Biotechnology Advances 36 (2018) 1730–1737

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Beyond malaria: The inhibition of viruses by artemisinin-type compounds

Thomas Efferth

Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Staudinger Weg 5, 55128 Mainz, Germany

ARTICLE INFO

Keywords:
Antiviral therapy
Artemisia annua
Phytochemicals
Sesquiterpene

ABSTRACT

Natural products represent valuable chemical scaffolds for drug development. A recent success story in this context was artemisinin, which is not only active against malaria but also to other diseases. This raised the interest of artemisinin's potential for drug repurposing. On the present review, we give an overview on artemisinin's antiviral activity. There is good *in vitro* and *in vivo* evidence for the activity of artemisinin and its derivatives against DNA viruses of the Herpesviridae and Hepadnaviridae families such as cytomegaloviruses, human herpesvirus 6, herpes simplex viruses 1 and 2, Epstein-Barr virus and Hepatitis B virus. The evidence is weaker for Polyomaviruses and papilloma viruses. Weaker or no inhibitory activity *in vitro* has been reported for RNA viruses such as human immunodeficiency viruses 1 and 2, hepatitis C virus, influenza virus and others. Interestingly, the artemisinin derivative artesunate did not exert cross-resistance to ganciclovir-resistant HCMV and exerted synergistic inhibition in combination with several clinically established antiviral standard drugs. The antiviral activity of first generation artemisinin derivatives (*e.g.* artesunate, artemether, *etc.*) was enhanced by novel derivatives, including dimer and trimer molecules. First results on patients indicating activity in a subset of HCMV patients. Novel developments in the field of nanotechnology and synthetic biology to bioengineer microorganisms for artemisinin production may pave the way for novel drugs to fight viral infections with artemisinin-based drugs.

1. Introduction

Natural products play an important role as scaffolds for the development of drugs. Innumerous chemical entities derived from natural sourced serve as clinically established drugs against many diseases (Newman and Cragg, 2012). Chemical derivatization, semi- or total synthesis, enzymatic modifications of chemical structures, plant cell and hairy root cultures, and other biotechnological techniques allowed to enlarge the chemical diversity of bioactive compounds, and to upscale their production for clinical utilization. Nowadays, novel technologies supplement the broad methodological range, including nanotechnological devices for natural products, coupling of natural toxins to disease-targeted vectors (*i.e.* antibody-drug conjugation, aptamer-drug conjugates, *etc.*). The heterologous expression of plant or microbial genes encoding entire biosynthetic pathways for pharmacologically active phytochemicals or antibiotics in microorganisms such as *E. coli*, *Bacillus subtilis*, *etc.* favored the emergence of an entire new field, termed synthetic biology (Mora-Pale et al., 2013). As a new branch, white biotechnology supplemented green biotechnology in agriculture and red biotechnology in medicine (Wenzel, 2006; Bouws et al., 2008; Gartland et al., 2013; Singh et al., 2017). It is expected that all these novel disciplines will speed up the drug development process in the years to come.

One of the most thriving showcase examples in natural product-based drug research represents artemisinin. It is a sesquiterpene lactone isolated from the medicinal plant, *Artemisia annua* L. It has been used since ages in traditional Chinese medicine to treat fever and chills. The chemical structure has been elucidated in the 1970s. The discovery of its antimalarial activity led to the conferment of the Nobel Prize for Medicine or Physiology in 2015 to the Chinese scientist, Youyou Tu (Efferth et al., 2015). Semisynthetic derivatives (*i.e.* artesunate, artemether) are now used worldwide for malaria treatment and saved millions of lifes during the past decades.

Intriguingly, the therapeutic spectrum of artemisinin-based compounds is much broader than initially estimated. There is an increasing number of investigations to show that this class of compounds reveal profound activities *in vitro* and *in vivo* against other diseases than malaria, including cancer, viral and other microbial diseases, diabetes, immunological diseases, *etc.* (Fig. 1) (Efferth et al., 2008; Michaelsen et al., 2015; Hou and Huang, 2016; Saeed et al., 2016; Daddy et al.,

Abbreviations: AIDS, acquired immunodeficiency syndrome; BKV, polyomavirus BK; BVDV, bovine viral diarrhea virus; EBTr, embryonic trachea tissue; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HCV, hepatitis C virus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HSV-1, human herpes simplex virus 1; IC_{50}, 50% inhibitory dose; JCPyV, human JC polyomavirus; ROS, reactive oxygen species

E-mail address: efferth@uni-mainz.de.

https://doi.org/10.1016/j.biotechadv.2018.01.001
Received 4 September 2017; Received in revised form 29 December 2017; Accepted 1 January 2018
Available online 03 January 2018
0734-9750/

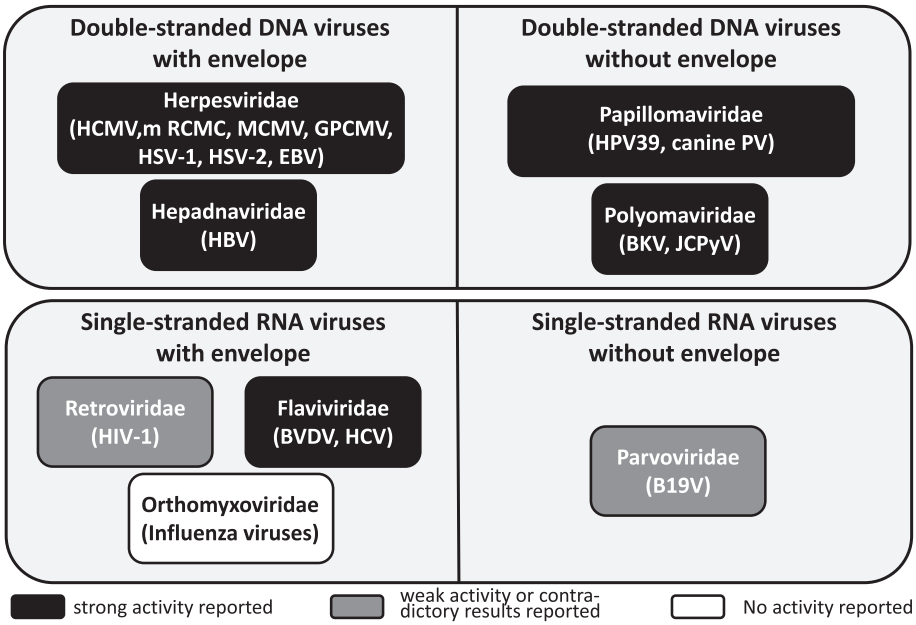

Fig. 1. Synopsis of the antiviral activity of artemisinin-type drugs *in vitro* towards diverse virus types.

2017; Li et al., 2017). While the anticancer activity (including clinical Phase I/II trials) is quite well documented (Efferth, 2017a,b), an updated review on artemisinin's antiviral activity is missing.

The aims of the present article were

(1) To give a comprehensive overview of bioactivities towards a broad range of viruses from diverse taxonomic genera and families;
(2) To critically discuss deficiencies and requirements to bring artemisinin-based therapies from the bench to the bedside; and
(3) To evaluate novel biotechnological approaches to improve and enhance the therapeutic potential of artemisinin-type compounds for antiviral treatment.

2. Human cytomegalovirus (HCMV)

2.1. Experimental data

Most of the investigations on the antiviral activity of artemisinin and its derivatives have been performed on double-stranded DNA viruses. In 2002, it was shown that artesunate inhibited the replication of the human cytomegalovirus (HCMV) in both sensitive and ganciclovir-resistant strains without cross-resistance. The HCMV-induced activation of the cellular transcription factor NF-κB and Sp-1 was also inhibited by artesunate. Furthermore, phosphorylation of the upstream kinase PI3K was downreglated by artesunate. These results have been confirmed in subsequent years (Table 1). The anti-HCMV activity has not been only shown for the widely distributed Towne and AD169 strains, but also for ganciclovir-resistant sublines and clinical isolates (Schnepf et al., 2011). Diverse artemisinin derivatives all revealed anti-HCMV activity, some of which at even lower IC_{50} values than the clinically approved compounds, artesunate and artemether (Arav-Boger et al., 2010; He et al., 2011). The fact that ganciclovir-resistant HCMV strains did not exhibit pronounced cross-resistance to artemisinin-type compounds indicate that clinically refractory HCMV-infections not responding to standard antiviral therapy may still be treatable with this class of compounds. An interesting finding was that fractional application of artesunate or dihydroartemisinin resulted in increased inhibitory activity as compared to application of corresponding single full doses (Flobinus et al., 2014). Reiter et al. (2015a,b) prepared artemisinin-based dimer and trimer molecules as well as 1,2,4-trioxane ferrocene hybrids. One of the trimers (trimer 6) revealed very strong anti-HCMV activity with an IC_{50} value of 0.04 μM, which was much lower than the ones of the control drug ganciclovir (2.6 μM), dihydroartemisinin (> 10 μM) and artesunic acid (3.8 μM). The trioxane ferrocene hybrids 5–7 were also better than the control drugs (IC_{50} values below 0.5 μM). Hutterer et al. (2015) also investigated dimers and trimers synthesized by the Reiter group and observed strong anti-HCMV activity. The anti-cytomegaloviral activity was not restricted to human CMV, but was also found in CMV strain from rat, guinea pig and mouse. Remarkably, the authors could not induce resistance of HCMV towards artesunate. The NF-κB activity upregulated during HCMV replication was partially inhibited by artesunate. The drug bound to NF-κB RelA/p65 *in vitro* and also *in silico* as shown by molecular docking.

The antiviral effectiveness of artemisinin-type compounds has been enhanced by combination therapy with ganciclovir, which revealed strong synergistic effects (Kaptein et al., 2006). A strong synergism of ganciclovir combined with artemisinin-based monomers or dimers was also reported by Cai et al. (2014). However, the same authors observed antagonistic effects with artemisinin dimers and sunitinib. The combination of artesunate plus maribavir also showed improved additive to synergistic anti-HCMV activities (Chou et al., 2011; Morère et al., 2015). Combinations of artesunate with cidofovir or foscarnet yielded additive anti-HCMV effects (Kaptein et al., 2006). Despite the numerous *in vitro* studies, only few authors *in vivo* investigations have been performed. Using a rat cytomegalovirus (RCMV) model, artesunate therapy decreased viral DNA load and virus titers in salivary glands of RCMV-infected rats (Kaptein et al., 2006). The synergistic effect of artesunate combined with maribavir observed *in vitro*, was not found in an *ex vivo* model of first trimester placental CMV infection. Here only additive effects were apparent (Morère et al., 2015).

In addition to cytomegaloviruses, a clinical isolate of human herpes simplex virus 1 (HSV-1) was strongly inhibited by artesunate *in vitro*, while no effect on cell viability on Vero host cells was seen (Efferth et al., 2002). The combination of artesunate and valacyclovir was compared with valacyclovir monotherapy in a murine model of herpes simplex encephalitis (Canivet et al., 2015). Intraperitoneal application to infected mice resulted in a significantly prolonged survival time of the combination regimen compared to valacyclovir alone. Immunological markers (IL-1β, IL-6, IFN-γ, CCL2/4/6) were also more reduced by the combination therpay than by valacyclovir alone.

Table 1
Activity of artemisinin derivatives against double-stranded DNA viruses.

Virus	Family	Drug	Effect	Reference
HCMV AD169	*Herpesviridae*	Artesunate	IC_{50}: 5.8 ± 0.4 μM (*in vitro*)	Efferth et al., 2002
HCMV Towne	*Herpesviridae*	Artesunate	99% inhibition at 15 μM *(in vitro*)	Efferth et al., 2002
HCMV clinical isolates	*Herpesviridae*	Artesunate	69% and 82% inhibition at 15 μM (*in vitro*)	Efferth et al., 2002
HCMV ganciclovir-resistant mutant	*Herpesviridae*	Artesunate	IC_{50}: 6.9 ± 0.2 μM (*in vitro*)	Efferth et al., 2002
HCMV, various sensitive and ganciclovir-resistant strains	*Herpesviridae*	Artesunate	Range of IC_{50} values: 1.5 ± 0.6 to 4.0 ± 0.16 μM no cross-resistance to gancliclovir	Schnepf et al., 2011
HCMV AD169 and clinical isolate VI1210	*Herpesviridae*	Artesunate, dihyroartemisinin, artemisinin	IC_{50} in a range of 3.46 ± 1.14 to 16.51 ± 7.29 μM (with FCS) and 9,50 ± 7.73 to 47.06 ± 13.79 (without FCS); fractional application increased activity of both drugs compared to single doses. Artemisin was inactive.	Flobinus et al., 2014
HCMV Towne	*Herpesviridae*	Artemisinin, artesunate, artemether, artefanilide, dimer sulfone carbonate, dimer primary alcohol	EC_{50} values artemisinin: 16.8 ± 4.0 μM; artesunate: 18.5 ± 5.2 μM; artemether 5.3 ± 2.7 μM; artefanilide 8.1 ± 2.2 μM; dimer sulfone carbonate 0.06 ± < 0.001 μM; dimer primary alcohol 0.15 ± 0.02 μM	Arav-Boger et al., 2010
HCMV Towne	*Herpesviridae*	Artemisinin dimers 832-4; 838; 760; 606; artesunate	EC_{50}: for dimers in the range of 0.04 ± 0.003 to 0.16 ± 0.008 μM; artesunate 6.6 ± 0.4 μM	He et al., 2011
HCMV Towne	*Herpesviridae*	Artemisinin dimers 838; 855; 836; 853; 907; 923; 895; 851; 762; artemether; artesunate	EC_{50} of 7 dimers in the range of 39 ± 3 to 78 ± 4 μM; artemether 5300 ± 2700 μM; artesunate 18.5 ± 5200 μM	Mott et al., 2013
HCMV Towne	*Herpesviridae*	Artemisinin dimer 606; artemisinin dimer 838; artemisinin dimer 762; artemisinin monomer 558; artemisinin monomer 482; artesunate	Dimer 606: 0.16 ± 0.01 μM; dimer 838: 0.039 ± < 0.001 μM; dimer 762: 1.57 ± 0.08 μM; dimer 558: 0.24 ± 0.01 μM; monomer 482: 38.3 ± 2.3 μM; artesunate6.6 ± 0.4 μM	He et al., 2013
HCMV ganciclovir-sensitive and resistant strains	*Herpesviridae*	Artesunate	IC_{50} in HEL cells: range from 3.26 ± 1.36 μM to 6.25 ± 1.22 μM; IC_{50} in HFF cells: range from 2.41 ± 0.84 μM to 4.93 ± 0.98 μM	Chou et al., 2011
HCMV Towne	*Herpesviridae*	Combination therapy of artmisinin dimer 838 plus ganiciclovir, artesunate plus ganciclovir	Both combinations were highly synergistic	He et al., 2012
HCMV Towne	*Herpesviridae*	Gancliclovir or sunitinib plus artemisinin monomers or dimers	Strong synergistic effects with ganciclovir; antagonistic effects with sunitinib	Cai et al., 2014
RCMV	*Herpesviridae*	Artesunate	38% inhibition at 15 μM (*in vitro*) reduction of DNA load and virus titers in salivary glands in rats	Kaptein et al., 2006
HCMV AD169-GFP	*Herpesviridae*	Artemisinin-derived dimer and trimer molecules	IC_{50} of trimer 6: 0.04 μM; IC_{50} of dihydroartemisinin: > 10 μM; IC_{50} of artesunic acid: 3,6 μM; IC_{50} of ganciclovir: 2.6 μM	Reiter et al., 2015a
HCMV AD169-GFP	*Herpesviridae*	1,2,4-Trioxane-ferrecene hybrids	IC_{50} of hybrids 5–7: < 0.5 μM	Reiter et al., 2015b
HCMV AD169-GFP; GPCMV v403-GFP; MCMV Smith-GFP; RCMV Maastricht	*Herpesviridae*	Artesunate	IC_{50} HCMV: 3.9 ± 0.6 μM; IC_{50} GPCMV: 1.2 ± 0.4 μM;IC_{50} MCMV: 9.9 ± 1.2 μM; IC_{50} RCMV: 4.1 ± 0.6 μM	Hutterer et al., 2015
HCMV AD169 and clinical isolate	*Herpesviridae*	Artesunate combination of artesunate plus maribavir	AD169 IC_{50}: 1.96 ± 1.32 μM; clinical isolate IC_{50}: 1.26 ± 0.13 μM AD169: reduction of infection by 40% clinical isolate: no synergistic effect	Morère et al., 2015
HCMV-Towne	*Herpesviridae*	Artesunate, artesunate dimer	Inhibition of HCMV; cell cycle arrest, down-regulation of CDK2/4/6 and hypophosphporylation of RB1 in HFF host cells.	Roy et al., 2015
HCMV-Towne-GFP	*Herpesviridae*	Artesunate	Artesunate treatment (> 12.5 μM) 1.5 h post-HCMV inoculation resulted in significantly reduced infection rates	Zhang et al., 2015
HCMV	*Herpesviridae*	Artesunate alone and in combination with ganciclovir, cidofovir, maribavir, or foscarnet	IC_{50} artesunate: 3.86 ± 1.25 μM; all combinations resulted in synergistic inhibition of HCMV	Drouet et al., 2016
HHV-6A U1102	*Herpesviridae*	Artesunate	IC_{50}: 3.80 ± 1.06 μM	Milbradt et al., 2009
HHV-6A GS	*Herpesviridae*	Artemisinin	Minimal cytotoxic concentration: 40 μg/mL	Naesens et al., 2006
HSV-1 clinical isolate	*Herpesviridae*	Artesunate	83% inhibition at 15 μM (*in vitro*)	Efferth et al., 2002
HSV-1 KOS and clinical isolates; HSV-2 clinical isolates	*Herpesviridae*	Artesunate, artemisinin derivative 838	No activity	He et al., 2012
EBV B95-8	*Herpesviridae*	Artesunate	IC_{50}: 7.21 ± 2.25 μM	Milbradt et al., 2009
EBV	*Herpesviridae*	Artesunate artemisinin dimer 838	No inhibition of lytic replication	He et al., 2012
EBV B95-8	*Herpesviridae*	Artesunate	IC_{50}: 6.4 ± 2.7 μM and 3.1 ± 0.9 μM	Auerochs et al., 2011
HBV	*Hepadnaviridae*	Artemisinin derivatives AD1 and AD4	Reduction of HBV-DNA to culture medium by > 50% at < 0.1 μM	Blazquez et al., 2013
HBV	*Hepadnaviridae*	Artemisinin artesunate	HB_sAg release IC_{50}: 55 μM; HBV-DNA release IC_{50}: > 100 μM HBsAg release IC_{50}: 2.3 μM; HBV-DNA release IC_{50}: 0.5 μM	Romero et al., 2005
BKV	*Polyomaviridae*	Artesunate	65% reduction of virus load by 10 μM artesunate after 72 h	Sharma et al., 2014a
JCPyV	*Polyomaviridae*	Artesunate	EC_{50}: 2.9 μM of viral DNA load 96 h post infection	Sharma et al., 2014b
Canine oral papillomavirus	*Papillomaviridae*	Dihydroartemisinin	No inhibition of virus replication, but inhibition of virus-induced tumor formation *in vivo*	Disbrow et al., 2005
HPV 39	*Papillomaviridae*	Artemisinin	Downregulation of E6 and E7 viral oncoproteins in ME-180 cervical carcinoma cells	Mondal and Chatterji, 2015

2.2. Clinical data

The anti-HCMV activity of artesunate and artemether has been investigated in only few observational case studies and clinical trials. The first hint that artesunate might reveal clinical activity came from stem cell transplant recipients, who experienced HCMV infection. Three patients revealed a rapid viral load decline during 7 days, whereas four other patients showed a continued, yet stalled viral growth slope during treatment (Shapira et al., 2008; Wolf et al., 2011). Another case report described a renal transplant recipient with valganciclovir resistance and mutations in the HCMV genome. Twenty days of intravenous artesunate therapy (180 mg/d) did not reduce the HCMV load (Lau et al., 2011). Another case series reported on two hematopoietic stem cell transplant recipients and three solid-organ transplant recipients with HCMV infection (Germi et al., 2014). Artesunate treatment led to virological and clinical response in two patients with mild infection, but failure in three other cases with fatal HCMV disease. Another hematopoietic stem cell transplantation patient developed a multidrug-resistant HCMV infection. Among other modalities, the patient was treated with artesunate, but he did not respond to this drug (Stuehler et al., 2015).

As yet, there is one larger clinical trial concerning artesunate treatment of HCMV-infected patients. However, viral infections frequently occur together with other diseases in co-morbid patients. In the course of malaria treatment, 11.4% out of 494 Ugandan children were diagnosed with HCMV infection prior to malaria treatment. All patients were randomized either for artesunate plus amodiaquine or sulfadoxine-pyrimethamine to manage their malaria infections. Concomitant determination of HCMV load revealed no measurable difference in either the frequency or quantity of HCMV detected in blood between patients of the two treatment arms. The author concluded that the artesunate-containing anti-malarial regimen exerted no detectable effect on HCMV viremia in children with malaria (Gantt et al., 2013). In 164 malaria patients with HCMV infection, the virus was shedded at high rates into the urine. Artemether-lumefantrine combination therapy decreased the urine virus loads (Barger-Kamate et al., 2016).

3. Human immunodeficiency virus (HIV)

In areas with high malaria incidence, *Artemisia annua* decoctions are frequently used to treat malaria. Since malaria patients occasionally may also suffer from AIDS due to infection with human immunodeficiency virus (HIV), it has been speculated that *A. annua* may also exert anti-HIV activity. Indeed, *A. annua* tea infusions inhibited HIV with an IC_{50} value of 2.9 μg/mL *in vitro* (Lubbe et al., 2012). Surprisingly, the isolated artemisinin did not show inhibitory effects up to concentrations of 25 μg/mL. Another *Artemisia* species, *A. afra*, also revealed anti-HIV activity *in vitro*. As *A. afra* is known not to contain artemisinin, this compound might not be responsible for the effects observed with *A. annua*. While Efferth et al. (2002) found only weak inhibition rates against two HIV-1 strains, Jana et al. (2017) synthesized six 1,5-disubstituted 1,2,3-triazole derivatives of dihydroartemisinin, and three of them showed considerable anti-HIV activity (IC_{50} values: 1.34–2.65 μM). In another study, artesunate at a concentration of 10 μM only modestly inhibited the NL4.3 HIV stain in peripheral blood mononuclear cells by 60% (Oguariri et al., 2010). In conclusion, the available *in vitro* data do not support a major role of the lead compound artemisinin for the management of HIV infections as of yet. However, novel derivatives may offer better alternatives.

4. Other viruses

4.1. Double-stranded DNA viruses (with envelope)

Human herpesvirus 6 (HHV6) is an AIDS-related virus that has been a frequent subject for investigations to identify antiviral compounds. The results on artemisinin and its derivatives are, however, somewhat controversial. Whereas Naesens et al. (2006) reported that artemisinin did not affect HHV-6A and -B replication in HSB2 and MOL3 cells, respectively, Milbradt et al. (2009) found an inhibition of HHV-6A replication as well as early and late protein synthesis at an IC_{50} value of 3.8 ± 1.06 μM.

Interestingly, the compassionate use of artesunate in a child suffering from HHV-6-related myocarditis improved the clinical status and heart function of the patient (Hakacova et al., 2013). The therapeutic improvement was correlated with a reduction of HHV-6B DNA in endomyocardial biopsies and lymphocytic infiltration. Side effects were not observed in the patient.

Using an Epstein-Barr virus (EBV)-based reporter construct, artesunate revealed inhibitory activity at low micromolar concentrations. The inhibition of EBV was due to suppression of biosynthesis of immediate early EBV proteins (Auerochs et al., 2011).

Romero et al. (2005) found that both artemisinin and artesunate inhibited hepatitis B virus (HBV) production at non-cytotoxic concentrations. The antiviral effects of several artemisinin derivatives were enhanced by iron-containing compounds (hemin, Ferrosanol™) (Blazquez et al., 2013).

4.2. Single-stranded RNA viruses (with envelope)

Artemisinin and its derivatives are also active against single-stranded RNA viruses. Using a luciferase assay, artemisinin inhibited the replication of a hepatitis C virus (HCV) genomic fragment in Huh 5-2 cell at an EC_{50} concentration of 78 ± 21 μM. Furthermore, the combination of artemisinin with hemin led to a synergistic HCV inhibition (Paeshuyse et al., 2006). The same authors also found that a panel of artemisinin derivatives inhibited full-length infectious HCV JFH1 in hepatoma cell with much better efficacy than the lead compound, artemisinin (Obeid et al., 2013). They suggest that reactive oxygen species (ROS) rather than carbon-centered radical molecules are responsible for the anti-HCV activity of artemisinin, since the ROS-inhibitor, L-*N*-acetylcysteine, inhibited artemisinin's activity. Huh7-5-1 and OR6 cells infected with the HCV strain JFH1 were used to test the effect of artesunate. The virus replication was inhibited, while the cell proliferation rates remained unchanged (Dai et al., 2016).

The Dengue virus is another single-stranded RNA virus. Interestingly, a malaria patient also suffering from Dengue shock syndrome-related acute renal failure was successfully treated with parenteral artemisinin (Thaha et al., 2008) in addition to standard multimodal therapy to manage the Dengue shock syndrome. The patient responded to treatment, but a specific effect of artemisinin on the Dengue virus load has not been demonstrated. The clinical activity of artemisinin against Dengue virus, therefore, remains elusive.

Artesunate was not active against influenza viruses. In MDCK cells infected with the InflVA (WSN/33) strain, neither replication nor host cell viability were affected (Efferth et al., 2002).

Artesunate was also active against bovine viral diarrhea virus (BVDV). Epithelial cells derived from embryonic trachea tissue (EBTr) infected with the BVDV-strain C24V were treated with artesunate. The drug prevented BVDV-induced death of EBTr cells in a similar fashion as the control drug ribavirin did (Romero et al., 2006).

4.3. Double-stranded DNA viruses (without envelope)

There are several reports on the activity of artemisinin-type compounds against double-stranded DNA viruses. Sharma et al. (2014a,b) investigated the antiviral effects of artesunate towards polyomavirus BK (BKV). This virus causes nephropathy and hemorrhagic cystitis in transplantation patients. Artesunate inhibited BKV replication in human primary renal proximal tubular epithelial cells. The antiviral effects were apparent in terms of viral DNA loads as well as early large T antigen in RNA and protein expression. However, artesunate also exerted anti-proliferative effects towards the host cells (Sharma et al.,

2014a).

Another polyomavirus, the human JC polyomavirus (JCPyV) was also inhibited by artesunate as measured by viral load and VP1 capsid protein expression (Sharma et al., 2014b). This virus causes progressive multifocal leukoencephalopathy.

Disbrow et al. (2005) analyzed the cytotoxicity of artemisinin, artesunate and dihydroartemisinin towards human papillomavirus (HPV)-immortalized and transformed cervix cells. Artesunate and dihydroartemisinin induced cellular apoptosis without affecting HPV-related oncogene expression. The oral mucosa of dogs infected with canine oral papillomavirus was topically treated with dihydroartemisinin, and the dogs developed antibodies against the viral L1 capsid protein. The authors concluded that dihydroartemisinin may exert antitumor, but not anti-papillomavirus effects. Mondal and Chatterji (2015) also investigated the effect of artemisinin on human papillomaviruses. They found that artemisinin downregulated the expression of HPV39 encoded E6 and E7 proteins in infected ME-180 cervical carcinoma cells. This might indicate that the antiproliferative effect of artemisinin on cervix cancer cells is caused by downregulation of the oncogenic viral E6 and E7 proteins. Studies with the high-risk HPV 18 and 16 are missing as yet.

4.4. Single-stranded DNA-viruses (without envelope)

Parvovirus infections occasionally occur together with malaria. Therefore, antimalarial drugs including artesunate have been investigated for their activity towards human parvovirus B19 (B19V). Artesunate exerted moderate activity at concentrations higher than 10 μM *in vitro* (Bönsch et al., 2010).

5. Conclusions and perspectives

As shown in Tables 1 and 2 and Fig. 1, artemisinin-based drugs inhibited some viruses. It seems that artemisinin and its derivatives act better against double-stranded DNA viruses with envelope (Herpesviridae and Hepadnaviridae) than against other virus types. However, much less has been published on single-stranded RNA viruses with or without envelope. It is still too early to draw a final conclusion, whether or not artemisinin is active against such virus-types. To my opinion, the future developments for research on the antiviral activity of artemisinin-type drugs are in the following fields:

5.1. Medicinal chemistry

Some studies demonstrated that artemisinin derivatives were strongly active against viruses, while the lead compound was considerably weakly active (Tables 1 and 2). This indicates that the antiviral potential may be improved by derivatization of the lead chemical scaffold. This is a main task of medicinal chemistry. While the number of artemisinin derivatives tested against viruses is limited, a plethora of artemisinin derivatives have been synthesized during the past two decades for the improvement of malaria and cancer therapy. All these derivatives await to be tested for their antiviral activity. In addition to modified artemisinin molecules, numerous dimer and trimer artemisinin-based complexes have been prepared. Besides homodimers (between two artemisinin molecules), heterodimers have also been published (*e.g.* artemisinin-ferrocene, and others) (Reiter et al., 2015b). It is beyond the scope of this review to give a detailed overview on the novel development in this field. Therefore, the reader is referred to the recent literature (O'Neill et al., 1999; Posner et al., 1999; Li et al., 2000; Vennerstrom et al., 2000; Ma et al., 2000; Ekthawatchai et al., 2001; Avery et al., 2002; Paik et al., 2006; Hencken et al., 2010; Njuguna et al., 2012; Fröhlich et al., 2016; Ren et al., 2016).

5.2. Nanotechnology

The hydrophobic artemisinin – like many other natural products too – is not well soluble in water. This is a challenge for pharmaceutical formulation, because the bioavailability is low. This and the drug's short half-life time causes suboptimal absorption and distribution in the body. Novel devices from nanotechnology may improve the therapeutic index of artemisinin-type drugs. Nanotechnology experienced a thriving development during the past years, because nano-sized drug formulations (below 200–300 nm) bear a tremendous therapeutic

Table 2
Activity of artemisinin derivatives against single-stranded RNA viruses.

Virus	Family	Drug	Effect	Reference
HIV-1 Ba-L HIV-1 NL4-3	*Retroviridae* *Retroviridae*	Artesunate	Inhibition of replication by 64 ± 1% of control at 600 nM; inhibition of replication by 52 ± 3% of control at 600 nM	Efferth et al., 2002
HIV-1 NL4-3	*Retroviridae*	Artemisinin	Inhibition of replication by 60% of control at 10 μM	Oguariri et al., 2010
iFIGS HIV-1 plasmid	*Retroviridae*	Artemisinin *Artemisia annua Artemisia afra*	Not active at 25 μg/mL IC_{50}: 2.0 μg/mL similar activity than *A. annua*	Lubbe et al., 2012
HIV-1 IIIb, HIV-1 ROD, HIV-2 RES056	*Retroviridae*	1,5-Disubstituted dihydroartemisinin derivatives	IC_{50} of 3 derviatives: 1.34 to 2.65 μM	Jana et al., 2017
BVDV	*Flaviviridae*	Dihydroartemisinin 8 artemisinin derivatives	Inhibition of BVDV-RNA release by AD1 and AD2	Blazquez et al., 2013
BVDV	*Flaviviridae*	Artemisinin	Reduction of BVDV-induced host cell death	Romero et al., 2006
HCV	*Flaviviridae*	Artesunate 6 artemisinin derivatives	EC_{50}: 75 ± 7 μM EC_{50}: 3.2 ± 2.4 to 36 ± 16 μM	Obeid et al., 2013
HCV	*Flaviviridae*	Artemisinin	EC_{50}: 14 ± 21 and 78 ± 21 μM	Paeshuyse et al., 2006
HCV JFH1	*Flaviviridae*	Artesunate	Dose-dependent inhibition of replication	Dai et al., 2016
Influenza virus A WSN/33	*Orthomyxoviridae*	Artesunate	No inhibition	Efferth et al., 2002
Influenza virus A	*Orthomyxoviridae*	Artesunate	No inhibition	Milbradt et al., 2009
B19V	Parvovirus	Artesunate	Moderate inhibition above 10 μM	Bönsch et al., 2010

potential. Novel nano-formulations have also been developed for artemisinin and it derivatives (Efferth et al., 2016; Aderibigbe, 2017):

- Liposomes are vesicles formed by lipid bilayers, which entrap drugs. Thereby, longer half-life times, reduced toxicity and better target specificity of the drugs can be yielded. The generation of artemisinin- and artemether-based liposomes has been reported (Isacchi et al., 2011; Chen et al., 2015).
- Ninosomes are liposomes consisting of non-ionic surfactants. They are more stable than liposomes. Artesunate and artemisone have been encapsuled in ninosomes (Asgharkhani et al., 2014; Dwivedi et al., 2015).
- Soft lipid vesicles are termed ethosomes. Artesunate has been used to prepare ethosomes (Shen et al., 2015).
- Nanocapsules have a shell composed of chitosan, gelatin, alginate and provide increased bioavailability of the enclosed drug. Nanocapsules have been reported for artemisinin and artesunate (Chen et al., 2009; Xiao and Hong, 2010).
- Micelles result from self-assembly of amphiphilic molecules in water leading to improved drug stability and increased bioavailability. Artemisinin- and artemether-containing micelles have been prepared (Bhadra et al., 2005; Wang et al., 2012).
- Nanotubes are carbon-based biodegradable drug delivery systems. Several nanotubes with encapsuled artemisinins have been reported (Rezaei et al., 2011; Zhang et al., 2015).
- Lipid nanoparticles are composed of triglycerides. They have been prepared with several artemisinin-type drugs, *e.g.* dihydroartemisinin, artemether, arteether, an artemisinin dimer, and artemisone (Aditya et al., 2010; Zhang et al., 2010, 2013; Dwivedi et al., 2014).
- Polymer-based nanoparticles consist of biodegradable polymers such as polyactic acid, chitosan, gelatin, poly-alkyl-cyanoacrylates, *etc.*) to provide controlled drug release. Artemisinin, artesunate and dihydroartemisinin have been incorporated into a number of different nanoparticles (Chadha et al., 2012; Ibrahim et al., 2015; Nguyen et al., 2015; Want et al., 2015).
- Polymer-based drug conjugates are biodegradable molecules that form a backbone for coalnet linkage with therapeutic drug molecules. Polymers used for drug conjugation are polyethylene glycol, cyclodextrins, chitosan, polyurethane, polyorganophosphazenes and others. Polymer-drug conjugates are known for artemisinin, dihydroartemisinin and artemether (Efferth et al., 2004; Yaméogo et al., 2012; Wang et al., 2013; Xiao et al., 2013; Kumar et al., 2015; Jabbarzadegan et al., 2017).

Nanotechnology for artemisinin-type drugs has been mainly used to treat malaria and cancer. A few studies reported on their use against leishmaniosis (Want et al., 2015). Artemisinin-based nano-drugs have not been used against viral infections as of yet. It can be expected that major improvements can be achieved by taking advantage of this technology, especially in animal experiment and clinical trials in patients, where the full potential of artemisinin-based drugs to combat viral diseases still remains to be convincingly demonstrated.

5.3. Synthetic biology

As the amount of artemisinin in wild-type *Artemisia annua* plants is only low (0.01–0.8%), there is a growing demand to increase sustainable artemisinin production. Although total synthesis is possible (Schmid and Hofheinz, 1983; Ravindranathan et al., 1990; Avery et al., 1992), it is not routinely done, because it is too time-consuming and not economical. Other approaches have been developed to increase the yield of artemisinin for the global market, *e.g.* the breeding of high-yield cultivars in field and greenhouses and the cultivation of transgenic plants that produce more artemisinin than wild-type plants (Laughlin, 1994; Delabays et al., 2001). An effective biotechnological technique is hairy root cultures of *Artemisia annua*, which can rapidly produce high amounts of artemisinin (Patra and Srivastava, 2016).

A revolutionary development in biotechnology is termed synthetic biology, which is the bioengineering of entire microorganisms to fulfill a specific function (Khalil and Collins, 2010; Weber and Fussenegger, 2011). A showcase example in synthetic biology has been the heterologous expression of the biosynthetic pathway for artemisinin in microorganisms (*E. coli*, *Saccharomyces cerevisiae*, *etc.*) (Martin et al., 2003; Ro et al., 2006; Withers and Keasling, 2007; Liu et al., 2011; Paddon et al., 2013). A precondition for this technical breakthrough was the elucidation of the biosynthesis pathway in *Artemisia annua* and the cloning of the genes encoding the corresponding biosynthetic enzymes (Kirby and Keasling, 2009). With the application of such high-tech methodologies, it is possible to upscale the artemisinin production without restrictions and to fulfill the global demand on artemisinin. It is to be hoped that artemisinin will not only be used for the malaria therapy market, but also to treat viral diseases in the future.

5.4. Clinical trials

Preliminary data have been generated on the treatment of viral diseases in patients. For HCMV, artesunate seemed to exert a positive effect in a subset of patients. The main challenge is that convincing clinical evidence is still missing for viral infections. In a comparable fashion as for cancer and schistosomiasis (Jansen et al., 2011; Krishna et al., 2014; Saeed et al., 2016; von Hagens et al., 2017), phase I/II trials should be performed for viral infections. Without placebo-controlled double-blind clinical trials, it cannot be finally decided, whether or not artemisinin-type drugs are useful weapons in the fight against viral diseases.

References

Aderibigbe, B.A., 2017. Design of drug delivery systems containing artemisinin and its derivatives. Molecules 22 (2) (pii: E323).

Aditya, N.P., Patankar, S., Madhusudhan, B., Murthy, R.S., Souto, E.B., 2010. Arthemeter-loaded lipid nanoparticles produced by modified thin-film hydration: Pharmacokinetics, toxicological and in vivo anti-malarial activity. Eur. J. Pharm. Sci. 40 (5), 448–455.

Arav-Boger, R., He, R., Chiou, C.J., Liu, J., Woodard, L., Rosenthal, A., Jones-Brando, L., Forman, M., Posner, G., 2010. Artemisinin-derived dimers have greatly improved anti-cytomegalovirus activity compared to artemisinin monomers. PLoS One 5 (4), e10370.

Asgharkhani, E., Najmafshar, A., Chiani, M., 2014. Artemisinin (ART) drug delivery using mixed non-ionic surfactants and evaluation of their efficiency in different cancer cell lines. Int. J. Drug Deliv. Technol. 4, 67–71.

Auerochs, S., Korn, K., Marschall, M., 2011. A reporter system for Epstein-Barr virus (EBV) lytic replication: anti-EBV activity of the broad anti-herpesviral drug artesunate. J. Virol. Methods 173 (2), 334–339.

Avery, M.A., Chong, W.K.M., Jennings-White, C., 1992. Stereoselective total synthesis of (dextro)-artzemisinin, the antimalarial constituent of *Artemisia annua* L. J. Am. Chem. Soc. 14, 974–979.

Avery, M.A., Alvim-Gaston, M., Vroman, J.A., Wu, B., Ager, A., Peters, W., Robinson, B.L., Charman, W., 2002. Structure-activity relationships of the antimalarial agent artemisinin. 7. Direct modification of (+)-artemisinin and in vivo antimalarial screening of new, potential preclinical antimalarial candidates. J. Med. Chem. 45 (19), 4321–4335.

Barger-Kamate, B., Forman, M., Sangare, C.O., Haidara, A.S., Maiga, H., Vaidya, D., Djimde, A., Arav-Boger, R., 2016. Effect of artemether-lumefantrine (Coartem) on cytomegalovirus urine viral load during and following treatment for malaria in children. J. Clin. Virol. 77, 40–45.

Bhadra, D., Bhadra, S., Jain, N.K., 2005. Pegylated lysine based copolymeric dendritic micelles for solubilization and delivery of artemether. J. Pharm. Pharm. Sci. 8 (3), 467–482.

Blazquez, A.G., Fernandez-Dolon, M., Sanchez-Vicente, L., Maestre, A.D., Gomez-San Miguel, A.B., Alvarez, M., Serrano, M.A., Jansen, H., Efferth, T., Marin, J.J., Romero, M.R., 2013. Novel artemisinin derivatives with potential usefulness against liver/colon cancer and viral hepatitis. Bioorg. Med. Chem. 21 (14), 4432–4441.

Bönsch, C., Kempf, C., Mueller, I., Manning, L., Laman, M., Davis, T.M., Ros, C., 2010. Chloroquine and its derivatives exacerbate B19V-associated anemia by promoting viral replication. PLoS Negl. Trop. Dis. 4 (4), e669.

Bouws, H., Wattenberg, A., Zorn, H., 2008. Fungal secretomes—nature's toolbox for white biotechnology. Appl. Microbiol. Biotechnol. 80 (3), 381–388.

Cai, H., Kapoor, A., He, R., Venkatadri, R., Forman, M., Posner, G.H., Arav-Boger, R., 2014. In vitro combination of anti-cytomegalovirus compounds acting through

different targets: role of the slope parameter and insights into mechanisms of Action. Antimicrob. Agents Chemother. 58 (2), 986–994.
Canivet, C., Menasria, R., Rhéaume, C., Piret, J., Boivin, G., 2015. Valacyclovir combined with artesunate or rapamycin improves the outcome of herpes simplex virus encephalitis in mice compared to antiviral therapy alone. Antivir. Res. 123, 105–113.
Chadha, R., Gupta, S., Pathak, N., 2012. Artesunate-loaded chitosan/lecithin nanoparticles: preparation, characterization, and in vivo studies. Drug Dev. Ind. Pharm. 38 (12), 1538–1546.
Chen, Y., Lin, X., Park, H., Greever, R., 2009 Sep. Study of artemisinin nanocapsules as anticancer drug delivery systems. Nanomedicine 5 (3), 316–322.
Chen, H.J., Huang, X.R., Zhou, X.B., Zheng, B.Y., Huang, J.D., 2015. Potential sonodynamic anticancer activities of artemether and liposome-encapsuled artemether. Chem. Commun. 51, 4681–4684.
Chou, S., Marousek, G., Auerochs, S., Stamminger, T., Milbradt, J., Marschall, M., 2011. The unique antiviral activity of artesunate is broadly effective against human cytomegaloviruses including therapy-resistant mutants. Antivir. Res. 92 (2), 364–368.
Daddy, N.B., Kalisya, L.M., Bagire, P.G., Watt, R.L., Towler, M.J., Weathers, P.J., 2017. *Artemisia annua* dried leaf tablets treated malaria resistant to ACT and i.v. artesunate: Case reports. Phytomedicine 32, 37–40.
Dai, R., Xiao, X., Peng, F., Li, M., Gong, G., 2016. Artesunate, an anti-malarial drug, has a potential to inhibit HCV replication. Virus Genes 52 (1), 22–28.
Delabays, N., Simonnet, X., Gaudin, M., 2001. The genetics of artemisinin content in *Artemisia annua* L. and the breeding of high yielding cultivars. Curr. Med. Chem. 8 (15), 1795–1801.
Disbrow, G.L., Baege, A.C., Kierpiec, K.A., Yuan, H., Centeno, J.A., Thibodeaux, C.A., Hartmann, D., Schlegel, R., 2005. Dihydroartemisinin is cytotoxic to papillomavirus-expressing epithelial cells in vitro and in vivo. Cancer Res. 65 (23), 10854–10861.
Drouot, E., Piret, J., Boivin, G., 2016. Artesunate demonstrates in vitro synergism with several antiviral agents against human cytomegalovirus. Antivir. Ther. 21 (6), 535–539.
Dwivedi, P., Khatik, R., Khandelwal, K., Taneja, I., Raju, K.S., Wahajuddin, Paliwal, S.K., Dwivedi, A.K., Mishra, P.R., 2014. Pharmacokinetics study of arteether loaded solid lipid nanoparticles: an improved oral bioavailability in rats. Int. J. Pharm. 466 (1–2), 321–327.
Dwivedi, A., Mazumder, A., Du Plessis, L., Du Preez, J.L., Haynes, R.K., Du Plessis, J., 2015. In vitro anti-cancer effects of artemisone nano-vesicular formulations on melanoma cells. Nanomed. Nanotechnol. Biol. Med. 11, 2041–2050.
Efferth, T., 2017a. From ancient herb to modern drug: Artemisia annua and artemisinin for cancer therapy. Semin. Cancer Biol. 46, 65–83.
Efferth, T., 2017b. Cancer combination therapies with artemisinin-type drugs. Biochem. Pharmacol. 139, 56–70.
Efferth, T., Marschall, M., Wang, X., Huong, S.M., Hauber, I., Olbrich, A., Kronschnabl, M., Stamminger, T., Huang, E.S., 2002. Antiviral activity of artesunate towards wild-type, recombinant, and ganciclovir-resistant human cytomegaloviruses. J. Mol. Med. (Berl.) 80 (4), 233–242.
Efferth, T., Benakis, A., Romero, M.R., Tomicic, M., Rauh, R., Steinbach, D., Häfer, R., Stamminger, T., Oesch, F., Kaina, B., Marschall, M., 2004. Enhancement of cytotoxicity of artemisinins toward cancer cells by ferrous iron. Free Radic. Biol. Med. 37 (7), 998–1009.
Efferth, T., Romero, M.R., Wolf, D.G., Stamminger, T., Marin, J.J., Marschall, M., 2008. The antiviral activities of artemisinin and artesunate. Clin. Infect. Dis. 47 (6), 804–811.
Efferth, T., Zacchino, S., Georgiev, M.I., Liu, L., Wagner, H., Panossian, A., 2015. Nobel Prize for artemisinin brings phytotherapy into the spotlight. Phytomedicine 22 (13), A1–3.
Efferth, T., Romero, M.R., Bilia, A.R., Osman, A.G., ElSohly, M., Wink, M., Bauer, R., Khan, I., Bergonzi, M.C., Marin, J.J.G., 2016. Expanding the therapeutic spectrum of artemisinin: activity against infectious diseases beyond malaria and novel pharmaceutical developments. World J. Tradit. Chin. Med. 2 (2), 1–23.
Ekthawatchai, S., Kamchonwongpaisan, S., Kongsaeree, P., Tarnchompoo, B., Thebtaranonth, Y., Yuthavong, Y., 2001. C-16 artemisinin derivatives and their antimalarial and cytotoxic activities: syntheses of artemisinin monomers, dimers, trimers, and tetramers by nucleophilic additions to artemisitene. J. Med. Chem. 44 (26), 4688–4695.
Flobinus, A., Taudon, N., Desbordes, M., Labrosse, B., Simon, F., Mazeron, M.C., Schnepf, N., 2014. Stability and antiviral activity against human cytomegalovirus of artemisinin derivatives. J. Antimicrob. Chemother. 69 (1), 34–40.
Fröhlich, T., Çapcı Karagöz, A., Reiter, C., Tsogoeva, S.B., 2016. Artemisinin-derived dimers: Potent antimalarial and anticancer agents. J. Med. Chem. 59 (16), 7360–7388.
Gantt, S., Huang, M.L., Magaret, A., Bunts, L., Selke, S., Wald, A., Rosenthal, P.J., Dorsey, G., Casper, C., 2013. An artesunate-containing antimalarial treatment regimen did not suppress cytomegalovirus viremia. J. Clin. Virol. 58 (1), 276–278.
Gartland, K.M., Bruschi, F., Dundar, M., Gahan, P.B., Viola Magni, M.P., Akbarova, Y., 2013. Progress towards the 'golden age' of biotechnology. Curr. Opin. Biotechnol. 24 (Suppl. 1), S6–13.
Germi, R., Mariette, C., Alain, S., Lupo, J., Thiebaut, A., Brion, J.P., Epaulard, O., Saint Raymond, C., Malvezzi, P., Morand, P., 2014. Success and failure of artesunate treatment in five transplant recipients with disease caused by drug-resistant cytomegalovirus. Antivir. Res. 101, 57–61.
Hakacova, N., Klingel, K., Kandolf, R., Engdahl, E., Fogdell-Hahn, A., Higgins, T., 2013. First therapeutic use of Artesunate in treatment of human herpesvirus 6B myocarditis in a child. J. Clin. Virol. 57 (2), 157–160.
He, R., Mott, B.T., Rosenthal, A.S., Genna, D.T., Posner, G.H., Arav-Boger, R., 2011. An artemisinin-derived dimer has highly potent anti-cytomegalovirus (CMV) and anticancer activities. PLoS One 6 (8), e24334.
He, R., Park, K., Cai, H., Kapoor, A., Forman, M., Mott, B., Posner, G.H., Arav-Boger, R., 2012. Artemisinin-derived dimer diphenyl phosphate is an irreversible inhibitor of human cytomegalovirus replication. Antimicrob. Agents Chemother. 56 (7), 3508–3515.
He, R., Forman, M., Mott, B.T., Venkatadri, R., Posner, G.H., Arav-Boger, R., 2013. Unique and highly selective anticytomegalovirus activities of artemisinin-derived dimer diphenyl phosphate stem from combination of dimer unit and a diphenyl phosphate moiety. Antimicrob. Agents Chemother. 57 (9), 4208–4214.
Hencken, C.P., Jones-Brando, L., Bordón, C., Stohler, R., Mott, B.T., Yolken, R., Posner, G.H., Woodard, L.E., 2010. Thiazole, oxadiazole, and carboxamide derivatives of artemisinin are highly selective and potent inhibitors of *Toxoplasma gondii*. J. Med. Chem. 53 (9), 3594–3601.
Hou, L., Huang, H., 2016. Immune suppressive properties of artemisinin family drugs. Pharmacol. Ther. 166, 123–127.
Hutterer, C., Niemann, I., Milbradt, J., Fröhlich, T., Reiter, C., Kadioglu, O., Bahsi, H., Zeitträger, I., Wagner, S., Einsiedel, J., Gmeiner, P., Vogel, N., Wandinger, S., Godl, K., Stamminger, T., Efferth, T., Tsogoeva, S.B., Marschall, M., 2015. The broad-spectrum antiinfective drug artesunate interferes with the canonical nuclear factor kappa B (NF-κB) pathway by targeting RelA/p65. Antivir. Res. 124, 101–109.
Ibrahim, N., Ibrahim, H., Sabater, A.M., Mazier, D., Valentin, A., Nepveu, F., 2015. Artemisinin nanoformulation suitable for intravenous injection: Preparation, characterization and antimalarial activities. Int. J. Pharm. 495 (2), 671–679.
Isacchi, B., Arrigucci, S., la Marca, G., Bergonzi, M.C., Vannucchi, M.G., Novelli, A., Bilia, A.R., 2011. Conventional and long-circulating liposomes of artemisinin: preparation, characterization, and pharmacokinetic profile in mice. J. Liposome Res. 21 (3), 237–244.
Jabbarzadegan, M., Rajayi, H., Mofazzal Jahromi, M.A., Yeganeh, H., Yousefi, M., Muhammad Hassan, Z., Majidi, J., 2017. Application of arteether-loaded polyurethane nanomicelles to induce immune response in breast cancer model. Artif. Cells Nanomed. Biotechnol. 45 (4), 808–816.
Jana, S., Iram, S., Thomas, J., Hayat, M.Q., Pannecouque, C., Dehaen, W., 2017. Application of the triazolization reaction to afford dihydroartemisinin derivatives with anti-HIV activity. Molecules 22 (2) (pii: E303).
Jansen, F.H., Adoubi, I., KC, J.C., DE Cnodder, T., Jansen, N., Tschulakow, A., Efferth, T., 2011. First study of oral Artenimol-R in advanced cervical cancer: clinical benefit, tolerability and tumor markers. Anticancer Res. 31 (12), 4417–4422.
Kaptein, S.J., Efferth, T., Leis, M., Rechter, S., Auerochs, S., Kalmer, M., Bruggeman, C.A., Vink, C., Stamminger, T., Marschall, M., 2006. The anti-malaria drug artesunate inhibits replication of cytomegalovirus in vitro and in vivo. Antivir. Res. 69 (2), 60–69.
Khalil, A.S., Collins, J.J., 2010. Synthetic biology: applications come of age. Nat. Rev. Genet. 11 (5), 367–379.
Kirby, J., Keasling, J.D., 2009. Biosynthesis of plant isoprenoids: perspectives for microbial engineering. Annu. Rev. Plant Biol. 60, 335–355.
Krishna, S., Ganapathi, S., Ster, I.C., Saeed, M.E., Cowan, M., Finlayson, C., Kovacsevics, H., Jansen, H., Kremsner, P.G., Efferth, T., Kumar, D., 2014. A randomised, double blind, placebo-controlled pilot study of oral artesunate therapy for colorectal cancer. EBioMedicine 2 (1), 82–90.
Kumar, S., Singh, R.K., Murthy, R.S., Bhardwaj, T.R., 2015. Synthesis and evaluation of substituted poly(organophosphazenes) as a novel nanocarrier system for combined antimalarial therapy of primaquine and dihydroartemisinin. Pharm. Res. 32 (8), 2736–2752.
Lau, P.K., Woods, M.L., Ratanjee, S.K., John, G.T., 2011. Artesunate is ineffective in controlling valganciclovir-resistant cytomegalovirus infection. Clin. Infect. Dis. 52 (2), 279.
Laughlin, J.C., 1994. Agricultural production of artemisinin - a review. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. 1), S21–2.
Li, Y., Zhu, Y.M., Jiang, H.J., Pan, J.P., Wu, G.S., Wu, J.M., Shi, Y.L., Yang, J.D., Wu, B.A., 2000. Synthesis and antimalarial activity of artemisinin derivatives containing an amino group. J. Med. Chem. 43 (8), 1635–1640.
Li, J., Casteels, T., Frogne, T., Ingvorsen, C., Honoré, C., Courtney, M., Huber, K.V., Schmitner, N., Kimmel, R.A., Romanov, R.A., Sturtzel, C., Lardeau, C.H., Klughammer, J., Farlik, M., Sdelci, S., Vieira, A., Avolio, F., Briand, F., Baburin, I., Májek, P., Pauler, F.M., Penz, T., Stukalov, A., Gridling, M., Parapatics, K., Barbieux, C., Berishvili, E., Spittler, A., Colinge, J., Bennett, K.L., Hering, S., Sulpice, T., Bock, C., Distel, M., Harkany, T., Meyer, D., Superti-Furga, G., Collombat, P., Hecksher-Sørensen, J., Kubicek, S., 2017. Artemisinins target $GABA_A$ receptor signaling and impair α cell identity. Cell 168 (1–2) (86-100.e15).
Liu, B., Wang, H., Du, Z., Li, G., Ye, H., 2011. Metabolic engineering of artemisinin biosynthesis in *Artemisia annua* L. Plant Cell Rep. 30 (5), 689–694.
Lubbe, A., Seibert, I., Klimkait, T., van der Kooy, F., 2012. Ethnopharmacology in overdrive: the remarkable anti-HIV activity of *Artemisia annua*. J. Ethnopharmacol. 141 (3), 854–859.
Ma, J., Katz, E., Kyle, D.E., Ziffer, H., 2000. Syntheses and antimalarial activities of 10-substituted deoxoartemisinins. J. Med. Chem. 43 (22), 4228–4232.
Martin, V.J., Pitera, D.J., Withers, S.T., Newman, J.D., Keasling, J.D., 2003. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nat. Biotechnol. 21 (7), 796–802.
Michaelsen, F.W., Saeed, M.E., Schwarzkopf, J., Efferth, T., 2015. Activity of *Artemisia annua* and artemisinin derivatives in prostate carcinoma. Phytomedicine 22 (14), 1223–1231.
Milbradt, J., Auerochs, S., Korn, K., Marschall, M., 2009. Sensitivity of human herpesvirus 6 and other human herpesviruses to the broad-spectrum antiinfective drug artesunate. J. Clin. Virol. 46 (1), 24–28.
Mondal, A., Chatterji, U., 2015. Artemisinin represses telomerase subunits and induces apoptosis in HPV-39 infected human cervical cancer cells. J. Cell. Biochem. 116 (9), 1968–1981.

Mora-Pale, M., Sanchez-Rodriguez, S.P., Linhardt, R.J., Dordick, J.S., Koffas, M.A., 2013. Metabolic engineering and in vitro biosynthesis of phytochemicals and non-natural analogues. Plant Sci. 210, 10–24.
Morère, L., Andouard, D., Labrousse, F., Saade, F., Calliste, C.A., Cotin, S., Aubard, Y., Rawlinson, W.D., Esclaire, F., Hantz, S., Ploy, M.C., Alain, S., 2015. Ex vivo model of congenital cytomegalovirus infection and new combination therapies. Placenta 36 (1), 41–47.
Mott, B.T., He, R., Chen, X., Fox, J.M., Civin, C.I., Arav-Boger, R., Posner, G.H., 2013. Artemisinin-derived dimer phosphate esters as potent anti-cytomegalovirus (anti-CMV) and anti-cancer agents: a structure-activity study. Bioorg. Med. Chem. 21 (13), 3702–3707.
Naesens, L., Bonnafous, P., Agut, H., De Clercq, E., 2006. Antiviral activity of diverse classes of broad-acting agents and natural compounds in HHV-6-infected lymphoblasts. J. Clin. Virol. 37 (Suppl. 1), S69–75.
Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J. Nat. Prod. 75 (3), 311–335.
Nguyen, H.T., Tran, T.H., Kim, J.O., Yong, C.S., Nguyen, C.N., 2015. Enhancing the in vitro anti-cancer efficacy of artesunate by loading into poly-D,L-lactide-co-glycolide (PLGA) nanoparticles. Arch. Pharm. Res. 38 (5), 716–724.
Njuguna, N.M., Ongarora, D.S., Chibale, K., 2012. Artemisinin derivatives: a patent review (2006 - present). Expert Opin. Ther. Pat. 22 (10), 1179–1203.
Obeid, S., Alen, J., Nguyen, V.H., Pham, V.C., Meuleman, P., Pannecouque, C., Le, T.N., Neyts, J., Dehaen, W., Paeshuyse, J., 2013. Artemisinin analogues as potent inhibitors of in vitro hepatitis C virus replication. PLoS One 8 (12), e81783.
Oguariri, R.M., Adelsberger, J.W., Baseler, M.W., Imamichi, T., 2010. Evaluation of the effect of pyrimethamine, an anti-malarial drug, on HIV-1 replication. Virus Res. 153 (2), 269–276.
O'Neill, P.M., Searle, N.L., Kan, K.W., Storr, R.C., Maggs, J.L., Ward, S.A., Raynes, K., Park, B.K., 1999. Novel, potent, semisynthetic antimalarial carba analogues of the first-generation 1,2,4-trioxane artemether. J. Med. Chem. 42 (26), 5487–5493.
Paddon, C.J., Westfall, P.J., Pitera, D.J., Benjamin, K., Fisher, K., McPhee, D., Leavell, M.D., Tai, A., Main, A., Eng, D., Polichuk, D.R., Teoh, K.H., Reed, D.W., Treynor, T., Lenihan, J., Fleck, M., Bajad, S., Dang, G., Dengrove, D., Diola, D., Dorin, G., Ellens, K.W., Fickes, S., Galazzo, J., Gaucher, S.P., Geistlinger, T., Henry, R., Hepp, M., Horning, T., Iqbal, T., Jiang, H., Kizer, L., Lieu, B., Melis, D., Moss, N., Regentin, R., Secrest, S., Tsuruta, H., Vazquez, R., Westblade, L.F., Xu, L., Yu, M., Zhang, Y., Zhao, L., Lievense, J., Covello, P.S., Keasling, J.D., Reiling, K.K., Renninger, N.S., Newman, J.D., 2013. High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 496 (7446), 528–532.
Paeshuyse, J., Coelmont, L., Vliegen, I., Van hemel, J., Vandenkerckhove, J., Peys, E., Sas, B., De Clercq, E., Neyts, J., 2006. Hemin potentiates the anti-hepatitis C virus activity of the antimalarial drug artemisinin. Biochem. Biophys. Res. Commun. 348 (1), 139–144.
Paik, I.H., Xie, S., Shapiro, T.A., Labonte, T., Narducci Sarjeant, A.A., Baege, A.C., Posner, G.H., 2006. Second generation, orally active, antimalarial, artemisinin-derived trioxane dimers with high stability, efficacy, and anticancer activity. J. Med. Chem. 49 (9), 2731–2734.
Patra, N., Srivastava, A.K., 2016. Artemisinin production by plant hairy root cultures in gas- and liquid-phase bioreactors. Plant Cell Rep. 35 (1), 143–153.
Posner, G.H., Ploypradith, P., Parker, M.H., O'Dowd, H., Woo, S.H., Northrop, J., Krasavin, M., Dolan, P., Kensler, T.W., Xie, S., Shapiro, T.A., 1999. Antimalarial, antiproliferative, and antitumor activities of artemisinin-derived, chemically robust, trioxane dimers. J. Med. Chem. 42 (21), 4275–4280.
Ravindranathan, R., Ani- Kumar, M., Nmenon, R.B., Hiremath, S.V., 1990. Stereoselective synthesis of artemisinin. Tetrahedron Lett. 31, 755–758.
Reiter, C., Fröhlich, T., Gruber, L., Hutterer, C., Marschall, M., Voigtländer, C., Friedrich, O., Kappes, B., Efferth, T., Tsogoeva, S.B., 2015a. Highly potent artemisinin-derived dimers and trimers: Synthesis and evaluation of their antimalarial, antileukemia and antiviral activities. Bioorg. Med. Chem. 23 (17), 5452–5458.
Reiter, C., Fröhlich, T., Zeino, M., Marschall, M., Bahsi, H., Leidenberger, M., Friedrich, O., Kappes, B., Hampel, F., Efferth, T., Tsogoeva, S.B., 2015b. New efficient artemisinin derived agents against human leukemia cells, human cytomegalovirus and *Plasmodium falciparum*: 2nd generation 1,2,4-trioxane-ferrocene hybrids. Eur. J. Med. Chem. 97, 164–172.
Ren, Y., Yu, J., Kinghorn, A.D., 2016. Development of anticancer agents from plant-derived sesquiterpene lactones. Curr. Med. Chem. 23 (23), 2397–2420.
Rezaei, B., Majidi, N., Noori, S., Hassan, Z.M., 2011. Multiwalled carbon nanotubes effect on the bioavailability of artemisinin and its cytotoxicity to cancerous cells. J. Nanopart. Res. 11, 6339–6346.
Ro, D.K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., Chang, M.C., Withers, S.T., Shiba, Y., Sarpong, R., Keasling, J.D., 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440 (7086), 940–943.
Romero, M.R., Efferth, T., Serrano, M.A., Castaño, B., Macias, R.I., Briz, O., Marin, J.J., 2005. Effect of artemisinin/artesunate as inhibitors of hepatitis B virus production in an "in vitro" replicative system. Antivir. Res. 68 (2), 75–83.
Romero, M.R., Serrano, M.A., Vallejo, M., Efferth, T., Alvarez, M., Marin, J.J., 2006. Antiviral effect of artemisinin from *Artemisia annua* against a model member of the Flaviviridae family, the bovine viral diarrhoea virus (BVDV). Planta Med. 72 (13), 1169–1174.
Roy, S., He, R., Kapoor, A., Forman, M., Mazzone, J.R., Posner, G.H., Arav-Boger, R., 2015. Inhibition of human cytomegalovirus replication by artemisinins: effects mediated through cell cycle modulation. Antimicrob. Agents Chemother. 59 (7), 3870–3879.
Saeed, M.E.M., Krishna, S., Greten, H.J., Kremsner, P.G., Efferth, T., 2016. Antischistosomal activity of artemisinin derivatives in vivo and in patients. Pharmacol. Res. 110, 216–226.
Schmid, C., Hofheinz, W., 1983. Total synthesis of qinghaosu. J. Am. Chem. Soc. 105, 624–625.
Schnepf, N., Corvo, J., Pors, M.J., Mazeron, M.C., 2011. Antiviral activity of ganciclovir and artesunate towards human cytomegalovirus in astrocytoma cells. Antivir. Res. 89 (2), 186–188.
Shapira, M.Y., Resnick, I.B., Chou, S., Neumann, A.U., Lurain, N.S., Stamminger, T., Caplan, O., Saleh, N., Efferth, T., Marschall, M., Wolf, D.G., 2008. Artesunate as a potent antiviral agent in a patient with late drug-resistant cytomegalovirus infection after hematopoietic stem cell transplantation. Clin. Infect. Dis. 46 (9), 1455–1457.
Sharma, B.N., Marschall, M., Henriksen, S., Rinaldo, C.H., 2014a. Antiviral effects of artesunate on polyomavirus BK replication in primary human kidney cells. Antimicrob. Agents Chemother. 58 (1), 279–289.
Sharma, B.N., Marschall, M., Rinaldo, C.H., 2014b. Antiviral effects of artesunate on JC polyomavirus replication in COS-7 cells. Antimicrob. Agents Chemother. 58 (11), 6724–6734.
Shen, S., Liu, S.Z., Zhang, Y.S., MB, Du, Liang, A.H.G., Song, L.H., Ye, Z.G., 2015. Compound antimalarial ethosomal cataplasm: preparation, evaluation, and mechanism of penetration enhancement. Int. J. Nanomedicine 10, 4239–4253.
Singh, R., Parihar, P., Singh, M., Bajguz, A., Kumar, J., Singh, S., Singh, V.P., Prasad, S.M., 2017. Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: current status and future prospects. Front. Microbiol. 8 (515).
Stuehler, C., Stüssi, G., Halter, J., Nowakowska, J., Schibli, A., Battegay, M., Dirks, J., Passweg, J., Heim, D., Rovo, A., Kalberer, C., Bucher, C., Weisser, M., Dumoulin, A., Hirsch, H.H., Khanna, N., 2015. Combination therapy for multidrug-resistant cytomegalovirus disease. Transpl. Infect. Dis. 17 (5), 751–755.
Thaha, M., Pranawa, Yogiantoro, M., Tanimoto, M., Tomino, Y., 2008. Acute renal failure in a patient with severe malaria and dengue shock syndrome. Clin. Nephrol. 70 (5), 427–430.
Vennerstrom, J.L., Dong, Y., Andersen, S.L., Ager Jr., A.L., Fu, H., Miller, R.E., Wesche, D.L., Kyle, D.E., Gerena, L., Walters, S.M., Wood, J.K., Edwards, G., Holme, A.D., McLean, W.G., Milhous, W.K., 2000. Synthesis and antimalarial activity of sixteen dispiro-1,2,4, 5-tetraoxanes: alkyl-substituted 7,8,15,16-tetraoxadispiro[5.2.5. 2] hexadecanes. J. Med. Chem. 43 (14), 2753–2758.
von Hagens, C., Walter-Sack, I., Goeckenjan, M., Osburg, J., Storch-Hagenlocher, B., Sertel, S., Elsässer, M., Remppis, B.A., Edler, L., Munzinger, J., Efferth, T., Schneeweiss, A., Strowitzki, T., 2017. Prospective open uncontrolled phase I study to define a well-tolerated dose of oral artesunate as add-on therapy in patients with metastatic breast cancer (ARTIC M33/2). Breast Cancer Res. Treat. 164 (2), 359–369.
Wang, Z., Yu, Y., Ma, J., Zhang, H., Zhang, H., Wang, X., Wang, J., Zhang, X., Zhang, Q., 2012. LyP-1 modification to enhance delivery of artemisinin or fluorescent probe loaded polymeric micelles to highly metastatic tumor and its lymphatics. Mol. Pharm. 9 (9), 2646–2657.
Wang, D., Li, H., Gu, J., Guo, T., Yang, S., Guo, Z., Zhang, X., Zhu, W., Zhang, J., 2013. Ternary system of dihydroartemisinin with hydroxypropyl-β-cyclodextrin and lecithin: simultaneous enhancement of drug solubility and stability in aqueous solutions. J. Pharm. Biomed. Anal. 83, 141–148.
Want, M.Y., Islamuddin, M., Chouhan, G., Ozbak, H.A., Hemeg, H.A., Dasgupta, A.K., Chattopadhyay, A.P., Afrin, F., 2015. Therapeutic efficacy of artemisinin-loaded nanoparticles in experimental visceral leishmaniasis. Colloids Surf. B: Biointerfaces 130, 215–221.
Weber, W., Fussenegger, M., 2011. Emerging biomedical applications of synthetic biology. Nat. Rev. Genet. 13 (1), 21–35.
Wenzel, G., 2006. Molecular plant breeding: achievements in green biotechnology and future perspectives. Appl. Microbiol. Biotechnol. 70 (6), 642–650.
Withers, S.T., Keasling, J.D., 2007. Biosynthesis and engineering of isoprenoid small molecules. Appl. Microbiol. Biotechnol. 73 (5), 980–990.
Wolf, D.G., Shimoni, A., Resnick, I.B., Stamminger, T., Neumann, A.U., Chou, S., Efferth, T., Caplan, O., Rose, J., Nagler, A., Marschall, M., 2011. Human cytomegalovirus kinetics following institution of artesunate after hematopoietic stem cell transplantation. Antivir. Res. 90 (3), 183–186.
Xiao, X.C., Hong, Z.G., 2010. Firstborn microcrystallization method to prepare nanocapsules containing artesunate. Int. J. Nanomedicine 5, 483–486.
Xiao, D., Yang, B., Chen, Y.J., Liao, X.L., Yang, X.L., Qin, Q.X., Yi, D., 2013. Synthesis of water soluble C-10-phenoxy artemisinin-chitosan conjugate. Asian J. Chem. 25, 4654–4656.
Yaméogo, J.B., Gèze, A., Choisnard, L., Putaux, J.L., Gansané, A., Sirima, S.B., Semdé, R., Wouessidjewe, D., 2012. Self-assembled biotransesterified cyclodextrins as Artemisinin nanocarriers - I: formulation, lyoavailability and in vitro antimalarial activity assessment. Eur. J. Pharm. Biopharm. 80 (3), 508–517.
Zhang, X., Qiao, H., Liu, J., Dong, H., Shen, C., Ni, J., Shi, Y., Xu, Y., 2010. Dihydroartemisinin loaded nanostructured lipid carriers (DHA-NLC): evaluation of pharmacokinetics and tissue distribution after intravenous administration to rats. Pharmazie 65 (9), 670–678.
Zhang, Y.J., Gallis, B., Taya, M., Wang, S., Ho, R.J., Sasaki, T., 2013. pH-responsive artemisinin derivatives and lipid nanoparticle formulations inhibit growth of breast cancer cells in vitro and induce down-regulation of HER family members. PLoS One 8 (3), e59086.
Zhang, H., Ji, Y., Chen, Q., Jiao, X., Hou, L., Zhu, X., Zhang, Z., 2015. Enhancement of cytotoxicity of artemisinin toward cancer cells by transferrin-mediated carbon nanotubes nanoparticles. J. Drug Target. 23 (6), 552–567.