Medicinal Chemistry

(Iso)Quinoline–Artemisinin Hybrids Prepared through Click Chemistry: Highly Potent Agents against Viruses

Aysun Çapcı,[a] Mélanie M. Lorion,[b] Christina Mai,[a] Friedrich Hahn,[c] Jan Hodek,[d] Christina Wangen,[c] Jan Weber,[d] Manfred Marschall,[c] Lutz Ackermann,*[b, e] and Svetlana B. Tsogoeva*[a]

Abstract: Viral infections cause life-threatening diseases in millions of people worldwide every year and there is an urgent need for new, effective antiviral drugs. Hybridization of two chemically diverse compounds into a new bioactive effector product is a successful concept to improve the properties of a hybrid drug relative to the parent compounds. In this study, (iso)quinoline–artemisinin hybrids, obtained through copper-catalyzed azide–alkyne cycloaddition or metal-free click reactions (in organic solvents or in the presence of water), were analyzed *in vitro*, for the first time, for their inhibitory activity against human cytomegalovirus (HCMV), relative to their parent compounds and the reference drug ganciclovir. EC_{50} (HCMV) values were obtained in a range 0.22–1.20 μM, which indicated highly potent antiviral properties in the absence of cytotoxic effects on normal cells ($CC_{50} > 100$ μM). The most active hybrid, **1** ($EC_{50} =$ 0.22 μM), is 25 times more potent than its parent compound artesunic acid ($EC_{50} = 5.41$ μM) and 12 times more efficient than the standard drug ganciclovir ($EC_{50} = 2.6$ μM). Interestingly, hybrid **1** also shows inhibitory activity against hepatitis B virus *in vitro* (EC_{50} (HBeAg) $= 2.57$ μM).

Introduction

Human cytomegalovirus (HCMV) is an opportunistic viral pathogen, which can take severe and sometimes life-threatening courses in immunocompromised people, such as transplant recipients; cancer or AIDS patients; and, most importantly, unborn children and neonates.[1] Notably, even after decades of intensive research, distinct medical questions concerning infection with HCMV remain highly relevant and have not yet been resolved. Ganciclovir (GCV; Figure 1C) is a deoxyguanosine analogue and, in 1988, was the first drug to be approved for the treatment of HCMV. The emergence of HCMV resistance to currently available antiviral drugs, among them GCV, represents a constant limitation of therapy success.[2] Another challenging virus, which leads to a wide spectrum of liver diseases, ranging from acute hepatitis to chronic (lifelong) hepatitis, cirrhosis, and hepatocellular carcinoma, is hepatitis B virus (HBV).[3] The World Health Organization (WHO) estimates that, of the 2 billion people who have been infected with HBV, more than 350 million have chronic infection.[4]

Hence, the development of new potent agents against HCMV and HBV are of high interest. For this task, hybridization can be used as a powerful concept to increase the biological potency or pharmacological efficacy (such as stability, distribution, or targeting) of the bioactive constituents of the hybrid molecule.[5] One reason for the increased activity of hybrids versus the individual constituents could be given by the simultaneous cellular uptake of covalently linked pharmacophores in a way that the inhibitory kinetics of the two constituents may potentiate each other.[6]

Since their discovery in the 19th century, quinoline and isoquinoline heterocycles have been ubiquitous in pharmaceutical compounds and drugs.[7] The class of quinolines shows a wide range of biological activities, such as anti-inflammatory[7a,e] anti-

[a] Dr. A. Çapcı, C. Mai, Prof. Dr. S. B. Tsogoeva
Organic Chemistry Chair I and Interdisciplinary Center for Molecular Materials (ICMM), Friedrich-Alexander University of Erlangen–Nürnberg, Nikolaus Fiebiger-Straße 10, 91058 Erlangen (Germany)
E-mail: svetlana.tsogoeva@fau.de

[b] Dr. M. M. Lorion, Prof. Dr. L. Ackermann
Institut für Organische und Biomolekulare Chemie
Georg-August-Universität Göttingen, Tammannstraße 2
37077 Göttingen (Germany)
E-mail: lutz.ackermann@chemie.uni-goettingen.de

[c] Dr. F. Hahn, C. Wangen, Prof. Dr. M. Marschall
Institute for Clinical and Molecular Virology
Friedrich-Alexander University of Erlangen-Nürnberg
Schlossgarten 4, 91054 Erlangen (Germany)

[d] Dr. J. Hodek, Dr. J. Weber
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo namesti 2
16610 Prague (Czech Republic)

[e] Prof. Dr. L. Ackermann
German Center for Cardiovascular Research (DZHK)
Potsdamer Str. 58, 10785 Berlin (Germany)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/chem.202001803.

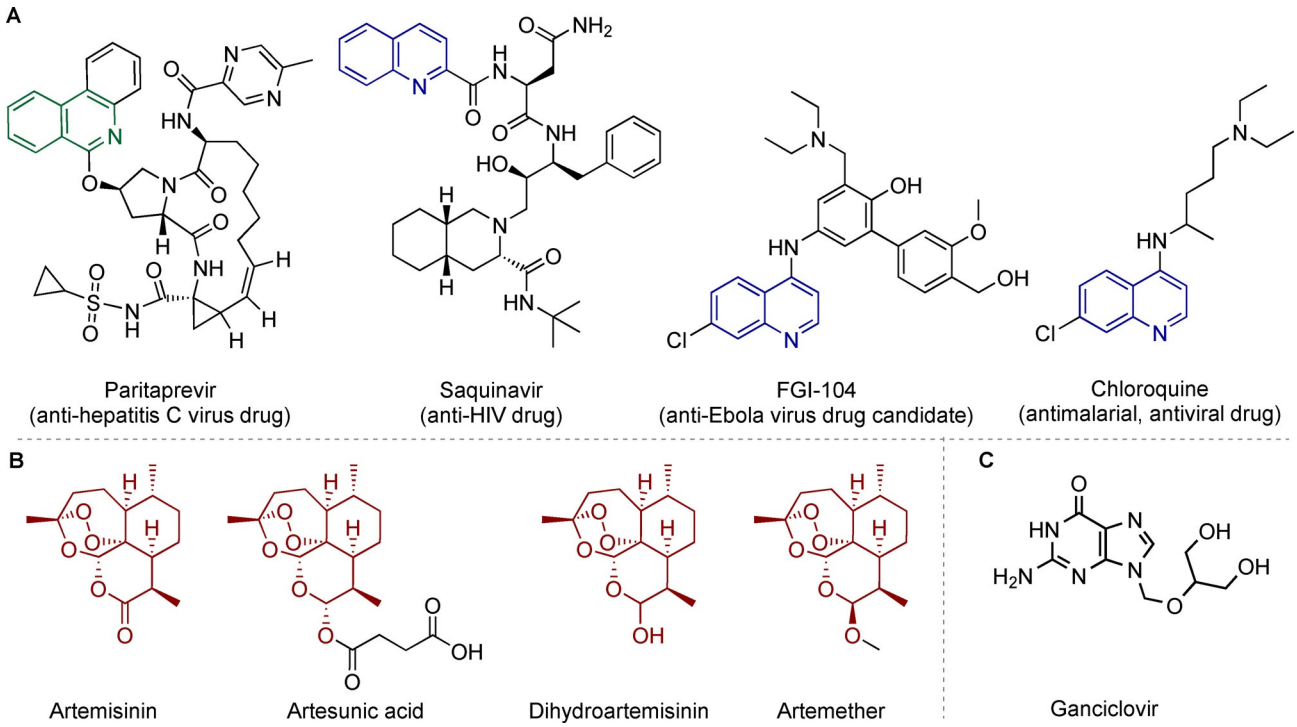


Figure 1. Structures of A) isoquinolines and quinolines with different bioactivities: antivirals Paritaprevir, saquinavir, FGI-104, and antimalarial/antiviral chloroquine; B) artemisinin (ARN, naturally occurring) and its semisynthetic derivatives artesunic acid (ART), dihydroartemisinin (DHA), and artemether; C) the anti-HCMV drug GCV, which is used as a reference compound in this study.

fungal,[7b] and antibacterial.[7c] Furthermore, quinolines are also active against a wide range of viruses, such as coronaviruses,[8] human immunodeficiency virus (HIV),[9] respiratory syncytial virus,[10] hepatitis C virus,[11] West Nile virus,[12] Zika virus,[13] and Dengue virus.[7e,13] Known representatives in the field of antiviral agents containing an (iso)quinoline core structure are depicted in Figure 1 A, namely, the protease inhibitors Paritaprevir (a drug against hepatitis C virus),[14] saquinavir (an anti-HIV drug),[15] FGI-104 (a drug candidate against Ebola virus),[16] and chloroquine (an antimalarial drug that is active against several viruses,[17] including HIV; hepatitis C virus; Ebola virus; and, as recently reported, against coronaviruses, including newly emerged SARS-CoV-2).[18]

ARN is an enantiopure sesquiterpene lactone/trioxane (Figure 1 B), which is an approved antimalarial drug originally been isolated from the plant *Artemisia annua L.* The herb has been used since ancient times; however, structural identification of ARN was first possible in 1972 through X-ray crystal structure analysis by Tu.[19] Artesunate (the sodium salt of ART (Figure 1 B)), a semisynthetic derivative of ARN, has recently been characterized as an active compound against wild-type, recombinant, GCV-sensitive, and GCV-resistant HCMVs, lacking cross-resistance with HCMV drugs *in vitro*.[20] In addition to artesunate, other derivatives, such as DHA and artemether (Figure 1 B), have been widely investigated as potential antiviral agents.

As alternatives to standard drugs, our ongoing search for highly active hybrid molecules,[6] which exceed their parent compounds in activity against HCMV and malaria parasites, has resulted in the synthesis of novel (iso)quinoline–ARN hybrids (Figure 2). Notably, in our recent study, we reported that these hybrids were able to combat multidrug-resistant malaria.[6b] Herein, we focus on their activities against HCMV. Twelve (iso)-quinoline–ARN hybrids from our previous work were, therefore, analyzed, for the first time, for their potency as quinoline–ARN HCMV agents. The hybrid compounds exhibited high anti-HCMV activities, without toxic effects on normal cells, and were more active than their parent compounds and more efficient than the standard drug GCV. In addition, the most potent anti-HCMV hybrid also showed inhibition activity against HBV *in vitro*.

Results and Discussion

Chemistry

The synthesis of hybrid compounds **1**–**12** (Figure 2) was described in our previous work (in which the compounds were studied against chloroquine-resistant malaria parasites).[6b] Copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reactions were applied to couple the corresponding (iso)quinolines **15**, **16**, or **22** with ARN derivatives **17**–**21** and **23**–**27**, giving hybrids **1**–**12** with different linkers, but all with a triazole moiety in common. The synthetic results are summarized in Scheme 1. The corresponding new isoquinolines **15** and **16**, with an alkyne moiety, were prepared through recently developed, facile C–H cobaltation of *O*-acetyl oximes with internal alkynes and a subsequent Sonogashira–Hagihara cross-coupling with

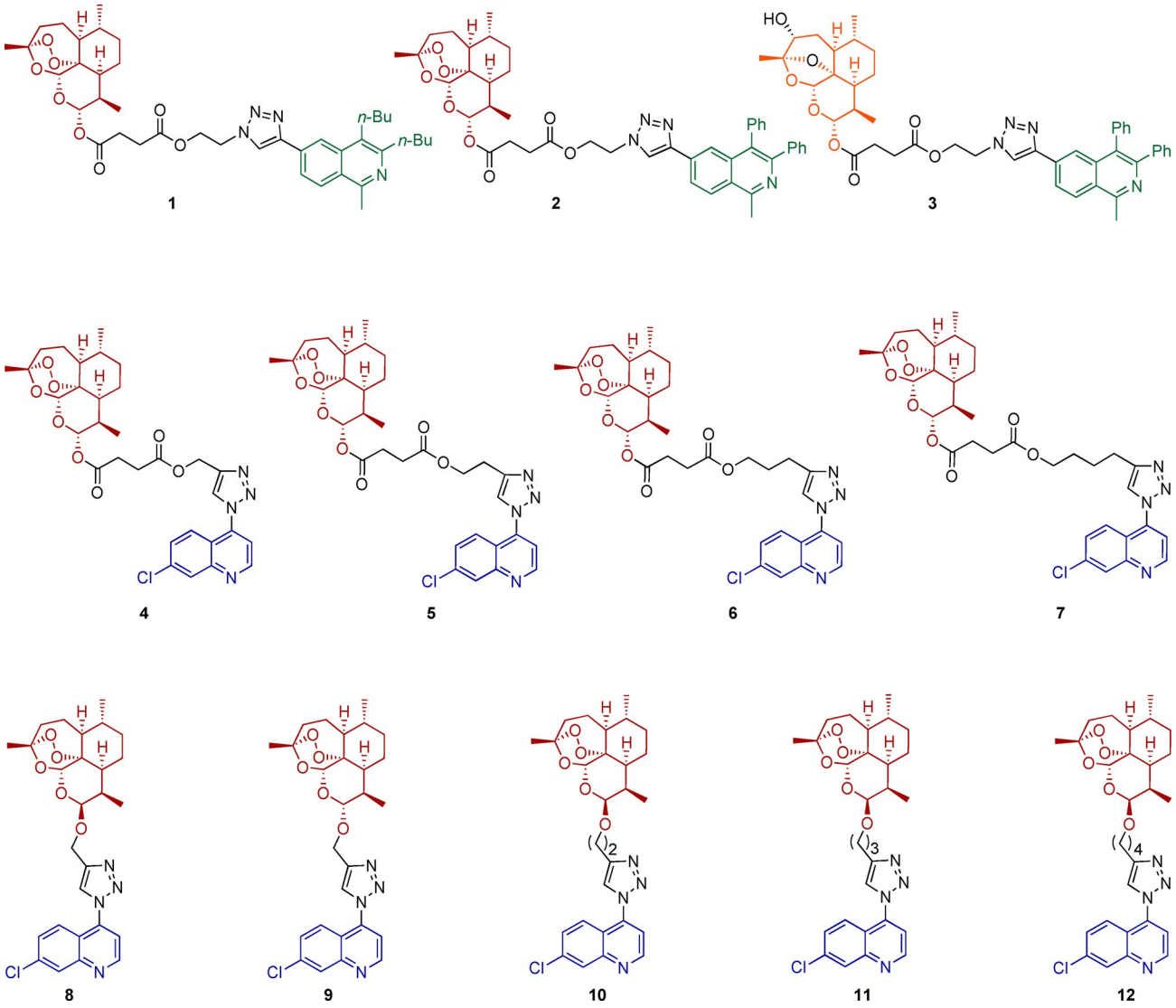
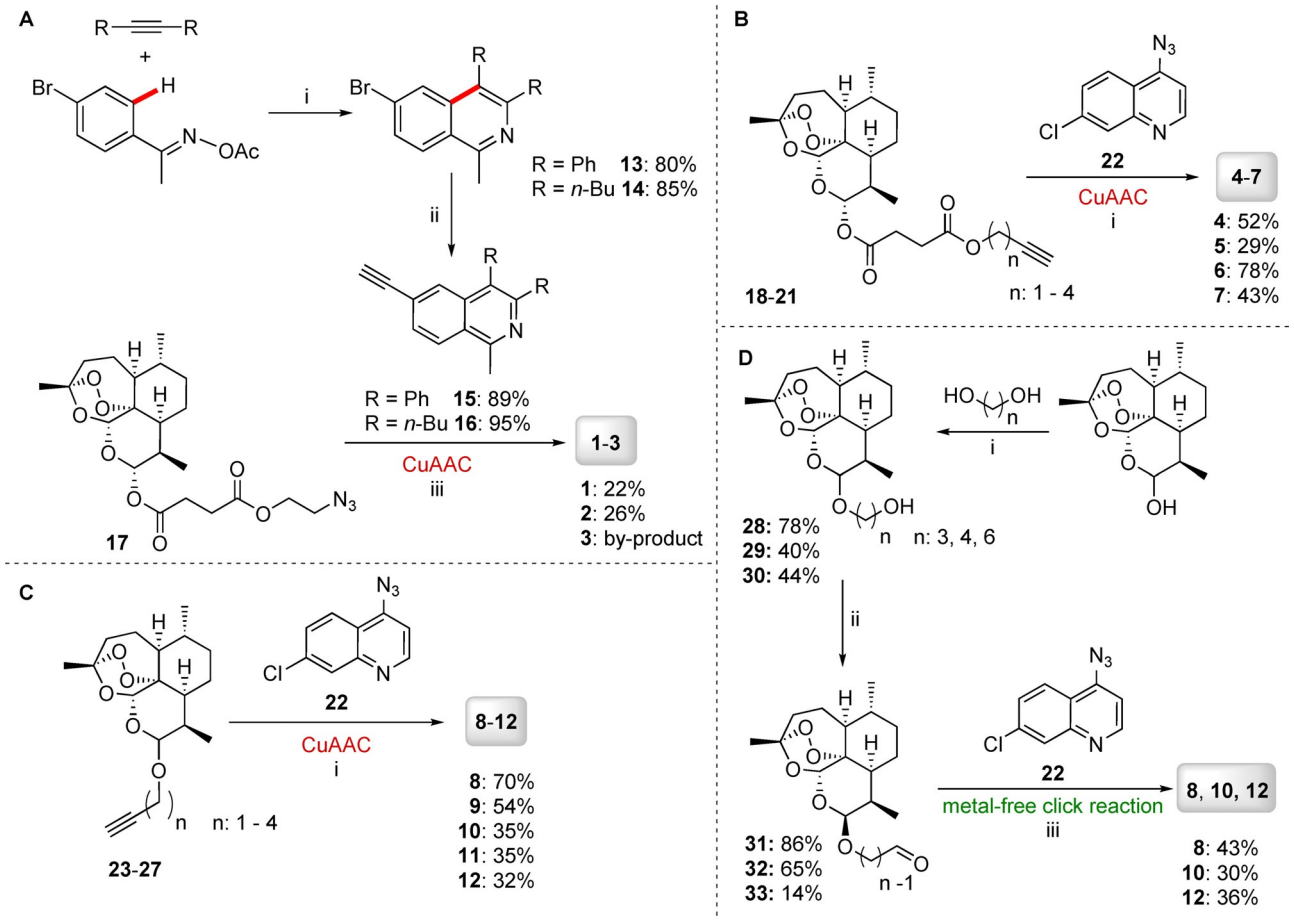


Figure 2. Structures of hybrids **1**–**12** applied for activity examination against HCMV. Red, blue, and green groups indicate parent pharmacophores of molecules. Black parts represent the varied linker groups of the molecules. The orange color indicates the 3-hydroxydesoxydihydroartemisinin unit of hybrid **3**.

trimethylsilylacetylene.[21] Additionally, hybrid **3** was isolated as a side product of hybrid **2** because the peroxide bridge of ARN was hydroxylated at the C3 position in the presence of copper(I) species, resulting in desoxydihydroartemisinin (Scheme 1 A).[22] Hybrids **4**–**12** were obtained by coupling ART- (**18**–**21**) or ARN-derived (**23**–**27**) alkynes with 4-azido-7-chloroquinline (**22**) in the presence of the catalyst system $CuSO_4 \cdot 5\,H_2O$ and sodium ascorbate (Scheme 1 B and C).

To open up the opportunity to apply the formation of our hybrid compounds in future research in the field of bioorthogonal chemistry and, for example, enable the generation of hybrids in situ directly in living cells, we additionally applied an alternative metal-free pathway, leading to the selected hybrid compounds **8**, **10**, and **12**. The first metal-free click reaction, which was reported independently in 2014 by the groups of Ramachary[23] and Paixão,[24] is a green method that might allow toxic copper(I) species[25] to be replaced with DBU. The mechanism of regioselective synthesis of 1,4-disubstituted-1,2,3-triazoles by applying a DBU/malononitrile co-catalyzed 1,3-dipolar cycloaddition strategy was proposed by Paixão et al.[24] It starts with a Knoevenagel condensation of malononitrile with the aliphatic aldehyde, giving the alkylidene malononitrile **A**, which is subsequently deprotonated by DBU, forming vinylogous carbanion **B** (Scheme 2). Electron-rich olefin **B** thereafter reacts with the aryl azide, proceeding via proposed transition-state **TS1**, to give cycloaddition adduct **D** after protonation. Hypothetical transition-states **TS2**, which could lead to isomer **F** (not observed), and **TS3**, which could give zwitterionic intermediate **E**, might be disfavored energetically. Finally, a *syn*-elimination step results in the 1,4-disubstituted-1,2,3-triazole products and recovers the malononitrile.[24]

Aldehydes are used as substrates instead of alkynes, which are applied in CuAAC. Thus, for metal-free click reaction, we used ARN-derived aldehydes **31**–**33** and the previously applied azide **22** to form hybrids **8**, **10**, and **12**. The aldehydes were obtained by etherification of DHA with the corresponding alco-

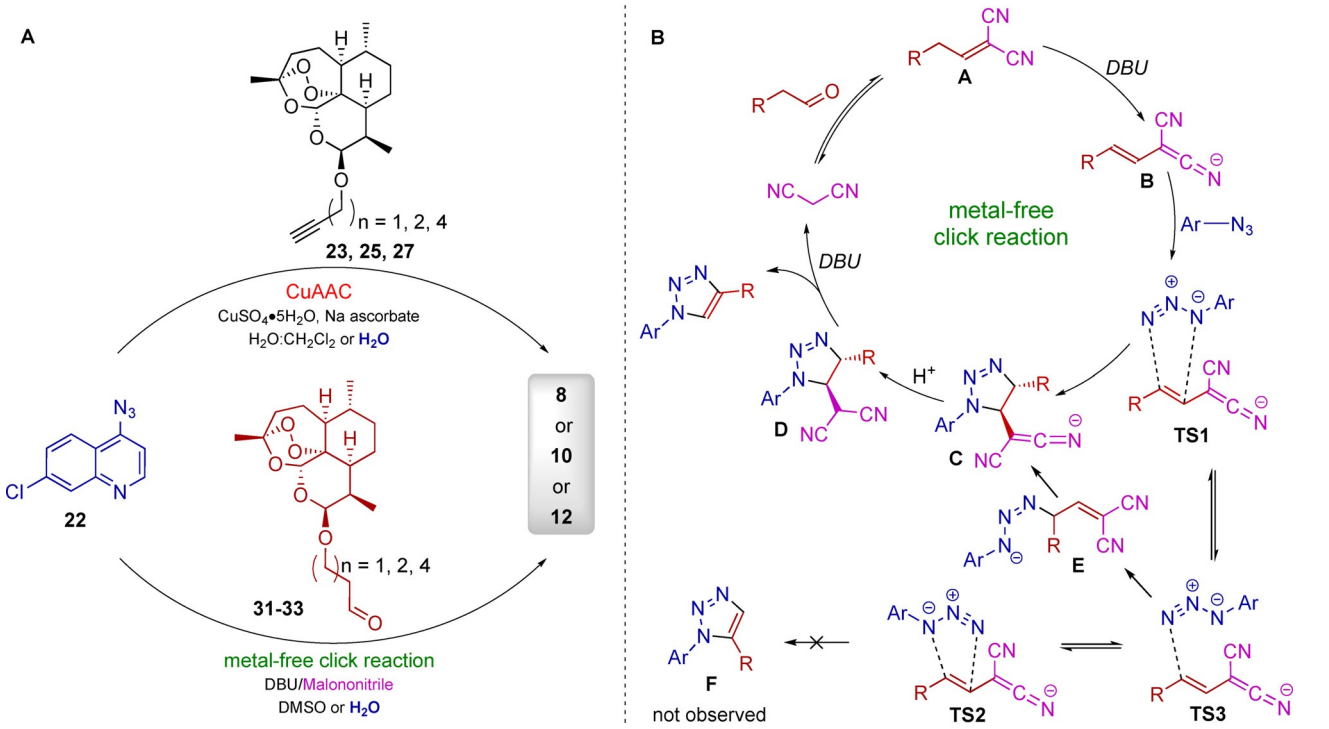


Scheme 1. A) Synthesis of isoquinoline–ARN hybrids **1**–**3** through the CuAAC reaction. B) Synthesis of 7-choloroquinoline–ART hybrids **4**–**7** through the CuAAC reaction. C) Synthesis of 7-chloroquinoline–ARN hybrids **8**–**12** through the CuAAC reaction. D) Synthesis of hybrids **8**, **10**, and **12** through metal-free click reactions. Reagents and conditions: A) i) $[Co(CO)Cp^*I_2]$ (10 mol%; Cp^* = 1,2,3,4,5-pentamethylcyclopentadiene), $AgSbF_6$ (20 mol%), NaOAc (20 mol%), 1,2-dichloroethane, 120 °C, 1 h; ii) 1) $[PdCl_2(PPh_3)_2]$ (1.0 mol%), trimethylsilylacetylene, triethylamine, 50 °C, 2 h; 2) K_2CO_3, MeOH, 25 °C, 4 h; iii) $CuSO_4$ (5 mol%), sodium L-ascorbate (10 mol%), CH_2Cl_2/H_2O (1:1), RT, o/n; R = Ph or *n*Bu. B) i) $CuSO_4{\cdot}5H_2O$ (20 mol%), sodium ascorbate (40 mol%), CH_2Cl_2/H_2O (1:1), RT, o/n; **18**: $n=1$; **19**: $n=2$; **20**: $n=3$; **21**: $n=4$. C) i) $CuSO_4{\cdot}5H_2O$ (20 mol%), sodium ascorbate (40 mol%), CH_2Cl_2/H_2O (1:1), RT, o/n; **23**: $n=1$ (C-10β); **24**: $n=1$ (C-10α); **25**: $n=2$ (C-10β); **26**: $n=3$ (C-10β); **27**: $n=4$ (C-10β). D) i) $H_3PW_{12}O_4{\cdot}H_2O$ (10 mol%), CH_2Cl_2/MeCN (1:1 ($n=3$), 5.5:4 ($n=4$), 8:2 ($n=6$)); ii) Dess–Martin periodinane (1.2 equiv), CH_2Cl_2; **28β**: $n=3$ (C-10β); **28α**: $n=3$ (C-10α); **29**: $n=4$ (C-10β); **30**: $n=6$ (C-10β); iii) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1 equiv), malononitrile (1 equiv), DMSO, RT, o/n; **31**: $n=3$ (C-10β); **32**: $n=4$ (C-10β); **33**: $n=6$ (C-10β).

hols,[26] catalyzed by $H_3PW_{12}O_4{\cdot}H_2O$ (Scheme 1 D), and subsequent Dess–Martin oxidation reaction to obtain aldehydes. Because a mixture of both DHA isomers was used, the alpha and beta isomers of the ARN-derived alcohols were formed. In the case of compound **28**, a mixture of both isomers (**28β** (C-10β)/**28α** (C-10α) = 5:4) were isolated and used further. In the case of compounds **29** and **30**, the β isomers were separately isolated in moderate yields (**29**: 40%, **30**: 44%). The corresponding Dess–Martin oxidation[27] gave the ARN-derived aldehydes **31**–**33** (**31**: 86%, **32**: 65%, **33**: 14%).

The DBU-mediated 1,3-dipolar cycloaddition was performed by stirring **22** with the corresponding ARN-derived aldehydes **31**–**33** at RT in DMSO in the presence of malononitrile (1 equiv) and DBU (1 equiv). Hybrids **8**, **10**, and **12** were obtained in 43, 30, and 36% yields, respectively (Scheme 1 D). Comparing the outcomes of the CuAAC and for metal-free cycloaddition reactions in organic solvents (Scheme 2 and Table 1), the CuAAC catalysis gives hybrid **8**, with the shortest linker, in a better yield (70%, entry 1) than that through metal-free click reaction (43%, entry 5). If the linker contains more CH_2 groups, the yields of hybrids **10** and **12** obtained in organic solvents through both synthetic pathways are almost identical: yields of CuAAC reactions were 35 (for hybrid **10**, entry 2) and 32% (for hybrid **12**, entry 3), whereas yields of the corresponding metal-free click reactions were 30 (hybrid **10**, entry 6) and 36% (hybrid **12**; Table 1, entry 7), respectively.

As an initial test case for a metal-free click reaction as a potential bioorthogonal reaction, we evaluated the tolerance of both catalytic systems to water as a solvent and studied how the yield of hybrid **8** was influenced by changing the solvent system from H_2O/CH_2Cl_2 (CuAAC; Table 1, entry 4) and DMSO (metal-free click reaction; Table 1, entry 8) to H_2O. The experiments showed that, for the formation of quinoline–ARN hybrid **8** under aqueous conditions, the metal-free system resulted in better yield (30% yield, Table 1, entry 8) than that of the CuAAC reaction system (9% yield, Table 1, entry 4). This higher yield of product **8**, obtained through the metal-free click reaction in the presence of water, could be explained by higher



Scheme 2. A) CuAAC and metal-free click reactions, leading to the same hybrid compounds **8**, **10**, and **12**. Detailed reaction conditions are described in Table 1. B) Proposed mechanism of metal-free click reactions based on recent reports.[24, 28]

Table 1. A comparison of CuAAC and metal-free cycloaddition reactions.

	Entry	Catalytic system[a]	Solvent[a]	Product	Yield[a] [%]
Cu^{I}-catalyzed	1	$CuSO_4$·5 H_2O (20 mol %), sodium ascorbate (40 mol %)	CH_2Cl_2/H_2O (1:1)	**8**	70
	2	$CuSO_4$·5 H_2O (20 mol %), sodium ascorbate (40 mol %)	CH_2Cl_2/H_2O (1:1)	**10**	35
	3	$CuSO_4$·5 H_2O (20 mol %), sodium ascorbate (40 mol %)	CH_2Cl_2/H_2O (1:1)	**12**	32
	4	$CuSO_4$·5 H_2O (1 equiv), sodium ascorbate (4 equiv)	H_2O	**8**	9
metal-free click reaction	5	DBU (1 equiv), malononitrile (1 equiv)	DMSO	**8**	43
	6	DBU (1 equiv), malononitrile (1 equiv)	DMSO	**10**	30
	7	DBU (1 equiv), malononitrile (1 equiv)	DMSO	**12**	36
	8	DBU (1 equiv), malononitrile (1 equiv)	H_2O	**8**	30

[a] Reaction conditions and product yields correspond to the reaction mechanism depicted in Scheme 2.

polarity of the aldehyde, relative to the alkyne, and thus, its higher solubility in water. In summary, such a metal-free click reaction in water has the potential to be developed as a bioorthogonal metal-free reaction under live-cell conditions or in live cells.

Antiviral activity

In our previous study, the set of hybrids **1**–**12** enabled us to investigate variation of the antimalarial activities, depending on the type of linkage between ARN and (iso)quinolones.[6b] Herein, the extension of this study to the *in vitro* quinoline–ARN HCMV activity is presented (Table 2).

Reference drug GCV; two quinoline compounds (**34** and **35**; see Table S1 in the Supporting Information); and the parent drugs ARN, ART, DHA, and artemether were used as comparative compounds in this analysis. Anti-HCMV EC_{50} values of the hybrids and of precursor **13** were determined. GCV and ART showed EC_{50} values of (2.60 ± 0.5) and (5.41 ± 0.61) μM (Table 2), respectively, whereas ARN, DHA, and artemether were mostly inactive against HCMV. Similarly, quinoline compounds **34** and **35**, as well as parent compound **13**, only comprising the 7-chloroquinoline unit, had no measurable effect on HCMV replication. Contrary to the antimalarial results, this antiviral analysis showed that dihydroartemisinin-7-chloroquinoline-based hybrids **8**–**12** and isoquinoline–ARN hybrids **1** and **2** achieved the highest activity, with EC_{50} values ranging from (0.22 ± 0.04) to (1.20 ± 0.11) μM, even exerting higher *in vitro* activities than those of the parent and reference compounds. Isomers **8** (C-10β-isomer) and **9** (C-10α-isomer) exhibited a similar structure–activity relationship against HCMV to that observed for the antimalarial activity; the β-isomer was

Table 2. EC_{50} values for reference compounds GCV, ART, ARN, DHA, artemether, tenofovir alafenamide fumarate (TAF); parent compound **13**; and hybrids **1**–**12**, which were analyzed for anti-HCMV and anti-HBV activities.[a]

Compound	HCMV EC_{50} [μM]	LDH CC_{50} [μM]	HepG2-hNTCP CC_{50} [μM]	HBeAg ELISA EC_{50} [μM]	HBV DNA qPCR EC_{50} [μM]
1	0.22 ± 0.04	> 100*	29.9 ± 1.1	2.57 ± 1.51	≈ 10
2	0.67 ± 0.03	> 100	> 50	> 10	> 10
3	none	> 100	n.d.	n.d.	n.d.
4	none (strong cytotox. **)	> 100**	n.d.	n.d.	n.d.
5	none (strong cytotox. **)	> 100**	> 50	> 10	> 10
6	none (strong cytotox. **)	> 100**	n.d.	n.d.	n.d.
7	none (strong cytotox. **)	> 100**	> 50	> 10	> 10
8	0.71 ± 0.03	n.d.	n.d.	n.d.	n.d.
9	1.20 ± 0.11	> 100	n.d.	n.d.	n.d.
10	1.08 ± 0.18	> 100*	n.d.	n.d.	n.d.
11	0.30 ± 0.02	> 100	> 50	> 10	> 10
12	0.38 ± 0.03	> 100	> 50	> 10	> 10
13	> 10	n.d.	n.d.	n.d.	n.d.
ARN[b]	> 10	> 100	n.d.	n.d.	n.d.
ART[c]	5.41 ± 0.61	n.d.	> 50	> 10	> 10
DHA[b]	> 10	n.d.	> 50	> 10	> 10
artemether	> 10	> 100	n.d.	n.d.	n.d.
GCV[d]	2.60 ± 0.5	> 100	n.d.	n.d.	n.d.
TAF	–	–	27.2 ± 0.7	3.93 ± 0.8	0.00024 ± 0.00004

[a] */** Microscopic inspection of cell morphology or cell lysis after 6–8 days, long-term cytotoxicity (* moderate, ** strong). LDH: lactate dehydrogenase release assay, 24 h, acute cytotoxicity; "n.d."—not determined. [b] EC_{50} values have been previously reported.[6a,29] [c] EC_{50} values have been previously reported.[30] [d] EC_{50} values have been previously reported.[31]

more active than the α-isomer. β-Isomer **8** showed an EC_{50} value of (0.71 ± 0.03) μM, whereas α-isomer **9** showed (1.20 ± 0.11) μM. Hybrids **10**, **11**, and **12** were particularly active, with EC_{50} values of (1.08 ± 0.18), (0.30 ± 0.02), and (0.38 ± 0.03) μM (Table 2). The two longest linkers (hybrids **11** and **12**), as spacers between pharmacophores, improved the activity; this was likely as a result of providing increased intramolecular flexibility.

Surprisingly, isoquinoline–ARN hybrids **1** and **2** showed 8- to 25-fold higher activities than that of ART, with EC_{50} values of (0.22 ± 0.04) and (0.67 ± 0.03) μM, respectively, whereas none of the other ART-based hybrids **4**–**7** were found to be active against HCMV (or the EC_{50} values could not be determined due to the induction of cytotoxicity). Of the three isoquinoline–ARN hybrids, only one, namely, hybrid **3**, was inactive; this can be ascribed to hydroxylation of the peroxide bridge of the ARN unit. This finding was comparable to the antimalarial activity of **3**, for which bioactivation was putatively based on the peroxide bridge. Notably, all active compounds were similar or even more active, in terms of *in vitro* anti-HCMV efficacy, than that of the parent compounds and reference drugs. Moreover, none of these compounds exerted acute cytotoxicity, according to the LDH release assay, with CC_{50} values of > 100 μM (Table 2).

Recently, the activities of ARN, artesunate, and whole extract of Chinese herb *Artemisia capillaris* were determined in HBV-transfected HepG2.2.15 cells.[32] Artesunate was shown to inhibit the HBV s antigen (HBsAg), with an IC_{50} value of 2.3 μM, and reduced the amount of HBV-DNA secreted from HepG2.2.15 cells to the culture medium. On the other hand, HBsAg reduction by ARN was weaker, with an IC_{50} value of around 50 μM, and no reduction in HBV-DNA was observed.[32a] Inspired by these results, we decided to assess the inhibitory potential of a selected subset of ARN-derived hybrid compounds **1**, **2**, **5**, **7**, **11**, and **12** against HBV. Inhibition of HBV was evaluated by measuring the extent to which the test compounds reduced the release of HBV e antigen (HBeAg) and HBV-DNA after infection of HepG2-hNTCP cells for 14 days.

The reference compounds TAF, DHA, and ART were used for comparison in the assays. Isoquinoline–ARN hybrid **1**, containing *n*-butyl moieties, inhibited HBeAg secretion in cell-free supernatant, with EC_{50} = (2.57 ± 1.51) μM, and showed a reduction in HBV-DNA, with an EC_{50} value of approximately 10 μM. This compound exhibited cytotoxicity in HepG2-hNTCP cells, with CC_{50} = (29.9 ± 1.1) μM. The reference compound TAF showed similar cytotoxicity and reduction in HBeAg secretion, but, as a selective inhibitor of reverse transcriptase activity, it showed 10 000 times better inhibition of HBV-DNA secretion in a medium than that of compound **1**. The introduction of phenyl moieties in the place of *n*-butyl in compound **2** abolished the anti-HBV activity. Similarly, there was no anti-HBV activity for dihydroartemisinin-7-chloroquinoline-based hybrids **11** and **12**.

Conclusion

Although HCMV infection is mostly inapparent in immunocompetent persons, it can be life-threatening for immunonaïve or immunocompromised individuals, so that anti-HCMV-specific drug research is an ongoing issue investigated worldwide. To this end, the present study characterized new (iso)quinoline–ARN hybrid compounds for anti-HCMV activity *in vitro*. Thus, three isoquinoline–ARN and nine quinoline–ARN hybrids, together with their precursors and reference drugs, were phar-

macologically evaluated. Hybrids were obtained by facile C–H activation and CuAAC reactions.[6b,21] Additionally, hybrids **8**, **10**, and **12** were, for the first time, synthesized through a metal-free cycloaddition reaction, with DBU and malononitrile as cocatalysts. Additionally, preliminary experiments of both cycloaddition reactions (CuAAC and metal-free) were performed to analyze their suitability for future application in the context of living cells, by performing the selected reactions in the presence of water. Metal-free click reaction proved to be more promising, since hybrid **8** could be isolated in 30% yield, whereas CuAAC catalysis gave the hybrid product in only 9% yield.

Remarkably, hybrids **1**, **2**, **8**, **11**, and **12** demonstrated pronounced activity against HCMV, that is, *in vitro* EC_{50} values in the sub-micromolar range (down to 0.22 μM for hybrid **1**). Notably, hybrid **1** also exhibited low micromolar activity against HBV (EC_{50} (HBeAg) = 2.57 μM). Moreover, the cytotoxicity profiles of all hybrids were almost negative within the relevant range of antiviral concentrations in primary HFFs, that is, CC_{50} values were low or undetectable at concentrations up to 100 μM. Thus, these compounds, exhibiting high antiviral activities combined with a low toxicity/high selectivity profile, illustrate the potential of the hybridization concept as an alternative drug-discovery approach, which can also be applied for current anti-coronavirus drug development.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: antiviral agents · artemisinins · click chemistry · cycloaddition · quinolines

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