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Supporting Information

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

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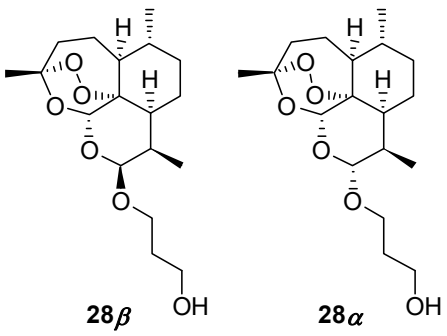
1. General information

All reactions were performed in distilled and dried or HPLC grade solvents under N_2. The reagents supplied from commercial sources were used without further purification. TLC chromatography was performed on precoated aluminium silica gel SIL G/UV254 plates (Macherey-Nagel & Co.) and the detection occurred via fluorescence quenching, development in a molybdato phosphate solution (10% in EtOH). The compounds were purified via column chromatography and the hybrid compounds were reprecipitated from CH_2Cl_2 in *n*-hexane to obtain a pure compound for elemental analysis and further biological tests. All compounds were dried in high-vacuum (10^{-3} mbar). ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance or JEOL JNM GX 400 spectrometer operating at 300, 400 or 600 MHz, 75, 100 or 125 MHz. All chemical shifts are given in the ppm-scale and refer to the nondeuterized proportion of the solvent. ESI, APPI and MALDI mass spectra were recorded on a Bruker Daltonik maXis 4G or Bruker Daltonik micrOTOF II focus. Elemental analysis (C, H, N) were carried out with an Euro EA 3000 (EuroVector) machine and an Elementar vario MICRO cube machine and calculated values confirm a purity of > 95% for the all biologically tested compounds. Artesunic acid and dihydroartemisinin were purchased from ABCR (Karlsruhe, Germany) and TCI (Deutschland GmbH). 4,7-dichloroquinoline was purchased from Sigma Aldrich (Germany). Experimental details and the spectra of the hybrids and their precursors can be found following in the supporting information.

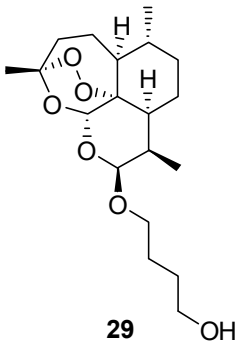
The synthesis of hybrids **1**-**12** and starting materials **13**-**27** was described earlier.[1] For the synthesis of compounds **28**-**30**[2] and **31**-**33**[3] literature known procedures were applied.

2. Synthesis and characterization of compounds



Compound **28*β*** and **28*α***: In a dried flask dihydroartemisinin (1.00 g, 3.50 mmol, 1.0 equiv.) was dissolved in anhydrous dichloromethane/acetonitrile (5:1) 60 mL. Phosphotungstic acid hydrate (1.01 g, 0.350 mmol, 10 mol%) was added and the mixture was stirred at room temperature for 5 min. 1,3-Propanediol (1.27 mL, 1.33 g, 3.50 mmol, 5.0 equiv.) dissolved in 40 mL dichloromethane/acetonitrile (1:3) was added in portions and the reaction mixture was further stirred at r.t. for 1 h 30 min. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography (hexanes/EtOAc, 6:4) to give a mixture of the two isomers in 65% (778 mg, 2.27 mmol, ***β***/***α*** 5:4, **28*β***: (467 mg, 1.36 mmol, 39%) and **28*α***: (311 mg, 0.908 mmol, 26%)).

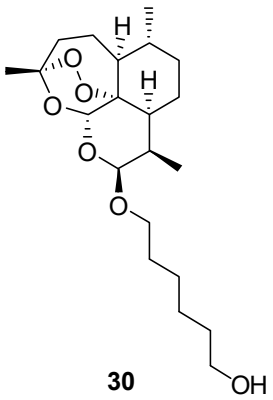
Compound **28*β***: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.37 (s, 1H), 4.77 (d, J = 3.4 Hz, 1H) ppm. Compound **28*α***: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.33 (s, 0.8H), 4.46 (d, J = 9.3 Hz, 0.8H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 103.8, 99.7, 90.7, 79.8, 68.7, 66.4, 51.2, 44.8, 36.9, 35.9, 33.8, 32.0, 25.5, 24.2, 21.7, 19.8, 19.4, 12.0 ppm. Compound **28*β*** + **28*α***: HRMS (ESI+): m/z calculated for $C_{18}H_{30}O_6$ $[M+Na]^+$: 365.1940, found: 365.1931.



29

Compound **29**: In a dried flask dihydroartemisinin (250 mg, 0.88 mmol, 1.0 equiv.) was dissolved in 10 mL anhydrous dichloromethane/ acetonitrile (8:2). Phosphotungstic acid hydrate (253 mg, 0.088 mmol, 10 mol%) was added and the mixture was stirred at room temperature for 5 min. 1,4-butanediol (311 µL, 317.2 mg, 3.52 mmol, 4.0 equiv.) dissolved in 1.0 mL acetonitrile was added in portions and the reaction mixture was further stirred at r.t. for approximately for 4 h (until TLC indicates no starting compound (DHA) was remaining). The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (hexanes/EtOAc 7:3) and the two isomers of the product were isolated. The *β*-isomer **29** was yielded in 40% (126 mg, 0.354 mmol) as colorless oil.

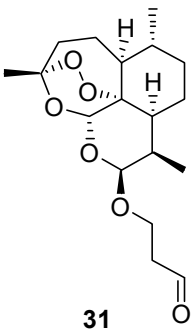
Compound **29:** ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 5.37 (s, 1H), 4.77 (d, *J* = 3.4 Hz, 1H), 3.93 – 3.81 (m, 1H), 3.69 – 3.61 (m, 2H), 3.45 – 3.33 (m, 1H), 2.67 – 2.55 (m, 1H), 2.42 – 2.27 (m, 1H), 2.08 – 1.95 (m, 2H), 1.93 – 1.70 (m, 5H), 1.68 – 1.57 (m, 5H), 1.54 – 1.44 (m, 2H), 1.42 (s, 3H), 1.27 – 1.12 (m, 3H), 0.93 (d, *J* = 6.1 Hz, 3H), 0.89 (d, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 104.2, 102.1, 88.0, 81.2, 68.4, 62.8, 52.7, 44.6, 37.6, 36.6, 34.8, 31.0, 29.9, 26.3 (2xC), 24.8, 24.6, 20.5, 13.1 ppm. HRMS (ESI+) m/z calculated for $C_{19}H_{32}O_6$ $[M+H]^+$: 357.2277, found: 357.2261.



30

Compound **30**: In a dried flask dihydroartemisinin (250 mg, 0.88 mmol, 1.0 equiv.) was dissolved in 10 mL anhydrous dichloromethane/ acetonitrile (8:2). Phosphotungstic acid hydrate (253 mg, 0.088 mmol, 10 mol%) was added and the mixture was stirred at room temperature for 5 min. 1,5-pentanediol (416 mg, 3.52 mmol, 4.0 equiv.) dissolved in 1.0 mL acetonitrile was added in portions and the reaction mixture was further stirred at r.t. for 4 h (until TLC indicates no starting compound (DHA) was remaining). The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (hexanes/EtOAc 7:3) and the two isomers of the product were isolated. The *β*-isomer **30** was yielded in 44% (150 mg, 0.39 mmol) as colorless oil.

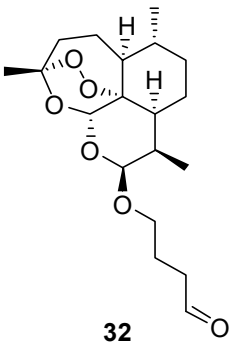
^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 5.30 (s, 1H), 4.68 (d, *J* = 3.3 Hz, 1H), 3.74 (d, *J* = 22.7 Hz, 1H), 3.53 (d, *J* = 13.2 Hz, 2H), 3.29 (d, *J* = 22.4 Hz, 1H), 2.57 – 2.47 (m, 1H), 2.28 (m, 1H), 2.17 (s, 1H), 1.98 – 1.65 (m, 4H), 1.57 – 1.40 (m, 6H), 1.42 – 1.13 (m, 11H), 0.84 (d, *J* = 23.8 Hz, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 103.8, 101.7, 87.7, 80.9, 68.1, 62.5, 52.4, 44.3, 37.3, 36.3, 34.5, 32.5, 30.7, 29.4, 26.0, 25.9, 25.3, 24.5, 24.3, 20.2, 12.8 ppm.



31

Compound **31**: To a suspension of Dess-Martin periodinane (62.6 mg, 0.148 mmol, 1.2 equiv.) in 0.5 mL anhydrous DCM the mixture of the DHA-alcohol-derivatives **28*β*** and **28*α*** (***β*/α** 5:4) (42.1 mg, 0.123 mmol, 1.0 equiv.) dissolved in 2.5 mL anhydrous DCM was added dropwise. The reaction mixture was stirred at room temperature and after complete consumption of the starting material the mixture was quenched with $Na_2S_2O_3$ (1M). The phases were separated, and the aqueous layer was extracted with DCM (2x). The combined organic layers were washed with 5% $NaHCO_3$ (2x) and brine (1x) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography (hexanes/EtOAc 2:1) to give 86% (refers to calculated yield of **28*β***) (20.2 mg, 0.0593 mmol) of the *β*-isomer **31** as a white solid.

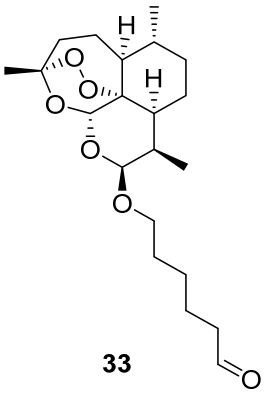
^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 9.79 (t, *J* = 1.9 Hz, 1H), 5.42 (s, 1H), 4.81 (d, *J* = 3.4 Hz, 1H), 4.27 – 4.19 (m, 1H), 3.75 – 3.67 (m, 1H), 2.70 – 2.58 (m, 4H), 2.42 – 2.31 (m, 1H), 2.07 – 2.00 (m, 1H), 1.92 – 1.84 (m, 1H), 1.71 – 1.46 (m, 5H), 1.44 (s, 3H, CH_3), 1.38 – 1.15 (m, 2H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 7.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 200.5, 103.9, 102.0, 87.8, 80.9, 62.0, 52.50, 44.3, 43.79, 37.3, 36.3, 34.5, 30.7, 26.0, 24.6, 24.2, 20.2, 12.8 ppm. HRMS (ESI+) m/z calculated for $C_{18}H_{28}O_6$ $[M+Na]^+$: 363.1784, found = 363.1778.



32

Compound **32**: To a suspension of Dess-Martin periodinane (157 mg, 0.370 mmol, 1.1 equiv.) in 10 mL anhydrous DCM the DHA-alcohol-derivative **29** (120 mg, 0,337 mmol, 1.0 equiv.) was added dropwise. The reaction mixture was stirred at room temperature and after complete consumption of the starting material the mixture was quenched with $Na_2S_2O_3$ (1M). The phases were separated, and the aqueous layer was extracted with DCM (2x). The combined organic layers were washed with 5% $NaHCO_3$ (2x) and brine (1x) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography (hexanes/EtOAc 7:3) to give 65% (78 mg, 0.22 mmol) of the product.

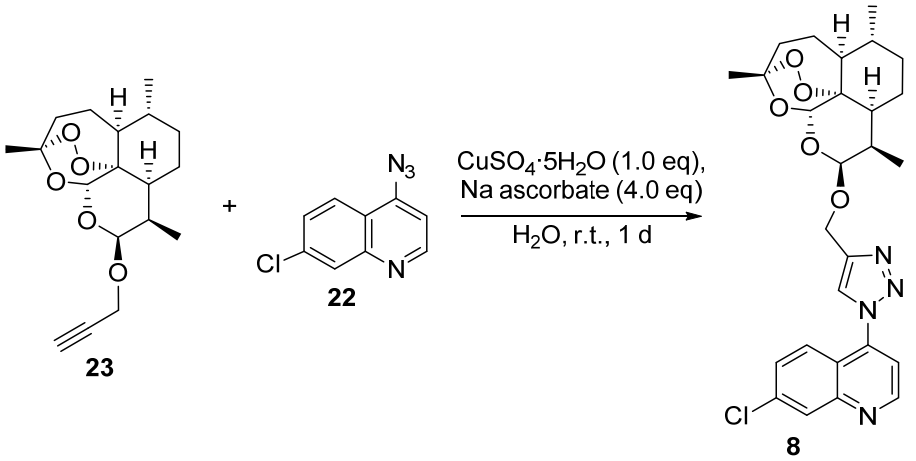
^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 9.74 (t, *J* = 1.6 Hz, 1H), 5.32 (s, 1H), 4.73 (d, *J* = 1.2 Hz, 1H), 3.89 (d, *J* = 21.8 Hz, 1H), 3.39 (d, *J* = 22.4 Hz, 1H), 2.63 (m, 1H), 2.49 (m, 2H), 2.37 (m, 1H), 2.02 (m, 4H), 1.72 (m, 3H), 1.48 (m, 5H), 1.31 (m, 3H), 0.92 (d, *J* = 25.6 Hz, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 201.6, 103.6, 101.6, 87.4, 80.5, 67.0, 52.0, 43.8, 40.5, 36.9, 35.9, 34.1, 30.4, 25.7, 24.2, 24.0, 22.0, 19.9, 12.5 ppm.



33

Compound **33**: To a suspension of Dess-Martin periodinane (182 mg, 0.43 mmol, 1.1 equiv.) in 12 mL anhydrous DCM the DHA-alcohol-derivative **30** (150 mg, 0.39 mmol, 1.0 equiv.) was added dropwise. The reaction mixture was stirred at room temperature and after complete consumption of the starting material the mixture was quenched with $Na_2S_2O_3$ (1M). The phases were separated, and the aqueous layer was extracted with DCM (2x). The combined organic layers were washed with 5% $NaHCO_3$ (2x) and brine (1x) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography (hexanes/EtOAc 7:3) to give 36% (53.7 mg, 0.14 mmol) of the product and was used further for organocatalyzed click reaction.

General procedure of the Cu(I) catalyzed cycloaddition reaction:



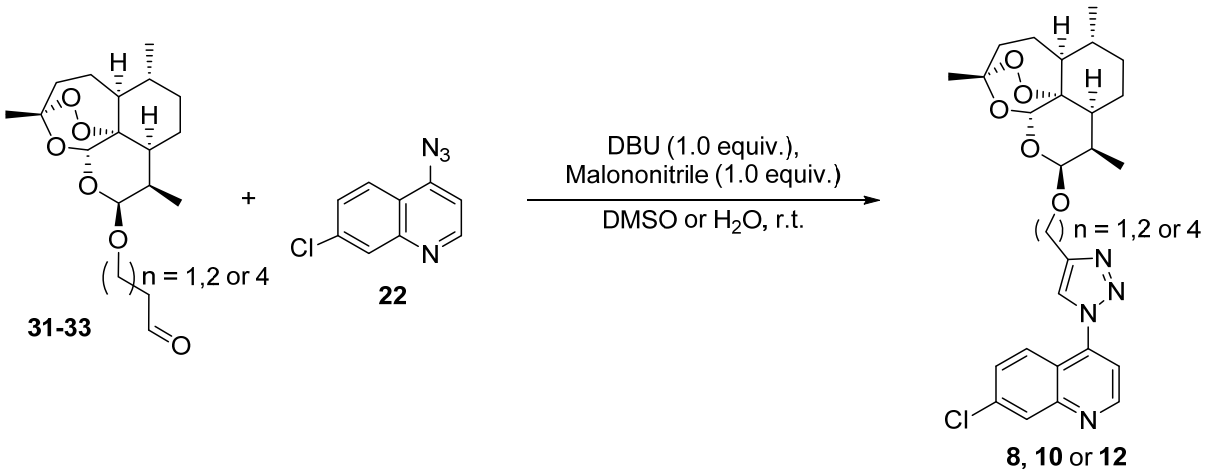
$CuSO_4 \bullet 5H_2O$ (23.2 mg, 93.1 µmol, 1.0 equiv.) and sodium ascorbate (73.7 mg, 0.372 mmol, 4.0 equiv.) were dissolved in 1 mL degassed H_2O to form the active catalyst species Cu(I). After the catalyst mixture turned brown and subsequently orange the DHA-derived alkyne **23** (30.0 mg, 93.1 µmol, 1.0 equiv.) and 4-azido-7-chloroquinoline **22** (38.1 mg, 0.186 mmol, 2.0 equiv.) were added and the reaction mixture was stirred at r.t. for 1 d. Afterwards the mixture was extracted with EtOAc and dried over Na_2SO_4. After removing the solvent under reduced pressure, the residue was purified by column chromatography (7:3 hexanes/EtOAc) to give the product in 9% (4.58 mg, 8.69 µmol) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.07 (d, J = 4.6 Hz, 1H), 8.25 (d, J = 2.0 Hz, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.98 (s, 1H, CH), 7.60 (dd, J = 9.1, 2.1 Hz, 1H), 7.51 (d, J = 4.6 Hz, 1H), 5.35 (s, 1H), 5.07 (d, J = 12.9 Hz, 1H), 5.02 (d, J = 4.6 Hz, 1H), 4.82 (d, J = 12.9 Hz, 1H), 2.58 – 2.46 (m, 1H), 1.94 – 1.77 (m, 4H), 1.75 – 1.64 (m, 4H), 1.58 (dd, J = 10.2, 4.4 Hz, 1H), 1.52 (s, 3H), 1.40 – 1.13 (m, 2H), 0.96 (d, J = 7.5 Hz, 3H), 0.88 (d, J = 5.9 Hz, 3H) ppm.

The spectral data are consistent with those previously reported.[1]

General procedure of the organocatalytic cycloaddition reaction:

The DHA derived-aldehyde **31**, **32** or **33**, 4-azido-7-chloroquinoline **22** and malononitrile were dissolved in the solvent. Then DBU was added dropwise. The reaction mixture was stirred at room temperature. Afterwards it was extracted with EtOAc and dried with Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (6:4 hexanes/EtOAc) to give the product as a colorless oil.



In DMSO:

Hybrid **8**: **31** (17.5 mg, 0.05 mmol, 1 equiv.), **22** (20.5 mg, 0.10 mmol, 2 equiv.) and malononitrile (3.30 mg, 0.05 mmol, 1 equiv.) were dissolved in 0.1 mL DMSO and DBU (7.46 µL, 7.60 mg, 0.05 mmol, 1 equiv.) was added dropwise. Hybrid **8** was obtained in 43% yield (11.4 mg, 0.02 mmol).

The spectral data are consistent with those previously reported.[1]

Hybrid **10**: **32** (77.0 mg, 0.22 mmol, 1 equiv.), **22** (88.4 mg, 0.43 mmol, 2 equiv.) and malononitrile (15.0 mg, 0.22 mmol, 1 equiv.) were dissolved in 0.6 mL DMSO and DBU (32.9 µL, 33.5 mg, 0.22 mmol, 1 equiv.) was added dropwise. Hybrid **10** was obtained in 30% yield (35.0 mg, 0.065 mmol).

The spectral data are consistent with those previously reported.[1]

Hybrid **12**: **33** (53.0 mg, 0.139 mmol, 1.0 equiv.), **22** (56.7 mg, 0.277 mmol, 2 equiv.) and malononitrile (9.17 mg, 0.139 mmol, 1.0 equiv.) were dissolved in 1 mL DMSO and DBU (20.8 µL, 21.0 mg, 0.139 mmol, 1 equiv.) was added dropwise. Hybrid **12** was obtained in 36% yield (29.1 mg, 0.051 mmol).

The spectral data are consistent with those previously reported.[1]

In H_2O:

Hybrid **8**: **31** (25.0 mg, 0.073 mmol, 1.0 equiv.), **22** (30.1 mg, 0.147 mmol, 2.0 equiv.) and malononitrile (4.85 mg, 0.073 mmol, 1.0 equiv.) were dissolved in 1 mL H_2O and DBU (11.0 µL, 11.2 mg, 0.073 mmol, 1.0 equiv.) was added dropwise. Hybrid **8** was obtained in 30% yield (11.6 mg, 0.022 mmol).

The spectral data are consistent with those previously reported.[1]

3. Stability experiments

Stability experiments of all biological evaluated compounds were reported in a previous work, where no decomposition to heat exposure was observed, showing that the hybrids are thermally stable.[1]

4. Biological methods

HCMV GFP-based replication assay

The HCMV GFP-based replication assay was performed over a period of seven days (multi-round HCMV infection) with primary human foreskin fibroblasts (HFFs) infected with a GFP-expressing recombinant human cytomegalovirus (HCMV AD169-GFP) as described before.[4] All data represent mean values of determinations in quadruplicate (HCMV infections performed in duplicate, GFP measurements of total cell lysates performed in duplicate). Processing and evaluation of data was performed by the use of Excel (mean values and standard deviations). Levels compound cytotoxicity were evaluated by routine microscopic inspection (cell morphology/lysis), after 6-8 days (long-term cytotoxicity) and by the use of a commercially available lactate dehydrogenase (LDH) release assay (24 hours, acute cytotoxicity; CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega).

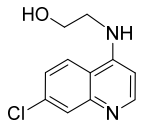
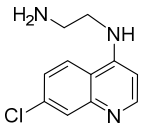
Evaluation of biological PAINS parameters

Table S1. Compound pools used for analyses[a]

Pool-1	Pool-2	Pool-3	Pool-4	Pool-5
Hybrid **4**	Compound **34**	Hybrid **9**	Artemether	Compound **13**
Hybrid **5**	Compound **35**	Hybrid **10**	DMSO	Hybrid **1**
Hybrid **6**		Hybrid **11**		Hybrid **2**
Hybrid **7**		Hybrid **12**		Hybrid **3**
Crystal/structure formation on cells[b]				
no	no	no	no	no
Colloidal aggregation[d]				
no	no	no	no	no
Autofluorescence[c]				
no	no	no	no	no
Irreversible binding[e]				
no	no	partial	partial	partial

[a] Compounds were analyzed in pools as indicated. Pools were diluted for the use in cell culture-based PAINS experiments to a final concentration of 1.25 µM for each compound, which chosen according to the determined antiviral EC_{50} values. [b] HFFs were incubated with the individual pools and formation of crystalline structures or precipitates was checked under a light microscope. [c] Autofluorescence was analyzed by treating uninfected HFFs with the compound pools to exclude compound-induced false positive GFP reads via the standard detection method described above. [d] Potentially formed colloidal aggregates were depleted by high speed centrifugation (20 000 × g, 2 min) and the biological activity of the remaining supernatants was compared to the setting without centrifugation. [e] For testing of irreversible inhibitor action, cells were treated for 30 min with the compound pools and treatment was stopped by replacing the cell culture supernatant with fresh media. Irreversibility was defined as sustained antiviral activity after compound withdrawal.

Table S2. Extended Table of EC_{50} values for reference compounds; ganciclovir (GCV), artesunic acid (ART), artemisinin (ARN), dihydroartemisinin, artemether, parent compound **13** and two quinoline derivatives **34** and **35** and hybrids **1**–**12** analyzed for anti-HCMV activity.

Compound	MW (g/mol)	HCMV EC_{50} (µM)	LDH CC_{50} (µM)
8	527.02	0.71 ± 0.03	n.d.
9	527.02	1.20 ± 0.11	>100
10	541.05	1.08 ± 0.18	>100*
11	555.07	0.30 ± 0.02	>100
12	569.10	0.38 ± 0.03	>100
13	374.281	>10	n.d.
 **34**	222.67	>10	>100
 **35**	221.69	>10	>100
artemisinin[a]	282.14	> 10	>100
artesunic acid[b]	384.42	5.41 ± 0.61	n.d.
dihydroartemisinin[a]	284.35	>10	n.d.
artemether	298.37	> 10	>100
ganciclovir[c]	579.98	2.60 ± 0.5	>100

[a] EC_{50} values have been previously reported.[5] [b] EC_{50} values have been previously reported.[6] [c] EC_{50} values have been previously reported.[7] */** Microscopic inspection of cell morphology or cell lysis after 6-8 days, long-term cytotoxicity (* moderate, ** strong). LDH**:** Lactate dehydrogenase (LDH) release assay, 24 hours, acute cytotoxicity; n.d., not determined.

Anti-HBV activity and cytotoxicity determination

HepG2-hNTCP cells (human liver cancer cells stably transduced with human sodium taurocholate co-transporting polypeptide[8]) were obtained from Dr. Stephan Urban, Heidelberg University Hospital (Heidelberg, Germany) and were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine, 10% fetal bovine serum, 100 U of penicillin/mL, 100 µg of streptomycin/mL and 0.05 mg/mL of puromycin (all Sigma-Aldrich, St. Louis, USA) in 5 % CO_2 at 37 °C. Cell line was mycoplasma negative (tested at Generi Biotech, Czech Republic).

For anti-HBV activity determination, 30,000 HEPG2-hNTCP cells in 100µL of DMEM with serially diluted compounds were infected with 2,000 genome equivalents of HBV per cell. One day after infection HBV virus was washed out and medium was replaced with fresh medium containing new compound. Next, medium was changed and fresh compound was added at day 3, 6 and 10. At day 14 amount of HBV e protein (HBeAg) and total HBV-DNA was determined by ELISA and

quantitative PCR, respectively. Briefly, level of HBeAg was measured using a commercial ELISA kit (Bioneovan, Beijing, China) according to the manufacturer's instructions. Amount of total HBV DNA in supernatants was determined by qPCR using primers and probe specific for HBV DNA as reported[9] with gb Elite PCR master Mix (Generi Biotech) and conditions as described.[10]

Cytotoxicity was evaluated in 30,000 HEPG2-hNTCP cells in 100µL of DMEM with serially diluted compounds from 50 µM. After 72 hours, the cell viability was determined by addition of XTT solution (Sigma-Aldrich) for 4 hours and the absorbance of newly formed orange formazan solution was measured using Victor X3 plate reader (Perkin Elmer, Waltham, USA).

Drug concentrations required to reduce HBeAg and total HBV-DNA levels by 50 % (EC50) or drug concentrations reducing the cell viability by 50% were calculated using nonlinear regression analysis with GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, USA).

Table S3. Extended Table of CC_{50} and EC_{50} values for reference compounds; dihydroartemisinin, artesunic acid, tenofovir alafenamide and hybrids **1, 2, 5, 7, 11,** and **12** analyzed for anti-HBV activity.

Compound	HepG2-hNTCP CC_{50} [µM]	95% CI of CC_{50}	EC_{50} [µM] ELISA E	95% CI of EC_{50} ELISA E	EC_{50} [µM] qPCR	95% CI of EC_{50} qPCR
1	29.9	27.8 - 32.2	2.57	0.96 – 6.9	~10	n.a.
2	>50	n.a.	>10	n.a.	>10	n.a.
5	>50	n.a.	>10	n.a.	>10	n.a.
7	>50	n.a.	>10	n.a.	>10	n.a.
11	>50	n.a.	>10	n.a.	>10	n.a.
12	>50	n.a.	>10	n.a.	>10	n.a.
dihydro-artemisinin	>50	n.a.	>10	n.a.	>10	n.a.
artesunic acid	>50	n.a.	>10	n.a.	>10	n.a.
tenofovir alafenamide (TAF)	27.2	25.8 – 28.6	3.93	2.66 - 5.8	0.00024	0.00017 - 0.00034

CI: confidence intervals; n.a.: not applicable.

5. Filtering PAINS elements among hybrid compounds 1 - 12

All seventeen hybrid compounds (**1 - 12**) were screened for pan assay interference compounds (PAINS) by FAF-Drugs4 filtering tool (server available at http://fafdrugs4.mti.univ-parisdiderot.fr).[11] The Results of the screening were reported recently.[1] All hybrids passed the PAINS filters.

6. References

[1] A. Çapcı, M. M. Lorion, H. Wang, N. Simon, M. Leidenberger, M. C. Borges Silva, D. R. M. Moreira, Y. Zhu, Y. Meng, J. Y. Chen, Y. M. Lee, O. Friedrich, B. Kappes, J. Wang, L. Ackermann, S. B. Tsogoeva, *Angew. Chem. Int. Ed. Engl.* **2019**, *58*, 13066-13079.

[2] P. P. Bora, N. Baruah, G. Bez, N. C. Barua, *Synth. Commun.* **2011**, *42*, 1218-1225.

[3] H. Ji, H. Li, P. Martásek, L. J. Roman, T. L. Poulos, R. B. Silverman, *J. Med. Chem.* **2009**, *52*, 779-797.

[4] a) M. Marschall, M. Freitag, S. Weiler, G. Sorg, T. Stamminger, *Antimicrob Agents Chemother* **2000**, *44*, 1588-1597; b) T. Fröhlich, F. Hahn, L. Belmudes, M. Leidenberger, O. Friedrich, B. Kappes, Y. Coute, M. Marschall, S. B. Tsogoeva, *Chem. Eur. J.* **2018**, *24*, 8103-8113; c) F. Hahn, C. Hutterer, C. Henry, S. T. Hamilton, H. Strojan, A. Kraut, U. Schulte, M. Schütz, S. Kohrt, C. Wangen, *J. Antivir. Res.* **2018**, *159*, 84-94.

[5] a) C. Hutterer, I. Niemann, J. Milbradt, T. Fröhlich, C. Reiter, O. Kadioglu, H. Bahsi, I. Zeitträger, S. Wagner, J. Einsiedel, P. Gmeiner, N. Vogel, S. Wandinger, K. Godl, T. Stamminger, T. Efferth, S. B. Tsogoeva, M. Marschall, *Antivir. Res.* **2015**, *124*, 101-109; b) F. E. Held, A. A. Guryev, T. Fröhlich, F. Hampel, A. Kahnt, C. Hutterer, M. Steingruber, H. Bahsi, C. von Bojničić-Kninski, D. S. Mattes, T. C. Foertsch, A. Nesterov-Mueller, M. Marschall, S. B. Tsogoeva, *Nat. Commun.* **2017**, *8*, 15071.

[6] S. Chou, G. Marousek, S. Auerochs, T. Stamminger, J. Milbradt, M. Marschall, *Antivir. Res.* **2011**, *92*, 364-368.

[7] S. J. F. Kaptein, T. Efferth, M. Leis, S. Rechter, S. Auerochs, M. Kalmer, C. A. Bruggeman, C. Vink, T. Stamminger, M. Marschall, *Antivir. Res.* **2006**, *69*, 60-69.

[8] Y. Ni, F. A. Lempp, S. Mehrle, S. Nkongolo, C. Kaufman, M. Falth, J. Stindt, C. Koniger, M. Nassal, R. Kubitz, H. Sultmann, S. Urban, *Gastroenterology* **2014**, *146*, 1070-1083.

[9] Y. T. Gao, T. Han, Y. Li, B. Yang, Y. J. Wang, F. M. Wang, X. Jing, Z. Du, *J Virol Methods* **2010**, *169*, 181-187.

[10] B. Lubyova, J. Hodek, A. Zabransky, H. Prouzova, M. Hubalek, I. Hirsch, J. Weber, *PLoS One* **2017**, *12*, e0186982.

[11] D. Lagorce, L. Bouslama, J. Becot, M. A. Miteva, B. O. Villoutreix, *Bioinformatics* **2017**, *33*, 3658-3660.