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RECEIVED 14 March 2023

ACCEPTED 16 June 2023

PUBLISHED 21 July 2023

CITATION

Rivero Berti I, Gantner ME, Rodriguez S,
Islan GA, Fávoro WJ, Talevi A, Castro GR
and Durán N (2023), Potential biocide
roles of violacein.
Front. Nanotechnol. 5:1186386.
doi: 10.3389/fnano.2023.1186386

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Potential biocide roles of violacein

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Violacein is a pigment produced by Gram-negative bacteria, which has shown several beneficial biological activities. The most relevant activities of violacein include the interference in the physiological activities of biological membranes, inhibition of cell proliferation, antioxidant, and anti-inflammatory activities. Moreover, the antiviral activities of violacein against some enveloped and non-enveloped viruses have also been reported. Violacein showed a wide spectrum of protease inhibition, both experimentally and *in silico*. Other *in silico* studies have suggested that violacein binds to the SARS-CoV-2 spike. Empirical physicochemical studies indicate that violacein (or, occasionally, its derivatives) may be administered orally to treat different disorders. In addition, different alternatives to product violacein, and molecular devices for delivery of this pigment are reviewed.

KEYWORDS

violacein, COVID-19, membrane interaction, antioxidant activity, anti-inflammatory activity, protease inhibition, violacein antiviral activities

1 Introduction

In the last five decades, viral infections have negatively affected the world population, mainly because of accelerated globalization and rapid urbanization, which are associated with strong changes in social habits. Many viral infections have spread worldwide through different vectors, such as dengue (DENV), Ebola, Hepatitis B virus, herpes virus, HIV/AIDS, Middle East respiratory syndrome, rotaviruses, and Zika. Recently, the global population has been in peril with the SARS-CoV-2 pandemic. The world death toll of SARS-CoV-2 overpassed 6.8 million people worldwide, and long-term deleterious consequences have been described in some of the 755 billion infected people based on the World Health Organization (WHO) website (WHO, 2023). Different therapeutic strategies have been developed worldwide for the treatment and prevention of SARS-CoV-2 infection. Many old and traditional antiviral drugs were on the battlefield trying to stop the viral pandemic but with poor or disappointing results (Das et al., 2020). The processes to develop SARS-CoV-2 vaccines, get the corresponding approval, and deliver them worldwide took approximately six to 12 months. Although large-scale worldwide vaccination has substantially reduced the death toll, new viral variants still challenge the effectiveness of marketed vaccines making their production more complex. Effective drug treatments are, however, still needed to complement vaccine development, especially for unvaccinated

people or groups that may not appropriately respond to vaccination (such as immunocompromised patients), and to cover viral mutations not covered by available vaccines (Mei and Tan, 2021).

Nature provides an enormous number of secondary metabolites with a wide variety of molecular scaffolds produced from different biological kingdoms. Among secondary metabolites, pigments have been used in many applications since the beginning of human society and are presently employed in many industrial areas, such as cosmetics, textiles, foods, and healthcare (Kulandaisamy et al., 2020). Microorganisms are one of the relevant pigment producers because they can be cultured under controlled conditions, have simple nutritional requirements, fast and reproducible growth, are easy to scale up, and provide well-established purification methods that are available in the market; they are also environmentally friendly (Mumtaz et al., 2019). *In silico* screening is among the modern approaches to find novel drug candidates; in particular, structure-based virtual screening departs from experimental or *in silico* predicted protein structures to select from large public chemical databases or relatively small in-house libraries, those compounds that are more likely to bind to a known or putative binding site (Rahman et al., 2022). Virtual screening has been extensively applied to find potential therapeutics against COVID-19. For example, using this approach 26 synthetic derivatives of coumarins and quinolines were analyzed by molecular docking and molecular dynamics; among them, six compounds were predicted to possess high binding capacity against SARS-CoV-2 main protease (M^{Pro}) (Yañez et al., 2021).

Several microbial secondary metabolites are currently marketed for therapeutic applications that started at the beginning of the last century with penicillin, diverse anthracyclines, mitomycins, etc., and have recently been reviewed (Abdelghani et al., 2021). Since the sanitary SARS-CoV-2 emergency, antiviral applications of many molecules and pigments, and their biological mechanisms have recently been revisited (Azman et al., 2018; Ma et al., 2020; Selim et al., 2021).

Violacein has recently attracted the attention of researchers owing to its wide variety of biological activities. During the last two decades, several reports have described numerous biological activities of this pigment, including immunomodulatory, antimicrobial, antiparasitic, antifungal, anticancer, and antiviral activities (Duran et al., 2021a; Duran et al., 2021b; Duran et al., 2022). The relevance of the antioxidant properties of violacein must be analyzed in the context of COVID-19 pathology, as acute infections of SARS-CoV-2 can produce cell death and long-term neurological pathologies. In general, most viral infections are related to the reduction of antioxidant physiological pathways mainly by the inhibition of Nuclear Factor Erythroid 2 (NRF2), a transcription factor (*i.e.*, leucine zipper), which triggers antioxidant proteins and hampers the NLRP3 inflammasome, which mediates the release of many cytokines (Zhu et al., 2021). Additionally, SARS-CoV-2 induces activation of Nuclear Factor kappa B (NF- κ B), promoting inflammation and oxidative stress. The direct consequence of SARS-CoV-2 is the development of elevated levels of inflammation and generalized oxidative processes. These processes are developed by the activation of pro-inflammatory cytokines (*i.e.*, Tumor Necrosis Factor- α or TNF- α , IL-1 β , and IL-6) produced by macrophages and monocytes, high recruitment of immune and endothelial cells, and platelets (Tay et al., 2020). The

NF- κ B activation induces high activities of cyclooxygenase 2 (COX2) and NOX2 (NADPH oxidase) responsible for ROS production and mitochondrial oxidative stress. Some of the postulated molecular mechanisms of SARS-CoV-2 infection have recently been reviewed (Chernyak et al., 2020). Besides, COVID-19 is not only considered a respiratory viral disease, but is also associated with endotheliopathy, triggering many molecular markers such as angiotensin 2 plasminogen activator inhibitor 1 (PAI-1) and Willebrand factor (vWF), among others, and compared to healthy individuals. These factors can be associated not only with fatigue and circulatory diseases (*i.e.*, immune thrombosis and myocardial infarction) but also with neuropsychiatric pathologies (*i.e.*, cognitive disorders and stroke) in acute COVID-19 infections and long-term COVID (Laforge et al., 2020; Fodor et al., 2021; Li et al., 2021). A recent study conducted in Nigeria showed that the levels of glutathione; vitamins A, C, and E; enzymes with antioxidant activities such as superoxide dismutase, catalase, and glutathione peroxidase; and the concentrations of Cu, Mg, Se, and Zn are lower in patients with COVID-19 than in healthy people (Muhammad et al., 2021). Intravenous administration of vitamin C, a well-known antioxidant compound, at high concentrations ameliorates inflammation and oxidative stress in patients with severe COVID-19 (Vollbracht and Kraft, 2022). COVID-19 pathology mediated by inflammation associated with the oxidative stress cascade unquestionably contributes to disease severity. Several trials of potential therapeutic antioxidant molecules to treat SARS-CoV-2 infection are in progress in the US (<https://clinicaltrials.gov/>).

It is of particular interest to analyze the antiviral activities of violacein against viruses since the emergence of SARS-CoV-19 is correlated with the scarce activity of many therapeutic molecules.

The present work endeavors to describe the potential mechanisms and properties of violacein as a potential antiviral agent against SARS-CoV-2. Analyses of the effects of violacein on cellular membranes, and antioxidant, anti-inflammatory, and antiviral activities are overviewed (Figure 1). Studies on protease inhibition and *in silico* studies have also been summarized. Finally, alternatives for the production and delivery of violacein are discussed.

2 Violacein properties and biological activities

2.1 Violacein general properties

Violacein is a low molecular weight (MW = 433.41 g/mol) bacterial purple pigment produced mainly by Gram (-) bacteria and has been recently reported (Duran et al., 2021a). *Chromobacterium* spp. and *Janthinobacterium* spp. are the most common violacein producers. (Duran et al., 2021b).

The IUPAC formula for violacein is 3-[2-hydroxy-5-(5-hydroxy-1H-indol-3-yl)-1H-pyrrol-3-yl]indol-2-one (C₂₀H₁₃N₃O₃). From a structural point of view, violacein is a bisindole molecule composed of three heterocycles and a 2-pyrrolidone molecule that links two indoles, an oxindole, and 5-hydroxyindole (Figure 2).

Violacein is a very hydrophobic molecule characterized by a P of 2.7 (PubChem) and Log Po/w = 3.34 (Choi et al., 2020).

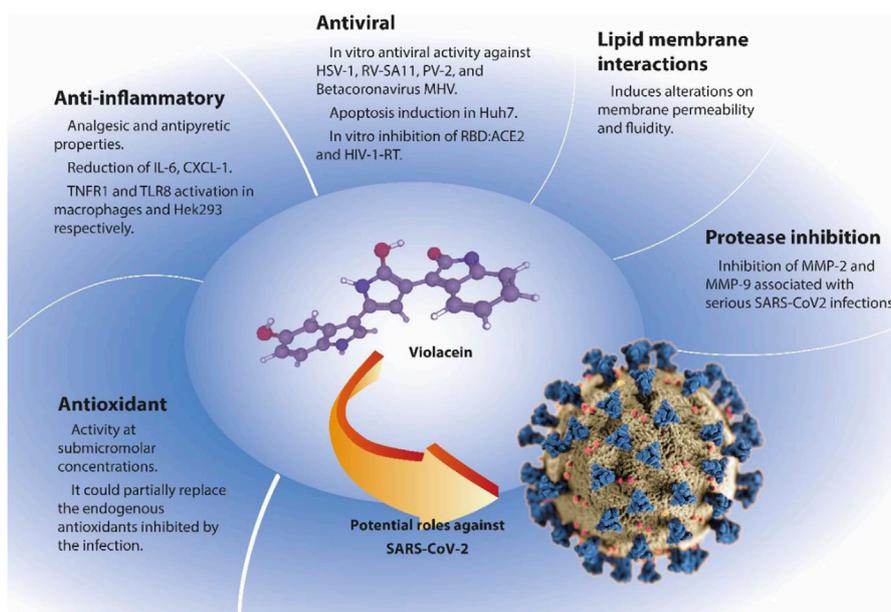


FIGURE 1

Potential antiviral violacein activities. Example: SARS-Cov-2. Abbreviations: IL-6 (Interleukin 6), CXCL-1 (chemokine ligand 1), TNFR1 (Tumor necrosis factor receptor 1), TLR8 (Toll-like receptor 8), HSV-1 (Herpes simplex virus 1), RV-SA11 (Simian rotavirus SA11), PV-2 (Poliovirus type 2), MHV (Murine hepatitis virus), Huh7 (human hepatocellular carcinoma cell line, which contains complete genome replicon of Hepatitis C virus), RBD:ACE2 (receptor-binding domain to angiotensin converting enzyme 2 [ACE2]), HIV-1-RT (human immunodeficiency virus -1 reverse transcriptase), MMP-2 and MMP-9 (matrix metalloprotease 2 and 9 respectively). Coronavirus COVID-19 structure illustration was created at the Centers for Disease Control and Prevention (CDC). Modified from the CDC, Alissa Eckert, MS; Dan Higgins, MAM (Public Domain).

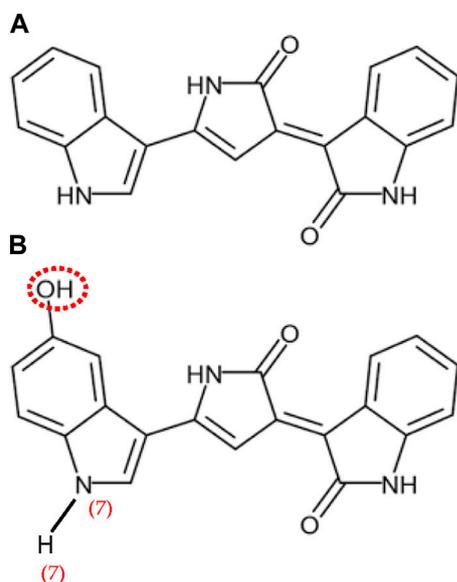


FIGURE 2

Chemical Structure of deoxyviolacein (A) and (B) violacein, adapted with permission from Nelson Durán et al., licensed under CC BY-SA 4.0.

Violacein is insoluble in aqueous media but is partially soluble in organic solvents such as acetone and dioxane, less soluble in ethanol and propanol, and soluble in ethyl acetate, DMSO, and methanol.

The UV-Visible spectra of violacein showed two maximum peaks at $\lambda_{\max} = 577\text{--}585\text{ nm}$ depending on the organic solvent, and a UV peak at $\lambda_{\max} = 260.3\text{ nm}$ in methanol (Abboud and Arment, 2013).

Violacein biosynthesis involves four genes arranged in a gene cluster *vioABCD* of 8 kb. The synthesis of violacein involves the condensation of two L-tryptophan molecules at the oxindole position, followed by intramolecular reorganization at the 5-hydroxy-indole ring (1 \rightarrow 2 shift) (Duran et al., 2021a).

The physicochemical properties of violacein were analyzed in relation to some typical empirical rules, namely: Lipinski and Veber (which predict whether a drug like compound is likely to be orally bioavailable via passive absorption) (Lipinski et al., 2001; Veber et al., 2002) and Pfizer 3/75 rule (which suggests that a drug candidates in the chemical space of low calculated log P and high topological surface area are not likely to cause significant toxicological effects at total plasma concentrations below 10 μM) (Hughes et al., 2008). Violacein fulfills all of them, thus being a good candidate for oral administration with likely low toxicity (Supplementary Table S1).

2.2 Violacein antioxidant activity

The antioxidant activity of violacein was reported for the first time in 1998 and confirmed by several extensive studies by Duran's research group during the 2000s and recently reviewed (Duran et al., 2021a; Durban et al., 2021b). The presence of several conjugated

double bonds and two peaks in the UV-visible spectra suggests a protective effect against visible and UV radiation. The experimental antioxidant activity of violacein has been confirmed by theoretical studies based on an electron density model and spectroscopic analysis (Cao et al., 2007; Jehlička et al., 2015). The estimated ionization potential (IP) of violacein is 146.88 kcal/mol which is about 5% lower than a well-known antioxidant *α*-tocopherol (IP = 154.90 kcal/mol). The antioxidant activity of violacein has been attributed to the N7-H7 band of 5-hydroxyindole (Cao et al., 2007).

In 2006, violacein antioxidant activity against lipid peroxidation was experimentally evaluated in three models of lipid membranes, including egg and soybean phosphatidylcholine liposomes, and also in rat liver microsomes. Protection of lipid membranes by violacein, either in solution or reconstituted within the liposomes, was evaluated against nitric oxide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and ascorbyl radicals in the presence or absence of the biodye showing IC₅₀ values of 21, 30, and 125 μM, respectively (Konzen et al., 2006). It was also observed that reconstitution of violacein into the liposomes enhanced its antioxidant activities. Liposomes in the absence of violacein were used as control. A more recent *in vitro* study on violacein antioxidant properties evaluated violacein partially purified from the isolate *Chromobacterium vaccinii* CV5 against common radicals. The IC₅₀ of violacein against DPPH, superoxide (produced from phenazine methosulphate), nitric oxide (produced from sodium nitroprusside), hydrogen peroxide, and hydroxyl (produced from ferrous ammonium sulfate) were 0.87 μM, 0.91 μM, 1.19 μM, 0.86 μM, and 0.85 μM, respectively (Vishnu and Palaniswamy, 2018). The lower *in vitro* IC₅₀ values reported in this study could be attributed to the partial purification of violacein. In any case, all these results suggested a protective effect mediated by violacein at micromolar or sub-micromolar concentrations against peroxidation produced by radical species.

In another study, the antioxidant activity of violacein produced by *C. violaceum* wild-type and mutants less- and non-biodye producers (*i.e.*, CV9, CV13, and CV14, respectively) was analyzed (Abboud and Arment, 2013). All *C. violaceum* strains were UV-irradiated at $\lambda = 253.7$ nm with 6,000 μW s⁻¹ cm⁻². Non-violacein-pigmented mutants did not grow, whereas the viability of the wild-type strain and violacein hyper-producer mutants was reduced by UV irradiation for 48 h. These results suggest the potential protective effects of Violacein against DNA UV-induced damage. Additionally, catalase activity in non-violacein-producing strains was enhanced compared with the enzymatic activity of violacein hyper-producer *C. violaceum* strains. This experiment strongly suggested an active scavenging role of violacein against reactive oxidative species (ROS). Violacein has been added to sunscreens to increase its protective effect against potential UV damage (Suryawanshi et al., 2015).

2.3 Violacein anti-inflammatory activity

The analgesic, antipyretic, and immunomodulatory effects of violacein produced by the newly isolated *C. violaceum* ESBV4400 were evaluated in Wistar albino rats and mice (Antonisamy and Ignacimuthu, 2010). The effects of violacein against non-inflammatory and inflammatory pain, anaphylactic

reactions and fever were assayed by injecting with ovalbumin, acetic acid, formalin, sheep red cells, and *Saccharomyces cerevisiae* yeast to create traumatic trials. The harmful effects in the treated animals were countered by using indomethacin, dexamethasone, disodium cromoglycate, naloxone, and morphine as control drugs. In the delayed hypersensitivity and provoked paw anaphylaxis tests employing red blood sheep cells and ovalbumin. Similar results were observed with 10 mg kg⁻¹ dexamethasone paired with 5 mg kg⁻¹ disodium cromoglycate and 40 mg kg⁻¹ violacein, with less than 2% difference. In the case of severe pain induced by acetic acid, the results showed a 93.9% and 78% reduction in writhing using 5 mg kg⁻¹ and 10 mg kg⁻¹ morphine and indomethacin. Similar response was elicited by 40 mg kg⁻¹ violacein. Comparable results were obtained using the formalin assay. Hyperthermia induced by the yeast *S. cerevisiae* injection in rodents showed normalization of temperature with 150 mg kg⁻¹ paracetamol after 60 min, and a similar response was observed with 20 mg kg⁻¹, and 40 mg kg⁻¹ violacein after 120 min and 60 min, respectively. The authors concluded that violacein exerts an immunosuppressive effect on inflammatory physiological responses through T Cells, antiallergic activity to the anaphylactic Ig E-mediated response, and antipyretic response by inhibiting prostaglandin synthesis.

In another report, the effect of violacein on acute and chronic inflammation in mice (C57BL/6) produced by intraperitoneal injection of 1.0 μg LPS from *Escherichia coli* 0111:B4 was analyzed (Verinaud et al., 2015). Dendritic cells and their CD80 and CD86 markers treated with LPS and violacein did not show variations compared to control cells. Comparable results were obtained for B- and T-cells, which did not show signs of toxicity. In addition, mice treated with LPS-violacein exhibited a significant decrease in neutrophil infiltration in the peritoneal cavity compared to control mice. In addition, cytokines IL-6 and CXCL-1 were reduced, whereas IL-10 was increased in the serum. An Experimental Autoimmune Encephalomyelitis mouse model was developed by induction with myelin oligodendrocyte glycoprotein (MOG35–55). The authors reported an increase in scurf protein levels (FoxP3, considered a primary regulator of T regulatory cells (Tregs) in mice treated with violacein.

A cellular study using immune lineages of human peripheral blood mononuclear cells (PBMCs) and two murine macrophage cells (ANA-1 and Raw 264.7, established by retroviral infections) treated with violacein was carried out (Venegas et al., 2019). Macrophages exposed to violacein displayed elevated levels of pro-inflammatory TNF α concomitant with TNF receptor 1 (TNFR1) activation, which induces cellular apoptosis. In addition, the authors suggested that Toll-like receptors (TLRs) could be activated by violacein in murine cells, which are strongly associated with PAMPs (*i.e.*, pathogen-associated molecular patterns). Particularly, human TLR8 was activated by violacein in the transfected HEK-293 cell line, and an *in silico* docking proposed a binding mode of violacein to the receptor similar to the antiviral drug imidazoquinolinone (Venegas et al., 2019). Members of the imidazoquinolines and imidazoles, which resemble the heterocycle structure of violacein, are known to activate TLR7/8, triggering cytokine production by the immune system associated with the clearance of virally infected cells and treatment of skin cancer. The activation of TLR7/8 by

imidazoquinolines and imidazoles depends on the electronic configuration of the heterocycles (Kaushik et al., 2021).

Briefly, the major anti-inflammatory effects of violacein were associated to the reduction of pro-inflammatory cytokines such as TNF- α , TGF- β , IL-1 β , and IL-6, and chemokines CXCL1 and CXCL12 involved in the cell recruitment but also increase the expression of IL-4 and IL-10 (Antonisamy and Ignacimuthu, 2010; Platt et al., 2014; Verinaud et al., 2015).

2.4 Violacein antiviral activities

In a pioneering study, May et al. (1989), May et al. (1991) reported the *in vitro* antiviral activity of violacein/deoxyviolacein (90/10) in HeLa cells infected with poliovirus and herpes simplex virus (HSV-1). The IC₅₀ value of violacein against HSV-1 infected cells was estimated to be 0.577 μ M. A decade later, another study challenged the *in vitro* antiviral activity of violacein against HSV-1, Simian rotavirus SA11 (RV-SA11), and Poliovirus type 2 (PV-2) strains with pigment concentrations approximately 1/3–1/2 below the cytotoxicity levels on the tested cell lines (*i.e.*, HEp-2, FRhK-4, MA104, or Vero cell cultures). The antiviral activity of 1.25 μ M violacein against the tested virus by the MTT assay ranged from 24.3% to 8.5%, depending on the virus and strain (Andrighetti-Fröhner et al., 2003).

The differences in violacein effectiveness against the tested viruses entail analyzing the main characteristics of the viral surface to underpin the potential antiviral mechanism of the pigment. Rotaviruses are non-enveloped viruses that contain a glycoprotein, VP7, attached to the cell surface. Glycoprotein VP7 is composed of 326 amino acids, with two hydrophobic domains in the amino-terminal group (Poruchynsky et al., 1985). In addition, polioviruses are non-enveloped viruses with three main proteins, VP1, VP2, and VP3, on the capsid surface, that are folded in a hydrophobic β -barrel (He et al., 2000). On the other hand, members of the herpes virus family are enveloped, having a lipid membrane structure obtained from the host covering the virion. Similarly, viral structures can be observed in coronaviruses and human immunodeficiency virus (HIV). Despite the different surface characteristics of the three tested viruses, all shared hydrophobic moieties on their surfaces that could interact with water-insoluble molecules, such as violacein. Since the interaction of violacein with molecules with hydrophobic motifs (*i.e.*, non-ionic surfactants, cyclodextrins, aromatic ionic liquids, lipid carriers) were previously reported (de Azevedo et al., 2000; Rivero Berti et al., 2019; Rivero Berti et al., 2020; Rivero Berti et al., 2022). It is expected that hydrophobic interactions appear to be the unspecific major mechanism of interaction between the virus and violacein.

Murine hepatitis virus (MHV), like SARS-CoV-2, belongs to the genus Betacoronavirus. Both are enveloped, with glycoproteins on the surface and a genome composed of positive-sense single-stranded RNA within a nucleocapsid. The inhibition of MHV-3 in L929 (mouse fibroblasts, ATCC CCL-1) infected cells by 20 μ M violacein was 42% after incubation at 37°C for 1 h (Gonçalves et al., 2023).

In a recent physicochemical study, the *in vitro* inhibitory effects of purified violacein and deoxyviolacein on CoV-2 spike RBD: ACE2 and HIV-1 Reverse transcriptase proteins were analyzed

(Supplementary Table S2). These results indicated that deoxyviolacein had a low inhibitory effect on both proteins at millimolar concentrations. Meanwhile, 1 mM violacein inhibited 94.3% of HIV-1 Reverse transcriptase, and CoV-2 spike RBD: ACE2 was inhibited by 53% at 2 mM violacein (Doganci et al., 2022). The concentration of the pigment needed to inhibit the activity of these proteins is approximately one thousand times higher than the high levels required to induce cytotoxicity in most of the reported cellular cell lines (de Sousa Leal et al., 2015; Duran et al., 2021a).

The low antiviral activity of violacein against HSV-1, Simian rotavirus 219 SA11, and Poliovirus type 2 reported previously (Andrighetti-Fröhner et al., 2003) could be attributed to its extremely poor solubility of the dye under physiological environmental conditions, which may be a major obstacle to replicate the conditions of *in vitro* assays (*e.g.*, the concentration of the dye) in cell assays or *in vivo*. The insolubility of violacein in aqueous media can be attributed to the lack of polar groups and the presence of aromatic motifs. The interaction of indole aromatic residues of different molecules that can pile on each other, which is attributed to π - π stacking, reduces violacein interactions with other molecules, including water. Consequently, molecular aggregates of violacein display reduced biological activities, similar to some low-solubility marketed drugs containing aromatic rings (Islan et al., 2012). It would be of most interest to study whether it is possible to generate violacein derivatives that retain or even potentiate the molecular interactions responsible for its antiviral effects (increasing potency) and, simultaneously, include structural modifications that diminish their π - π stacking potential, reducing their tendency to aggregate. It is important to bear in mind, however, that π - π stacking can be exploited in the design of last-generation drug delivery systems, including high purified carbon- or graphene-based nanomaterials, nanocomposites, targeted delivery systems, and prodrug delivery systems such as self-assembled polyprodrug amphiphiles (Zhuang et al., 2019). The key question appears to be: Can violacein molecular structure be modified in a way that the derivatives retain the ability to interact with viral constituents (which, as explained later, also requires π - π stacking) and load the dye into state-of-the-art drug delivery systems, and at the same time display enhanced aqueous solubility? Are these mutually exclusive properties? In any case, fine-tuning would likely be required at the molecular optimization step.

Besides that, the antiviral mechanisms of violacein were not reported yet, based on the biophysical properties of the dye it could be speculate that the hydrophobic interaction between the virus moieties and violacein will be the major driven force for virus inactivation.

2.5 Violacein and membrane interactions

Enveloped viruses are causative agents of diverse infections. Enveloped viruses have a lipid membrane covering the viral structure (*i.e.*, the capsid) that originates from the infected host. Among the advantages of encapsulated viruses, the fusion of the viral membrane with the cell membrane is considered to be one of the most effective strategies for infecting cells. In particular, many coronaviruses have a bilayer of lipids and proteins, forming an

envelope around their capsids. This envelope is derived from host membranes during virion formation but also plays a crucial role during infection when virus particles adhere to the host cell membrane (Nardacci et al., 2021). Therefore, the role of lipids in SARS-CoV-2 infection and pathogenesis cannot be underestimated. In a previous study, the existence of pockets of high hydrophobicity in the SARS-CoV-2 protein S (spike), which firmly bind linoleic acid, was described. This binding seemingly locks the S protein in a state that decreases its association with the ACE2 receptor (Toelzer et al., 2020). These types of hydrophobic pockets have previously been used to develop drugs to treat rhinoviruses (Casanova et al., 2018).

Additionally, lipid membranes play a key role in the replication of all RNA (+) viruses, including coronaviruses. These viruses manipulate the host membranes to form viral replication organelles. In addition to sequestering the cellular machinery necessary for viral multiplication, these organelles may also play a role in evading the immune response. It is known that coronaviruses alter the cellular metabolism of lipids, favoring the synthesis of sterols and fatty acids that are propitious to them in an analogous way to that in which tumor cells reprogram lipid metabolism to ensure their survival (Borella et al., 2022; Rudiansyah et al., 2022). Furthermore, coronaviruses interfere with exosome formation, autophagy, and lipid rafts (Casari et al., 2021). This reorganization of lipid metabolism and membrane mechanisms ensures or promotes viral multiplication and is, therefore, a potential target for new drugs or therapeutic strategies.

Therefore, studies of violacein-related lipids and membrane systems are of interest. While violacein does not have a typical amphiphilic head-tail structure, it is hydrophobic and has some polar groups; therefore, direct interaction with cell or viral membranes is highly probable (de Souza et al., 2017). Cauz et al. (2019) described some interactions between violacein and membranes, initially, to explain its antimicrobial activity. However, these findings may be useful in explaining its antiviral activity. *In vitro* experiments have shown that the addition of violacein to large unilamellar vesicles (LUVs) disrupts these vesicles and produces leaks, thereby affecting their permeability. Additionally, because of the low concentration of violacein required to produce these effects, they hypothesized that violacein does not act as a tensioactive, like many antimicrobials, but rather produces defects and discontinuities in phospholipid organization that alter membrane permeability (Cauz et al., 2019). This partially contrasts with previous results; de Souza et al. (2017) explored violacein interactions with phospholipid thin films to model eukaryotic plasma membranes and found that violacein effectively interferes with the correct ordering of phospholipids, affecting their compressibility and viscoelasticity, and therefore fluidity, but does not produce changes in permeability in the tested models (de Souza et al., 2017). In a recent study, the effect of violacein on synthetic monolayer lipid membranes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-ethylphosphocholine chloride salt (DSEPC), and 1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DPPG) was studied using the Langmuir-Blodgett technique. The isotherms of violacein in the cationic and zwitterionic lipid monolayers showed a decrease in lipid molecular area, suggesting compaction. However, compaction was not observed in negatively charged lipid

monolayers. Further analyses with X-rays indicate that violacein decreases the lipid tilt angle, which consequently induces the thickening of lipid monolayers (Gupta et al., 2021; Gupta and Ghosh, 2023). These results indicate the interaction of the aromatic electrons of violacein with the positive residues of lipid-like π - σ non-specific molecular interactions.

A recent study showed that the release of violacein from the cells in one of the main bacterial pigment producers, *Chromobacterium violaceum* (ATCC 12472), occurred through approximately 100 nm extracellular membrane vesicles. The study revealed that 79.5% of the violacein encapsulated in the membrane vesicles remained in solution, and the membrane vesicles provided an increase of violacein estimated in 1740 folds (Choi et al., 2020). The strategy of violacein release in *C. violaceum* possesses two relevant characteristics: first, the pigment will not be attached to the cell surface because the bacteria grow in aqueous media and violacein is a very hydrophobic molecule; and second, the released membrane vesicles can be attached to the hydrophobic surfaces of several molecules and structures, such as the VP7 glycoprotein of rotaviruses and the β -barrel structure of VP1-VP3 of poliovirus. The interaction of viral glycoproteins present on the virus surface handles virus-cell interactions, leading to causative infections (Cosset and Lavillette, 2011). Consequently, membrane vesicles containing violacein can easily merge with other membranous lipid structures of enveloped viruses (HSV-1, SARS-CoV-2, HIV, etc.).

The main cellular target of violacein are biological membranes and molecules because of violacein hydrophobicity (*i.e.*, low solubility in watery environments). Additionally, the recent finding of violacein which is released from the producer cells in the form of membranous structures will favor the interaction within mammalian cell membranes and also facilitates the entrance of violacein to the cytoplasm.

2.6 Violacein inhibition activity on proteases

The traditional mechanism of SARS-CoV-2 infection involves the binding of the spike (*i.e.*, S glycoprotein) to ACE2 (*i.e.*, angiotensin-converting enzyme type 2) to produce a membrane fusion by a serine protease (*i.e.*, TMPRSS-2) or by the cysteine protease pathways (*i.e.*, cathepsins). In recent studies, the spike in SARS-CoV-2 variants was found with a wider spectrum of action by activating the transmembrane serine protease 13 (*i.e.*, TMPRSS-13) and the matrix metalloproteases 2 and 9 (*i.e.*, MMP-2 and MMP-9). High MMP levels were found in patients with serious SARS-CoV-2 infections. Supporting the role of proteases in viral infection, protease inhibitors can reduce the infection of the SARS-CoV-2 α in the human fibrosarcoma HT1080 cell line (Benlarbi et al., 2022).

In previous work, the inhibition of matrix metalloproteases (MMPs) by violacein was reported (Platt et al., 2014). The authors reported that violacein inhibited MMP-2 in the MCF-7 cell line, and the metalloprotease played a relevant role in the secretion of CXCL12, an inflammatory cytokine. Additionally, violacein inhibited MMP-9, which downregulates the α -chemokine receptor (CXCR4) specific for stromal-derived factor-1, a chemotactic molecule for the recruiting lymphocytes (Platt et al., 2014).

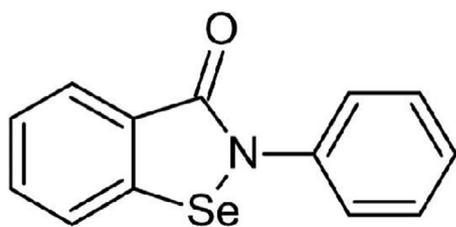


FIGURE 3
Ebselen molecular structure, reprinted from Benjah-bmm 27, Public Domain, via Wikimedia Commons

Recently it was suggested that violacein presumably acted as a protease inhibitor against the ACE2 receptor, and as an immunomodulator against COVID-19 (Durán et al., 2022). M^{pro} , also called 3CL pro , and also the papain-like protease (PL pro) is responsible for viral polyprotein disruption, a process important for the virus to survive and replicate. M^{pro} is a decisive coronavirus enzyme that plays essential roles in modulating two key processes: viral transcription and replication, which makes it an attractive pharmacological target against SARS-CoV-2.

Then, one strategy could be protease inhibition as a target to M^{pro} , once it was shown that inhibition of many proteases was discovered by researching the cytotoxic actions of violacein. Decease caused in CD34+/c-Kit+/P-glycoprotein+/MRP1+ TF1 leukemia progenitor cells was moderated by calpain (calcium-dependent protease-cysteine protease) suppression and by decease-associated protein kinase 1 (DAPK1). Comparative analysis showed that violacein also induced several protein kinases activities, such as protein kinase A (PKA), pyruvate dehydrogenase kinase (PDK), and protein kinase B (AKT), that were monitored by structural modifications induced by endoplasmic reticulum stress and Golgi apparatus collapse, as led to cell decease (Queiroz et al., 2012). It is attainable concluding some chemical structural comparison between violacein activity and that of protease suppressors such as Ebselen (Figure 3).

Following the suppression data, the HIV-1 RT suppression rate of violacein (1 mM) from the *Janthinobacterium* sp. GK strain was higher than 90%, and the SARS-CoV-2 spike RBD: ACE2 suppression rate of violacein (2 mM) was higher than 50%. *In silico* studies were performed to explore the potential interactions among deoxyviolacein and violacein and three reference compounds with the target proteins: ACE2, SARS-CoV-2 spike RBD (*i.e.*, receptor binding domain), and HIV-1 RT (*i.e.*, reverse transcriptase). Violacein seems to bind strongly to the three receptors as showed by their docking binding energies: -9.94 kcal/mol for ACE2, -9.32 kcal/mol for HIV-1 RT, and -9.32 kcal/mol for SARS-CoV-2 spike RBD. Comparable results were obtained for deoxyviolacein: -10.38 kcal/mol for ACE2, -9.50 kcal/mol for HIV-1 RT, and -8.06 kcal/mol for SARS-CoV-2 spike RBD. Following these outcomes, deoxyviolacein and violacein seem to bind to all the receptors with high efficiency. HIV-1-RT and SARS-CoV-2 spike protein suppression searches with deoxyviolacein and violacein were reported for the first time in the literature (Doganci et al., 2022).

The inhibition of diverse proteases involved in the viral infection process by violacein observed experimentally and by *in silico*

molecular docking is a relevant advantage from therapeutic point of view because it is additional feature to the many biological activities of violacein which will reduce the chance of drug multi-resistance displayed by many molecules available in the market.

2.7 Violacein cytotoxicity

In vitro cell toxicity of violacein was evaluated in four mammalian cell lines (*i.e.*, Vero, MA104, FRhK-4, and HEP-2) using cell morphology by light microscopy, cell viability by Trypan blue and by the MTT assays (Andrighetti-Fröhner et al., 2003). The violacein cytotoxic concentration 50% was in the range of 2.07 a 3.55 μ M, which depends on the used technique and the cell line. While the concentrations of violacein showed the best antiviral activity at 1.25 μ M against HSV-1 (*i.e.*, strains KOS, VR-733, RV-SA11) with inhibition percentages in the range of 17.75%–24.27% and compared with acyclovir with inhibition percentages close to 100% Besides, the antiviral violacein concentration assayed was 35.5 to 8.9 times lower compared to the acyclovir (Andrighetti-Fröhner et al., 2003). In general, violacein cytotoxicity in non-tumoral cell lines is in the range of 5 μ M–10 μ M, meanwhile tumoral cell lines are more sensitive to the biodye with a cytotoxic range of 0.71 μ M to about 5 μ M (de Sousa Leal et al., 2015; Rivero Berti et al., 2020). Several mechanisms of violacein cytotoxicity including disturbance of mammalian cell membrane and polarization of mitochondrial membrane, disruption of actin function, p44/42 mitogen activated protein kinase (MAPK), apoptosis mediated by caspase 3, etc. were reviewed recently (Duran et al., 2016; Durban et al., 2021a). Besides, it is currently accepted that violacein displays low toxicity *in-vitro* against non-tumoral cell lines.

2.8 Violacein *in silico* binding studies

In an attempt to further study the potential binding site of violacein within the SARS-CoV-2 Spike protein, five potential binding sites reported in the literature were explored through molecular docking using AutoDock4 (Morris et al., 2009). The binding sites analyzed in this study are shown in Figure 4. Two sites are located within the S2 subunit: site 1 refers to the arbidol site previously published (Shuster et al., 2021) and site 2 corresponds to the fusion peptide binding site described in a recent report (Hu et al., 2021). The other sites are located within the S1 subunit: site 3 is a potential allosteric site reported by Wang et al. (2022), site 4 corresponds to the experimentally determined biliverdin site in the N-terminal domain (NTD) (Rosa et al., 2021), and site 5 addresses two possible reported sites in the RBD subunit, which are the Spike: ACE2 protein-protein interaction (PPI) interface (site 5a) (Bojadzic et al., 2021) and the site reported by Doganci et al. (2022) for violacein from the blind docking study on the isolated RBD subunit (site 5b). In addition, the entire RBD subunit was considered for the docking calculations to explore other possible binding sites within such subunit (site 5y). The biliverdin site has not been considered an inhibitor site, because biliverdin binding helps Spike evade antibody immunity (Rosa et al., 2021). Nevertheless, it was decided to include it in the present study

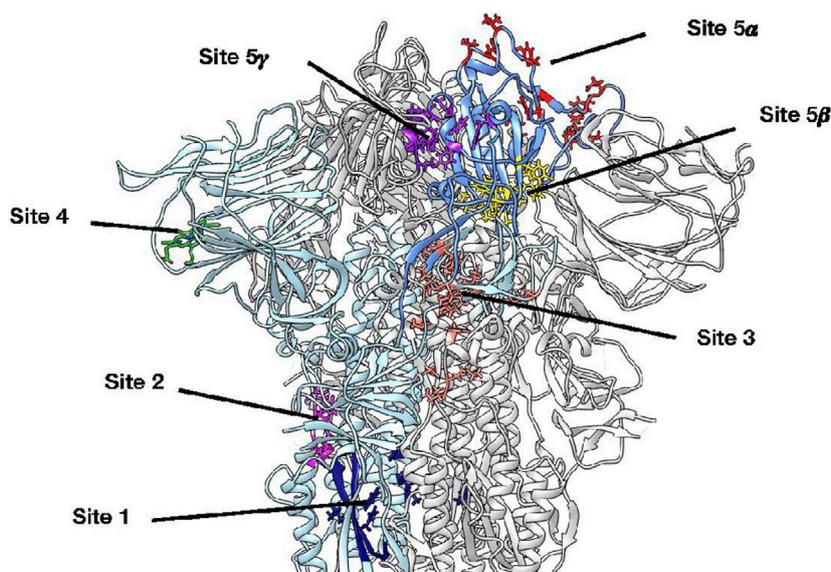


FIGURE 4

3D structure of the SARS-CoV-2 Spike protein, where all potential violacein binding sites studied by molecular docking are highlighted on protomer A. Protomer A is colored light blue while the corresponding RBD subunit is cornflower blue. Site 1 (navy blue) refers to the arbidol site located within the S2 subunit, site 2 (magenta) corresponds to a fusion peptide site, site 3 (salmon) is a potential allosteric site within the S1 subunit, site 4 (lime green) corresponds to the biliverdin site in the NTD, site 5 α (red) refers to the Spike: ACE2 PPI interface, site 5 β (yellow) corresponds to the site reported by Doganci et al. (2022) for violacein from a blind docking study on the RBD subunit, and site 5 γ (purple) emerged by considering the entire RBD subunit for docking calculations; to the best of our knowledge, this is the first report on this putative binding site.

because it is an experimentally validated binding site for small molecules.

The docking results obtained are shown in [Supplementary Table S3](#). The most favorable score considering the three protomers was obtained for the 5 γ site in the RBD subunit. While site 5 γ is likely to be the most probable binding site, site 2 and site 3 are also probable candidates.

Considering site 5 γ in protomer A, violacein was located in an environment defined by residues Phe338A, Tyr365A, Tyr369A, Ala372A, Ser373A, Phe374A, Asp405C, Glu406C, Arg408C, Gln409C, Thr415C, Gly416C, Lys417C ([Figure 5A](#)), finding a parallel-displaced π -stacking with Tyr365A, a T-shaped π -stacking with Tyr369A, hydrogen bonds with Tyr369A, Ser373A, and Thr415C, and a π -cation interaction with Arg408C ([Figure 5B](#)).

To further study the different interactions between violacein and the 5 γ binding site in the protomer A, 50 nanoseconds of molecular dynamics (MD) simulation was carried out using GROMACS 2020.4 Molecular Dynamics engine ([Abraham et al., 2015](#)).

[Figure 5C](#) shows the root-mean-square deviation (RMSD) for violacein as a function of the simulation time. The average RMSD value was 0.645 Å with a standard deviation of 0.152 Å, indicating high ligand stability at the 5 γ site. By exploring the interactions that stabilize the complex during the MD simulation through the measurement of the distances between violacein atoms and the key residues of the protein defined by docking ([Supplementary Figures S1–S5](#)), it can be concluded that the stabilization of violacein at the 5 γ site appears to be mediated mainly by π -stacking and π -cation interactions, with a coordinated contribution from hydrogen bonding. The docking and MD procedure are described in the [Supplementary Material](#) together with a detailed analysis of the interactions observed in this study.

Finally, due to the novelty of this site, the SARS-CoV-2 Spike protein was further analyzed through the DoGSiteScorer web server, a grid-based method that uses a Difference of Gaussian filter to the binding pocket prediction, characterization, and druggability estimation ([Volkamer et al., 2012](#)). This analysis ranked the 5 γ site in third place out of more than 100 potential sites found in the protein, with a druggability score of 0.85 (0.86 being the highest druggability score obtained).

2.9 Violacein production

The large-scale production of microbial pigments is seriously limited by the production-purification costs and regulations in dominant pharmaceutical countries. In 2018, the world market for organic dyes was valued at USD 3.5 billion, with an estimated growth of about 37% by 2024 ([Cassarini et al., 2021](#)). The most representative studies on violacein production are briefly described below, including the use of recombinant microorganisms and the new trend of recycling waste in the frame of the circular economy.

Violacein production was initially studied in the 2000s. The first work on violacein production was conducted in *C. violaceum* and *Janthinobacterium lividum* in synthetic liquid media ([Mendes et al., 2001](#); [Nakamura et al., 2003](#)). The main guidelines for the production of violacein from *C. violaceum* can be found in the websites of the American Type Culture Collection (ATCC, United States, <https://www.atcc.org/products/12472>), and the National Collection of Type Cultures (NCTC, United Kingdom, <https://www.culturecollections.org.uk/products/bacteria/detail.jsp?collection=nctcandrefld=NCTC+9757>).

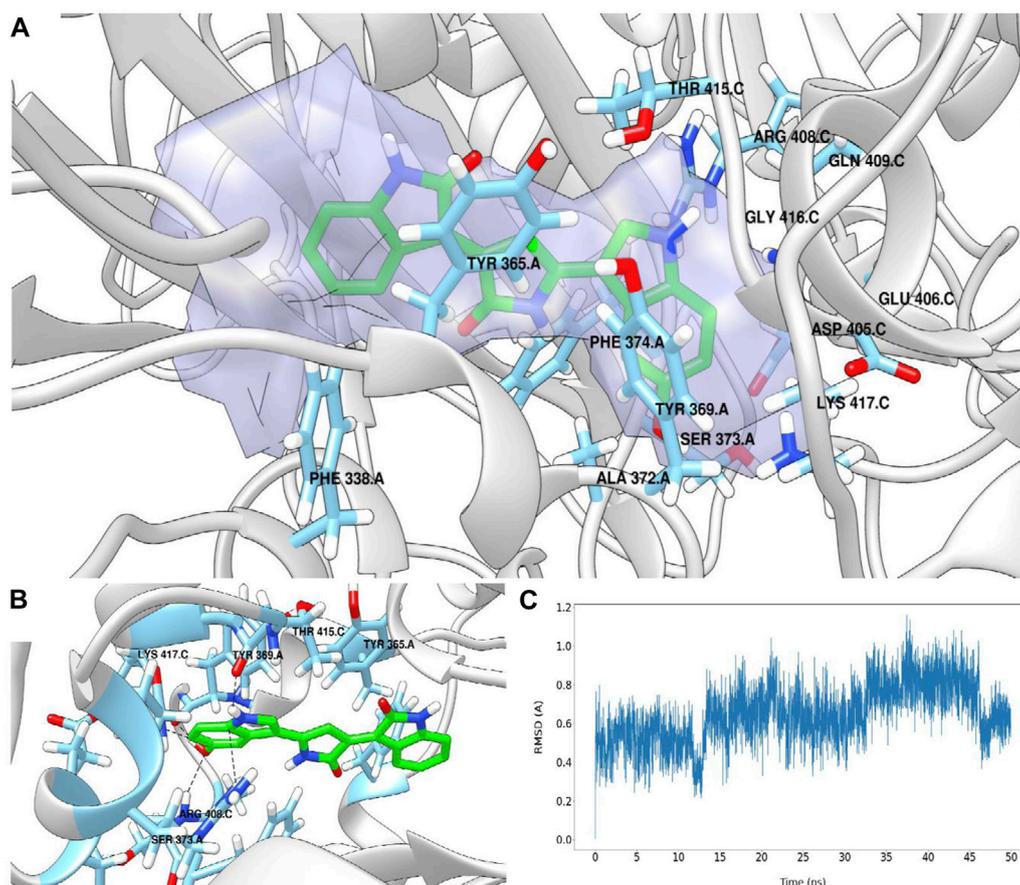


FIGURE 5

Binding site 5 γ in protomer A predicted for violacein by molecular docking and characterized by the DoGSiteScorer web server (A). Pose prediction for violacein. Some relevant interactions are shown with black dashed lines (B). Violacein's RMSD (Å) vs. simulation time plot (C).

In general, it is currently accepted that carbon sources play a crucial role in pigment synthesis; in particular, glycerol increases while glucose represses its production (Pantanello et al., 2007; Duran et al., 2021). These results indicate shared pathways for simple carbon sources, as well as the presence of catabolic repression by glucose. A systematic statistical study of violacein production by *C. violaceum* CCT 3496 cultured in a 50 mL flask containing glucose-rich media supplemented with tryptophan was conducted using a fractional factorial design followed by a central composite design. The violacein production increased from 7.5 g L⁻¹ dry cell mass and 0.17 g L⁻¹ crude violacein concentration to 21 g L⁻¹ and 0.43 g L⁻¹, respectively (Mendes et al., 2001). The ratios of dry cell mass/crude violacein concentration were approximately 44 and 49 for initial and optimized production, respectively. These results indicate that coupled violacein production is related to bacterial cell mass.

The violacein producer *Duganella* sp. B2, isolated from a glacier in China, was statistically optimized using a two-level Plackett-Burman, Box-Behnken, and surface response for biodye production. The experiments were carried out in 250 mL stirred flasks containing minimal saline medium and starch as a carbon source supplemented with tryptophan. The highest violacein concentration produced in the optimized medium was 1.62 g L⁻¹ after 32 h of culture (Wang et al., 2009).

The production of violacein from a wild-type isolated psychotropic bacterium RT102, close to *J. lividum*, cultivated in a 3-L fermenter containing rich media (*i.e.*, glucose, casein, and yeast extract) under physicochemical controlled parameters resulted in a maximum violacein concentration and productivity of 3.7 g L⁻¹ and 0.12 g L⁻¹ h⁻¹ at 40 h culture (Nakamura et al., 2003).

Several efforts have been made to obtain recombinant bacterial strains that are capable of producing high concentrations of violacein. Most studies have focused on *E. coli* (Rodrigues et al., 2012; Rodrigues et al., 2013; Fang et al., 2015). The *E. coli* TOP10 strain transformed with the plasmids pJP1000 and pPSX-Vio + which are harboring the violacein operon *pBvioABCDE* from *C. violaceum* showed low levels of violacein production ranging from 0.006 to 0.025 g L⁻¹, which was comparable to the biodye production by *J. lividum* DSM 1522 under the same experimental conditions (Rodrigues et al., 2012). In another study by the same research group, different *E. coli* strains were transformed with plasmids and the integrated gene responsible for violacein production. The mutant *E. coli* dVio-1 and dVio-6 expressing the *vioABCDE* gene cluster accumulate 0.18 g L⁻¹ and 320 mg L⁻¹ of deoxyviolacein intracellularly, respectively. Integration of the *vioD* gene into the *E. coli* genome, *E. coli* Vio-4, produced 0.71 g L⁻¹ of intracellular violacein by fed-batch fermentation (Rodrigues et al.,

2013). In future work, different recombinant *E. coli* BL21 strains were used as a chassis for the expression of violacein genes inserted in a plasmid associated with the enhanced production of tryptophan. Flask culture in glucose saline medium of the recombinant strain *E. coli* B2/pED + pVio produces 0.60 g L⁻¹ violacein at a concentration of 48 h. Scale-up of violacein production in a fermenter with an optimized glucose medium showed a titer of 1.75 g L⁻¹ associated with 36 mg L⁻¹ h⁻¹ biodye productivity (Fang et al., 2015).

In another microbial recombinant strategy, *Citrobacter freundii* containing the violacein gene cluster in a plasmid (i.e., pCom10vio) was cultured in a 2 L minimal salt medium supplemented with glycerol and tryptophan in a 5-L fermenter using a fed-batch technique. The optimized yield parameters were 3.34 g L⁻¹ dry cell mass, maximum violacein concentration of 4.13 g L⁻¹ with violacein productivity of 0.083 mg L⁻¹ h⁻¹ (Yang et al., 2011).

However, some issues must be taken into account for potential commercial applications of violacein in pharmaceutical applications because the high production and/or productivity of violacein in recombinant strains depends on the strain stability during scale-up and successive microbial cultures, violacein purification complexity (i.e., steps and procedures) because the biodye is located intracellularly in most recombinant microorganisms, and the presence of violacein precursors and/or cometabolites (i.e., protoviolaceinic acid, deoxyviolacein, etc.). Alternatively, the use of agricultural food waste can be an alternative to reduce violacein-producing costs as well as co-production with other metabolites of industrial interest. Pioneering investigations reported in the literature for violacein production were developed by Ahmad's research group at the University Technical of Malaysia. A wild-type strain of *C. violaceum* isolated from a waste plant has been extensively studied for the production of violacein in liquid media supplemented with local agricultural wastes and/or cheap substrates, such as brown sugar, molasses, solid pineapple waste, sugarcane bagasse, and commercial-rich media. Violacein production in the rich medium was very low or negligible. While the high biodye productions were 0.19 g L⁻¹ and 0.82 g L⁻¹ obtained in a medium containing 1% and 3% sugarcane bagasse supplemented with tryptophan, a pigment precursor, respectively (Ahmad et al., 2012).

Violacein production in *C. violaceum* UTM5 was studied by combining static and stirred growth conditions, and violacein production was between 0.17 g L⁻¹ to 0.26 g L⁻¹. However, high production was observed when the microorganism was first cultivated under static conditions followed by stirring. After environmental optimized culture conditions, the microorganism produced 16.26 ± 0.44 g L⁻¹ of violacein in a 50 L fermenter containing 45 L of the medium of filtered pineapple waste supplemented tryptophan at 30°C for 24 h (Aruldass et al., 2015).

Microparticulate wheat bran, a low-cost substrate with high lignocellulose content, was used to supplement the Luria-Bertani medium for violacein production using *C. vaccinii* in batch culture (Cassarini et al., 2021). The optimal violacein production was 0.208 g L⁻¹ after 73 h of culture.

In a recent study, the simultaneous production of poly hydroxybutyrate (PHB) and violacein was reported (Kumar et al., 2021). The synthesis of PHB and violacein by wild-type *Iodobacter* sp. PCH194 was optimized in a 22-L fermenter containing glucose and tryptone in a saline medium. The maximum production of PHB

was 11.0 ± 1.0 g L⁻¹, and a mixture of violacein (50%–60%) and deoxyviolacein (40%–50%) was obtained. The highest concentration of violacein was 1.5 g L⁻¹ at 48 h of cultivation.

The main data on violacein production using different microorganisms, methodologies, and brief culture conditions are displayed in Supplementary Table S4. Another factor to be considered is related to the obtention of violacein from the culture media. Briefly, at the late stationary phase, the bacterial cells were centrifuged, and the violacein was extracted from the pellet with ethanol (Mendes et al., 2001).

Among the advantages of violacein production are the standard conditions of production, short times for production, the facile bacterial cultivation in fermenters, and scale up with traditional strains, the low nutritional requirements of the microorganism for the biodye production which could include wastes (e.g. with the advantage of the circular economy), easy recovery of the pigment and purification because of high hydrophobicity.

2.10 Development of violacein devices

Violacein attached to different materials has two main applications: as a filter barrier with antiviral activity or loaded into particulate systems for drug delivery (Khaksar et al., 2021; Fu et al., 2023).

Because the human coronavirus is mainly transmitted by aerosols caused by coughing, sneezing, breathing, or speaking, there is a need to establish a physical barrier for the dispersion of these aerosols. However, the infectivity over time of the coronavirus in a droplet of aerosol depends greatly on the surface where it is deposited, being able to remain active for several hours in some cases (Fu et al., 2023). This is why a functional material must not only retain droplets but also inactivate the virus.

Strategies have been developed to use violacein as a biodye in the development of functional fabrics, taking advantage of its antiviral properties. Three different methods for dyeing polyamide fabrics with violacein have been tested (Kanelli et al., 2018). Direct fermentation of *J. lividum* on the fabric, exposure of the fabric to the culture after fermentation, and exposure of the fabric to an acellular extract of the culture. Improved color retention and antimicrobial and biofilm inhibition activities were obtained using the first method (Kanelli et al., 2018). With the same goal of producing functional fabrics, Gao et al. (2019) developed a silk-based composite that takes advantage of the antimicrobial synergy between violacein and silver nanoparticles (Gao et al., 2019). Electrospinning techniques have also been tested to design fabrics based on violacein-containing synthetic polymers, such as Nylon-66 and Polyvinyl-alcohol/polyvinyl-pyrrolidone (Osman and Setu, 2018; Rosli and Setu, 2018). Also, by using electrospinning, Lee et al. (2022) have recently taken this concept further; developing prototype respiratory masks from polyacrylonitrile nanofibers with violacein, demonstrating aerosol retention from 0.8 to 3.4 μm (human-produced aerosols being between 0.74 and 2.12 μm), antimicrobial activity against *S. aureus* and antiviral activity against human influenza A and human coronavirus. Violacein also provides selective UV protection to the fabric (Lee et al., 2022). In particular, these violacein-loaded materials retain the inactivating activity of coronaviruses and influenza and could constitute products with real applications, such as surgical masks.

In another study, viscose fabric was incubated in the presence of violacein, followed by the incorporation of silver and titanium dioxide nanoparticles by sonication. The antimicrobial activity of the fabric containing violacein and nanoparticles showed a 3- to 6-log reduction in the growth of *S. aureus*, *B. cereus*, and *E. coli*, indicating the effectiveness of the fabric derivatized with violacein and nanoparticles (Khaksar et al., 2021).

Another potential application of violacein is to include biodye in 3D matrices for molecular delivery. In 2000, violacein was added to a β -CD solution and precipitated. The violacein complexed in cyclodextrin showed the same biocide activity against *E. coli*, but the cytotoxicity in lung fibroblasts of Chinese hamster V-79 cell cultures was reduced, and lipid peroxidation was fully inhibited at 500 μ M of the violacein- β -cyclodextrin complex (). Later, a proof of concept was developed by the encapsulation of violacein in nanoparticles of poly-(D, L-lactic-co-glycolic acid) for assaying antimicrobial activity. PLGA nanoparticles with 116 and 139 nm diameter containing violacein efficiently inhibit five strains of *S. aureus*, including antibiotic-resistant (MRSA) strains, at micromolar concentrations three to five times lower than free violacein (Martins et al., 2011). In a subsequent study by Durán et al., violacein was encapsulated in nanoparticles made of poly (ϵ -caprolactone) covered with chitosan to produce a positively charged device and increase mucoadhesiveness (Berni et al., 2013). Violacein nanoparticles of 201–320 nm were tested to prevent bovine mastitis against *S. aureus* MRSA with a low minimal inhibitory concentration compared to free biodye. Additionally, violacein encapsulation diminished ecotoxicity by approximately 10 to 5 times using *Daphnia similis* according to the OCDE guidelines.

Another strategy for violacein encapsulation is the use of Arabic gum as a matrix (Venil et al., 2015). Violacein microparticles can be used to provide color to foods, such as jellies and yogurts. Violacein encapsulated in Arabic gum was spray-dried and displayed high stability in the range of 25°C–60°C for 30 days.

Similarly, based on the low violacein solubility in aqueous media, the biodye was first suspended in a polyoxyethylene sorbitan monolaurate solution and showed high stability at room temperature for 6 days. Later, the violacein emulsion was successfully encapsulated in gelatin-pectin coacervate and tested against HCT-116 colon cancer (Rivero Berti et al., 2019). More recently, violacein was encapsulated in a nanostructured lipid carrier with an active release by lipase and 3D printed using a hydroxypropyl cellulose-chitosan matrix. The formulation was tested against the A549 and HCT-116 cancer cell lines (Rivero Berti et al., 2022).

Other strategies have been developed to increase the biocidal activity of violacein by using metallic nanoparticles. Silver nanoparticles with violacein adsorbed in the surface displayed high antibacterial activities against multiresistant bacterial strains of *P. aeruginosa*, *E. coli*, and *S. aureus*, also tested against *Aspergillus tamari*, *Aspergillus tubingensis*, and *Fusarium proliferatum*, and also algicidal activities against *Dictyosphaerium* sp. strains DHM1 and DHM 2, and *Pectinodesmus* sp. strain PHM 3. In all cases, the biocide activity of AgNPs capped with violacein against bacteria, fungi, and algae were enhanced by the presence of the biodye (Arif et al., 2017). Similar results were obtained for silk coated with AgNPs and violacein, as previously described (Gao et al., 2019).

A novel strategy for repositioning old antibiotics is to develop a combination of antibiotics and violacein. The authors developed a 1:1 individual mixture of 20 antibiotics with violacein and tested these

mixtures against *Salmonella typhi*, *Vibrio cholerae*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*. In most cases, the biocidal activity of violacein is synergistic and/or additive concerning the antibiotic (Subramaniam et al., 2014). This strategy offers several advantages. First, the antibiotic-violacein mixtures can increase the biocide activity against pathogens because of different molecular targets, decreasing the amount of the antibiotic concomitantly with a decrease in potential toxicities, and increasing the bioavailability of the components.

Additionally, the interaction of ten imidazole ionic liquids with violacein was analyzed. Imidazole ionic liquids and violacein form stable micellar solutions that are encapsulated in solid lipid nanoparticles. The anticancer activity of the violacein-imidazole ionic liquids formulation against A549, HeLa, and HCT116 cells was tested. These results indicated that the imidazole group of the ionic liquids associated with violacein displayed strong anticancer activity (Rivero Berti et al., 2020). Further computational studies have demonstrated the effectiveness of imidazole derivatives against SARS-CoV-2 (Belhassan et al., 2020). Data from both studies showed promising results for the use of violacein-imidazole ionic liquid formulations for SARS-CoV-2 therapy.

Taking into consideration what is stated in this section, not only should the biological activity of violacein be considered, but also its ability to form functional materials. The latter one is a relevant challenge that will result in an improved biocide and antiviral activities.

3 Conclusion

Many biological activities of violacein were extensively described in the literature. The previous focus was to use violacein in the treatment of different types of cancer. However, recently found violacein properties such as its effect on cellular membranes, and anti-inflammatory, antioxidant, and antiviral activities suggest that another application is possible. Other detailed studies of violacein activities revealed a wide spectrum of protease inhibition. Additionally, *in silico* studies through molecular docking confirmed the binding activities of violacein within the SARS-CoV-2 spike in several sites. However, in some cases, the antiviral titers of violacein are not enough to be considered as a potential virucide agent. These results could be attributed to two factors, first the presence of other molecules in the violacein extracts because of a lack of appropriate purification steps and quality controls, and second because of violacein low bioavailability due to its very low solubility in physiological media. Besides, the production of violacein from different strains and procedures is in *the state of the art*, but it is essential to standardize pigment purification procedures. Also, the potential oral administration of violacein will require the development of novel strategies for molecular delivery systems to obtain more efficient biocide activities.

Author contributions

IR, writing, drawing, reviewing. MG, docking and molecular studies, writing. SR, docking and molecular studies, writing. GI, editing, reviewing. WF, editing, reviewing. AT, critical review, and text edition, discussion, and manuscript revision. ND, writing, text edition, and discussion. GC, organization, writing, editing, critical

review, discussion, and manuscript revision. All authors contributed to the article and approved the submitted version.

Funding

Financial support by UNLP (X815), CONICET (PIP 0034), and ANPCyT (PICT 2016-4597; PICT 2017-0359) is gratefully acknowledged.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnano.2023.1186386/full#supplementary-material>

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