CELL CYCLE
2021, VOL. 20, NO. 22, 2321–2336
https://doi.org/10.1080/15384101.2021.1982509

Taylor & Francis
Taylor & Francis Group

REVIEW

Therapeutic potential of chelerythrine as a multi-purpose adjuvant for the treatment of COVID-19

Mehdi Valipour [a], Afshin Zarghi[b], Mohammad Ali Ebrahimzadeh[a], and Hamid Irannejad [a]

[a]Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran; [b]Department of Pharmaceutical Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Multifunctional nature of phytochemicals and their chemical diversity has attracted attention to develop leads originated from nature to fight COVID-19. Pharmacological activities of chelerythrine and its congeners have been studied and reported in the literature. This compound simultaneously has two key therapeutic effects for the treatment of COVID-19, antiviral and anti-inflammatory activities. Chelerythrine can prevent hyper-inflammatory immune response through regulating critical signaling pathways involved in SARS-CoV-2 infection, such as alteration in Nrf2, NF-κB, and p38 MAPK activities. In addition, chelerythrine has a strong protein kinase C-α/-β inhibitory activity suitable for cerebral vasospasm prevention and eryptosis reduction, as well as beneficial effects in suppressing pulmonary inflammation and fibrosis. In terms of antiviral activity, chelerythrine can fight with SARS-CoV-2 through various mechanisms, such as direct-acting mechanism, viral RNA-intercalation, and regulation of host-based antiviral targets. Although chelerythrine is toxic in vitro, the in vivo toxicity is significantly reduced due to its structural conversion to alkanolamine. Its multifunctional action makes chelerythrine a prominent compound for the treatment of COVID-19. Considering precautions related to the toxicity at higher doses, it is expected that this compound is useful in combination with proper antivirals to reduce the severity of COVID-19 symptoms.

ARTICLE HISTORY
Received 7 May 2021
Revised 2 June 2021
Accepted 14 September 2021

KEYWORDS
Chelerythrine; signaling pathways; SARS-CoV-2; anti-inflammatory; antiviral; protein kinase C

1. Introduction

New emerged β-coronavirus SARS-CoV-2 has caused global unprecedented problems. According to the WHO statistics, this virus has infected more than 170,000,000 cases so far (from December 31, 2019, to June 1, 2021), and has resulted in more than 3,500,000 deaths (https://covid19.who.int). However, given the fact that many infected people are asymptomatic, the actual number seems to be much higher. To date, no definite treatment has been identified for the COVID-19 patients infected with the SARS-CoV-2. Clinical management of COVID-19 patients is generally directed toward experimental therapies using FDA-approved antiviral drugs and symptomatic therapy [1]. Given the devastating effects of the SARS-CoV-2 on the human life, it seems necessary to discover effective drugs. Natural products and phytochemicals have been always of a great importance in the treatment of various human diseases. Natural compounds are among the rich sources of biologically active compounds and can be used to find new agents for the treatment of COVID-19. Chelerythrine is a quaternary ammonium alkaloid with 2,3,7,8-tetrasubstituted benzophenanthridine structure (Figure 1) isolated from the roots of some medicinal plant families such as Fumariaceae, Papaveraceae, and Rutaceae [1–2]. This compound has a wide range of therapeutic effects used in traditional Chinese medicine (TCM). Chelerythrine has broad-spectrum antiviral and anti-inflammatory activities that could potentially be used in the treatment of COVID-19 patients. Although the therapeutic effects of chelerythrine have been studied well, many of its analogs have not yet been evaluated. In this article, we attempt to review the therapeutic potential of chelerythrine for the treatment of COVID-19. Briefly, Table 1 summarizes all the biological effects, pharmacokinetics and structural features of chelerythrine discussed later in the text.

CONTACT Hamid Irannejad irannejadhamid@gmail.com Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Payambar Azam University Campus, 18th km Khazarabad St., Sari, Iran; Mehdi Valipour vp.mehdi4@gmail.com Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Payambar Azam University Campus, 18th km Khazarabad St., Sari, Iran

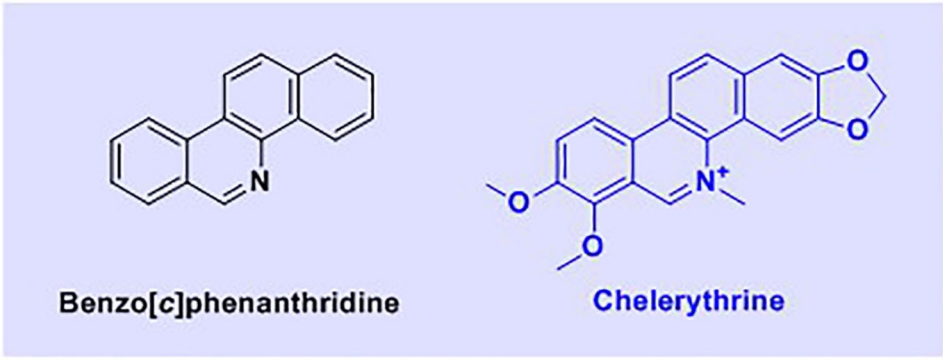

Figure 1. Chemical structure of chelerythrine and its backbone benzo[c]phenanthridine.

2. Chelerythrine has protein kinase C inhibitory activity

Many FDA-approved protein kinase inhibitors have valuable antiviral potencies against different types of viruses through the inhibition of viral entry, metabolism, or reproduction of viral particles [3–6]. Protein kinase C regulates a wide range of cellular processes involved in different stages of virus replication, and is considered as a potential host-based target for inhibiting the replication of viruses. Hence, protein kinase C has been introduced as a valuable target for treating viral diseases [7,8]. In this case, regulation of the protein kinase C pathway by small molecules has been considered as a viable mechanism toward HIV-1 reservoir eradication [9]. Also, since the Influenza virus exploits host protein kinase C to regulate its replication machinery, modulation of this target was suggested as a sensible strategy for treating the Flu [8]. Therapeutically, kinase inhibitors could be used as dual function agents through direct reduction of viral infection and also suppressing disease symptoms. These agents can potentially be useful due to their anti-inflammatory effects, cytokine suppression, and anti-fibrotic activities [10]. Some older reports, which have been cited by many recent studies, claimed that chelerythrine has a selective and potent inhibitory activity against protein kinase C-α/-β *in vitro* and *in vivo* [11,12]. Although the validity of this chelerythrine effect has raised questions by some other independent studies [13–15], there are numerous studies, which resulted that chelerythrine is effective on the modulation of protein kinase C. Since, protein kinase C is basically documented to be potentially involved in SARS-CoV-2 infection, and chelerythrine as a protein kinase C-α/-β inhibitor may be beneficial in the blockade of the virus pathogenic processes.

A recent review has emphasized on the vitality and protective role of chelerythrine on the human erythrocytes as anti-inflammatory cells through the suppression of protein kinase C unlike the anemic and eryptosis effects of chloroquine. Human erythrocytes have protective and immunemodulatory role in bacterial infection and inflammatory response. In the case of respiratory viral infection, protein kinase C activation facilitates nuclear transport of the viral ribonucleoproteins in the infected cells and their subsequent budding at the cell membrane. Hence, protein kinase C is an ideal choice for drug targeting and chelerythrine as an inhibitor of protein kinase C-α/-β mediated viral genome transport would be of a great importance in the induction of apoptosis in the SARS-CoV-2 infected cells [16].

3. Chelerythrine has anti-inflammatory effects

The anti-inflammatory effects of chelerythrine have been evaluated in various studies. In a study by Zdenek Dvorak et al., who examined the effects of chelerythrine and some other alkaloids berberine, sanguinarine, and colchicine, on the glucocorticoid receptors (an important anti-inflammatory target) and nuclear factor kappa B (NF-κB), (a pro-inflammatory factor) in HeLa cells. The results showed that both chelerythrine and sanguinarine exert their anti-inflammatory effects by different

Table 1. Summary of chelerythrine activities.

Activity	Descript.	Ref
Protein kinase C inhibition	• Protein kinases C is a host-based antiviral target and can regulate some cellular processes involved in virus replication. Chelerythrine is a potent, selective and a standard PKC inhibitor. Suppression or inhibition of PKC has protective role in erypthosis and prevention of nuclear transport of the viral ribonucleoproteins in infected cells and also dysregulation of the viral replication.	[11,12,16,48]
Anti-inflammatory activity	• Proper inhibition of the body's inflammatory response in patients with COVID-19 is crucial in the treatment. Chelerythrine has anti-inflammatory activity and can exert this effect by different mechanisms. This compound acts through the nuclear translocation of the p65 subunit of NF-κB and binding to glucocorticoid receptors. It shows TNF-α inhibitory activity. It also modulates prostaglandin H synthase and COX-2 to reduce prostaglandin E2 (PGE2).	[17–19]
General anti-viral activity	• Chelerythrine has antiviral activity against a wide range of viruses such as HBV, RSV, WNV, and TMV. Also, it has antiviral activity through the PKC inhibition and high affinity DNA/RNA intercalation property.	[48–54]
Modulation of the process involved in SARS-CoV-2 infection	• Chelerythrine has significant inhibitory effects on the p38 MAPK signaling pathway involved in the inflammatory cytokine storm.	[26,27]
	• Chelerythrine has strong effects on the regulation of NF-κB pathway and suppression of the cytokine storm.	[17]
	• Activation of the Nrf2 by chelerythrine leads to the reduction of nuclear translocation of the NF-kB p65, thereby reducing inflammation. It also increases the expression of Nrf2, and hemeoxygenase-1.	[46]
Special therapeutic effects suitable for COVID-19 complications	• Chelerythrine could potentially reduce cerebral vasospasm and eryptosis in COVID-19 patients, and positively suppress pulmonary inflammation and fibrosis. • Chelerythrine significantly reduced the mean arterial pressure and renal vasoconstriction produced by Ang II. • Chelerythrine effectively inhibits the increase of myofilament calcium sensitivity induced by endothelin-1 in ventricular myocytes thus preventing cardiac disturbances. • Chelerythrine can reduce arrhythmias and myocardial ischemia by increasing the Na-K-ATPase activity. It also activates cystathionine γ-lyase/hydrogen sulfide via PKC/NF-κB Pathway.	[57–59,69–71,73–77]
Pharmacokinetic and toxicity	• Chelerythrine caused marked parenchymal damage in the liver and showed acute hepatotoxicity at dose of 10 mg/kg/day. While, chronic administration of 0.2 mg/kg (i.p.) did not result in any liver damage or necrosis over 56 days. • Chelerythrine did not cause any genotoxicity and hepatotoxicity and an average daily dose of up to 5 mg/kg was completely safe. • Chelerythrine has a significant sarco/endoplasmic reticulum Ca^{2+}-ATPase inhibitory activity. • The *in vivo* toxicity of chelerythrine is considerably less than it's *in vitro* toxicity due to its structural changes.	[83,84,88]
Structural features	• Chelerythrine exists in two forms: "charged iminium" and "neutral alkanolamine" at neutral to mildly alkaline pH. • Chelerythrine exists almost exclusively in the form of "charged iminium" in the pH range 1 to 6, and "alkanolamine" form in the pH range 8.5 to 11. • The two forms have different biological and biochemical behaviors *in vivo*. While the charged iminium form binds more strongly to DNA/RNA, but the alkanolamine has more binding affinity to the bovine serum albumin. • The carbon adjacent to the quaternary nitrogen is the most active part of chelerythrine, attacked by the nucleophiles and is responsible for the observed toxicity of this compound. • Hydroxylated derivatives of chelerythrine such as decarinium, fagaridine and fagaronine seem to be better tolerated and less toxic in patients.	[92–94]

mechanisms compared to the other two alkaloids colchicine and berberine. These structurally similar alkaloids strongly elicited nuclear translocation of the p65 subunit of NF-κB in both non-stimulated cells and in cells challenged with TNF-α, while regardless of the presence or absence of TNF-α, berberine and colchicine had no effects on p65 nuclear translocation. Other complementary analyzes revealed that none of the alkaloids had the effects to trigger glucocorticoid receptor and/or NF-κB transcriptional activities. The ligand-binding studies also displayed that colchicine and berberine did not affect glucocorticoid receptors, whereas chelerythrine and sanguinarine interacted significantly with this receptor [17]. In a new study, the anti-inflammatory activity of chelerythrine and sanguinarine was also assessed via interaction with glucocorticoid receptor *in vitro* using fluorescence polarization (FP) and luciferase reporter (LR) assays. FP results stated that both compounds could bind well to the glucocorticoid receptors (GR) receptors with potent affinities, while LR results showed that chelerythrine did not stimulate GR transcription in HeLa cells, nor did sanguinarine. In the same study, both chelerythrine and sanguinarine showed comparable TNF-α inhibitory activity to dexamethasone as a standard drug in RAW 264.7 cells, indicating their remarkable anti-inflammatory effects [18]. In another study, Xiao-Feng Niu et al, evaluated the anti-inflammatory activities and mechanisms of action of this alkaloid *in vivo* and *in vitro*. Confirming the anti-inflammatory properties of chelerythrine, the researchers suggested that this activity may be relevant to the prostaglandin E2 (PGE2) through the regulation of cyclooxygenase-2 (COX-2). They also demonstrated that chelerythrine effectively inhibits the protein expression of prostaglandin H synthase (PGHS), a critical enzyme in inflammatory responses [19].

4. Chelerythrine can modulate critical process involved in SARS-CoV-2 infection

4.1. Inhibition of p38 MAPK signaling pathway

The mitogen-activated protein kinases (MAPKs) are serine-threonine protein kinases divided into three subfamilies called extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 mitogen-activated protein kinases (p38s), which are involved in the modulation of the subcellular processes during pathophysiological and stressed situations [20]. Basically, involvement of the above three pathways in the infection of coronaviruses have been documented [21–23]. Interestingly, the P38 MAPK mediated cross-talk activation of the NF-κB pathway, another critical signaling pathway related to SARS-CoV-2 infection has been determined [24]. Recently, inhibition of the p38 MAPK pathway involved in inflammatory cytokine storm was introduced as a promising target for the treatment of COVID-19. It is argued that the increase in angiotensin II (Ang II) levels due to ACE2 down-regulation is a stimulus for the disproportionate and severe activation of the P38 MAPK pathway and the development of severe inflammatory reactions. Therefore, inhibition of the P38 MAPK pathway can be a rational strategy to reduce the severity of the COVID-19 symptoms [25]. Some studies have reported that chelerythrine has significant inhibitory effects on this target. The effect of chelerythrine on p38, JNK1, and ERK/MAPK signaling pathways has been studied by Rong Yu et al. Correspondingly, they treated HeLa cells with chelerythrine by sub-micromolar concentrations around 0.66 μM (reported IC_{50} for protein kinase C inhibition) and the results revealed that chelerythrine strongly regulated p38 and JNK1 activity (but not ERK2) in a dose-dependent manner, through an oxidative stress mechanism, and without any inhibitory effects on protein kinase C [26]. In another study, Weifeng Li et al., stated that the suppressing effects of chelerythrine on LPS-induced TNF-α level and nitric oxide (NO) production is related to the selective effect of this compound on the inhibition of p38 MAPK pathway, and regulation of inflammatory mediator's expression [27].

4.2. Inhibition of NF-κB signaling pathway

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) has a critical role in cytokine production [28]. NF-κB pathway disruption is associated with inflammatory and autoimmune

diseases, as well as viral infections [29]. In prior epidemics of coronavirus SARS and MERS, some studies suggested that certain viral proteins such as spike and nucleocapsid proteins may play an important role in disease severity through over-activation of the NF-κB pathway [30,31]. With the elucidation of the SARS-CoV-2 pathogenesis, the role of the NF-κB pathway in patients with severe COVID-19 has also received much attention. Some studies have suggested that hyper-activation of the NF-κB pathway is critical in the pathogenesis of the severe COVID-19 phenotype [32,33]. The "cytokine storm," which occurs in severe forms of COVID-19, triggers the release of cytokines/chemokines, leading to severe inflammation and tissue damage in some organs, especially the lungs. Accordingly, it seems that proper regulation of the NF-κB pathway using NF-κB-inhibitors is a sensible strategy to reduce cytokine storm and COVID-19 severity. As mentioned in the earlier section, chelerythrine has strong effects on regulating the NF-κB pathway [17].

4.3. Activation of Nrf2 signaling pathway

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcription factor involved in regulating some important processes such as metabolism, immune response, and inflammation. Nrf2 also prevents oxidative damage caused by inflammation and injuries, through regulating the expression of antioxidant proteins [34]. Due to the decrease in Nrf2 activity with increasing age, body resistance to diseases such as viral infections decreases over time [35]. Some other evidence suggest that Nrf2 activity may be involved in regulating mechanisms affecting virus susceptibility and replication [36]. The role of Nrf2 signaling pathway in controlling inflammatory processes is crucial. Nrf2 regulates heme oxygenase-1 (HO-1), an important enzyme with significant anti-inflammatory and antioxidant properties [37]. In addition, the Nrf2 controls and regulates the release of cytokines, matrix metalloproteinases (MMPs), COX-2, iNOS production and etc [38]. These mediators can affect other pathways associated with inflammation, such as NF-κB and MAPKs [39,40]. Because patients infected by SARS-CoV-2 often have signs of systemic inflammation and oxidative stress, some studies suggest using Nrf2-inducers to prevent these pathologic complications [41]. Their rationale is that activation of Nrf2 could protect the airway epithelium against disassembly of tight intracellular junctions during the inflammatory phase and acute respiratory distress syndrome (ARDS). They also state that the use of Nrf2-inducers protects the vascular endothelium against damage caused by inflammatory cytokines or oxidative stress, increases glutathione content, and it helps more vulnerable COVID-19 patients (such as the elderly and those with hyperglycemia) for better resistance [42]. Some recent experimental studies also suggest that Nrf2 activators may be effective in treating COVID-19. In a study by Joe M. McCord et al., the therapeutic potential of a three-component Nrf2 activator PB125 was assessed against COVID-19. Using GeneChip microarray and RNA-seq methods, they reported that the potent Nrf2-activator PB125 can downregulate ACE2 and TMPRSS2 mRNA gene expression in HepG2 cells. They have also reported the remarkable downregulation of some genes encoding cytokines in endotoxin-stimulated primary human pulmonary artery endothelial cells, such as IL-1-beta, IL-6, TNF-α. Based on the results, the researchers suggested that Nrf2 activation may significantly reduce the severity of COVID-19 cytokine storm [43].

Plants have been identified as rich sources of anti-inflammatory Nrf2-inducers. Many natural compounds have been identified as Nrf2-dependent anti-inflammatory agents that can exert their therapeutic effects by activating the Nrf2 transcription factor and regulating cytoprotective genes [44,45]. Chelerythrine is a natural compound and can effectively activate the Nrf2/ARE signaling pathway. In a study, Lu Fan et al., examined the anti-inflammatory effects of chelerythrine on acute lung injury caused by lipopolysaccharide *in vitro* and *in vivo*. They reported that chelerythrine significantly ameliorated pathological injuries in the lungs by suppressing the interleukin 6 (IL-6), interleukin 1b (IL-1b), and tumor necrosis factor-alpha (TNF- α) induced by lipopolysaccharides. Furthermore, they showed that modulation of the nuclear factor kappa-B (NF-κB) pathway by chelerythrine is an important

mechanism for suppressing the inflammation in RAW264.7 cells. In fact, the activation of the Nrf2 by chelerythrine leads to the reduction of nuclear translocation of the NF-kB p65, thereby reducing inflammation [46]. In another study by Ling Peng et al., the effect and mechanism of chelerythrine on pulmonary fibrosis were evaluated. In conclusion, this study reported that chelerythrine can significantly alleviate bleomycin-induced pulmonary fibrosis via activating the Nrf2/ARE signaling pathway. Results showed that chelerythrine can effectively reduce the expression levels of alpha-smooth muscle actin (α-SMA), fibronectin, and transforming growth factor-beta (TGF-β), increase the expression of Nrf2, hemeoxygenase-1 (HO-1), and quinone oxidoreductase (NQO1), upregulate the levels of superoxide dismutase (SOD) and glutathione (GSH), and also decrease the levels of hydroxyproline (HP) and 4-hydroxynonenal (4-HNE) [47].

5. Anti-viral activity of chelerythrine

5.1. Broad spectrum anti-viral activity

Chelerythrine has a moderate antiviral activity against a wide range of viruses. Most related studies indicate that chelerythrine prevents the replication of viruses. Especially, viruses that use the protein kinase C during their pathogenesis, chelerythrine can be a potential inhibitor. So far, few studies have been performed on the antiviral effects of chelerythrine. Most of these studies were not designed to investigate the antiviral effects of chelerythrine but used chelerythrine as a standard protein kinase C inhibitor. In a study by LAN LIU, effects of hepatitis B core antigen (HBcAg) on cell proliferation and apoptosis were assessed against DC2.4 cells using chelerythrine as a standard positive control. The results showed that chelerythrine causes blockade of the NF-κB pathway, and also eliminates the effects of HBcAg on p-IκB, p-P65, p-PKC, and Bcl-2 levels. Results of MTT assay also showed that chelerythrine significantly reduced the effect of HBcAg on DC2.4 cell proliferation compared to untreated cells. These results suggest that chelerythrine reduced the effects of HBcAg in increased proliferation and apoptosis through regulation of the PKC/NF-κB signaling pathway [48]. In another study, Homero San-Juan-Vergara et al., stated that protein kinase C-α activity is necessary for the fusion of the Respiratory Syncytial Virus (RSV) with human bronchial epithelial cells. This study showed that chelerythrine can inhibit RSV infection with an IC_{50} of 7.5 μM in NHBE cells, and did not exhibit significant cytotoxicity in both crystal violet toxicity and MTT assays [49]. Some studies showed that chelerythrine can significantly inhibit flaviviruses multiplication. In this case, a new study by Ana B. Blazquez et al., showed that chelerythrine effectively inhibits West Nile Virus (WNV) replication, without affecting cell viability. Quantitative RT-PCR evaluations in this study represented that the amount of the viral RNA released to the supernatant in chelerythrine-treated infected cells significantly decreased. Also, assessment of the number of mouse monoclonal antibody J2 against double-stranded RNA (dsRNA) intermediates using the immunofluorescence method confirmed the significant disruption in WNV viral replication exerted by chelerythrine [50]. It has also been reported that chelerythrine has significant effects against plant viral diseases. In a recent study, anti-Tobacco Mosaic Virus (TMV) effects and mode of action of chelerythrine and some other alkaloids having antiviral activity such as chelidonine and sanguinarine were evaluated by Wenhui Guo et al. The results showed that chelerythrine exhibits the best effects against TMV (at 0.5 mg mL^{-1} concentration) compared to other tested alkaloids [51].

5.2. Chelerythrine as a viral-DNA/RNA intercalating agent

The genetic material of viruses has always been one of the most attractive targets for the discovery and development of antiviral drugs. Whereas most of the direct-acting antiviral agents are active against the structural proteins (SPs) of the viruses, antiviral DNA/RNA-intercalators can potentially suppress the viral replication inside the host cell by interacting only with the nucleic acids of the genome. In most cases, these compounds have planar structures with multiple and fused aromatic rings that can be inserted and stacked in between the DNA base pairs or paired regions of RNAs. Depending on the concentration and affinity of the intercalators for binding to DNA/RNA, nucleic acid strands are stabilized in such a way that replication is suppressed.

Quaternary isoquinoline alkaloids are high-affinity DNA binders and their potential ability to bind human G-quadruplex (G4) and dsDNA helix have been reported in two studies performed by the research group of Sissi C. and Gratteri P. using of various techniques such as fluorescent intercalator displacement assay, fluorescence melting, crystallization, X-ray diffraction and molecular modeling [52,53]. The two natural alkaloids chelerythrine and coptisine, respectively, with benzophenanthridine and phenylisoquinoline structures are strong DNA intercalators and have superior G4 stabilizing properties and also G4 versus dsDNA binding selectivity. The role of the benzo-1,3-dioxolo group in the interaction of guanine residues was studied comprehensively and showed that the oxygen atoms of this group has key hydrogen bonding to the guanine atoms to stabilize the G4 structure. The results showed that the benzodioxolo group is preferred than the two methoxy groups in the same position for binding and promotes the interaction with G4. These alkaloids also showed a significant selectivity to G4 over the random double-helix DNA for binding. Chelerythrine has slightly higher stabilization ability of G4 over coptisine and benzophenanthridine structure has better interaction with the human telomeric G quadruplex than the phenylisoquinoline scaffold in coptisine. The presence of large aromatic surface and also positive nitrogen of chelerythrine are important features for DNA interaction and intercalation. Some π-π stacking interactions and salt bridge formed by charged nitrogen are fundamental bonding forces to stabilize the complex. The results also highlighted that the planarity of the scaffold is not only a determinant factor, but the presence of an extra benzodioxolo group can constitute stronger interactions with guanine residues. The results also indicated that a degree of flexibility is important in selectivity toward G4 vs dsDNA, which is seen in the structure of coptisine with partially reduced isoquinoline scaffold. Coptisine can adopt a bent conformation with a dihedral angle of around 18° between its phenyl and quinoline parts and confers higher selectivity than chelerythrine to G4.

In conclusion, DNA intercalators not only stop DNA replication, but also inhibit DNA polymerase, reverse transcriptase, RNA polymerase and protein biosynthesis at ribosomal level. Hence, these compounds are potential antiviral agents against various panels of viral infections including coronaviruses. A recent review on chelerythrine and similar alkaloids (Figure 2), has introduced them as a potential strong nucleic acid intercalators of the single-stranded RNA SARS-CoV-2 virus [54].

6. Some special therapeutic effects of chelerythrine for COVID-19 patients

6.1. Protective effects on renal-vascular system

Since ACE2 is highly expressed in renal tubular cells and podocytes, the kidney is a vital organ which can be seriously endangered by SARS-CoV-2. Many patients with COVID-19 have kidney abnormalities such as proteinuria, hematuria, and acute kidney damage [55]. In addition to direct infection, this organ can be severely damaged by secondary problems caused by COVID-19, such as cytokine storm and hypoxia [56]. On the other hand, heavily administered drugs and renal excretion of their metabolites during COVID-19 treatment exert extra strain on this hard-working organ. Therefore, continuous protection of this vital organ by using drugs with nephron-protective effects and/or minimal renal-complications seems essential during the treatment of COVID-19. The significant effect of chelerythrine on the renal arteries clearly was confirmed in several studies. In fact, chelerythrine inhibits the vasoconstriction effect of Ang II. An earlier study reported that chelerythrine at 1 μM concentration can attenuate myogenic vasoconstriction of the renal afferent arteriole (in a dose-dependent manner) in the rat model [57]. A study reported that chelerythrine significantly reduced the mean arterial pressure (MAP) and renal vascular resistance (RVR) responses to Ang II [58]. Another study also stated that chelerythrine can effectively reduce the renal vasoconstriction produced by Ang II (with a maximum inhibition of 60%) in a dose-dependent manner [59]. T. Nagahama et al., showed in a direct manner that chelerythrine can block the Ang II–induced arteriolar constriction in both Ang II–induced afferent (AFF) and efferent (EFF) of renal microvessels, with higher responsiveness for EFF. Since, chelerythrine was found to have no effect on KCl-induced AFF contraction but tend to cause vasodilation in the presence of calcium antagonists. Hence, researchers concluded that the vasodilatory

effect of this compound has not been exerted by acting on voltage-gated calcium channels (VGCCs) [60].

Interestingly, the abnormal increase in the Ang II levels caused by downregulation of ACE2 (by SARS-CoV-2), has been one of the main causes of severe complications of COVID-19 and activation of the p38 MAPK and consequent hyper-inflammatory immune response [61,62]. Therefore, the inhibitory effects of chelerythrine on Ang II-induced vascular contractions could potentially be beneficial in protecting the renal vascular system.

6.2. Anti-cerebral vasospasm

Some acute neurological and cerebrovascular symptoms such as headache, speech confusion, epilepsy, and limb paralysis have been reported by neurologists during the visit and treatment of COVID-19 patients [63,64]. This indicates that COVID-19 severely affects the central nervous system (CNS) in some patients. Also, clinical data show that COVID-19 patients with underlying cerebrovascular involvement are among the patients with the highest morbidity and mortality rates [65,66]. Although the lungs are more directly affected by COVID-19, acute respiratory distress syndrome can cause some life-threatening secondary problems such as hypoxemia. While COVID-19 patients experience hypoxemia due to severe respiratory dysfunction, the brain is the first organ to be affected and a series of pathophysiological changes occur. Hypoxia causes major CNS-problems such as dilation of cerebral blood vessels (increasing intracranial blood flow and capillary blood pressure), production of free radicals, and increasing cerebral vasospasm [67,68].

Protein kinase C (PKC) has a well-known role in cerebral circulation by the regulation of myogenic tone through phosphorylation of ion channels. Numerous studies confirm that PKC activation is involved in cerebral vasoconstriction [69,70]. In a study, Shigeru Nishizawa et al., showed that chelerythrine could effectively prevent cerebral vasospasm and protein kinase C translocation, and suggested that this compound could be a useful agent in preventing cerebral vasospasm after subarachnoid hemorrhage (SAH) [70]. In another study, the role of chelerythrine in the inhibition of cerebral vasospasm after experimental SAH was evaluated in a rat model by Arif Aladag et al. These researchers displayed that chelerythrine has beneficial therapeutic effects and significant systemic activities on cerebral vasospasm [71]. This evidence suggests that chelerythrine has valuable therapeutic effects in the treatment of cerebrovascular patients with COVID-19.

6.3. Effects on cardiovascular system

Since angiotensin-converting enzyme 2 (ACE2) which is the gateway for the SARS-CoV-2 to enter the cell is highly expressed in the heart, people with cardiovascular illnesses are among the most COVID-19 patients at-risk. This risk elevates when the medications used by these patients have cardiovascular side effects. Therefore, it is important that the drugs prescribed for these patients have minimal cardiovascular adverse effects [72]. In addition, it makes sense to use compounds that have protective effects on this vital system.

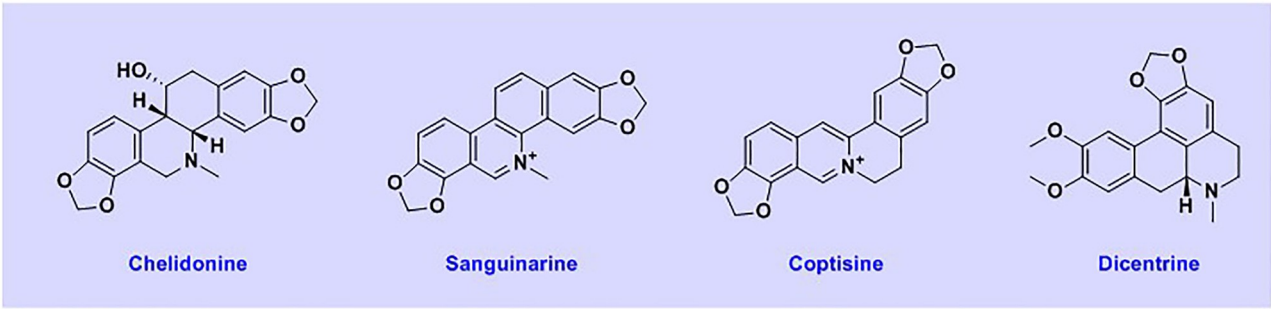

Figure 2. Chemical structure of some DNA/RNA intercalating alkaloids with antiviral activity.

Endothelin-1 (ET-1) is a potent vasoconstrictor and its disproportionate activity can cause some cardiac disturbances. A study showed that chelerythrine effectively inhibits the increase of myofilament calcium sensitivity induced by endothelin-1 in ventricular myocytes [73]. An earlier study about the effect of endothelin-1 on cardiac fibroblast proliferation also showed that chelerythrine completely abolished the effects of endothelin-1 on DNA synthesis and cell proliferation [74]. Chelerythrine can also have some antiarrhythmic effects [75]. A study reported that chelerythrine could prolong cardiac action potentials in rat ventricular myocytes [76]. Also, it has been shown that chelerythrine can reduce myocardial ischemia by increasing the Na-K-ATPase activity [77]. A recent study reported that chelerythrine has a protective role on the myocardium, and can attenuate renal ischemia/reperfusion-induced myocardial injury by activating cystathionine γ-lyase/hydrogen sulfide (CSE/H_2S) via modulating the protein kinas C/NF-κB Pathway [78]. It is obvious that chelerythrine has a significant effect on the cardiovascular system. The possible and beneficial therapeutic application of chelerythrine for COVID-19 associated cardiac complications, should be further evaluated by cardiologists.

6.4. Anti-pulmonary inflammation

Because the SARS-CoV-2 virus attacks the lungs more than any other organs, treating patients with pulmonary fibrosis who develop COVID-19 infection would be difficult. Furthermore, clinical data indicate that significant fibrotic consequences can occur for COVID-19 patients. Therefore, the role of anti-fibrotic therapy in these patients would be very prominent [79,80]. Chelerythrine has beneficial effects in suppressing pulmonary inflammation and fibrosis, which may be useful in treating COVID-19 patients. These effects of chelerythrine have been considered and evaluated in a study by Ling Peng described above (section 4.3) [47].

7. Pharmacokinetic and toxicological properties of chelerythrine

Toxicity and safety are important parameters that determine whether a drug candidate can be used in clinic or not. Chelerythrine and its structural analogue sanguinarine have a long history of usage in traditional Chinese medicine (TCM). The toxicological properties of these alkaloids have been evaluated in several studies *in vitro* and *in vivo*. Hepatotoxicity is one of the most important toxicities studied for these compounds. A study reported that sanguinarine (single *i.p.* dose, 10 mg/kg) had significant hepatotoxic effects manifested by increasing the activity of SGPT and SGOT, and reducing the activity of cytochrome P450 [81]. While the short-term toxicity of sanguinarine which was assessed by Peter J. Becci et al., showed that sanguinarine chloride does not have remarkable toxicity expressed as parameters: acute oral LD_{50} of 1658 mg/kg and acute *iv* LD_{50} of 29 mg/kg in rats, and acute dermal LD_{50} > 200 mg/kg in rabbits. The researchers also stated that there was no statistically or toxicologically significant inhibition of the enzyme in rats fed by sanguinarine diet for 14 days (up to 150 ppm) and in rats treated for 30 days by gavage (up to 0.6 mg/kg body weight) [82]. In another study, hepatotoxicity effect of the three alkaloids chelerythrine, sanguinarine, and fagaronine (structural analog of chelerythrine having hydroxyl group) was assessed. Evaluation of the acute hepatotoxicity at a dose of 10 mg/kg/day (*i.p.*), showed that chelerythrine and sanguinarine caused marked parenchymal damage in the liver, while fagaronine was not found to be hepatotoxic. Chronic administration of 0.2 mg/kg (*i.p.*) did not cause any liver damage or necrosis over 56 days [83]. In terms of the structure–activity relationship (SAR), this difference in toxicity due to the presence of a hydroxyl group can be a valuable point in the future design of novel analogs and their structural modifications to have safer drugs. In a study by Pavel Kosina et al., the safety and toxicity of chelerythrine and sanguinarine was evaluated in a feeding experiment in pigs for 3 months. The results showed that these compounds did not cause any genotoxicity and hepatotoxicity and that an average daily dose of up to 5 mg/kg was completely safe [84]. Some other studies suggest that chelerythrine analogues interact with cytochrome P450 CYP1A through the metabolization processes [85,86]. After investigating the effect of chelerythrine and some of its analogues on the expression and

activity of CYP1A1 in HepG2 cells, Zdarilova et al., reported that although CYP1A modulates the cytotoxicity and genotoxicity of these structures, they don't affect CYP1A1 expression. The researchers also stated that metabolism of these compounds by hepatic microsomes can lead to the generation of DNA adducts [87]. A study conducted by S. M. Vieira et al., also reported that chelerythrine has a significant sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA1)-inhibitory activity, and this effect could be a possible cause of the chelerythrine cytotoxicity by the resulting loss of the cell calcium homeostasis [88].

The *in vivo* toxicity observed for chelerythrine is considerably less than it's *in vitro* toxicity [89]. This toxicity difference may be related to the structural changes of chelerythrine in vivo, which is explained in the next section. Overall, quaternary benzo[c]phenanthridine alkaloids (cationic derivatives) bearing a quaternary nitrogen atom have higher toxicity compared to the neutral benzo[c]phenanthridine derivatives such as chelidonine.

The pharmacokinetic parameters of chelerythrine and its metabolite dihydrochelerythrine after oral and intramuscular administrations in pigs have recently been reported. Results showed that chelerythrine and its metabolite rapidly reached peak plasma concentration (C_{max}) less than an hour after IM administration. The time to reach peak plasma concentration in a single oral administration was also rapid and was obtained in less than 2 hours. Half-life (T ½) of chelerythrine was calculated to be 2.03 hours and it is metabolized rapidly after a single oral administration [90].

8. Structural evaluation of chelerythrine

Chelerythrine is a benzophenanthridine alkaloid by the IUPAC name 1,2-dimethoxy-12-methyl-[1,3]benzodioxolo[5,6-c]phenanthridin-12-ium, has a rigid structure bearing four fused rings. Previously, the chemical structure of the chelerythrine was examined by various methods such as elemental analysis, infrared spectroscopy (IR), 1D, and 2D nuclear magnetic resonance (NMR), electron ionization mass spectrometry (EI-MS), chemical ionization mass spectrometry (CI-MS) and etc. Under near-neutral pH to mildly alkaline conditions, chelerythrine derivatives have two "charged iminium" and "neutral alkanolamine" forms that convert to each other in an equilibrium reaction (Figure 3) [91,92]. Structural analysis by Motilal Maiti et al., also showed that the chelerythrine exists almost exclusively as an iminium form in the pH range 1 to 6, and alkanolamine form only exists in the pH range 8.5 to 11 [93]. These different structures can have different biological and biochemical behaviors *in vivo*. For example, a multi-spectroscopic investigation by Sutanwi Bhuiya et al., revealed that alkanolamine form has a greater binding affinity on bovine serum albumin (BSA) compared to that of the iminium form [94]. In another study by Motilal Maiti, when the interaction of these different structures with single and double-stranded DNA was evaluated using spectrofluorimetric, spectrophotometric, and circular dichroic methods, the results showed that iminium form binds strongly to all DNA structures, while the alkanolamine form does not bind to DNA [93].

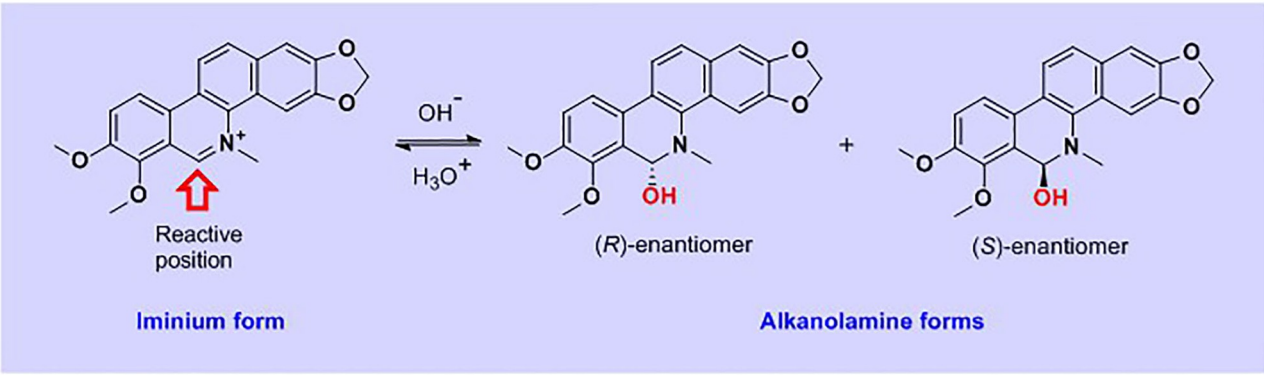

Figure 3. Chemical structure of chelerythrine and its equilibrium forms iminium and alkanolamine under near-neutral pH.

In terms of the reactivity, the carbon adjacent to the quaternary nitrogen is the most active part of chelerythrine, which is well attacked by nucleophiles and produces neutral structures. Because there are many nucleophiles *in vivo*, the iminium form can react with these agents to produce neutral structures. This could be a plausible reason for the difference in toxicity of chelerythrine in *in vivo* and *in vitro*.

Chelerythrine has many famous structural analogues such as berberine, palmatine, and sanguinarine, all of which have significant antiviral activities [95]. In the minimized free energy state, these compounds have perfectly planar structures that make them suitable for viral-DNA/RNA intercalation (Figure 4) [96].

The pharmacological effects of some other derivatives have not yet been well studied. Some of them such as decarinium and fagaridine contain hydroxyl groups that seem to be better tolerated and less toxic in patients (Figure 5). An animal study showed that fagaronine (10 mg/kg/day; *i.p.*) had no liver toxicity effects, whereas chelerythrine and sanguinarine showed remarkable hepatotoxicity at this dose [83]. Some derivatives are structurally polymethoxylated, reminiscent of colchicine alkaloid (Figure 4), which is now recommended for the treatment of COVID-19 due to its promising anti-inflammatory and anti-viral effects [97]. Structural analogues of chelerythrine can be used as a valuable source by researchers to find new agents for the treatment of COVID-19.

9. Conclusion

Today, research to find agents with high therapeutic potential against COVID-19 is one of the most essential human needs. Because natural compounds are rich sources of bioactive agents, they can be used for this purpose. In the past years, therapeutic profile of some natural compounds have been appropriate so that they have been approved by competent authorities for the treatment of diseases. In some other cases, despite having valuable therapeutic effects, several adverse effects prevented them from entering the clinical phases. These compounds were often used as leads in drug discovery and development processes, and their efficacy or potency shortcomings was eliminated by various methods such as semi-synthetic approaches. As stated, chelerythrine is an interesting compound, which can potentially be used for the treatment of COVID-19 infections. In addition to the therapeutic effects reviewed here, chelerythrine can also prevent eryptosis as an important side effect of drugs used for COVID-19 treatment. Despite having valuable therapeutic effects, high doses of this compound can be a limiting factor. Therefore, any clinical use of chelerythrine is recommended to be in a low and safe dosage range. We also draw the attention of scientists to investigate the therapeutic effects of less toxic derivatives of this compound such as decarinium, agaridine, decarine, and isodecarine which can be used in higher doses.

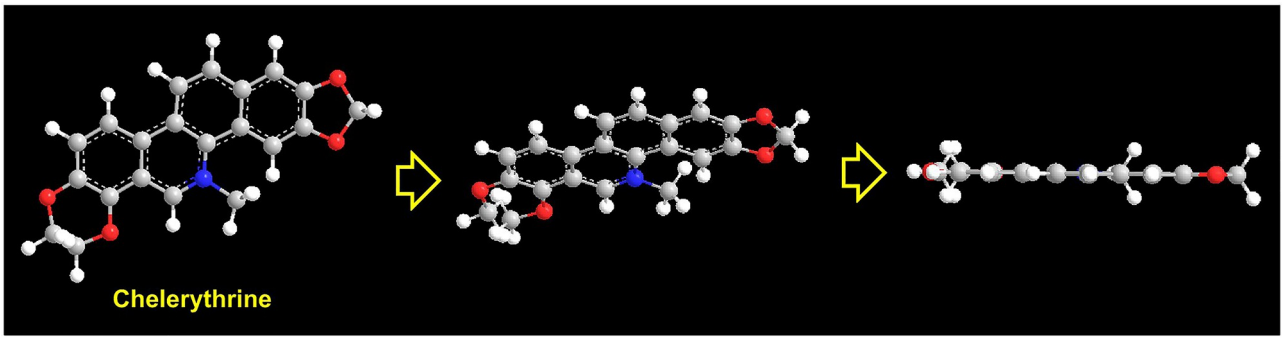

Figure 4. Rotation of the 3D optimized structure of chelerythrine around X-axis shows the planar structure suitable for DNA/RNA intercalation.

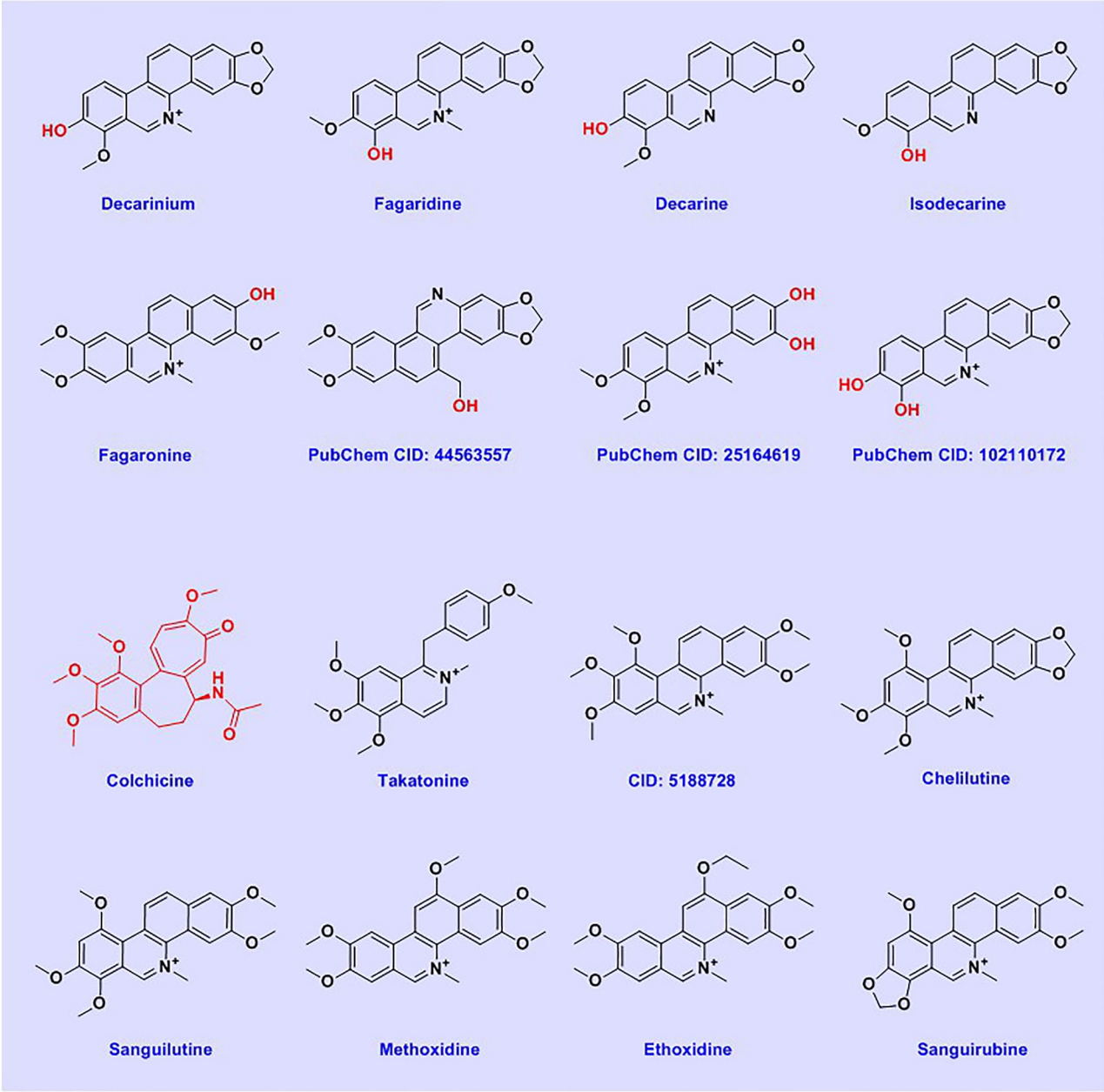

Figure 5. Chemical structure of some of the less-studied chelerythrine analogues containing the hydroxyl functional group and poly-methoxy substituent analogues.

Acknowledgements

We thank to the research council of the Mazandaran University of Medical Sciences for their support in accessing the literature.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Disclosure statement

The authors declare no conflict of interest.

Author contributions

Mehdi Valipour contributed in conceptualization and writing the original draft, Afshin Zarghi contributed in review and

editing the manuscript, Mohammad Ali Ebrahimzadeh contributed in review and editing and Hamid Irannejad contributed in supervision and writing the original draft of the manuscript.

ORCID

Mehdi Valipour http://orcid.org/0000-0001-6689-3743
Hamid Irannejad http://orcid.org/0000-0001-7513-6162

References

[1] Sanders JM, Monogue ML, Jodlowski TZ, et al. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. Jama. 2020;323 (18):1824–1836.
[2] Wink M, Roberts MF. Alkaloids: biochemistry, ecology, and medicinal applications. New York: Plenum Press; 1998.
[3] Marschall M, Stein-Gerlach M, Freitag M, et al. Direct targeting of human cytomegalovirus protein kinase pUL97 by kinase inhibitors is a novel principle for antiviral therapy. J Gen Virol. 2002;83(5):1013–1023.
[4] Chen W-C, Simanjuntak Y, Chu L-W, et al. Benzenesulfonamide derivatives as calcium/calmodulin-dependent protein kinase inhibitors and antiviral agents against dengue and zika virus infections. J Med Chem. 2020;63(3):1313–1327.
[5] Garcia JG, Sharma A, Ramaiah A, et al. Antiviral drug screen of kinase inhibitors identifies cellular signaling pathways critical for SARS-CoV-2 replication. Available at SSRN 3682004. 2020.
[6] Perwitasari O, Yan X, O'Donnell J, et al. Repurposing kinase inhibitors as antiviral agents to control influenza A virus replication. Assay Drug Dev Technol. 2015;13 (10):638–649.
[7] Hoffmann -H-H, Palese P, Shaw ML. Modulation of influenza virus replication by alteration of sodium ion transport and protein kinase C activity. Antiviral Res. 2008;80(2):124–134.
[8] Mondal A, Dawson AR, Potts GK, et al. Influenza virus recruits host protein kinase C to control assembly and activity of its replication machinery. Elife. 2017;6: e26910.
[9] McKernan LN, Momjian D, Kulkosky J. Protein kinase C: one pathway towards the eradication of latent HIV-1 reservoirs. Adv Virol. 2012;2012:8. https://doi.org/10.1155/2012/805347
[10] Weisberg E, Parent A, Yang PL, et al. Repurposing of kinase inhibitors for treatment of COVID-19. Pharm Res. 2020;37(9):1–29.
[11] Herbert J, Augereau J, Gleye J, et al. Chelerythrine is a potent and specific inhibitor of protein kinase C. Biochem Biophys Res Commun. 1990;172(3):993–999.
[12] Chmura SJ, Dolan ME, Cha A, et al. In vitro and in vivo activity of protein kinase C inhibitor chelerythrine chloride induces tumor cell toxicity and growth delay in vivo. Clin Cancer Res. 2000;6(2):737–742.
[13] Davies SP, Reddy H, Caivano M, et al. Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J. 2000;351(1):95–105.
[14] Anastassiadis T, Deacon SW, Devarajan K, et al. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. Nat Biotechnol. 2011;29(11):1039–1045.
[15] Lee SK, Qing WG, Mar W, et al. Angoline and chelerythrine, benzophenanthridine alkaloids that do not inhibit protein kinase C. J Biol Chem. 1998;273 (31):19829–19833.
[16] Ghashghaeinia M, Dreischer P, Wieder T, et al. Coronavirus disease 2019 (COVID-19), human erythrocytes and the PKC-alpha/-beta inhibitor chelerythrine–possible therapeutic implication. Cell Cycle. 2020;19(24):3399–3405.
[17] Dvořák Z, Vrzal R, Maurel P, et al. Differential effects of selected natural compounds with anti-inflammatory activity on the glucocorticoid receptor and NF-κB in HeLa cells. Chem Biol Interact. 2006;159(2):117–128.
[18] Zhang J, Liang Y, Ren L, et al. In vitro anti-inflammatory potency of sanguinarine and chelerythrine via interaction with glucocorticoid receptor. eFood. 2021;1(6):392–398. 10.2991/efood.k.210118.001.
[19] Niu X-F, Zhou P, Li W-F, et al. Effects of chelerythrine, a specific inhibitor of cyclooxygenase-2, on acute inflammation in mice. Fitoterapia. 2011;82(4):620–625.
[20] Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science. 2002;298(5600):1911–1912.
[21] Banerjee S, Narayanan K, Mizutani T, et al. Murine coronavirus replication-induced p38 mitogen-activated protein kinase activation promotes interleukin-6 production and virus replication in cultured cells. J Virol. 2002;76(12):5937–5948.
[22] Kopecky-Bromberg SA, Martinez-Sobrido L, Palese P. 7a protein of severe acute respiratory syndrome coronavirus inhibits cellular protein synthesis and activates p38 mitogen-activated protein kinase. J Virol. 2006;80 (2):785–793.
[23] Kono M, Tatsumi K, Imai AM, et al. Inhibition of human coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: involvement of p38 MAPK and ERK. Antiviral Res. 2008;77(2):150–152.
[24] Saccani S, Pantano S, Natoli G. p38-dependent marking of inflammatory genes for increased NF-κB recruitment. Nat Immunol. 2002;3(1):69–75.
[25] Grimes JM, Grimes KV. p38 MAPK inhibition: a promising therapeutic approach for COVID-19. J Mol Cell Cardiol. 2020;144:63–65.
[26] Yu R, Mandlekar S, Tan T-H, et al. Activation of p38 and c-Jun N-terminal kinase pathways and induction of apoptosis by chelerythrine do not require inhibition of protein kinase C. J Biol Chem. 2000;275 (13):9612–9619.

[27] Li W, Fan T, Zhang Y, et al. Effect of chelerythrine against endotoxic shock in mice and its modulation of inflammatory mediators in peritoneal macrophages through the modulation of mitogen-activated protein kinase (MAPK) pathway. Inflammation. 2012;35 (6):1814–1824.
[28] DiDonato JA, Hayakawa M, Rothwarf DM, et al. A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. Nature. 1997;388 (6642):548–554.
[29] Santoro MG, Rossi A, Amici C. NF-κB and virus infection: who controls whom. EMBO J. 2003;22 (11):2552–2560.
[30] DeDiego ML, Nieto-Torres JL, Regla-Nava JA, et al. Inhibition of NF-κB-mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol. 2014;88(2):913–924.
[31] Qj LIAO, Lb YE, Timani KA, et al. Activation of NF-κB by the full-length nucleocapsid protein of the SARS coronavirus. Acta Biochim Biophys Sin (Shanghai). 2005;37(9):607–612.
[32] Kircheis R, Haasbach E, Lueftenegger D, et al. NF-κB pathway as a potential target for treatment of critical stage COVID-19 patients. Front Immunol. 2020;11:598444.
[33] Hariharan A, Hakeem AR, Radhakrishnan S, et al. The role and therapeutic potential of NF-kappa-B pathway in severe COVID-19 patients. Inflammopharmacology. 2020; 29(1), 91–100.
[34] Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem. 2009;284 (20):13291–13295.
[35] Ramezani A, Nahad MP, Faghihloo E. The role of Nrf2 transcription factor in viral infection. J Cell Biochem. 2018;119(8):6366–6382.
[36] Huang H, Falgout B, Takeda K, et al. Nrf2-dependent induction of innate host defense via heme oxygenase-1 inhibits Zika virus replication. Virology. 2017;503:1–5.
[37] Loboda A, Damulewicz M, Pyza E, et al. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci. 2016;73(17):3221–3247.
[38] Ahmed SMU, Luo L, Namani A, et al. Nrf2 signaling pathway: pivotal roles in inflammation. Biochim Biophys Acta Mol Basis Dis. 2017;1863(2):585–597.
[39] Buelna-Chontal M, Zazueta C. Redox activation of Nrf2 & NF-κB: a double end sword? Cell Signal. 2013;25(12):2548–2557.
[40] Ren J, Li L, Wang Y, et al. Gambogic acid induces heme oxygenase-1 through Nrf2 signaling pathway and inhibits NF-κB and MAPK activation to reduce inflammation in LPS-activated RAW264. 7 cells. Biomed Pharmacother. 2019;109:555–562.
[41] Cuadrado A, Pajares M, Benito C, et al. Can activation of NRF2 be a strategy against COVID-19? Trends in pharmacological sciences. 2020;41(9):598–610.
[42] Zinovkin R, Grebenchikov O. Transcription factor Nrf2 as a potential therapeutic target for prevention of cytokine storm in COVID-19 patients. Biochemistry (Moscow). 2020;85(7):833–837.
[43] McCord JM, Hybertson BM, Cota-Gomez A, et al. Nrf2 activator PB125® as a potential therapeutic agent against COVID-19. Antioxidants. 2020;9(6):518.
[44] Ooi BK, Chan K-G, Goh BH, et al. The role of natural products in targeting cardiovascular diseases via Nrf2 pathway: novel molecular mechanisms and therapeutic approaches. Front Pharmacol. 2018;9:1308.
[45] Kumar H, Kim I-S, More SV, et al. Natural product-derived pharmacological modulators of Nrf2/ARE pathway for chronic diseases. Nat Prod Rep. 2014;31(1):109–139.
[46] Fan L, Fan Y, Liu L, et al. Chelerythrine attenuates the inflammation of lipopolysaccharide-induced acute lung inflammation through NF-κB signaling pathway mediated by Nrf2. Front Pharmacol. 2018;9:1047.
[47] Peng L, Wen L, Shi Q, et al. Chelerythrine ameliorates pulmonary fibrosis via activating the Nrf2/ARE signaling pathway. Cell Biochem Biophys. 2021;79(2):337–347.
[48] Liu L, Huang Y, Zhang K, et al. Hepatitis B core antigen regulates dendritic cell proliferation and apoptosis through regulation of PKC/NF-κB signaling pathway. Mol Med Rep. 2018;18(6):5726–5732.
[49] San-Juan-Vergara H, Peeples ME, Lockey RF, et al. Protein kinase C-α activity is required for respiratory syncytial virus fusion to human bronchial epithelial cells. J Virol. 2004;78(24):13717.
[50] Blázquez AB, Á V-C, Martín-Acebes MA, et al. Pharmacological inhibition of protein kinase C reduces West Nile virus replication. Viruses. 2018;10(2):91.
[51] Guo W, Lu X, Liu B, et al. Anti-TMV activity and mode of action of three alkaloids isolated from Chelidonium majus. Pest Manag Sci. 2021;77 (1):510–517.
[52] Papi F, Ferraroni M, Rigo R, et al. Role of the benzodioxole group in the interactions between the natural alkaloids chelerythrine and coptisine and the human telomeric G-quadruplex DNA. A multiapproach investigation. J Nat Prod. 2017;80(12):3128–3135.
[53] Bessi I, Bazzicalupi C, Richter C, et al. Spectroscopic, molecular modeling, and NMR-spectroscopic investigation of the binding mode of the natural alkaloids berberine and sanguinarine to human telomeric G-quadruplex DNA. ACS Chem Biol. 2012;7 (6):1109–1119.
[54] Wink M. Potential of DNA intercalating alkaloids and other plant secondary metabolites against SARS-CoV-2 causing COVID-19. Diversity. 2020;12(5):175.
[55] Martinez-Rojas MA, Vega-Vega O, Bobadilla NA. Is the kidney a target of SARS-CoV-2? Am J Physiol Renal Physiol. 2020;318(6):F1454–F1462.

[56] Delsante M, Rossi GM, Gandolfini I, et al. Kidney involvement in COVID-19: need for better definitions. J Am Soc Nephrol. 2020;31(9):2224–2225.
[57] Kirton CA, Loutzenhiser R. Alterations in basal protein kinase C activity modulate renal afferent arteriolar myogenic reactivity. Am J Physiol Heart Circ Physiol. 1998;275(2):H467–H475.
[58] Song J, Eyster KM, Kost JCK, et al. Involvement of protein kinase C-CPI-17 in androgen modulation of angiotensin II-renal vasoconstriction. Cardiovasc Res. 2010;85(3):614–621.
[59] Ruan X, Arendshorst WJ. Role of protein kinase C in angiotensin II-induced renal vasoconstriction in genetically hypertensive rats. Am J Physiol Renal Physiol. 1996;270(6):F945–F952.
[60] Nagahama T, Hayashi K, Ozawa Y, et al. Role of protein kinase C in angiotensin II-induced constriction of renal microvessels. Kidney Int. 2000;57(1):215–223.
[61] Yu X, Cui L, Hou F, et al. Angiotensin-converting enzyme 2-angiotensin (1-7)-Mas axis prevents pancreatic acinar cell inflammatory response via inhibition of the p38 mitogen-activated protein kinase/nuclear factor-κB pathway. Int J Mol Med. 2018;41(1):409–420.
[62] Park J-K, Fischer R, Dechend R, et al. p38 Mitogen-activated protein kinase inhibition ameliorates angiotensin II–Induced Target Organ Damage. Hypertension. 2007;49(3):481–489.
[63] Ahmad I, Rathore FA. Neurological manifestations and complications of COVID-19: a literature review. J Clin Neurosci. 2020;77:8–12.
[64] Bridwell R, Long B, Gottlieb M. Neurologic complications of COVID-19. Am J Emerg Med. 2020;38(7):1549.e3.
[65] Li B, Yang J, Zhao F, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clin Res Cardiol. 2020;109(5):531–538.
[66] Hernández-Fernández F, Sandoval VH, Barbella-Aponte RA, et al. Cerebrovascular disease in patients with COVID-19: neuroimaging, histological and clinical description. Brain. 2020;143(10):3089–3103.
[67] Xie J, Covassin N, Fan Z, et al., editors. Association between hypoxemia and mortality in patients with COVID-19. Mayo Clinic Proceedings; 2020;95(6):1138–1147.
[68] Coen M, Allali G, Adler D, et al. Hypoxemia in COVID-19; Comment on:"The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients". J Med Virol. 2020;92(10):1705–1706.
[69] Laher I, Zhang JH. Protein kinase C and cerebral vasospasm. J Cereb Blood Flow Metab. 2001;21(8):887–906.
[70] Nishizawa S, Obara K, Koide M, et al. Attenuation of canine cerebral vasospasm after subarachnoid hemorrhage by protein kinase C inhibitors despite augmented phosphorylation of myosin light chain. J Vasc Res. 2003;40(2):169–178.
[71] Aladağ M, Yıldız A, Türköz Y, et al. The ınhibition of cerebral vasospasm by using chelerythrine after experimental subarachnoidal haemorrhage in rats. 2017;6(1):18–22.
[72] Boukhris M, Hillani A, Moroni F, et al. Cardiovascular implications of the COVID-19 pandemic: a global perspective. Can J Cardiol. 2020;36(7):1068–1080.
[73] Wang H, Endoh M. Chelerythrine and genistein inhibit the endothelin-1-induced increase in myofilament Ca2 + sensitivity in rabbit ventricular myocytes. Eur J Pharmacol. 2001;424(2):91–96.
[74] Piacentini L, Gray M, Honbo NY, et al. Endothelin-1 stimulates cardiac fibroblast proliferation through activation of protein kinase C. J Mol Cell Cardiol. 2000;32(4):565–576.
[75] Wang S, Xu D-J, Cai J-B, et al. Rapid component IKr of cardiac delayed rectifier potassium currents in Guinea-pig is inhibited by α1-adrenoreceptor activation via protein kinase A and protein kinase C-dependent pathways. Eur J Pharmacol. 2009;608(1–3):1–6.
[76] Voutilainen-Myllylä S, Tavi P, Weckström M. Chelerythrine and bisindolylmaleimide I prolong cardiac action potentials by protein kinase C-independent mechanism. Eur J Pharmacol. 2003;466(1–2):41–51.
[77] Lundmark JL, Ramasamy R, Vulliet PR, et al. Chelerythrine increases Na-K-ATPase activity and limits ischemic injury in isolated rat hearts. Am J Physiol Heart Circ Physiol. 1999;277(3):H999–H1006.
[78] Hu B, Xu G, Zheng Y, et al. Chelerythrine attenuates renal ischemia/reperfusion-induced myocardial injury by activating CSE/H2S via PKC/NF-κB pathway in diabetic rats. Kidney Blood Pressure Res. 2017;42(2):379–388.
[79] George PM, Wells AU, Jenkins RG. Pulmonary fibrosis and COVID-19: the potential role for antifibrotic therapy. Lancet Respir Med. 2020;8(8):807–815.
[80] Ojo AS, Balogun SA, Williams OT, et al. Pulmonary fibrosis in COVID-19 survivors: predictive factors and risk reduction strategies. Pulm Med. 2020;2020. Article ID 6175964.
[81] Dalvi R. Sanguinarine: its potential, as a liver toxic alkaloid present in the seeds of Argemone mexicana. Experientia. 1985;41(1):77–78.
[82] Becci PJ, Schwartz H, Barnes HH, et al. Short-term toxicity studies of sanguinarine and of two alkaloid extracts of sanguinaria canadensis L. Journal of Toxicology and Environmental Health, Part A Current Issues. 1987;20(1–2):199–208.
[83] Ulrichová J, Walterová D, Vavrečková C, et al. Cytotoxicity of benzo [c] phenanthridinium alkaloids in isolated rat hepatocytes. Phytother Res. 1996;10(3):220–223.
[84] Kosina P, Walterova D, Ulrichová J, et al. Sanguinarine and chelerythrine: assessment of safety on pigs in ninety days feeding experiment. Food Chem Toxicol. 2004;42(1):85–91.

[85] Williams M, Dalvi S, Dalvi R. Influence of 3-methylcholanthrene pretreatment on sanguinarine toxicity in mice. Veterinary and human toxicology. 2000;42(4):196–198.
[86] Vrba J, Kosina P, Ulrichová J, et al. Involvement of cytochrome P450 1A in sanguinarine detoxication. Toxicol Lett. 2004;151(2):375–387.
[87] Zdařilová A, Vrzal R, Rypka M, et al. Investigation of sanguinarine and chelerythrine effects on CYP1A1 expression and activity in human hepatoma cells. Food Chem Toxicol. 2006;44(2):242–249.
[88] Vieira SM, de Oliveira VH, do Carmo Valente R, et al. Chelerythrine inhibits the sarco/endoplasmic reticulum Ca2+-ATPase and results in cell Ca2+ imbalance. Arch Biochem Biophys. 2015;570:58–65.
[89] Gao L, Schmitz H-J, Merz K-H, et al. Characterization of the cytotoxicity of selected Chelidonium alkaloids in rat hepatocytes. Toxicol Lett. 2019;311:91–97.
[90] Zhao N-J, Wang -L-L, Liu Z-Y, et al. Pharmacokinetics of chelerythrine and its metabolite after oral and intramuscular administrations in pigs. Xenobiotica. 2021:1–24. 10.1080/00498254.2021.1882714.
[91] Basu P, Bhowmik D, Kumar GS. The benzophenanthridine alkaloid chelerythrine binds to DNA by intercalation: photophysical aspects and thermodynamic results of iminium versus alkanolamine interaction. J Photochem Photobiol B Biol. 2013;129:57–68.
[92] Dostál J, Táborská E, Slavík J, et al. Structure of chelerythrine base. J Nat Prod. 1995;58(5):723–729.
[93] Maiti M, Das S, Sen A, et al. Influence of DNA structures on the conversion of sanguinarine alkanolamine form to iminium form. J Biomol Struct Dyn. 2002;20 (3):455–464.
[94] Bhuiya S, Pradhan AB, Haque L, et al. Molecular aspects of the interaction of iminium and alkanolamine forms of the anticancer alkaloid chelerythrine with plasma protein bovine serum albumin. J Phys Chem A. 2016;120(1):5–17.
[95] Warowicka A, Nawrot R, Goździcka-Józefiak A. Antiviral activity of berberine. Arch Virol. 2020;165 (9):1935–1945.
[96] Sinha R, Kumar GS. Interaction of isoquinoline alkaloids with an RNA triplex: structural and thermodynamic studies of berberine, palmatine, and coralyne binding to poly (U). poly (A)* poly (U). J Phys Chem A. 2009;113(40):13410–13420.
[97] Parra-Medina R, Sarmiento-Monroy JC, Rojas-Villarraga A, et al. Colchicine as a possible therapeutic option in COVID-19 infection. Clin Rheumatol. 2020;39(8):2485–2486.