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Review

Secondary metabolites as potential drug candidates against Zika virus, an emerging looming human threat: Current landscape, molecular mechanism and challenges ahead

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ABSTRACT

Nature has given us yet another wild card in the form of Zika virus (ZIKV). It was found in 1947, but has only recently become an important public health risk, predominantly to pregnant women and their unborn offspring. Currently, no specific therapeutic agent exists for ZIKV and treatment is mainly supportive. Natural products (NPs) can serve as a major source of potent antiviral drugs. To create this review, a comprehensive search was conducted from different databases (PubMed, ScienceDirect, Google scholar). A statistical analysis on the number of publications related to NPs and ZIKV was conducted to analyse the trend in research covering the period 1980–2020. From the data collated in this review, a number of NPs have been found to be inhibitive towards different stages of the ZIKV lifecycle in in vitro studies. For instance, baicalin, (-)-epigallocatechin gallate, curcumin, nanchangmycin, gossypol, cephaeline, emetine, resveratrol, berberine, amongst others, can prevent viral entry by attacking ZIKV E protein. Compounds luteolin, myricetin, astragalin, rutin, (-)-epigallocatechin gallate, carnosine, pedalitin, amongst others, inhibited NS2B-NS3 protease activity which consequently hamper replication. Interestingly, a few NPs had the ability to arrest both viral entry and replication, namely baicalin, (-)-epigallocatechin gallate, curcumin, cephaeline, emetine, and resveratrol. To the best of our knowledge, we obtained only one *in vivo* study conducted on emetine and results showed that it decreased the levels of circulating ZIKV by approximately 10-fold. Our understanding on NPs exhibiting anti-ZIKV effects in in vivo testing as well as clinical trials is limited. Our trend analysis showed that interest in searching for a cure or prevention against Zika in NPs is negligible and there are no publications yet covering the clinical evaluation. NPs with anti-ZIKV property can a winning strategy in controlling the bio-burden of an epidemic or pandemic. We therefore opine that in the future, more research should be devoted to ZIKV. This review attempts to provide baseline data and roadmap to pursuit detailed investigations for developing potent and novel therapeutic agents to prevent and cure ZIKV infection.

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Contents

Introduction

Natural products (NPs) represent the golden key in medicinal chemistry and drug discovery. They offer special features compared to conventional synthetic compounds which present both benefits and limitations to the pharmaceutical industries. They predominantly have a higher molecular mass, a larger number of sp^3 carbon atoms and oxygen atoms but fewer nitrogen and halogen atoms, higher numbers of H-bond acceptors and donors, lower calculated octanol-water partition coefficients and greater molecular rigidity in comparison to synthetic compounds [1]. These dissimilarities can be favourable while designing drugs to challenge protein-protein interactions [2]. The importance of NPs is not recent but dates back to years ago. Historically, human beings have been using NPs as a treatment strategy to fight numerous diseases. They represent a wide spectrum of diverse chemical entities with potential biological activities that have multiple applications, notably in human medicine [3]. The great structural diversity and complexity possess by NPs have allowed the production of nearly 50 % of today's pharmaceutical drugs [4]. Drugs derived from NPs have some real success stories and many accounts of these can be found in the literature. Some notable examples include the anti-inflammatory drug, salicin (isolated from *Salix alba* L.), the anti-malaria drug, quinine (derived from *Cinchona succirubra* Pav. ex Klotsch), the anti-glaucoma drug, pilocarpine (present in *Pilocarpus jaborandi* Holmes) which are among the most important pharmaceutical products in the world [5]. Apart from plants, microorganisms (bacteria, fungi) are also an untapped resource of valuable producers of bioactive NPs [6,7].

While scientific advances have led to large-scale production and widespread distribution of therapeutic drugs, development of antiviral drugs is still at an infancy stage despite viruses still represent a major threat to human health today as witnessed by the current COVID-19 pandemic. The first 30 years of NP discovery was spent on antibacterial and antifungal medications but little efforts were directed towards the discovery and development of antiviral compounds [3]. This research trend can be devastating for the well-being of future generations. The rise of a novel viral outbreak in the future is not a matter of 'if', but instead a matter of 'when'. Thus, it is crucial that the a priori development of antiviral drugs and prophylactic vaccines with pandemic potential is given a careful thought. Even though, viruses are equally deadly as chronic diseases, a lack of effort is noted in antiviral drug discovery. Antiviral development is not considered to be an economically wise investment for the pharmaceutical industry as these drugs may not be as profitable as drugs that treat chronic conditions, such as cancer, diabetes, neurodegenerative disorders, or cardiovascular diseases [8].

Viruses continue to create havoc to public health globally. In 2016, a global epidemic was declared as a Public Health Emergency of International Concern by the World Health Organisation (WHO) when a bevy of cases was recorded in 27 countries in America. The viral outbreak was caused by a mosquito-borne flavivirus known as the Zika virus (ZIKV). Between the year 2015 and 2016, ZIKV caused a pandemic with more than one million cases reported only in Latin America [9]. Since then, ZIKV has rapidly been transmitted to 86 countries or territories with a total of 223, 477 cases confirmed [10]. Further details on the origin, interactions and transmission of the virus are given later in the different sections of this review. So far, developing an effective anti-ZIKV drug or vaccine has been a failure. However, effort should remain a continuing need to remove the lacunae present in the development of successful antivirals. There is an urgency for global-preparedness programmes that potentiate our ability to rapidly screen and test NPs as future anti-ZIKV drugs, and for developing safe, effective, easy-to-produce and cost-effective therapeutics in a reasonably short timeframe. Existing literature supported that NPs can serve as a major source of antiviral drugs as reviewed elsewhere [4,11]. The unprecedented speed of research and development presently focused on COVID-19 may serve as a template to prevent the impact of future viral pandemics from disturbing socioeconomic activities [12].

Nature acts as a major stockpile of potential phytochemicals that require in-depth exploration in order to develop drugs for treating numerous maladies including viral diseases [13]. Until now, a considerable number of herbal medicines or their respective NPs have exhibited promising antiviral properties [11]. However, as we wade through literature, a dearth of intensive research on the development of anti-ZIKV agents from such NPs is noted. Using our multidisciplinary expertise and information gathered from existing literature, we want to provide an overview on the central role of NPs in the discovery and development of potential anti-ZIKV drugs or

vaccines. In 2022, a review article compiled using in silico data suggesting glycocin F as a natural biomolecule with high potential to be developed as a broad-spectrum antiviral agent [14] and a research article concluding plant extracts as a valuable source of antiviral compounds against ZIKV [15] had formed a foundation of support and hastened the urgent research on Zika that was required. The hallmark of this review is to scrutinise current understanding of the anti-ZIKV aspects of NPs and their underlying mechanism of actions. The current snapshot of in vitro, in vivo studies and clinical trials are also reviewed. In the last section of this review, we criticise on the way research are being conducted on ZIKV to determine whether we are going in the right direction or not by analysing the trends in PubMed publications.

Zika virus: origin, structure, transmission, interaction and mechanism of action

ZIKV, an arbovirus originating from the Flavivirus genus of the *Flaviviridae* family, has close resemblance to other viruses namely Dengue, Yellow Fever, West Nile, Japanese Encephalitis and Tick-borne Encephalitis Virus [16]. ZIKV genome has about 10.7 kb, encoding 3423 amino acids, with two flanking 5′ and 3′ noncoding regions and a single long structure encoding a polyprotein, which is cleaved into capsid protein (C), precursor protein (prM), envelope protein (E) and seven non-structural proteins (NS): 50-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5–30 [16]. Fig. 1 illustrates the structure and genome of ZIKV. It was in the year 1947 in Uganda that the ZIKV was isolated for the first time from the serum of a sentinel Rhesus macaque monkey in the Zika forest [17]. ZIKV can be transmitted by mosquito bites (27 species), tick bites (12 species) or by unknown arthropod vectors (14 species) [18]. However, *Aedes* mosquitoes are the main vectors of this virus namely *Aedes africanus*, *Ae. aegypti, Ae. albopictus*, and *Ae. hensilli* [10]. Little attention was given to this virus until the outbreaks reported in Yap Island in 2007 and in French Polynesia in 2013. Severe complications of ZIKV infection were observed in a patient in the French Polynesia with an autoimmune disease that affect the peripheral nervous system known as Guillain-Barre Syndrome [19].

ZIKV has recently garnered a fair amount of media attention internationally as a major emerging pathogen as ZIKV fever is transitioning from epidemic to a global pandemic. The speed of transmission is alarming and do impart because 80 % of infections are asymptomatic [20]. While massive media coverage highlights the growing threat and attempts to battle the disease, very little is known about the biology of ZIKV. Presently, research breakthroughs are beginning to unmask the mystery of Zika and could be the first step to finding a treatment or even a cure. The ZIKV is transmitted through the *Aedes* mosquitoes as mentioned before. Transmission occurs in several ways. First, when a female *Aedes* mosquito is probing for a blood vessel, ZIKV travels from the mosquito saliva into the skin. Also, as ZIKV is similar to other flaviviruses, a small amount of the virus enters directly into the bloodstream while the mosquito is feeding. Second, transmission can also happen via non vector approaches such as sexual, congenital transmission, blood transfusion and organ transplant [21]. In the skin, the virus primarily infects the fibroblasts but can also infect immature dendritic cells and epidermal keratinocytes. Through a series of processes, ZIKV hijacked the cells to serve as virus factories followed by disruption of the cells. In pregnant women, the threats to fetal and child health posed a challenging problem and consequently cause a lot of damage. How the ZIKV affect the foetus is elucidated in Section 2.2 of this review.

How Zika attacks in cells?

The ZIKV is believed to enter cells through one or more receptors. Some of the identified receptors including DC-SIGN, TIM1, TIM4, AXL and TYRO3 [22]. However, AXL is believed to be a key receptor for ZIKV entry. AXL appears on both fibroblasts and hNPCs. AXL is also highly expressed on some retinal cells and microcephaly cases have shown significant ocular abnormalities [23]. Interestingly, AXL has also showed to mediate dengue virus suggesting close similarity between the pathways used by these two viruses. Once inside the cells, the ZIKV is believed to follow a route similar to other envelope viruses. ZIKV is delivered to an endosome which provides the virus transport into the cytoplasm. The virus envelope then fuses with the

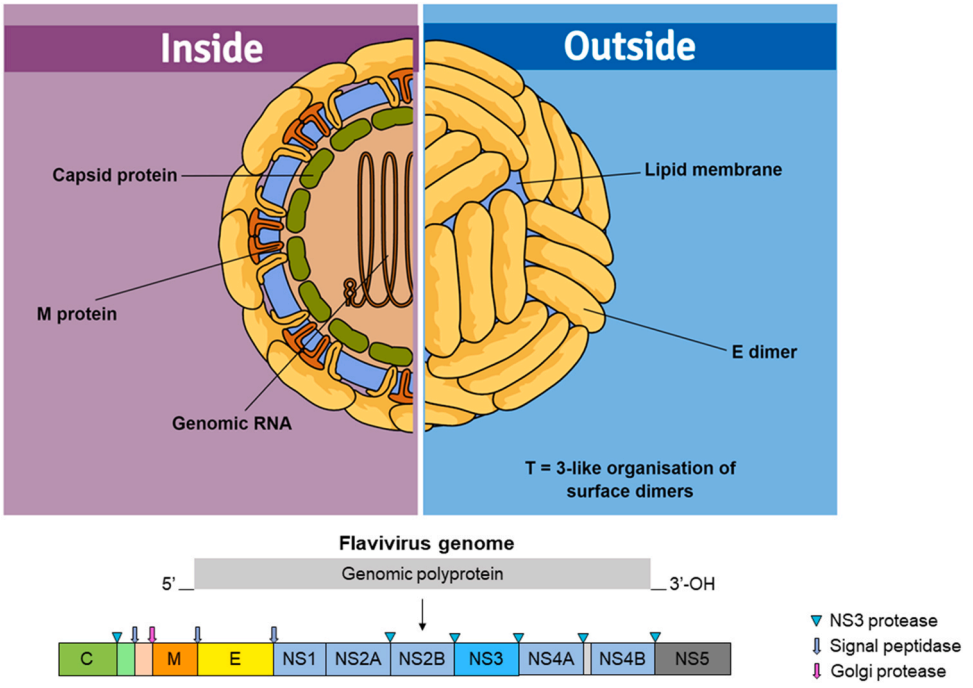


Fig. 1. Zika virus (ZIKV) structure and genome.

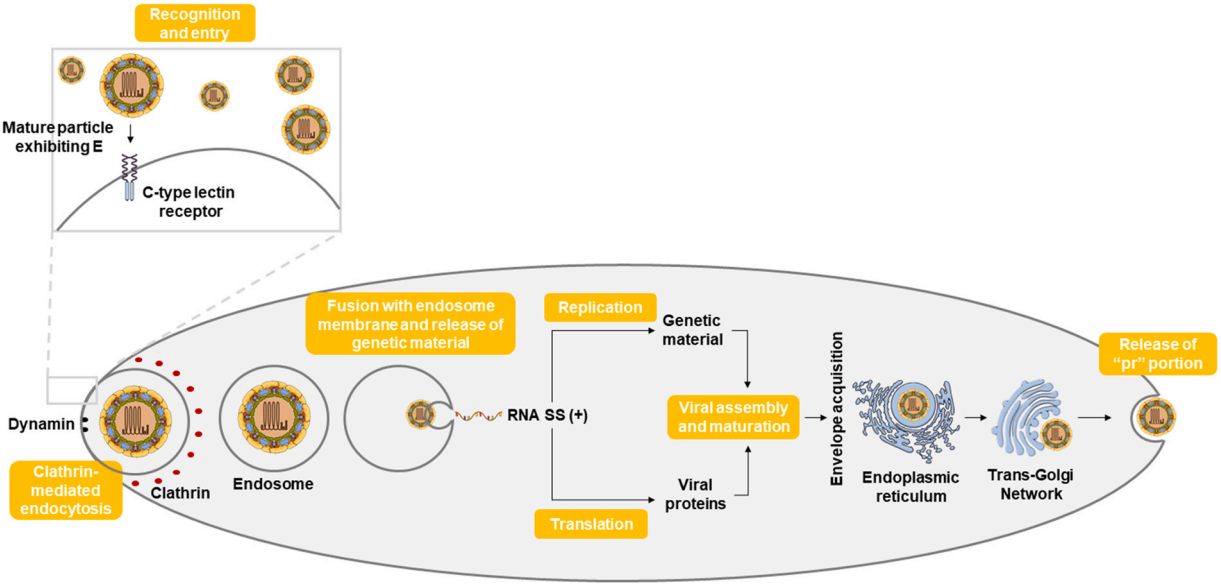


Fig. 2. ZIKV processes of attachment and entry, replication, translation, assembly, maturation and release from infected cells (Adapted from [16]).

endosomal membrane releasing virus RNA into the cytoplasm. ZIKV then interferes with cellular autophagy; a process by which cells control the death of cellular components in the cytoplasm. Zika turns the cell into a virus factory by hijacking cellular pathways to create copies of the viral genome and virions. Eventually, the cell is destroyed in a process known as apoptosis and virus molecules are released that can infect other cells [24]. Fig. 2 illustrates the mechanism of ZIKV-host interaction at cellular level.

In the skin, this causes oedema; a symptom reported in clinical examination of Zika patients [25]. In the foetus, the rapid depletion of hNPCs might be intricately involved with a clinically observed microcephaly or with many of the other neurological anomalies linked to Zika [26]. Much is still a mystery with the ZIKV but structural similarities with other envelope viruses and flaviviruses and the growing understanding of the pathogenesis of ZIKV using cell structure and animal models are giving hope for effective treatment strategies and for vaccine development. The use of natural compounds has been an alternative antiviral approach. Section 3 will elaborate in more details promising NPs showing inhibitory activity against ZIKV.

In 2015 and 2016, large ZIKV outbreak occurred in the Americas resulting in 1300, 000 suspected Zika cases in Brazil [9] (Fig. 3). ZIKV was easily transmitted from Brazil to Puerto Rico and US Virgin Islands while few cases were reported in Texas and Florida. In 2017, the number of Zika cases started to decline in the United States. In 2018 and early 2019, no report of ZIKV transmission was registered in the United States [27]. However, the virus recently re-emerged and the Ministry of Health in Brazil has reported 579 new ZIKV cases between December 2019 and February 2020 [28,29].

Effect of Zika virus on the foetus and other organs

Syncytiotrophoblasts (SYNs) are specialised layer of epithelial cells that are responsible for the protection of the foetus from microbes. After the implantation process which usually lasts for approximately seven days, the SYN forms a layer around the embryo acting as a protective barrier. The placenta is the sole barrier that stops pathogens from entering the fetal membrane. Vertical transmission of microorganisms from a mother to her foetus occurs via different pathways namely (i) ascending infections, (ii) trafficking of

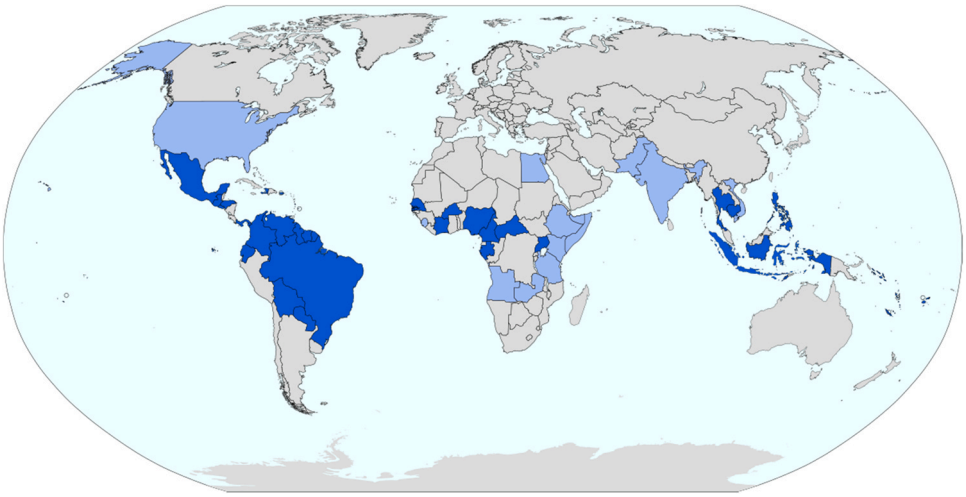


Fig. 3. Distribution of Zika virus (ZIKV) infection worldwide in 2016 (Wikimedia Commons, distributed under a CC-BY 2.0 license).

and/or signalling from maternal immune cells, (iii) paracellular or transcellular transport, (iv) breaching in trophoblast layers, (v) direct crossing or infection of SYNs, and (vi) infection of extra villous trophoblast via maternal microvasculature [30]. ZIKV can infect or damage developing foetuses solely by its ability to cross and/or bypass the placental barrier. It is known that other flaviviruses, such as dengue virus, are not linked with vertical transmission or congenital disorders, which implies that this type of mechanism is specific to ZIKV [30].

In order to reach the fetal brain tissue, the ZIKV has to cross two physiological barriers namely the placental barrier and the blood-brain barrier. However, the mechanism of ZIKV crossing those barriers to reach the central nervous system is still elusive [31]. Scientists have determined that the ZIKV readily infects human neural progenitor cells (hNPCs) which are key components for fetal development and are prevalent during the second trimester of pregnancy. The virus destroys these cells preventing the pathway that leads to the production of neurons [26].

Microcephaly was the first congenital malformation in correlation with ZIKV infection, most probably because it is strikingly and visually unappealing [32]. ZIKV-associated microcephaly involves a 'fetal brain disruption sequence', during which the growth of the cerebellum is arrested causing partial collapsing of the skull and consequently forms distinctive folds from redundant scalp skin. ZIKV causes numerous other developmental disabilities in addition to microcephaly, namely placental insufficiency, fetal growth restriction, ocular abnormalities, other central nervous system disorders and fetal demise is also possible (Fig. 3) [33]. All these abnormalities together are referred as 'congenital Zika syndrome' [32]. As a consequent of a lack or even no laboratory experiment was conducted before the present epidemic, the mechanism of ZIKV is now beginning to be understood (Fig. 4). The mechanisms through which ZIKV crosses the placenta and causes fetal damage are indeed of paramount importance and of particular interest to the scientific community. How ZIKV gains access to the fetal membrane has not yet been fully unravelled despite some possible mechanisms have been mentioned above. A recent study suggested that extra villous trophoblasts may be the entry portal for pathogens that allow them to cross and/or bypass the placental barrier [34]. But still, this is not sufficient to unmask the mystery behind how foetuses are damaged by ZIKV. The understanding of how pathogens reach the foetus is a hot topic that requires intensive research and in-depth investigations are needed to shed more light on this matter.

Natural products inhibiting Zika virus: *in vitro* and *in silico* studies

The arthropod-borne flavivirus, ZIKV, is an emerging global health challenge. As mentioned in former sections, ZIKV is responsible for drastic neurological complications, namely Guillain-Barre Syndrome in adult patients and congenital anomalies such as microcephaly in foetuses. However, there is currently no antiviral drugs or suitable vaccines available for the prevention and treatment of ZIKV and its associated complications. Presently, only palliative care is given to patients to relieve symptoms. But medicinal plants have showed significant potential as natural deposits of anti-ZIKV phytomedicine compounds. Therefore, as a treatment strategy, ZIKV infection can be countered by developing an effective vaccine and screening of numerous biomolecules that can hinder the different stages of the viral lifecycle. In this section, we attempt to gather all possible NPs reported in literature that possess inhibitory effect against ZIKV infection.

Several host cells targets have recently been identified. The neural RNA-binding protein, Musashi-1, was found to bind with ZIKV genome and permits viral replication [35]. The genome-wide CRISPR/Cas9-based screens showed that the endoplasmic reticulum-

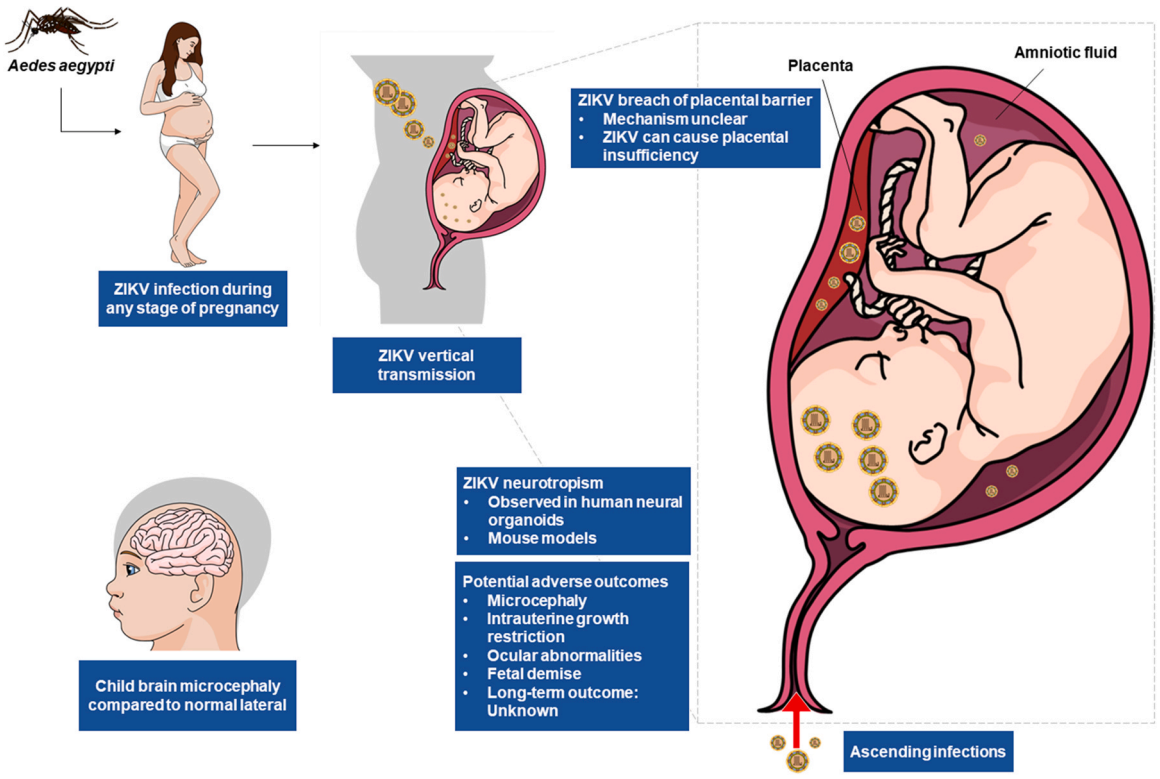


Fig. 4. The ZIKV can have highly destructive effects on the developing foetus after crossing the placental barrier. Unlike other viruses, the ZIKV can induce fetal disease and/or several adverse outcomes during pregnancy including microcephaly, hydranencephaly, growth restriction, ocular abnormalities and death. On the other hand, ZIKV causes only mild or no symptoms in the pregnant woman. Human organoids and in vivo studies demonstrated how ZIKV infections can be devastative to fetal brains.

associated signal peptidase complex (SPCS) also can be a pharmacological target for inhibiting ZIKV activity [36]. Additionally, the habitual drug targets such as NS2B-NS3 viral protease and NS5 RNA polymerase can be considered as promising targets for inactivating ZIKV [37]. Small molecules that inhibit binding with C protein or prevent capsid formation have the ability to stop viral assembly and release. Meantime, the right expression and processing of nascent proteins in host cells are vital for proper viral replication. A number of host proteins, namely ER membrane complex, a-glucosidase, cyclophilin, and proteasome elements, are responsible for controlling synthetisation, folding, and degradation of protein. Impairment of these functions tend to reduce viral assembly and budding. Hence, the aforementioned host proteins may also serve as pharmacological targets for developing small molecule inhibitors against ZIKV [37].

The majority of antiviral drugs are small-molecule inhibitors that target different stages of the viral life cycle by interacting with virus or host proteins critical for virus replication [38]. For example, inhibiting AXL function can protect cells from infection and, thus, may be a potential target for the production of entry inhibitors. However, destroying AXL function may also have many adverse consequences [39]. In addition, the proteases crucial for ZIKV replication are potential targets for developing ZIKV replication inhibitors. Therefore, more effective and appropriate targets need to be developed by scientists.

ZIKV entry inhibitors

ZIKV inhibitors targeting viral E protein

The envelope E protein is the main viral protein responsible for cell receptor binding and entry and is thus considered as one of the major factors in ZIKV pathogenesis [40]. Since recognition and cell receptor binding marks the beginning of ZIKV infection; thus, searching and developing inhibitors that can effectively prevent binding can be a first step in stopping viral fever [41].

Several NP inhibitors that can specifically attack ZIKV E protein have been documented. NP inhibitors appear to be one of the most promising compounds in in vitro studies solely due to their potent antiviral efficacy and broad-spectrum antiviral activity. **Baicalin (5, 6-dihydroxy-7-O-glucuronide flavone) (1)** (Fig. 5) is the most dominant flavonoid in the roots of *Scutellaria baicalensis* Georgi, a Chinese herb exhibiting significant pharmacological properties namely antioxidant, antiviral, anti-inflammatory, anti-HIV, antiproliferative effects [42]. The flavonoid has high binding affinity towards the E protein of the virus and was shown to inhibit ZIKV from entering host cells at negligible toxicity [43]. **(-)-Epigallocatechin gallate (EGCG) (2)**, a polyphenol rich in green tea, is reported by a number of publications to protect organs from a pile of viruses including Zika [44]. Correspondingly, EGCG has the ability to bind with the E protein blocking the virus from entering host cells [45]. Nonetheless, since EGCG has a catechol group, the compound may not specifically inhibit many different targets [46]. From the study of Sharma et al. (2017), EGCG at a concentration of 100 µM, inhibited ZIKV entry with a percentage of more than 90 %. This result proposed that EGCG can be used as a promising treatment strategy to hamper or decrease ZIKV infection load. However, further investigations are required to evaluate the efficacy of EGCG as a therapeutic drug molecule [47]. Despite no adverse effects have been reported with EGCG during organogenesis period in rat models, more safety studies are needed to implement its use for pregnant women [48].

Turmeric and its most active ingredient **curcumin (3)** have a plethora of scientifically-proven health benefits. **Curcumin (3)** has been extensively studied for its antioxidant, anti-inflammatory and anti-tumour properties. Interestingly, a wealth of scientific reports indicated that this NP possess an inhibitory property against many viral infections. The polyphenol inhibits ZIKV infection in a dose-dependent manner and blocks the E protein of the virus from binding to the cell surface [49]. **Nanchangmycin (4)**, a NP formed from the fermentation of *Streptomyces nanchangensis*, can prevent the growth of gram-positive bacteria and also exhibit insecticidal properties. A study demonstrated that the compound could blocked the early stage of the lifecycle of ZIKV which is viral entry. **Nanchangmycin (4)** inhibited clathrin-mediated endocytosis at IC50 values ranging from 0.1 to 0.4 µM at low toxicity in U2OS host cells, human brain microvascular endothelial cells (HBMEC), and human jeg-3 cells, accordingly [50]. Another important inhibitor of viral entry is **gossypol (5)**. It is a natural phenol produced by cotton plants (*Gossypium* species). For the first time, Goa et al. (2019) demonstrated that **gossypol (5)** could overcome ZIKV infection by its strong binding affinity with the E protein, particularly the conserved E domain III [51].

Carapichea ipecacuanha (Brot.) L.Andersson (ipecac) is a medicinal plant native to Brazil, Costa Rica, Nicaragua, Panama and Colombia. Its roots contain the emetic alkaloids **cephaeline (6)** and **emetine (7)** [52]. Since cellular cholesterol haemostasis and autophagy are considered as important factors in viral entry and replication, therefore a study was designed to assess the effect of **emetine (7)** on the accumulation of cholesterol and lysosome. The study showed that **cephaeline (6)** and **emetine (7)** increased the level of cholesterol in lysosomes, most probably due to a change in pH of the organelles and consequently caused a disruption in their functions resulting in a reduction in viral entry [53]. Recently, Sharma and coworkers investigated on three NPs: **pentagalloylglucose (8)**, **parishin A (9)** and **stevioside (10)** on their ability to hamper the early stage of viral lifecycle. **Pentagalloylglucose (8)** is a hydrolysable tannin belonging to the gallotannin group naturally occurring in many plants including *Anacardium occidentale* L. [54,55]. **Parishin A (9)** is present in the rhizomes of *Gastrodia elata* Blume while **stevioside (10)** is a phytocompound that can be extracted from *Stevia* leaves. Interestingly, all three compounds demonstrated high binding affinity with E envelope protein preventing ZIKV infection [56]. However, docking studies suggested that **pentagalloylglucose (8)** presented the best scoring phytochemical with docking score − 15.696 kcal/mol. Another study also suggested that the compound was a good inhibitor against many flaviviruses namely ZIKV and hepatitis C [57].

Resveratrol (11) exhibited virucidal activities by acting directly against extracellular ZIKV particles. **Resveratrol (11)** treatment lowered more than 70 % virus titer in the anti-adsorption assay, indicating the possibility that the compound interfered with ZIKV binding to the cell surface [58]. The virucidal assay conducted on **berberine (12)** and **emodin (13)** showed that both compounds have the ability to inhibit pre-entry of ZIKV by directly acting on the Zika particles at non-toxic concentrations. Compounds like **berberine (12)** and **emodin (13)** that can exhibit inhibitory effect on a virus before its attachment on a cell surface can be used at low concentrations since it is known that the number of infectious particles present in primary infection is relatively low. Also, it is worthy to note that **berberine (12)** can be easily spread to numerous organs such as liver, brain, kidney, muscles, and stay in these locations for a prolonged period of time [59]. **Thymohydroquinone dimethyl ether (14)**, a major component of *Ayapana triplinervis* (Vahl) R.M.King & H.Rob. essential oil, was showed to be a good inhibitor in the early stage of viral lifecycle. The ZIKV-attached particles were unable to be absorbed into the host cells in the presence of the compound [60].

ZIKV replication inhibitors

ZIKV inhibitors targeting NS2B-NS3 protease

The NS3 helicase of ZIKV plays a crucial role in viral RNA replication by unwinding the RNA after the hydrolysis of NTP. In vitro study showed that **EGCG (2)** substantially inhibited NTPase activity

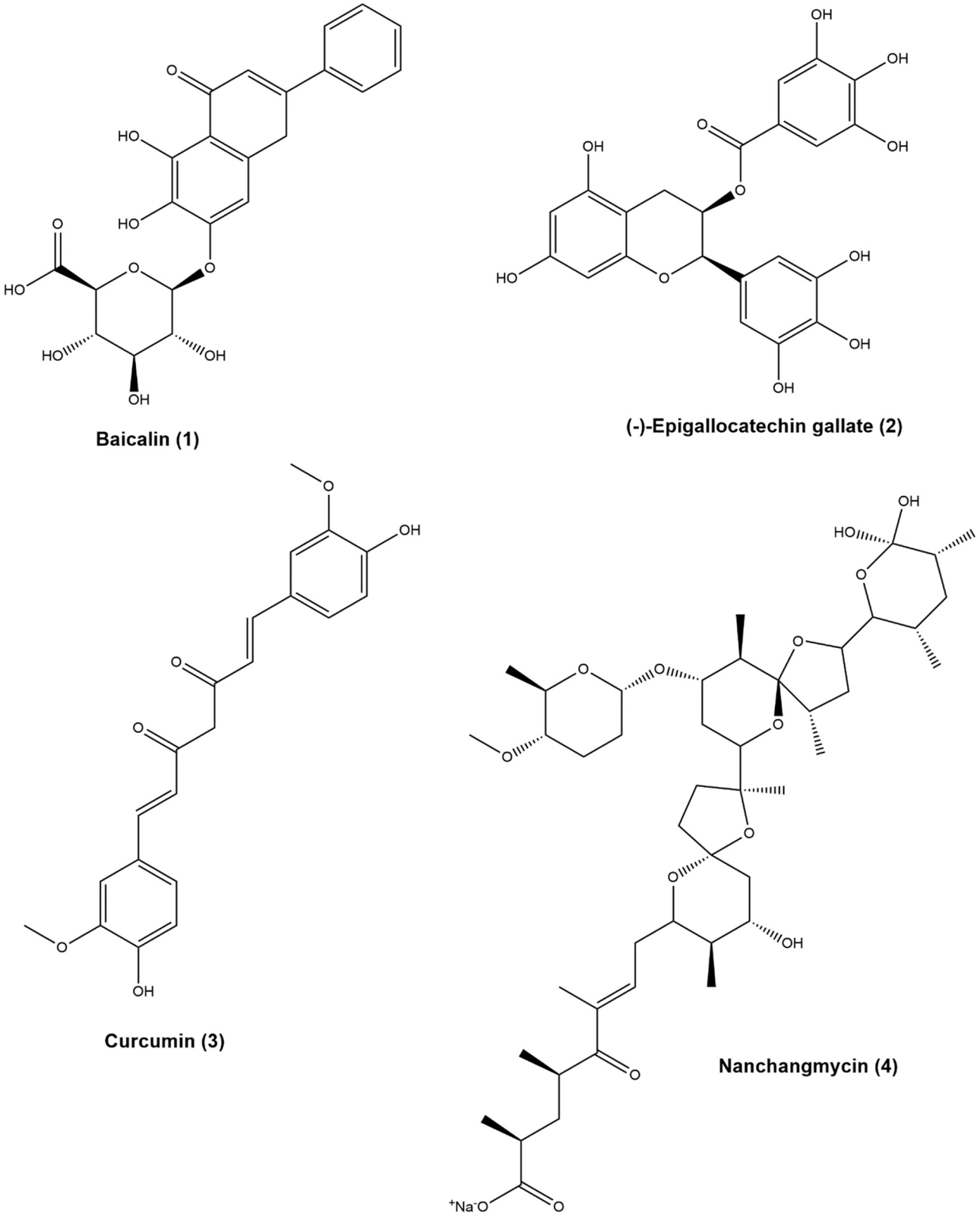


Fig. 5. Chemical structures of compounds 1–4 exhibiting anti-ZIKV activity in in vitro studies.

at in IC50 of 295.7 nM. The result from the study suggested that **EGCG (2)** can be a backbone molecule for developing further broad-spectrum inhibitor to tackle ZIKV and also other flaviviruses [61]. **Myricetin (15)** is a hexahydroxyflavone isolated from the leaves of *Myrica rubra* (Lour.) Siebold & Zucc. and can also be commonly found in tea, berries, nuts, fruits, vegetables and medicinal herbs. The pharmacological activity of the flavonoid has been adequately documented in literature. Lim et al. (2017) investigated on the inhibitory effects of a list of flavonoids [luteolin, chrysin, myricetin, quercetin, ampelopsin, astragalin, rutin, icaritin, hesperidin, naringin, EGCG, epicatechin gallate (ECG), gallocatechin gallate (GCG), catechin gallate (CG), epicatechin (EC), EGCG-7-O-α-

glucopyranoside, EGCG-4′-O-α-glucopyranoside, epigallocatechin (EGC), catechin] and non-flavonoid (pyrogallol, pyrocatechol, caffeine, gallic acid) compounds against NS2B-NS3. Among the compounds, seven compounds [**luteolin (16), myricetin (15), astragalin (17), rutin (18), EGCG (2), ECG (19), GCG (20)**] have IC50 values ranging from 22 to 112 µM with **myricetin (16)** reporting the lowest IC50. Since the latter compound exhibited the strongest inhibitory activity, it was subjected to kinetic studies. Results showed that **myricetin (15)** was reported to inhibit NS2B-NS3 protease activity in a non-competitive manner [62]. The chemical structures of the compounds exhibiting inhibitory effects can be found in Figs. 8 and 9. In another study, **myricetin (15)** (500 µM) was found to bind to the pockets on the back of the active site of NS2B-NS3 protease resulting in an IC50 of 1.3 µM and inhibitory constant Ki of 0.8 µM [63]. Interestingly, in the same study, in addition to **myricetin (15)**, NPs **quercetin (21), isorhamnetin (22), apigenin (23)** also showed good inhibitory activity against the protease with IC50 values 2.4, 15.5 and 56.3 µM, respectively. It was observed that the short β-sheet formed by NS2B residue Leu74-Leu78 and Asp83-Leu86 in the phytocompounds has direct interactions with the active site inhibitor cn-716 on one side, and with on another side of the four aforementioned compounds [63]. However, it is worthy to note that **curcumin (3)** and **quercetin (21)** are unselective inhibitors due to colloidal aggregation. **Curcumin (3)** contains reactive Michael acceptors and **quercetin (21)** possesses a catechol group, a commonly known PAINS substructure, which could be the reason why these NPs can be unfavourable [64].

Carnosine (24) is a protein naturally produced in the body and is present in high concentration in muscle and brain tissues. ZIKV is associated with multiple organ dysfunction. For instance, in Zika patients, the normal functioning of several organs such as liver, brain, and kidney is disrupted, which in turn affect the production of **carnosine (24)**. It is therefore proposed that exogenous administration of **carnosine (24)** may be needed to incite antiviral activity in Zika patients [65]. A study showed that the naturally occurring protein could interact with NS2B-NS3 protease inhibiting viral genome replication at IC50 of 63.7 µM [66]. **Pedalitin (25)** is a flavonoid with the four hydroxy groups at C-3', − 4', − 5 and 6, and the methoxy group at C-7. It can be isolated from a few plant species, namely *Eremosparton songoricum* (Litv.) Vassilcz, *Rabdosia japonica* (Burm.f.) H.Hara and *Ruellia tuberosa* L [67]. Interestingly, **pedalitin (25)** isolated from a Brazilian medicinal plant, *Pterogyne nitens* Tul. was reported to inhibit NS2B-NS3 protease at an IC50 value of 5 µM and Ki value of 4.5 µM [68].

A panel of 2263 plant-derived NPs have been docked against NS2B-NS3 protease and NS3 helicase. Several phytochemicals that are present in common medicinal plants displayed good selective docking properties. For instance, **balsacone B (26)** is found in *Populus balsamifera* L., **kanzonol V (27)** is found in licorice root (*Glycyrrhiza glabra* L.), **cinnamoylechinaxanthol (28)** is present in *Echinacea* root; and **rosemarinic acid (29)** is found in various common herbs including rosemary (*Rosmarinus officinalis* L.), lemon balm (*Melissa officinalis* L.), and common sage (*Salvia officinalis* L.) [69].

ZIKV inhibitors targeting NS5

In addition to the inhibitors of NS2B-NS3 polymerase, inhibitors of the viral NS5 protein demonstrated a good potential for the therapeutic development. **Baicalin (5,6,7-trihydroxyflavone) (1)**, another flavone found in the Chinese herb, *Scutellaria baicalensis*, has strong binding affinity towards ZIKV NS5 and therefore can downregulate ZIKV replication up to 10 h post infection [43]. Apart from the inhibition of cell entry, **curcumin (3)** can also prevent virus replication as reported by a study of Mounce et al. (2017) [49]. The mechanism of actions involves either a direct interference of viral replication machinery or suppression of cellular signalling pathways essential for viral replication, such as PI3K/Akt, NF-κB [70]. A recent study demonstrated that by encapsulating **curcumin (3)** with poly (lactic-co-glycolic acid) nanoparticles substantially reduced the synthesis of viral macromolecules within host cells. The researchers of the study suggested that this combination of curcumin with nanoparticle can be a relevant drug formulation to fight pathogenic flaviviruses. However, studies in animal models still need to be conducted to confirm their curative potential [71]. **Cavinafungin (30)** isolated from the fungus *Colispora cavincola* is a potent inhibitor of the NS5 RNA polymerase activity [72].

In addition to viral entry inhibition, **cephaeline (6)** and **emetine (7)** are active at viral post-entry stages by sequestering the replicase system. Data from Yang et al. (2018) showed that **emetine (7)** directly bound to the NS5 RNA polymerase which subsequently inhibited the polymerase activity at IC50 value of 121 nM. The study also reported that **emetine (7)** prevented the polymerase from thermally-induced aggregation. The inhibitory effect was observed in various host cells including SNB-19, HEK293, and Vero E6 cell lines, indicating that the effect is not specific to particular cell lines. **Cephaeline (6)** exhibited similar potent activity against ZIKV replication. It is proposed that more studies should be conducted to investigate on the efficacy of both NPs in arresting vertical transmission and other ZIKV associated neurological complications in new-borns [53]. A plant-secreted phytoalexin, **resveratrol (11)**, was assessed for its ability to inhibit ZIKV replication. Experimental data showed that **resveratrol (11)** exhibited antiviral effects on ZIKV in a dose-dependent manner with > 90 % inhibition at a concentration of 80 µM. Negligible cytotoxicity effects were recorded in the host cells, Huh7 and Vero cell lines [58].

Figs. 5–11 illustrate the chemical structures of compounds exhibiting anti-ZIKV activity.

Shortcomings and challenges in conducting in vivo studies and clinical trials of natural products to tackle Zika virus

The proportion of NPs that will make it to clinical trials through in vivo studies from the in vitro list remains unclear (Fig. 12). Nonetheless, due to the standard procedures of drug discovery process, lead compounds must usually first demonstrate significant *in vitro* activity to be able to obtain the necessary attention among biological researchers to warrant further in vivo exploration followed by clinical trials. Despite promising results were obtained from several NPs in in vitro tests, to the best of our knowledge, only **emetine (7)** reached in vivo study. The anti-ZIKV activity of **emetine (7)** was tested using *in vivo* models. The compound (1 mg/kg/day) was delivered to the three-month old SJL male mice infected with $ZIKV^{BR}$ (Brazilian strain) via intraperitoneal for six days. **Emetine (7)** treatment was observed to decrease the levels of circulating ZIKV by approximately 10-fold. The level of ZIKV infection was determined by qPCR analysis of blood samples [53]. It is well understood that there is a real gap in the corpus of knowledge on NPs exhibiting anti-ZIKV effects in in vivo testing and also clinical trials. Usually, from the compounds tested in in vivo models, the percentage of drug candidates that make it to the clinic is even smaller. Until now, there is only a handful of clinical trials in progress for ZIKV treatments. Yet none of these so far involve NPs but are instead trials on ZIKV vaccine candidates. Vaccines and antivirals that are presently in preclinical development are not naturally derived and have not yet approved for human use [73].

Taken together, the lacunae in the development of safe, potent, and selective anti-ZIKV therapeutics based on NPs and the lack of in vivo studies and clinical trials provide strong evidence that either researchers are not interested in such work or there are too many challenges to face in including NPs in the production of medications against ZIKV. To bridge the gap between in vitro studies and clinical trials, we suggest that it is high time to

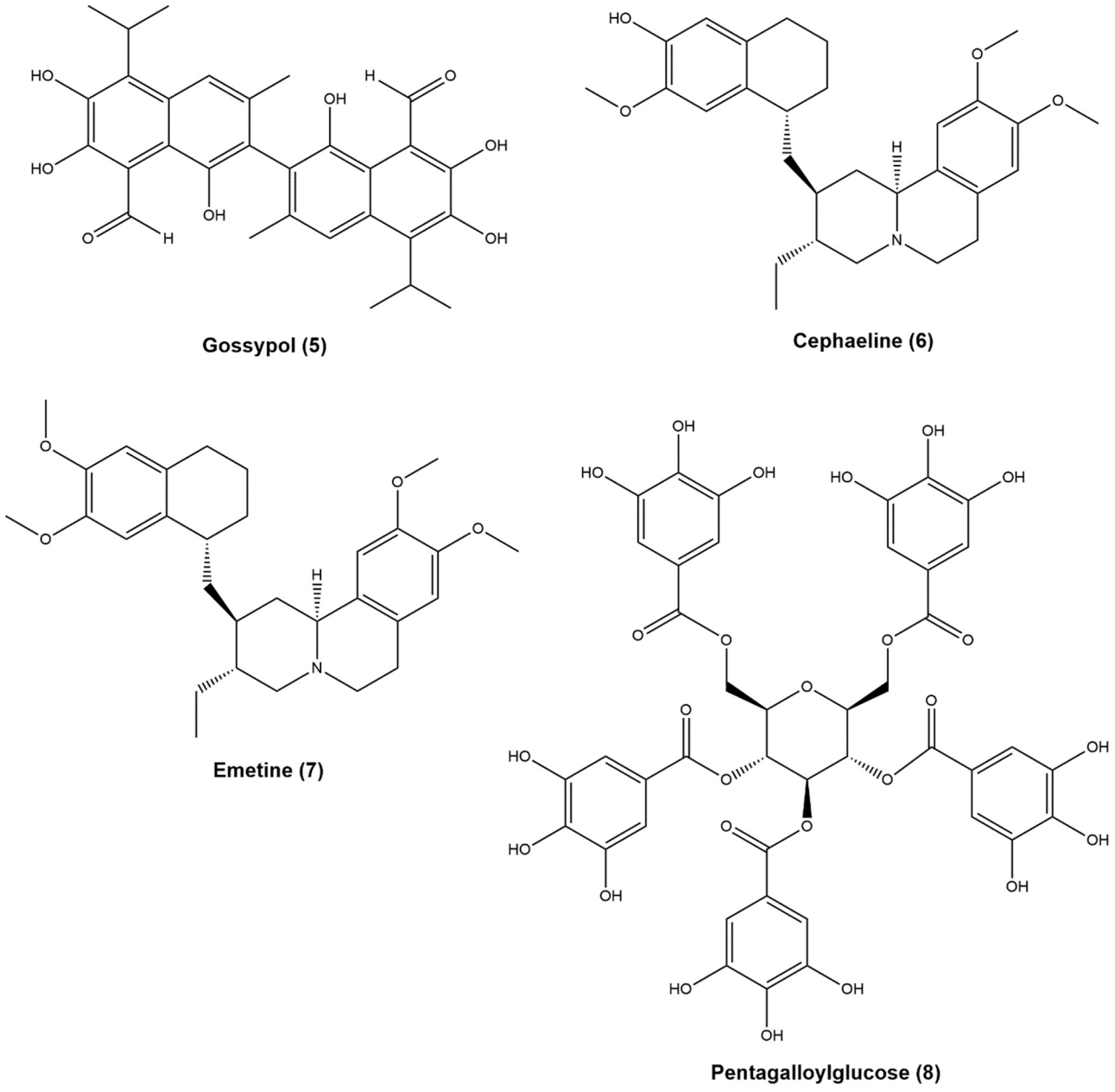


Fig. 6. Chemical structures of compounds 5–8 exhibiting anti-ZIKV activity in in vitro studies.

improve NP research translation from its source to the clinic. Based on its unique pathology, ZIKV is an elusive virus for any drug discovery program.

By definition, NP can be derived from living organisms such as plants/herbs, bacteria, fungi, animals. If a compound is present in low quantity in these living things, it can be difficult for mass production of a particular compound for drug development. Additionally, if NPs are yielded by plants that grow in remote or difficult to access areas, or by marine organisms that reside in great depth of the ocean, thus delivering these NPs in in vivo models or administering this NPs to humans for trials can be a problem as the amount needed can be high [74]. Because of the danger of extinction caused by deforestation, the risk of losing potentially useful natural resources of pharmacologically active ingredients is continuously growing. To satisfy the increasing demand of NPs, to preserve wild supplies, and to reduce the possible diversity of active ingredients in medicinal plants due to variation in collection areas, it is vital that better cultivation systems must be implemented to ascertain consistency and protect resources [75]. Similarly, extracting promising compounds from marine organisms can pose a problem because of the high salinity and water content and also to be able to provide large quantities of these compounds, tons of raw materials are required [76]. As a consequent, these difficulties may be the reason that discourage clinical researchers in conducting in vivo studies as well as clinical trials on NPs to fight ZIKV.

Intellectual property rights can be another challenge in exploring NPs in *in vivo* studies and clinical evaluations. In some cases, it can be a financial issue in negotiating and obtaining agreements to collect and develop NPs-based products from species that live in foreign countries [74]. Another challenge that the drug discovery and

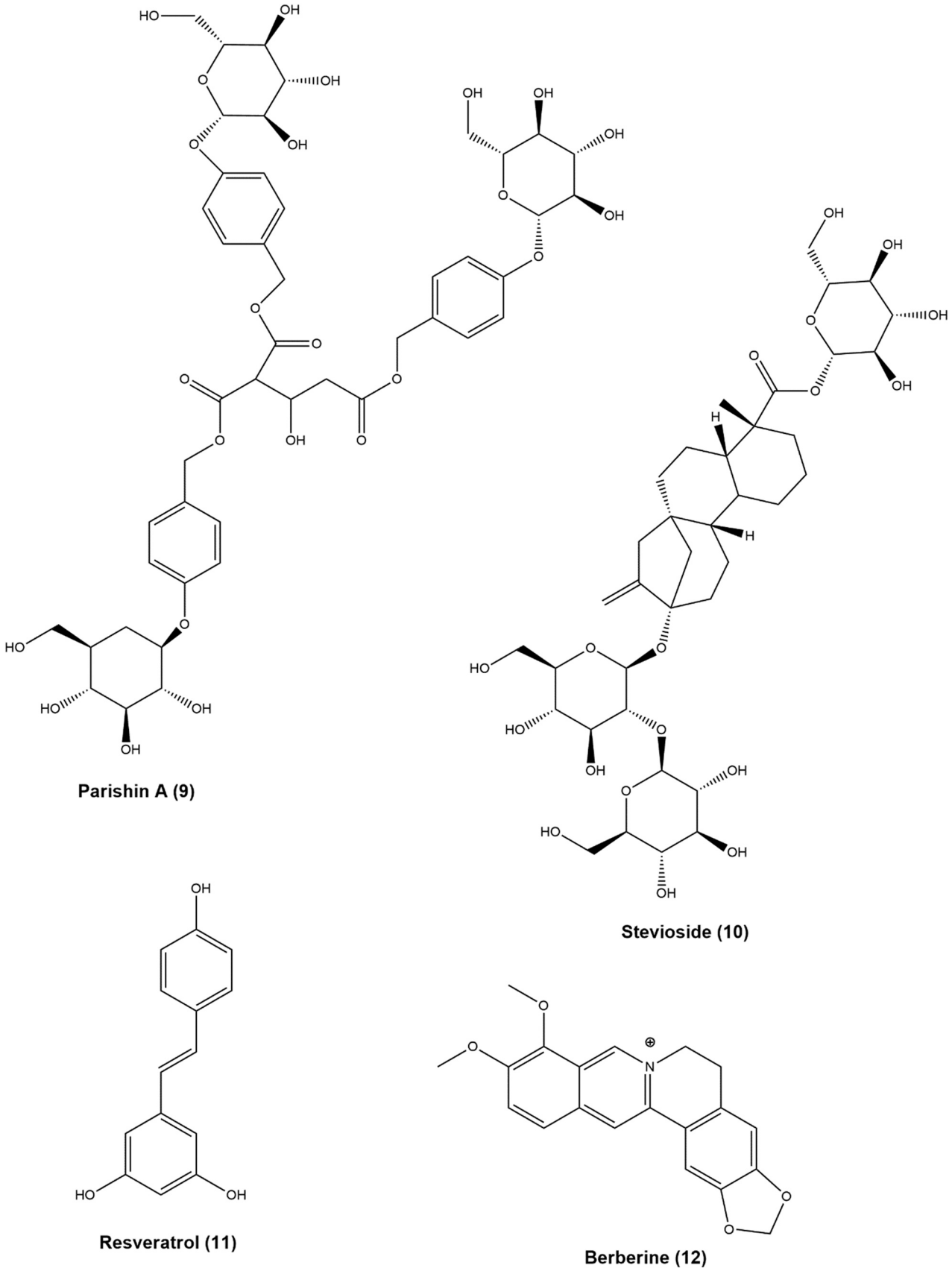
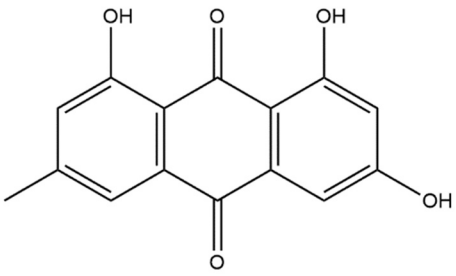
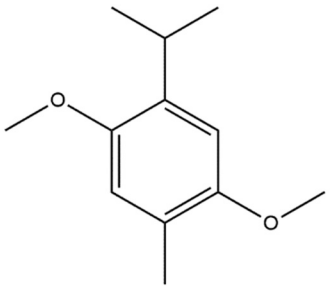


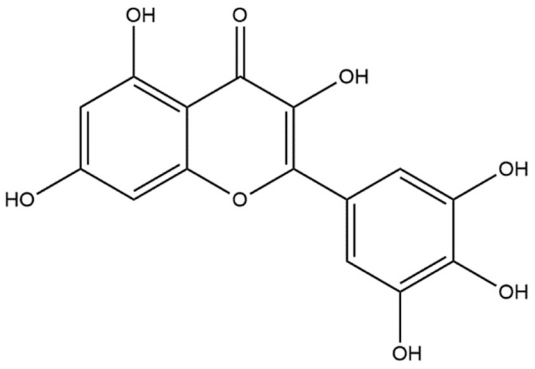
Fig. 7. Chemical structures of compounds 9–12 exhibiting anti-ZIKV activity in in vitro studies.



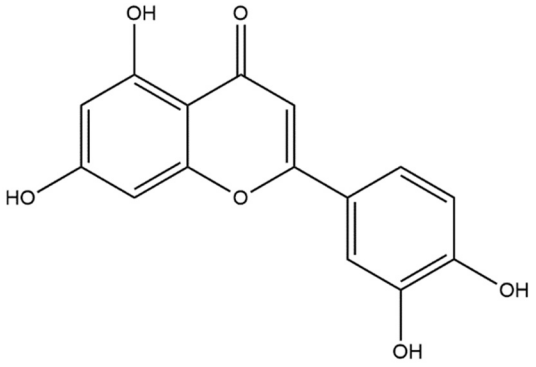
Emodin (13)



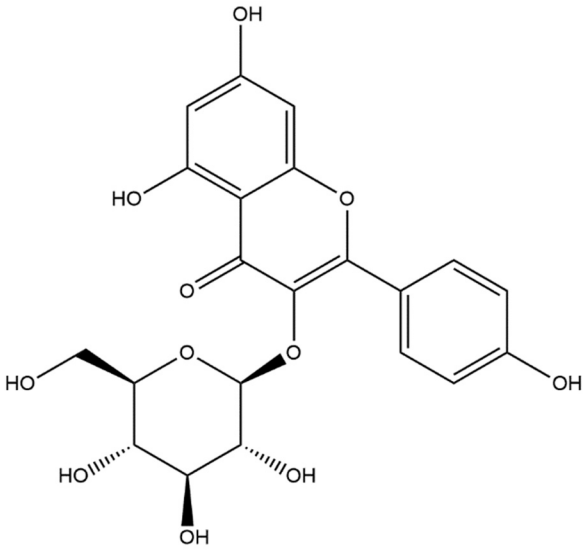
Thymohydroquinone dimethyl ether (14)



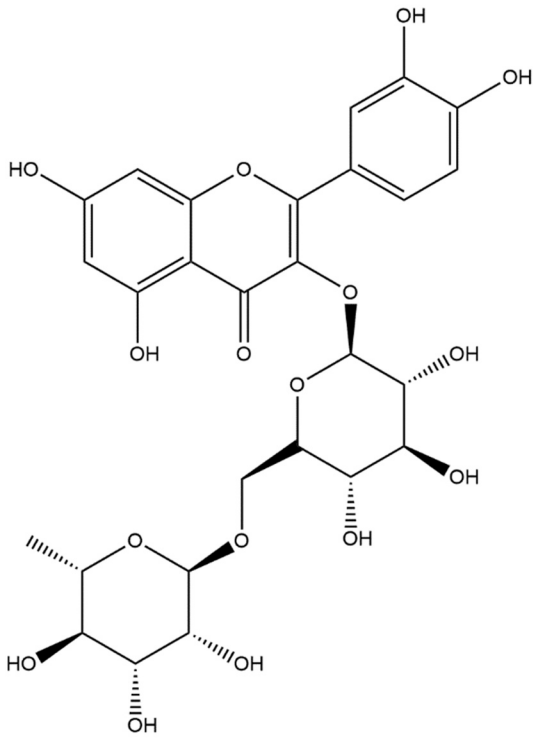
Myricetin (15)



Luteolin (16)

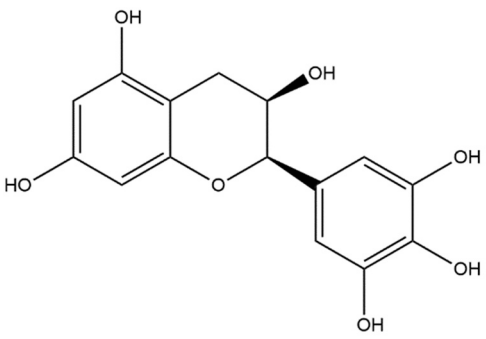


Astragalin (17)

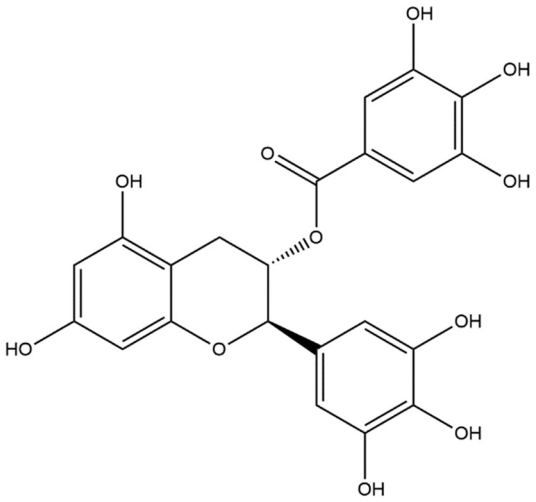


Rutin (18)

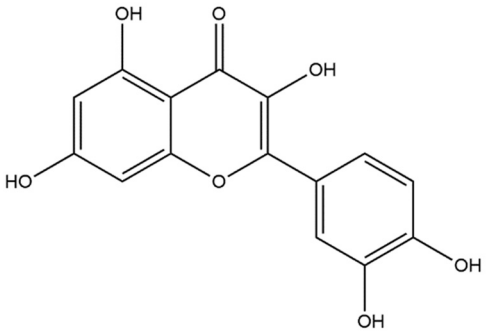
Fig. 8. Chemical structures of compounds 13–18 exhibiting anti-ZIKV activity in in vitro studies.



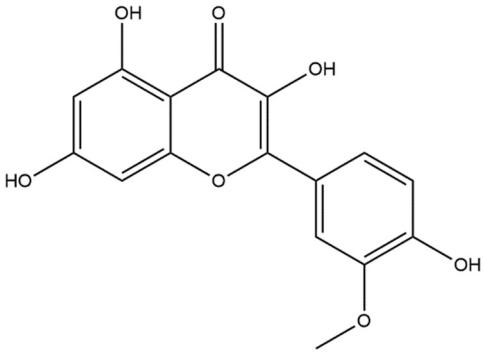
Epigallocatechin (19)



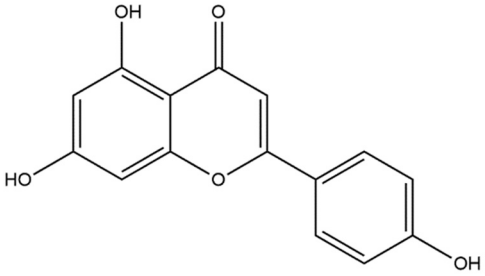
Gallocatechin gallate (20)



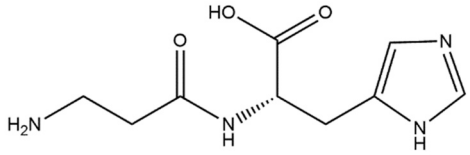
Quercetin (21)



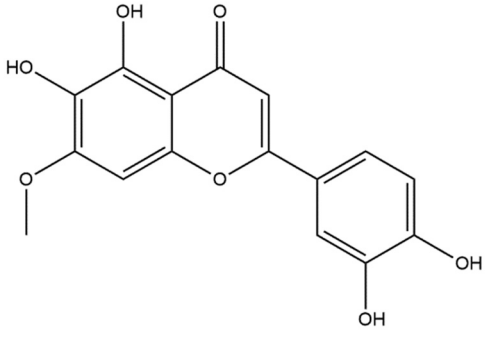
Isorhamnetin (22)



Apigenin (23)



Carnosine (24)



Pedalitin (25)

Fig. 9. Chemical structures of compounds 19–25 exhibiting anti-ZIKV activity in in vitro studies.

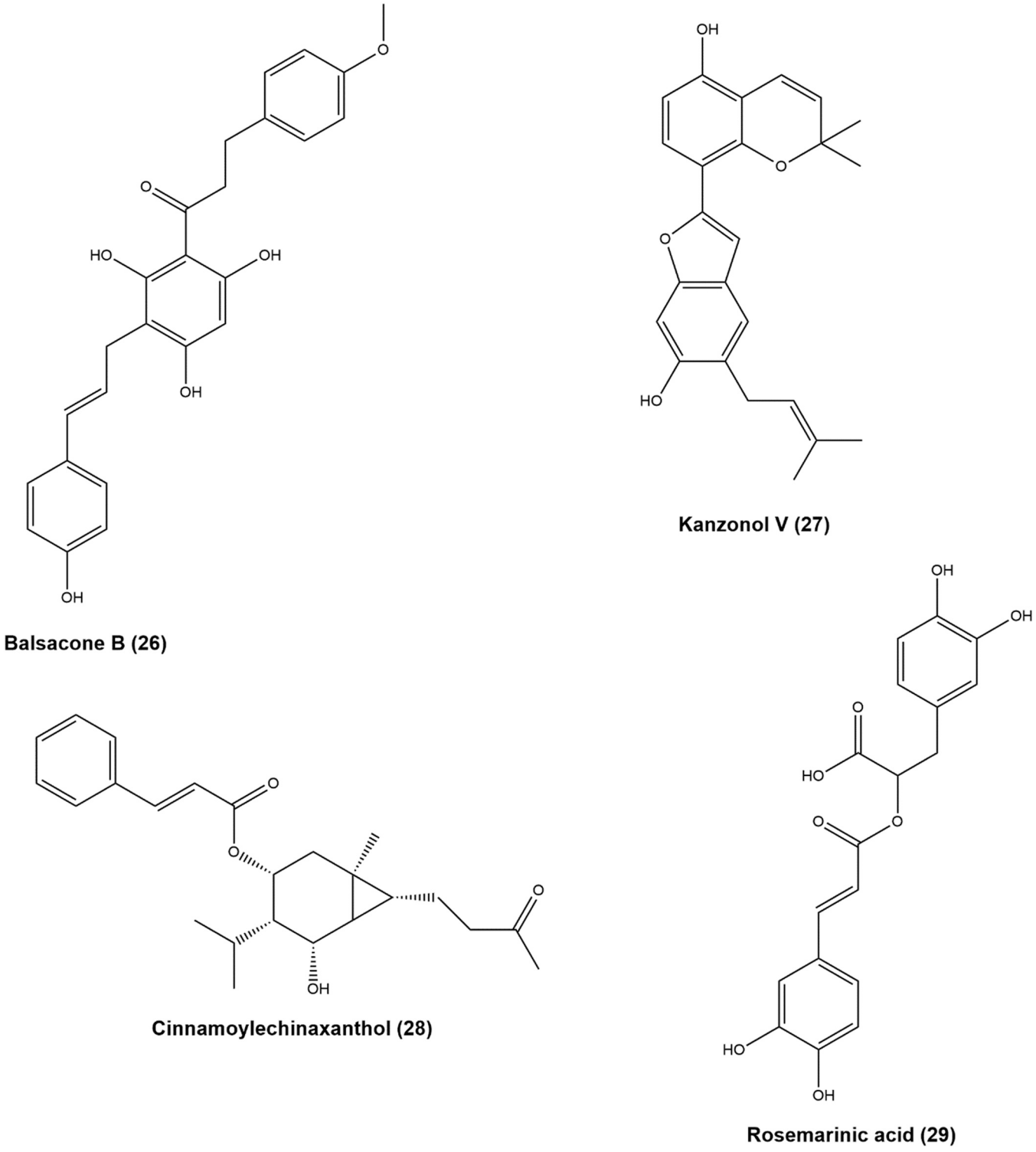


Fig. 10. Chemical structures of compounds 26–29 exhibiting anti-ZIKV activity in in vitro studies.

development projects might be confronting in clinical phases is the difficulty in recruiting subjects for the trials. The presently low number of ZIKV infection cases combined with unpredictable geographical epidemics can jeopardise the on-going and future clinical evaluations of ZIKV [77]. However, by developing a controlled human infection model, ZIKV research can speed up and down-selection of effective NPs can be completed rapidly [73]. Bearing in mind that Zika affect pregnant women and caused fetal and placental infections, conducting such trials on pregnant women is not possible as they are usually excluded from most pre- and clinical trials [78]. Thus, understanding how will the selected NPs affect a pregnant woman is a question that remains unanswered despite pregnant women represent a priority population that needs safety and immunogenicity against ZIKV.

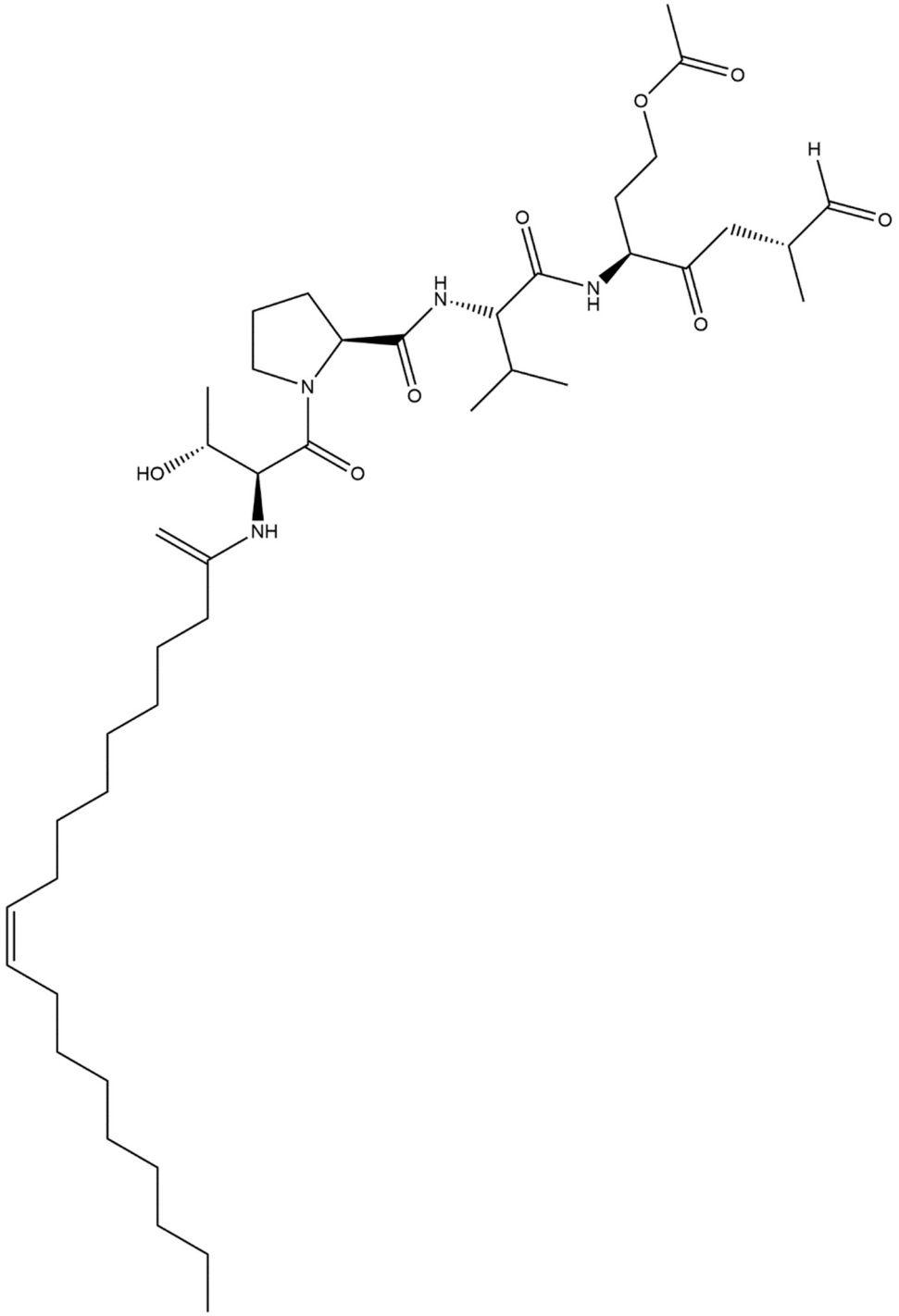


Fig. 11. Chemical structure of compound 30 exhibiting anti-ZIKV activity in in vitro studies.

With all these possible shortcomings and challenges presented here, we find out that the development of ZIKV therapeutics is still at its infancy. Therapeutics fighting ZIKV might well be devised alongside vaccines as they can play a key role in decreasing the risk of Zika infection in populations that are most vulnerable to serious complications associated. However, large amount of work still to be done in relation to develop a proper NPs that can inhibit ZIKV fever. We understand that clinical trials can be expensive, timely and risky but combating a pandemic with no remedy can be costlier and riskier as witnessed by the current situation of COVID-19. Therefore, developing NP-based drugs is essential and may be used reasonably for prophylaxis or post-exposure prophylaxis to deter or reduce the effects of ZIKV. Also, they may be particularly useful where low endemicity does not warrant universal immunisation [77].

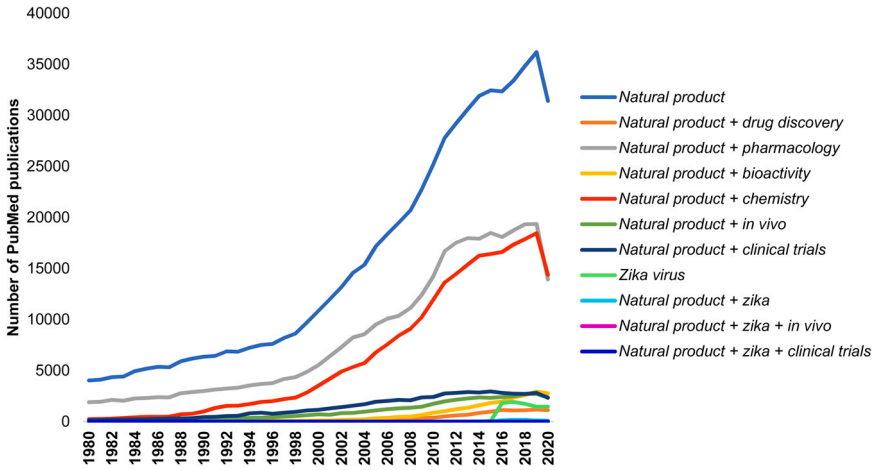


Fig. 12. PubMed publication trend analysis, demonstrating increased scientific interest in natural product, natural product pharmacology, natural product chemistry until 2019. As from 2016, scientists started to show interest in research on Zika. However, little research involved natural product in in vivo studies and clinical trials. The data were retrieved from PubMed database (https://pubmed.ncbi.nlm.nih.gov/) on 22 April 2021, covering the period 1980–2020. As mentioned, the used search keywords were *Natural product, natural product + drug discovery, natural product + pharmacology, natural product + bioactivity, natural product + chemistry, natural product +* in vivo*, natural product + clinical trials, zika virus, natural product + zika, natural product + zika + in vivo, natural product + zika + clinical trials.* The trend analysis indicates that research on natural product in general is faster than research conducted on Zika involving natural product.

Charting the future of pharmacological research on Zika virus by analysing research trend using statistics. Are we doing well in Zika research?

Resulting from the discussion above on the ability of some reported NPs in inhibiting entry of ZIKV into host cells or preventing replication of the virus in cells, we attempted to analyse in more details the trend of publications on Zika involving NPs. Fig. 6 is plotted from data retrieved from PubMed using keywords such as *natural product, natural product + drug discovery, natural product + pharmacology, natural product + bioactivity, natural product + chemistry, natural product +* in vivo*, natural product + clinical trials, zika virus, natural product + zika, natural product + zika + in vivo,* and *natural product + zika + clinical trials*. We wanted to do a search using only the keyword *natural product* with the aim to later compare the trend with the number of PubMed publications involving NPs and Zika. As evident from Fig. 6, increasing slopes were observed when we searched for reports on *natural product*, *natural product + pharmacology,* and *natural product + chemistry*. The interest was noted as from the year 2000 until 2019, but suddenly decrease as from 2019. The decline of interest in 2020 may be the result of a lack of prioritisation, unavailability of appropriate tools and equipment, lack of interest, poor financial resources, absence of novel analytical methods, or emergence of a new topic of interest. Indeed, in 2020, a scads of publications were devoted to COVID-19 due to the urgency of finding a treatment which could eventually curb research on NPs related to ZIKV. The slopes plotted from data using the keywords *natural product + zika, natural product + zika +* in vivo, and *natural product + zika + clinical trials* were barely visible indicating their low and/or no contributions to the scientific literature. The number of publications on *natural product* are substantially larger than reports on *natural product + zika*. This trend shows that interest in searching for a cure or prevention against zika in NPs is negligible. Furthermore, research work on in vivo studies and clinical trials of NPs is very little; and our statistical data showed that in vivo studies and clinical evaluations of NPs exhibiting anti-ZIKV properties is none. If this trend persists, we presume that in the coming years, we could be facing another viral pandemic unprepared. To respond to the question: 'Are we doing well in Zika research'? the answer is 'no'. Therefore, concerted effort is indispensable to identify, screen and evaluate as much as NPs as possible in in vivo models and clinical settings respectively to prepare a better pandemic action plan if there is widespread outbreak of ZIKV (Fig. 12).

Conclusion

The new unparalleled coronavirus pandemic announced by the World Health Organization (WHO) has shocked the world and due to unpreparedness, millions of people have died and still counting. This pandemic should serve as an initiative to strengthen global preparedness against threats of another emerging and re-emerging infections by focussing attention on surveillance, prevention, developing ways on how to contain an outbreak and most importantly finding treatment of the disease. The ZIKV has re-emerged in Brazil in 2020 and thus the search for effective therapeutic approaches to curtail the effect of the virus on global health are urgently needed. Currently there is no cure for ZIKV. The ongoing outbreak of ZIKV highlights the need not only to better understand how ZIKV breach the placental barrier and induce congenital disease, but also the urgent need to assess the safety and efficacy of therapeutics in pregnant women.

Because the field of antiviral research is growing, several NPs have been identified *in vitro*. However, only one biomolecule (emetine) has been tested so far *in vivo* for efficacy and none have been evaluated in clinic yet. A lack of scientific interest in natural product-based drug discovery is evident from the analysis of PubMed publications trends. From the trend, we presumed that researchers are not making enough efforts to find cure and prevention to combat ZIKV infection. The COVID-19 pandemic has shown the world of the consequence of neglecting research on infectious diseases. The absence of *in vivo* tests and clinical trials deeply limit our understanding on the efficacy of NPs in treating and preventing ZIKV infection although they displayed interesting inhibitory activity in in vitro studies. It is hoped that researchers will be guided by the information presented here in the process of developing safe, effective anti-ZIKV therapeutic drugs from naturally derived compounds. To conclude, it can be said that we are not ready yet to combat another infectious disease and we uphold the need for more studies with appropriate study designs.

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Ethical approval

Not applicable.

CRediT authorship contribution statement

All authors were involved in the design and data mining process. All authors read and gave approval for submission.

Competing interests

None declared.

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