholar. Reported anti-SARS-CoV-2 potency, cytotoxicity and possible biological targets of AAs were summarized and classified into different skeletal subclasses. Then, the structure-activity relationship (SAR) was explored, pinpointing the key pharmacophore-related structural moieties. To study the mechanism of action of anti-SARS-CoV-2 AAs, possible biological targets were discussed.

Results

In total, fourteen research articles about anti-SARS-CoV-2 was selected. From the SAR point of view, four skeletal subclasses of AAs (lycorine-, galanthamine-, crinine- and homolycorine-types) appear to be promising for further investigation as anti-SARS-CoV-2 agents despite experimental inconsistencies in determining *in vitro* half maximal inhibitory effective concentration (EC_{50}). Narciclasine, haemanthamine- and montanine-type skeletons were cytotoxic and devoid of anti-SARS-CoV-2 activity. The lycorine-type scaffold was the most structurally diverse in this study and preliminary structure-activity relationships revealed the crucial role of ring C and substituents on rings A, C and D in its anti-SARS-CoV-2 activity. It also appears that two enantiomeric skeletons (haemanthamine- and crinine-types) displayed opposite activity/toxicity profiles regarding anti-SARS-CoV-2 activity. Pharmacophore-related moieties of the haemanthamine/crinine-type skeletons were the substituents on rings B, C and the dioxymethylene moiety. All galanthamine-type alkaloids in this study were devoid of cytotoxicity and it appears that varying substituents on rings C and D could enhance the anti-SARS-CoV-2 potency. Regarding MOAs, initial experimental results suggested Mpro and RdRp as possible viral targets. Dual functionality between anti-inflammatory activity on host cells and anti-SARS-CoV-2 activity on the SARS-CoV-2 virus of

isoquinoline alkaloids, including AAs, were suggested as the possible MOAs to alleviate severe complications in COVID-19 patients. This dual functionality was proposed to be related to the p38 MAPK signaling pathway.

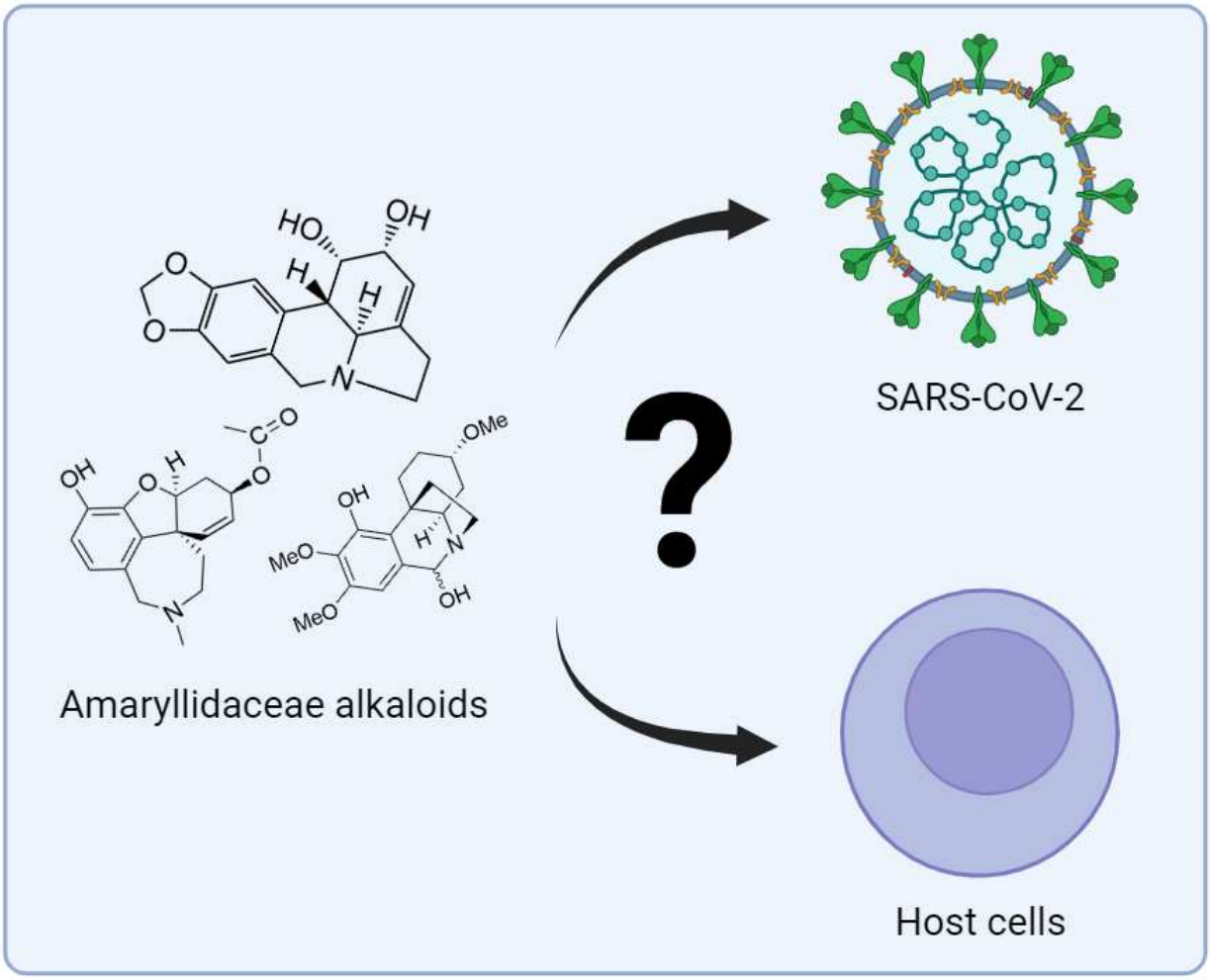
Conclusion

Overall, Amaryllidaceae alkaloids appear to be promising for further investigation as anti-SARS-CoV-2 agents. The skeletal subclasses holding the premise for further investigation are lycorine-, crinine-, galanthamine- and homolycorine-types.

Keywords: Amaryllidaceae alkaloids, COVID-19, cytotoxicity, SARS-CoV-2, structure-activity relationship

Abbreviations: Amaryllidaceae alkaloids (AAs), Absorption - distribution - metabolism - excretion - toxicity (ADMET), Coronavirus disease - 2019 (COVID-19), 50% Cytotoxic concentration (CC_{50}), 50% Effective concentration (EC_{50}), Human coronavirus OC43 (HCoV-OC43), Human coronavirus NL63 (HCoV-NL63), 50% Inhibitory concentration (IC_{50}), Molecular dynamics (MD), Middle East respiratory syndrome coronavirus (MERS), Mechanism of action (MOA), Main protease (Mpro), p38 mitogen-activated protein kinase (p38 MAPK), Papain-like protease (PLpro), RNA-dependent RNA polymerase (RdRp), Structure-activity relationship (SAR), Severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2), Cleavage activating protein (SCAP), Selectivity index (SI), Sterol regulatory element-binding protein (SREBP), Trimethylamine N-oxide (TMAO).

Graphical abstract



Introduction

Viral infections in humans cause millions of deaths around the globe and are accountable for many human diseases like HIV/AIDS, hepatitis, influenza, herpes, common cold, etc. Most recently, the COVID-19 (coronavirus disease-2019) pandemic has threatened humanity since 2019. SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus-2), responsible for COVID-19, shares 79% genetic sequence similarity with SARS-CoV, belonging to the genus Sarbecovirus (Lamers and Haagmans, 2022). Since the onset of the SARS-CoV-2 outbreak in December 2019, the investigation and implementation of medicinal plants as complementary and alternative medicines for addressing SARS-CoV-2, or as sources of new lead compounds, has remained a compelling research subject and a prevalent therapeutic approach worldwide, concurrently with the deployment of vaccines and synthetic pharmaceutical agents (Christy et al., 2021) (Raman et al., 2022). In addition, the pandemic has boosted the testing of plant extracts and libraries of natural compounds for potential activity against the virus (Kim, 2021).

Viruses have been more resistant to treatment or prevention than any other infectious agent due to their ability to mutate constantly. Compared to antibacterial antibiotics, relatively few drugs are on the market to treat viral diseases, like acyclovir (Chakravarti et al., 2021). Natural products as antiviral substances have been a subject of study for decades (El Sayed, 2000). Extensive reviews have demonstrated that several plant families could offer a rich reserve for drug discovery of antiviral natural products, such as Acanthaceae, Amaranthaceae, Amaryllidaceae, Apocynaceae, Asphodelaceae, Combretaceae, Lamiaceae, Fabaceae, Rutaceae, Piperaceae, etc. Among these, weak-to-potent antiviral activities were reported for many Amaryllidaceae alkaloids (AAs) against viruses in the Arenaviridae, Retroviridae, Piconaviridae, Togaviridae, Flaviviridae, Phenuiviridae, Paramyxoviridae, Coronaviridae, Rhabdoviridae, Filoviridae, Virgaviridae, Orthomyxoviridae, Herpesviridae and Poxviridae families (Nair and van Staden, 2022).

AAs are compounds belonging to the large group of isoquinoline alkaloids, derived from L-tyrosine and L-phenylalanine, and they are almost exclusively found in the Amaryllidaceae family (Ding et al., 2017). These alkaloids show various pharmacological activities such as anticancer, antiplasmodial, anti-inflammatory, among others (Ding et al., 2017). In the pharmaceutical domain, galanthamine is marketed worldwide for treating cognitive decline in mild to moderate Alzheimer's disease and other memory impairments (Marco and Do Carmo Carreiras, 2006). To date, more than 600 compounds of the Amaryllidaceae family have been isolated and characterized, and the number is still increasing (Berkov et al., 2020). From 2019 to 2023, numerous studies reported AAs as potential natural products for treating SARS-CoV-2 infections. Thus, this mini-review offers an update on the state-of-the-art about the anti-SARS-CoV-2 potency of AAs from 2019 to 2023. Prospects are also discussed.

Methodology

Search strategy

To systematically cover the state-of-the-art findings, the following libraries and scientific webpages were used: ScienceDirect, PubMed, Web of Science and Google Scholar. Manual research was completed at the Department of Pharmaceutical Sciences, University of Antwerp (Belgium). Systematic search was performed applying two methods; a search with "Medical Subject Headings (MeSH) terms" and a search with "free text" terms. First, we defined the MeSH terms related to species and chemical skeletons: "Amaryllidaceae", "Amaryllidaceae alkaloids", "lycorine", "homolycorine" (or "lycorenine"), "narciclasine", "norbelladine", "haemanthamine", "crinine", "galanthamine" and "montanine". The classification of AAs' skeletal subclasses followed the conventional acknowledgement in pharmacognosy (Ding et al., 2017; Berkov et al., 2020). Then, the MeSH terms related to SARS-CoV-2 were included: "SARS-CoV-2", "anti-SARS-CoV-2", "COVID-19", and "antiviral". The MeSH terms related to the Coronaviridae family and the Coronaviruses causing diseases in humans "SARS-CoV", "SARS", "MERS-CoV", "MERS", "HCoV" were also used to cover the possible broad-spectrum antiviral activities of AAs. Each MeSH term defining species and chemical groups was combined with each MeSH term designating disease, separated by "AND". For "free text" search, we used the term "SARS-CoV-2" to define the disease AND the word "Amaryllidaceae" OR "Amaryllidaceae alkaloids" to define plants.

Eligibility criteria

The coverage period of this study was from 2019 to 2023 (the onset of the COVID-19 pandemic was in December 2019). Only papers containing related content published in Q1, Q2, Q3 and Q4 journals were selected for this review. *In silico* studies were also considered valid since investigating anti-SARS-CoV-2 activity *in vitro* and *in vivo* were limited during the first two years of the pandemic (2020 – 2021). The journal ranking system of Scimago was used for this purpose (https://www.scimagojr.com/journalrank.php, last check December 2023). Reported anti-SARS-CoV-2 potency, cytotoxicity and possible biological targets of AAs were summarized and classified into different skeleton-types. Furthermore, the structure-activity relationship (SAR) was explored, pinpointing the key pharmacophore-related structural moieties. Next, to study the mechanism of action (MOA) of anti-SARS-CoV-2 AAs, reports of possible biological targets were discussed, covering both viral and host targets. The inclusion of host targets aims at providing possible explanations for the multi-functions and cytotoxicity of AAs. First, a screening of article titles was conducted, followed by the reading of eligible abstracts to assess their conformity with the eligibility criteria. Abstracts meeting the inclusion criteria were subsequently retrieved for thorough examination of the full text articles.

Results and discussion

Anti-SARS-CoV-2 potency of AAs and structure-activity relationship study

In total, fourteen research articles were collected for this mini-review, covering 9 *in vitro* studies, 2 *in vivo* and 5 *in silico*. Literature data was classified according to known skeletal subclasses of AAs to study structure-activity relationship (SAR). The assessment of SAR was established using a library of 37 AAs isolated from three Amaryllidaceae plant species (*Pancratium maritimum* L., *Scadoxus multiflorus* (Martyn) Raf., *Hymenocallis littoralis* Salisb.), since all compounds were evaluated in one run by a high-throughput system, creating an optimal setting for comparative results to be produced. Dose-dependent antiviral activities and cytotoxicity were reported by Le et al. from 2022 to 2023 (Le et al., 2022, 2023a, 2023b, 2023c). This represents the largest and the most chemically diverse library of anti-SARS-CoV-2 AAs assessed against SARS-CoV-2 till the end of 2023.

Lycorine-type skeleton

The lycorine-type represents the most ubiquitous and chemically diverse scaffold of AAs. In the course of 2019-2021, lycorine itself, a main and widespread alkaloid in the Amaryllidaceae family, was reported as a highly potent anti-SARS-CoV-2 agent by various independent studies with an EC_{50} ranging from 0.31 - 0.87 µM (Jin et al., 2021; Zhang et al., 2020). Apart from lycorine, anti-SARS-CoV-2 activity has been reported only for pseudolycorine. However, inconsistencies in bioassay results were observed, as summarized in **Table 1**. Lycorine was found to be a highly potent anti-SARS-CoV-2 agent in several studies in which tests were performed on Vero-E6 cells. However, according to results published by the authors, lycorine was not active against SARS-CoV-2, tested on the same cell line (**Fig. 1**).

Among the fourteen tested lycorine-type alkaloids (**Fig. 1**), only lycorine and pseudolycorine were cytotoxic to the Vero-E6 cell line with CC_{50} values of 1.21 and 3.40 µM, respectively. The four most potent anti-SARS-CoV-2 analogs from our work were 2-epi-lycorine, zephyranthine, 2-*O*-methyl-11-hydroxypseudolycorine, 1-*O*-acetyl-10-*O*-methyl-pseudolycorine with EC_{50} of approximately 50 µM and with selectivity indices (SI) of at least 2.

With regard to the SAR, in pseudolycorine, the dioxolane ring present in lycorine is opened. However, this structural difference was not observed to affect the antiviral activity or cytotoxicity. Next, opposite to lycorine, 2-epi-lycorine exhibited a slightly selective antiviral effect, implying that the configuration of position C-2 greatly affected the activity and cytotoxicity. A similar observation could be made for the pair of pseudolycorine and 2-epi-pseudolycorine, where the sole change of stereochemistry of C-2 resulted in the absence of cytotoxicity. Interestingly, the methylation of the 2-hydroxy group also led to the absence of cytotoxicity, as evidenced by comparing 2-*O*-methylpseudolycorine to pseudolycorine. Therefore, it

appears that 2-OH as a hydrogen donor may be responsible for the cytotoxicity, and the change of the configuration at C-2 might remove the interaction causing the cytotoxic effect. Further activity enhancement was observed when C-11 was hydroxylated, as seen in the case of 2-*O*-methyl-11-hydroxypseudolycorine (EC_{50} = 53 µM) compared to 2-*O*-methylpseudolycorine (EC_{50} = 82 µM). It appears that acetylation of 1-OH also resulted in the absence of cytotoxic effects, as observed for 1-*O*-acetyl-norpluviine and 1-*O*-acetyl-10-*O*-methyl-pseudolycorine.

Double bond rearrangement between C-3 and C-4 on ring C to between C-4 and C-11 on ring D appears to not influence, since ungiminorine, ungiminorine *N*-oxide and narcissidine were devoid of activity and cytotoxicity. This could be explained by the rigid fused ring system of rings B, C and D: when the conformation of ring D changes by the double bond, the conformation of ring C is affected accordingly. Furthermore, ungiminorine and its N-oxide derivative displayed insignificant activity, indicating that the oxidation of the nitrogen may not affect the activity. The flexibility and configuration of ring C are required for the antiviral activity, as evidenced by ungeremine – when ring C is fully aromatized, the antiviral activity was lost.

Overall, it appears that ring C and its substituents play a pivotal role in both the antiviral activity and cytotoxicity, and the configuration of ring C is important for the activity. In addition, substituents on rings A and D may also be involved. However, it is noteworthy that the observations as discussed above are mostly limited to the following minor structural modifications: (1) – chirality change of C-2, (2) – substitution by two small functional groups (methyl, acetyl), and (3) – rearrangement of the double bond. There are many other possibilities for further chemical modifications via semi-/total synthesis: What would be the effect of a chemically diverse library of substituents? What would be the effect of the same substituent at different positions from R_1 to R_5? What would be the effect of a change in the stereochemistry of C-1? What would be the effect of opening ring C or D? Most recently, a semi-synthesis study demonstrated the reduction of cytotoxicity, while maintaining the anti-coronavirus activity both *in vitro* and *in vivo,* by varying the substituents on C1 and C2 of lycorine (Shen et al., 2023).

Galanthamine-type skeleton

With regard to the galanthamine-type, as can be seen in **Fig. 2**, the anti-SARS-CoV-2 potency of 3-*O*-acetylsanguinine (EC_{50} = 49 µM) was higher than the activity of sanguinine (EC_{50} = 92 µM), indicating that substitution of the 3-OH group by an acetyl group increases the activity. The structure of 9-*O*-demethyl-11-hydroxygalanthamine differs from sanguinine at two positions: the saturation of the double bond between C-1 and C-2, and the addition of the 11-OH group. This compound also had a stronger activity compared to sanguinine and comparable activity to 3-*O*-acetylsanguinine. Therefore, ring C appears to be involved in the pharmacophore of galanthamine-type alkaloids, and the effects of various substituents are

significant. The saturation of the double bond seems responsible for the increased activity of *O*-demethyl-norlycoramine (EC_{50} = 45 µM) compared to galanthamine and sanguinine. Therefore, it is hypothesized that the increased flexibility of ring C favors the formation of interactions with its biological target at the pharmacophore.

Similar to the lycorine-type, the influence of different types of substitution is of interest. In particular, the role of substitution on C-9 and on nitrogen remains to be determined. Noticeably, the galanthamine-type scaffold appears devoid of cytotoxicity at the tested concentrations and seems promising for further exploration. Should the configuration of C-10b be fixed, as depicted in **Fig. 2** to maintain this selective antiviral activity? This could be further verified by testing (semi-)synthetic derivatives.

Haemanthamine- and crinine-type skeletons

The haemanthamine- and crinine-type differ from each other in the orientation of the ethylene bridge: α-orientation in case of haemanthamine and β-orientation of crinine. In general, the haemanthamine-type skeleton appears to be cytotoxic. Structurally, when C-11 is substituted with a hydroxy group, cytotoxic effects were observed for haemanthamine, 6α- and 6β-haemanthidine and 11-hydroxyvittatine with CC_{50} < 10 µM (**Fig. 3**). This observation is supported by the fact that vittatine, in which the 11-hydroxy is absent, was devoid of cytotoxicity. The epimeric mixture of 6α- and 6β-hydroxyhippeastidine was the most active against SARS-CoV-2 (EC_{50} = 45 µM) and is also the sole crinine-type AAs. Would the crinine-type skeleton be an interesting scaffold for further research on this topic? Also, the effect of various substitutions and the opening of the dioxymethylene moiety remains unknown.

Other skeletal subclasses

Although only three homolycorine-type AAs have been tested against the SARS-CoV-2 virus, this scaffold appears promising for further investigations. 9-*O*-demethylhomolycorine exhibited weak activity with an EC_{50} value of 44 µM. The cytotoxic effect observed for 2β,10bα-dihydroxy-9-*O*-demethylhomolycorine might be related to the hydroxylation of C-2 and/or C-10b (**Fig. 4**).

All three compounds possessing the narciclasine-type skeleton were cytotoxic with CC_{50} < 1 µM (**Fig. 5**), indicating that this scaffold is not interesting for further research on anti-SARS-CoV-2 activity.

The two analogs of norbelladine did not display antiviral activity nor cytotoxicity, while montanine was cytotoxic to the Vero-E6 cell line (**Fig. 6**).

Possible biological targets and mechanism of actions

Viral targets

Lycorine remains the most studied AA in the literature. Prior to the COVID-19 outbreak, lycorine was reported to inhibit SARS-CoV by Li et al., 2005 and Shen et al., 2019. Shen et al. (2019) reported lycorine as a potent *in vitro* inhibitor of four CoV strains (HCoV-OC43, HCoV-NL63, MERS-CoV, MHV-A59) with EC_{50} values < 5 µM. Among these, antiviral efficacy against the lethal HCoV-OC43 was verified *in vivo*. After the outbreak, in mid-2020, lycorine was first reported as anti-SARS-CoV-2 agent by (Zhang et al., 2020) with EC_{50} = 0.31 µM and CC_{50} > 40 µM (**Table 1**), together with three other broad-spectrum alkaloids. Later on Jin et al. (2021) reported *in vitro* antiviral inhibition of lycorine against MERS-CoV, SARS-CoV, and SARS-CoV-2 and lycorine was proposed as a potent non-nucleoside RNA-dependent RNA polymerase (RdRp) inhibitor. One year later, by applying a multi-targeting drug design strategy, Ren et al. (2022) proposed that the antiviral mechanism of lycorine may stem from interaction with the host ribosome and inhibiting SARS-CoV-2 RdRp at the same time. The authors then proposed that its interaction with the human ribosomes caused the toxic effects of lycorine. According to Ren et al. (2022), lycorine exhibited potent antiviral activity in Vero-E6 cell lines, but showed toxic effects in Huh-7 cells and HEK293T cells.

A marked progress of *in vitro* testing was reported by Narayanan et al. (2022) where the HCl salt of lycorine (lycorine.HCl) displayed potent inhibition of SARS-CoV-2 replication in Huh-7.5 cells (EC_{50} = 0.01 µM, CC_{50} = 19 µM, SI = 1878) by specifically blocking Mpro activity (**Table 1**). The Mpro inhibition activity of lycorine.HCl was more potent than that of nelfinavir mesylate (EC_{50} = 0.07 µM, CC_{50} = 25.95 µM, SI = 370) and MG-101 (EC_{50} = 0.038 µM, CC_{50} = 36.95 µM, SI = 972). This result aligns with an *in silico* prediction effort of Kurt et al. (2022) which also pointed out lycorine as a Main protease (Mpro) inhibitor (**Table 1**).

In 2022, Musila et al. performed an *in silico* study, combining molecular docking, ADMET (absorption, distribution, metabolism, excretion, toxicity) screening and molecular dynamics simulation for 150 AAs obtained from online databases. This study identified four galanthamine-type AAs as drug-like and lead-like Mpro inhibitors: *O*-demethyllycoramine, 3,11-dimethoxy-lycoramine, epilycoramine and narwedine. No experimental verification was performed. (**Table 1**).

Dual functionality of anti-inflammatory and anti-SARS-CoV-2 agents

In recent years, inflammation has been considered a major cause for increased severe complications in COVID-19 patients (Grimes and Grimes, 2020). Dual functionality (anti-inflammatory and antiviral activities) was suggested as the possible mechanism of action for naturally occurring anti-SARS-CoV-2 isoquinoline alkaloids, including AAs (Valipour et al., 2023). Several studies suggested the combined effect

to be mainly related to a host-based anti-SARS-CoV-2 target and the p38 mitogen-activated protein kinase (MAPK) signaling pathway (Grimes and Grimes, 2020; Valipour et al., 2023). As SARS-CoV-2 binds to ACE receptors to enter the host cell, this eventually affects the normal activity of the renin angiotensin system. This disruption subsequently induces the activation of the p38 MAPK pathway, leading to the occurrence of inflammatory complications (Jackson et al., 2022). Regarding the dual functionality, lycorine was proven experimentally as a 'cleavage activating protein – sterol regulatory element-binding protein'(SCAP-SREBP) inhibitor, where lycorine induced SCAP degradation in lysosomes independent of the ubiquitin-proteasome pathway (Li et al., 2021). The recent study of Liu et al. (2024) shed some light on the mechanism underlying this dual effect, since they discovered that SARS-CoV-2 nucleocapsid protein promoted trimethylamine N-oxide (TMAO)-induced lipogenesis and NLRP3 inflammasome activation by SCAP-SREBP signaling pathway (Liu et al. 2024). More research on this topic and on other AAs are thus of great interest.

Cytotoxicity

In this study, cytotoxicity on commonly used cell lines (Vero-E6, HEK293T, Huh-7, Huh-7.5) of eleven compounds was reported: (Figures 1 – 6, Table 1). Structurally, cytotoxicity was observed for several lycorine-type, homolycorine-type, haemanthamine-type, narciclasine-type and montanine-type compounds. In literature, comprehensive reviews exploring possible mechanisms of actions for the cytotoxicity of AAs on these host cells were recently published (Nair et al., 2015; Nair et van Staden, 2022; Jayawardena et al., 2023), and therefore, this is not covered in the current mini-review.

Conclusions and perspectives

COVID-19 has been an emerging contagious disease in humans since 2019. During the peak of the pandemic (2020 and 2021), the use of traditional medicine as complementary medicines has played an important role in many countries worldwide. This mini-review focuses on the anti-SARS-CoV-2 potency of Amaryllidaceae alkaloids (AAs), alongside their cytotoxicity, since AAs have been long investigated as a rich source of broad-spectrum antiviral compounds.

Generally, the obtained results proved the potential of AAs as anti-SARS-CoV-2 agents despite experimental inconsistencies in determining *in vitro* EC_{50} and CC_{50} values. This mini-review first provides recent insights into the structure-activity relationship of anti-SARS-CoV-2 AAs, classified by their main skeletal subclasses. This revealed that lycorine-, galanthamine-, crinine- and homolycorine-type skeletons appear to be promising for further investigation of anti-SARS-CoV-2 activity. On the other hand, the haemanthamine-, narciclasine-, montanine- and norbelladine-type AAs in this study did not display anti-

SARS-CoV-2 potency. Although this study consists of 37 natural AAs, lycorine-type compounds account for approximately half of the studied AAs, and thus, the numbers of compounds possessing other skeletons are limited. Therefore, as a perspective, in order to obtain a broader view of the anti-SARS-CoV-2 potential of Amaryllidaceae alkaloids, research on a larger and more chemically diverse library of compounds is required.

Within the scope of this study, the lycorine-type scaffold stood out as the most structurally diverse chemical class, and an initial analysis of its structure-activity relationship underlined the crucial role of ring C and substituents on rings A, C and D in its anti-SARS-CoV-2 activity. Interestingly, it was noticeable that the two enantiomeric skeletons (haemanthamine and crinine) generally displayed opposite activity/toxicity profiles. Pharmacophore-related moieties of the haemanthamine/crinine-type skeletons were the substituents on rings B, C and the dioxymethylene moiety. Regarding the galanthamine-type skeleton, all six analogs were not cytotoxic on Vero-E6 cells, while varying substituents on rings C and D affected the observed anti-SARS-CoV-2 activity.

Up to now, possible viral targets of AAs were Mpro and RdRp with several *in vitro* and *in vivo* evidences. Most recently, the dual inflammatory/ antiviral functionality has been suggested as a possible mechanism of action of isoquinoline alkaloids, including AAs, in alleviating severe complications in COVID-19 patients through the p38 MAPK signaling pathway. Experimentally and historically, lycorine has always been the first AA to be studied, and thus, studying the other AAs remains an intriguing research topic.

Cytotoxicity of many AAs was also highlighted in this study, since this is the main challenge to overcome in order to advance the development of natural and/or synthetic AAs as therapeutic anti-SARS-CoV-2 agents. It can be observed in this study that lycorine, pseudolycorine and many compounds possessing haemanthamine, narciclasine and montanine-type skeletons were highly cytotoxic. Therefore, monitoring the activity/toxicity profile of AAs is important for research on antiviral AAs. Recently published promising results of semi-synthetic AAs were also summarized, where a lycorine analog displayed more potent broad-spectrum anti-coronavirus activity, while it was found to be less cytotoxic.

Finally, three key aspects remain undiscovered. Firstly, since SARS-CoV-2 causes a respiratory infection, physiologically more relevant cell lines (lungs cells) would be preferred to explore the anti-SARS-CoV-2 potency of AAs. All assays reported in the present review use general cell types (Vero, Huh, HEK). Thus, this may require the development and validation of new *in vitro* models. The commonly used lung cancer cell lines (Calu-3 and A549) may be of interest to study the anti-SARS-CoV-2 activity. Secondly, testing different *in vivo* models can provide more insights regarding *in vivo* efficacy. Lastly, confirming AA-protein interactions by molecular biology and structural biology (crystallography, and/or electronic microscopy techniques) is required to fully elucidate the mechanisms of actions.

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CRediT authorship contribution statement

N. T. H. L.: conceptualization, discussion, data compilation and analysis, and manuscript writing; J.K., K.J.: discussion and manuscript writing. L. P., E. T.: supervision, discussion and manuscript writing.

Declaration of competing interest

The authors declare no conflicts of interest.

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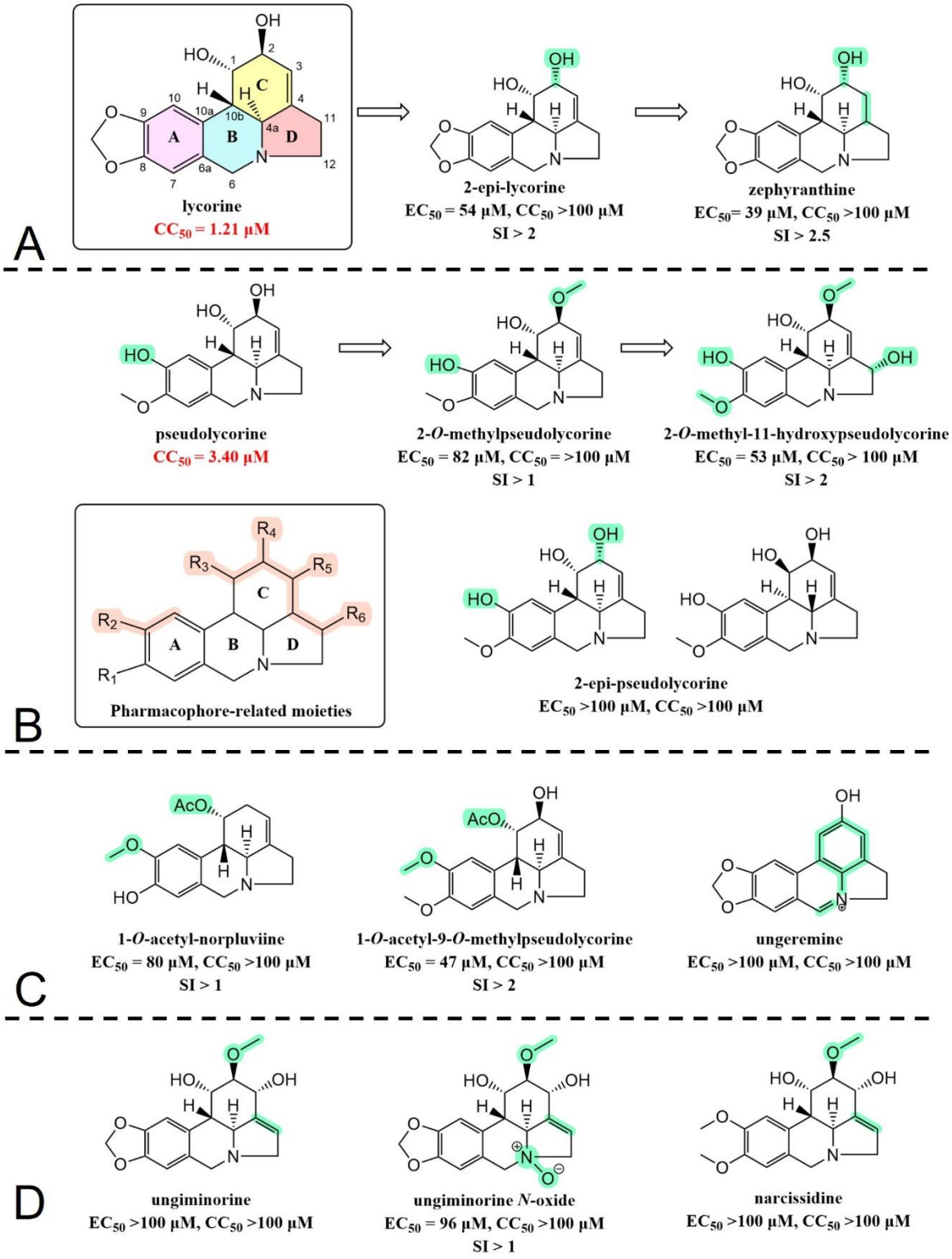

Fig. 1. Anti-SARS-CoV-2 activity and cytotoxicity of lycorine-type analogs. (A) – lycorine analogs. (B) – pseudolycorine analogs. (C) – 1-OH modified analogs. (D) – Analogs with rearrangement of the double bond between C-3 and C-11.

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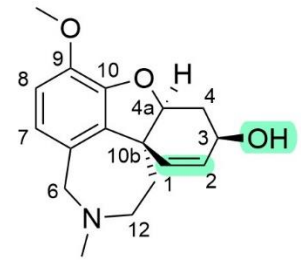
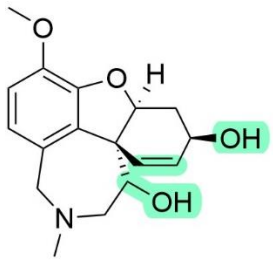
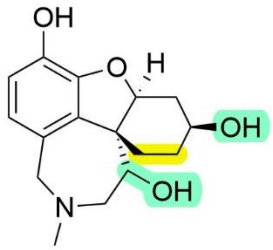


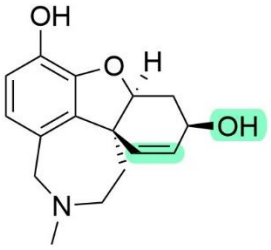
galanthamine
EC_{50} = 70 μM, CC_{50} >100 μM



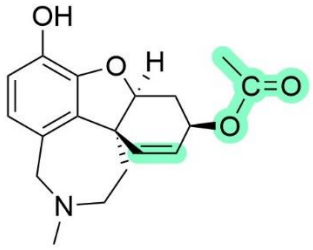
habranthine
EC_{50} >100 μM, CC_{50} >100 μM



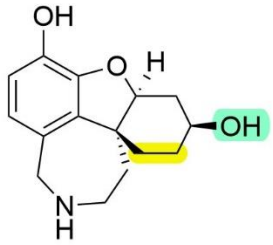
9-*O*-demethyl-11-hydroxygalanthamine
EC_{50} = 62 μM, CC_{50} >100 μM



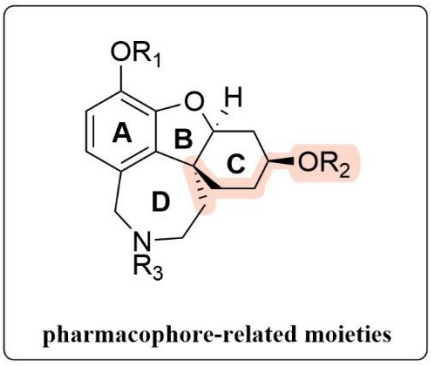
sanguinine
EC_{50} = 92 μM, CC_{50} >100 μM



3-*O*-acetylsanguinine
EC_{50} = 49 μM, CC_{50} >100 μM



***O*-demethyl-norlycoramine**
EC_{50} = 45 μM, CC_{50} >100 μM



pharmacophore-related moieties

469

470 **Fig. 2.** Anti-SARS-CoV-2 activity and cytotoxicity of galanthamine-type analogs.

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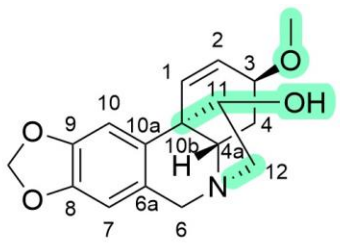
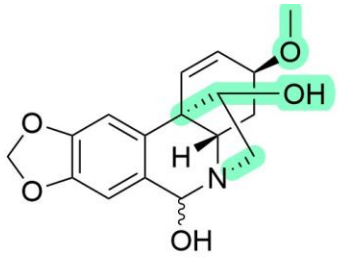
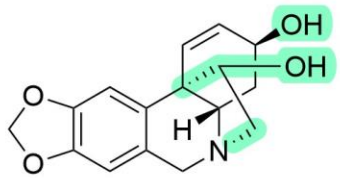


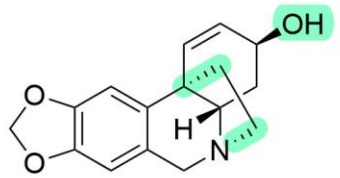
haemanthamine
EC_{50} >100 μM, CC_{50} = 0.76 μM



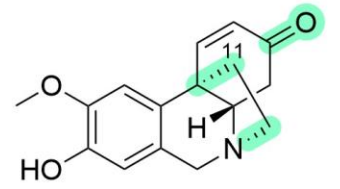
6*α*- and 6β-haemanthidine
EC_{50} >100 μM, CC_{50} = 5.43 μM



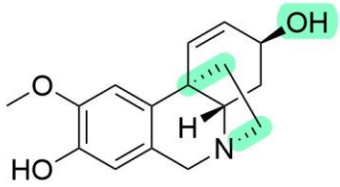
11-hydroxyvittatine
EC_{50} >100 μM, CC_{50} = 10.57 μM



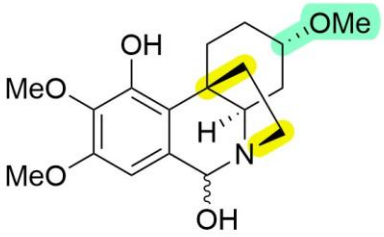
vittatine
EC_{50} >100 μM, CC_{50} >100 μM



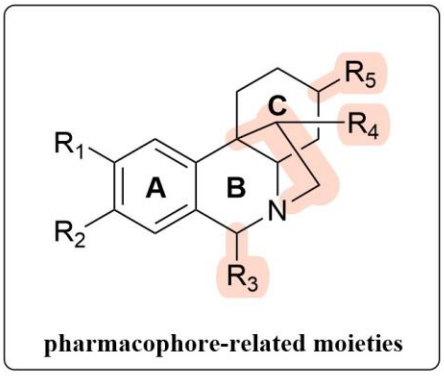
8-*O*-demethyloxomaritidine
EC_{50} = 70 μM, CC_{50} >100 μM



8-*O*-demethylmaritidine
EC_{50} >100 μM, CC_{50} = 8.80 μM

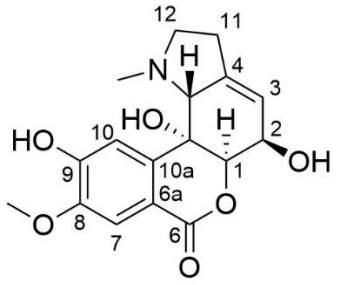


6*α*- and 6β-hydroxyhippeastidine
EC_{50} = 45 μM, CC_{50} >100 μM

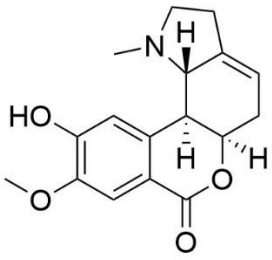


pharmacophore-related moieties

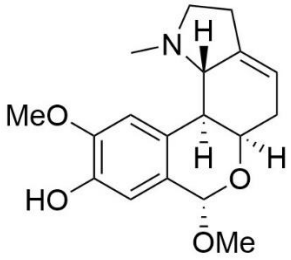
Fig. 3. Anti-SARS-CoV-2 activity and cytotoxicity of haemanthamine- and crinine-type analogs.



2β,10bα-dihydroxy-9-*O*-demethylhomolycorine
EC_{50} >100 μM, CC_{50} = 13.28 μM



9-*O*-demethylhomolycorine
EC_{50} = 44 μM, CC_{50} >100 μM



8-*O*-demethyl-6-*O*-methyllycorenine
EC_{50} = 77 μM, CC_{50} >100 μM

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479 **Fig. 4.** Anti-SARS-CoV-2 activity and cytotoxicity of homolycorine-type analogs.

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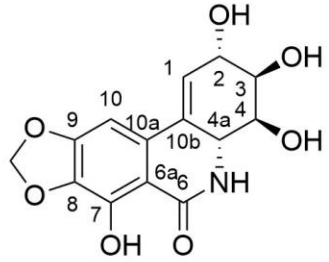
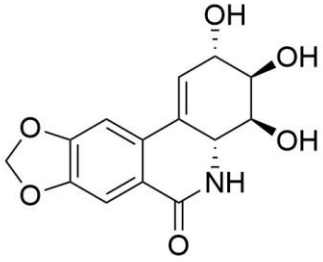
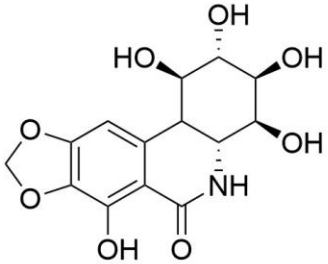


narciclasine
EC_{50} >100 μM, CC_{50} < 0.05 μM



lycoricidine
EC_{50} >100 μM, CC_{50} = 0.33 μM



pancratistatin
EC_{50} >100 μM, CC_{50} = 0.13 μM

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484 **Fig. 5.** Anti-SARS-CoV-2 activity and cytotoxicity of narciclasine-type analogs.

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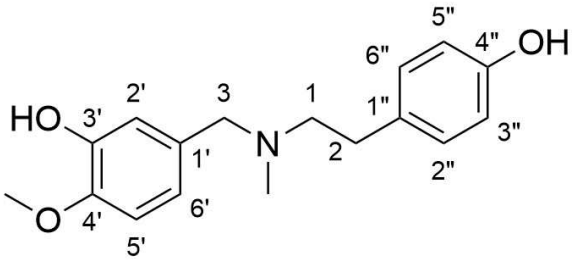
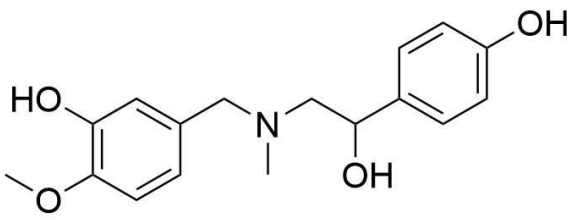
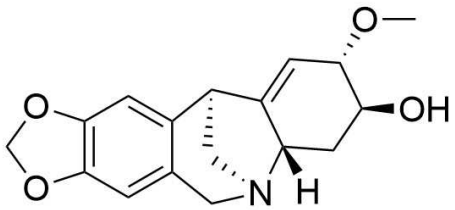


4'-*O*-methylnorbelladine
EC_{50} >100 µM, CC_{50} >100 µM



2-hydroxy-*O,N*-dimethylnorbelladine
EC_{50} >100 µM, CC_{50} >100 µM



montanine
EC_{50} >100 µM, CC_{50} = 0.55 µM

Fig. 6. Anti-SARS-CoV-2 activity and cytotoxicity of norbelladine-type analogs and montanine.

Table 1. *In silico* and *in vitro* screening results of lycorine, pseudolycorine and four galanthamine-type AAs (*O*-demethyllycoramine, 3,11-dimethoxy-lycoramine, epilycoramine and narwedine) against SARS-CoV-2.

Compound	**Targets**	**EC_{50} (µM)**	**Cell line**	**CC_{50} (µM)**	**Cell line**	***In silico* study**	**References**
Lycorine	-	0.31	Vero-E6	> 40	Vero-E6	-	Zhang et al. (2020)
Lycorine	RdRp	0.878 ± 0.022	Vero-E6	> 50 µM	Vero-E6	yes	Jin et al. (2021)
Lycorine	Host ribosomes RdRp	0.439 ± 0.122	Vero-E6	> 1000 µM 0.834 ± 0.06 1.044 ± 0.07	Vero-E6 Huh-7 HEK293T	yes	Ren et al. (2022)
Lycorine	Mpro	-	-	-	-	yes	Kurt et al. (2022)
Lycorine	-	> 100	Vero-E6	1.21	Vero-E6	-	Le et al. (2023b)
Lycorine.HCl	Mpro	0.01	Huh-7.5	19	Huh-7.5	yes	Narayanan et al. (2022)
Lycorine	-	0.30	Vero cells	3.14	Vero cells	-	Shen et al. (2023)
Ly-8	-	0.39	Vero cells	6.2	Vero cells	-	Shen et al. (2023)
Pseudolycorine	-	1.1	Vero-E6	5.2	Vero-E6	-	Ripoche et al. (2023)
Narwedine Epilycoramine *O*-demethyllycoramine 3,11-dimethyl-lycoramine	Mpro	-	-	-	-	yes	Musila et al. (2022)

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