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Synthesis of Novel Hybrids of Quinazoline and Artemisinin with High Activities against *Plasmodium falciparum*, Human Cytomegalovirus, and Leukemia Cells

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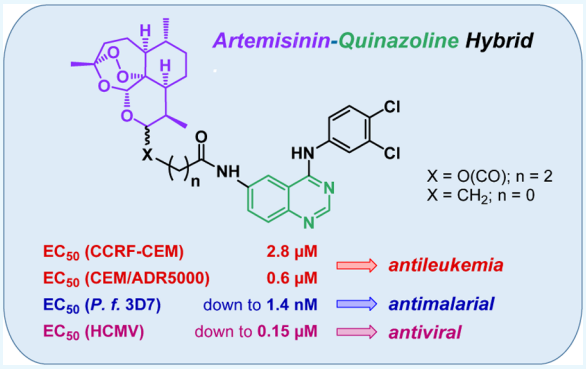
Supporting Information

ABSTRACT: Many quinazoline derivatives have been synthesized over the last few decades with great pharmacological potential, such as antimalarial, anti-inflammatory, antimicrobial, anticancer, and antiviral. But so far, no quinazoline−artemisinin hybrids have been reported in the literature. In the present study, five novel quinazoline−artemisinin hybrids were synthesized and evaluated for their in vitro biological activity against malarial parasites (*Plasmodium falciparum* 3D7), leukemia cells (CCRF-CEM and CEM/ADR5000), and human cytomegalovirus. Remarkably, hybrid **9** (EC_{50} = 1.4 nM), the most active antimalarial compound of this study, was not only more potent than artesunic acid (EC_{50} = 9.7 nM) but at the same time more active than the clinically used drugs dihydroartemisinin (EC_{50} = 2.4 nM) and chloroquine (EC_{50} = 9.8 nM). Furthermore, hybrids **9** and **10** were the most potent compounds with regard to anticytomegaloviral activity (EC_{50} = 0.15−0.21 μM). They were able to outperform ganciclovir (EC_{50} = 2.6 μM), which is the relevant standard drug of antiviral therapy, by a factor of 12−17. Moreover, we identified a new highly active quinazoline derivative, compound **14**, that is most effective in suppressing cytomegalovirus replication with an EC_{50} value in the nanomolar range (EC_{50} = 50 nM). In addition, hybrid **9** exhibited an antileukemia effect similar to that of artesunic acid, with EC_{50} values in the low micromolar range, and was 45 times more active toward the multidrug-resistant CEM/ADR5000 cells (EC_{50} = 0.5 μM) than the standard drug doxorubicin.



1. INTRODUCTION

The quinazoline scaffold, which consists of two fused six-membered aromatic rings benzene and pyrimidine, plays a fundamental role in today's discovery and design of new drugs due to its wide-ranging pharmacological properties, such as antimicrobial,[1−3] antimalarial,[4,5] anti-inflammatory,[6−8] anti-cancer,[9−11] and antiviral.[12,13] One of the first quinazoline derivatives, which was found to have very promising biological efficacy, was the natural occurring alkaloid febrifugine (**1**) (Figure 1). The compound was isolated 60 years ago from the Chinese plant aseru (*Dichroa febrifuga* Lour), which has been applied as an antimalarial agent in traditional Chinese medicine for over 2000 years.[14−16] Since the discovery of febrifugine (**1**), the quinazoline moiety became one of the most studied motifs in medicinal chemistry, and as a consequence, a great variety of quinazoline derivatives exhibiting diverse biological activities have been synthesized in the past few decades.[17−21] Selected examples are displayed in Figure 1: gefitinib (**2a**), an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), which is already used clinically for the treatment of non-small-

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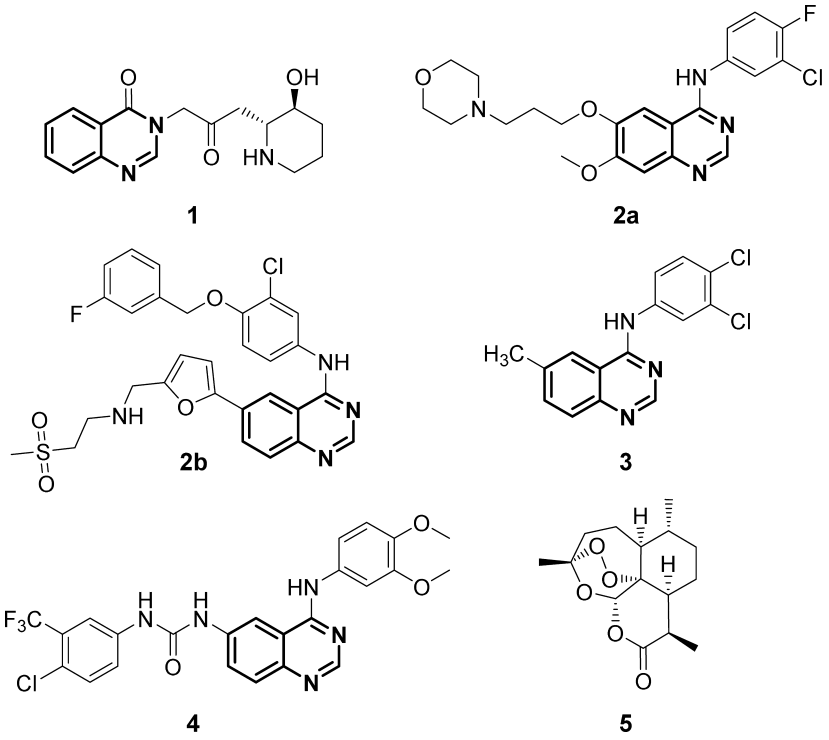

Figure 1. Biologically active quinazoline derivatives: febrifugine (**1**) (antimalarial), gefitinib (**2a**), lapatinib (**2b**) (anticancer), and two other synthetic 4-anilinoquinazoline derivatives **3** (anti-human cytomegalovirus (anti-HCMV)) and **4** (antimalarial), as well as the structure of artemisinin (**5**).

cell lung carcinoma;[22,23] lapatinib (**2b**), an orally active drug against breast cancer;[24] and two other synthetic 4-anilinoquinazoline derivatives: compound **3**, which possesses anticytomegaloviral activity,[13] and compound **4**, which is effective even in vivo against malarial parasites.[5]

To enhance the pharmacological properties of the available quinazoline drugs and to counter the spread of resistance, especially toward malaria and cancer, we intended to apply the so-called molecular hybridization, which is a relatively new concept in drug design and development, wherein at least two different pharmacophoric moieties originating from different bioactive substances are combined into one single structure.[25−28] The resulting hybrids have, in many cases, improved pharmacological properties compared to those of their parent drugs, such as increased biological efficacy, reduced undesired side effects, a modified selectivity profile (lower toxicity), better bioavailability, and sometimes even completely new biological features that were absent in the parent compounds.[28−32] The second pharmacologically active substance we chose besides quinazoline for the hybridization was artemisinin (**5**), which is an enantiomerically pure sesquiterpene with a 1,2,4-trioxane heterocycle and was first isolated in 1972 from the Chinese medicinal plant *Artemisia annua* L. by Youyou Tu (Nobel Prize 2015).[33] Artemisinin was selected by us not only because of its known antimalarial[34−37] and anticancer potential[38,39] but also due to our promising experiences in the past with artemisinin-based hybrids.[40−44]

In this study, we report the synthesis of five novel quinazoline−artemisinin hybrids **6**−**10** (Figure 2), which were investigated for their inhibitory potency against the malarial parasite *Plasmodium falciparum* 3D7 strain, the leukemia cell lines CCRF-CEM and CEM/ADR5000, and HCMV.

2. RESULTS AND DISCUSSION

2.1. Chemistry. Quinazoline derivatives **11**, **12**, and **14** (Figure 3, Scheme 1), which were necessary for the synthesis of hybrids **6**−**10** (Scheme 2), were prepared in analogy to a literature-known procedure developed by Wang et al. in 2014.[45] Applying the Steglich esterification reaction, it was possible to couple artesunic acid (**16**), a semisynthetic derivative of artemisinin (**5**), directly with quinazoline derivative **11** containing a phenol moiety, yielding the desired hybrid **6** in 60% (Scheme 2). The reaction was performed using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP) as coupling agents and a mixture of CH_3CN and ethyl acetate (EtOAc) as the solvent.

To be able to synthesize the other quinazoline−artemisinin hybrids, **7**−**10** (Scheme 2), further modifications of quinazoline derivatives **12** and **14** were required (Scheme 1). Quinazoline **12** was converted into an alcohol, **13**, by *N*-alkylation of the secondary amine using 3-bromopropanol as the reagent, K_2CO_3 as the base, TBAI as the additive, and CH_3CN as the solvent. After refluxing the reaction mixture overnight, the product could be isolated in 22% yield. Conversion of the aromatic nitro group of new quinazoline **14** to the corresponding primary amine was realized by hydrazine monohydrate-mediated reduction catalyzed by Raney-Ni.

This procedure, which is already reported in the literature in context of the synthesis of other simple aromatic amino compounds,[46] afforded quinazoline amine **15** in 87% yield. These two quinazoline precursors, **13** and **15**, were then coupled with either artesunic acid (**16**) or artemisinin-derived carboxylic acid **17** to furnish hybrids **7**−**10**: Steglich conditions (DCC, DMAP) afforded ester hybrids **7**/**8** in 35/49% yield, and standard amide coupling (EDCI/1-hydroxybenzotriazole (HOBt), DIPEA) gave amide hybrids **9**/**10** in 64/68% yield. Artemisinin-derived acid **17** was synthesized from dihydroarte-

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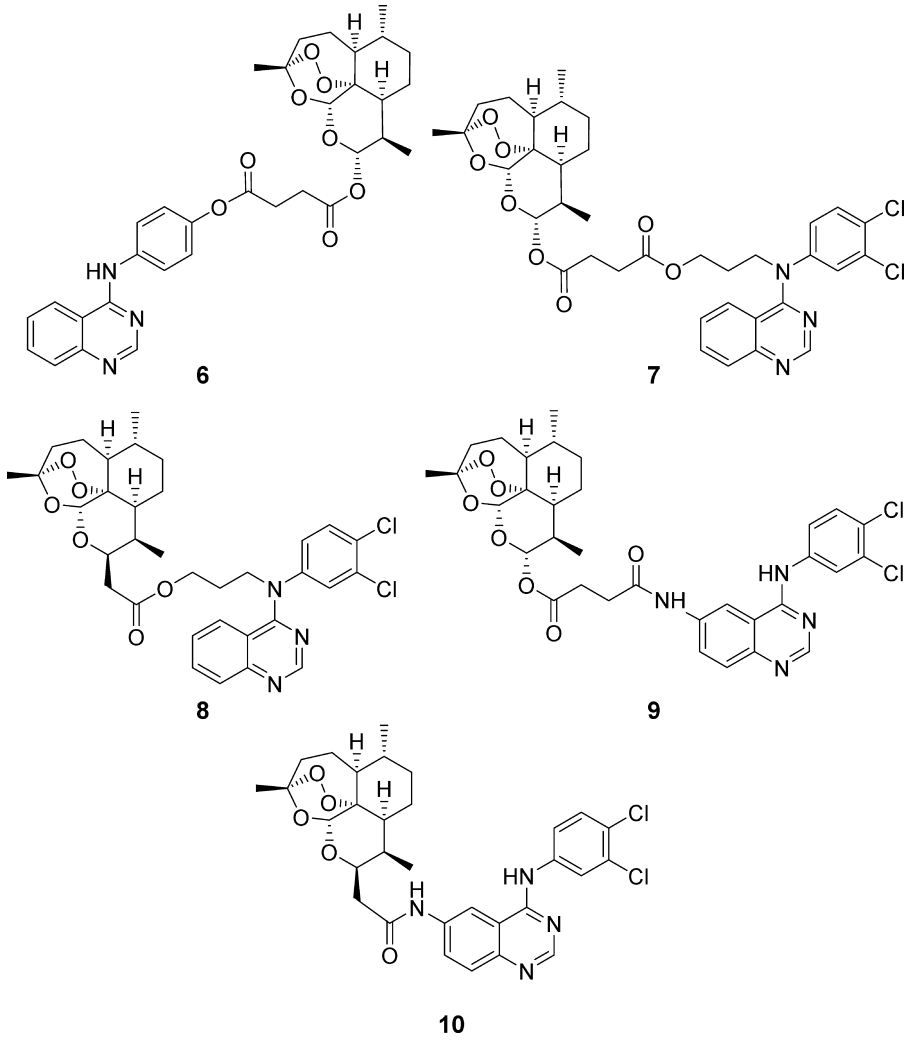

Figure 2. Hybrids **6**–**10** applied in this work for biological tests against *P. falciparum* 3D7 parasites, HCMV, and leukemia cell lines CCRF-CEM and CEM/ADR5000.

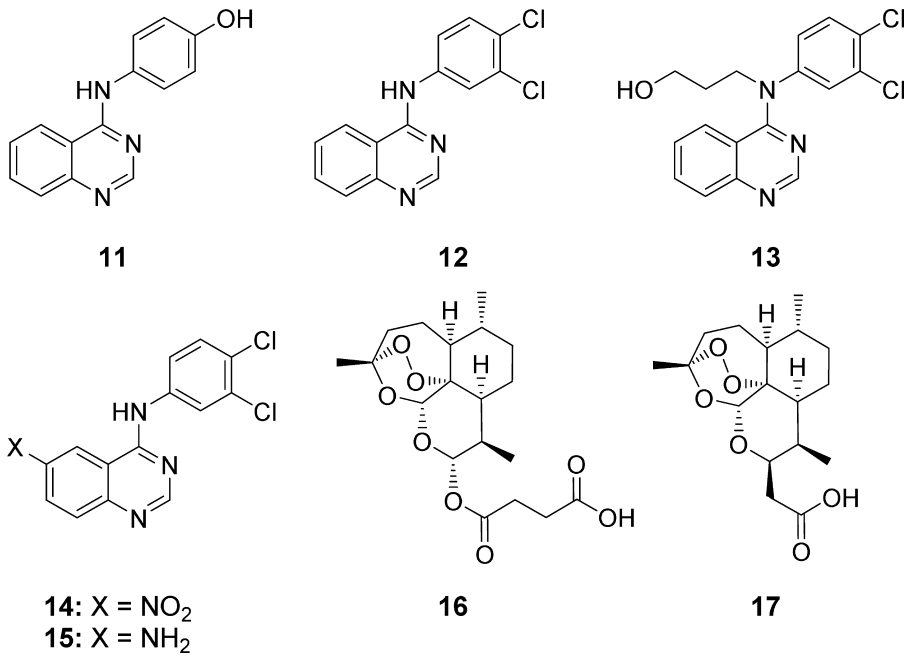

Figure 3. Precursors for hybrids presented in this work.

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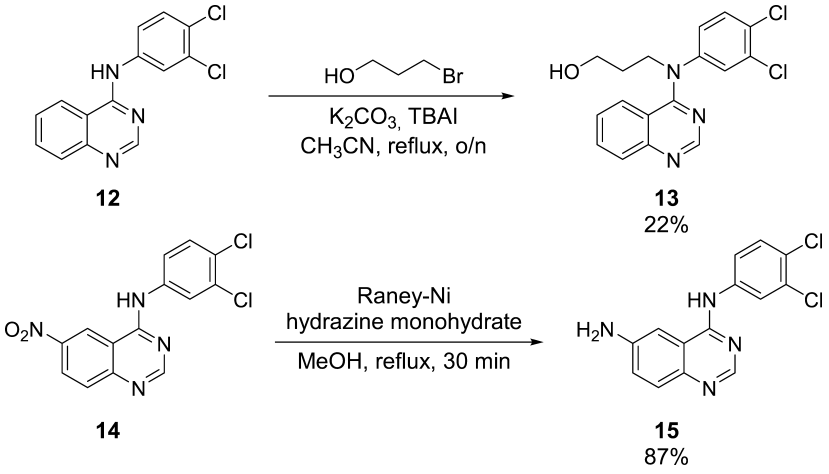
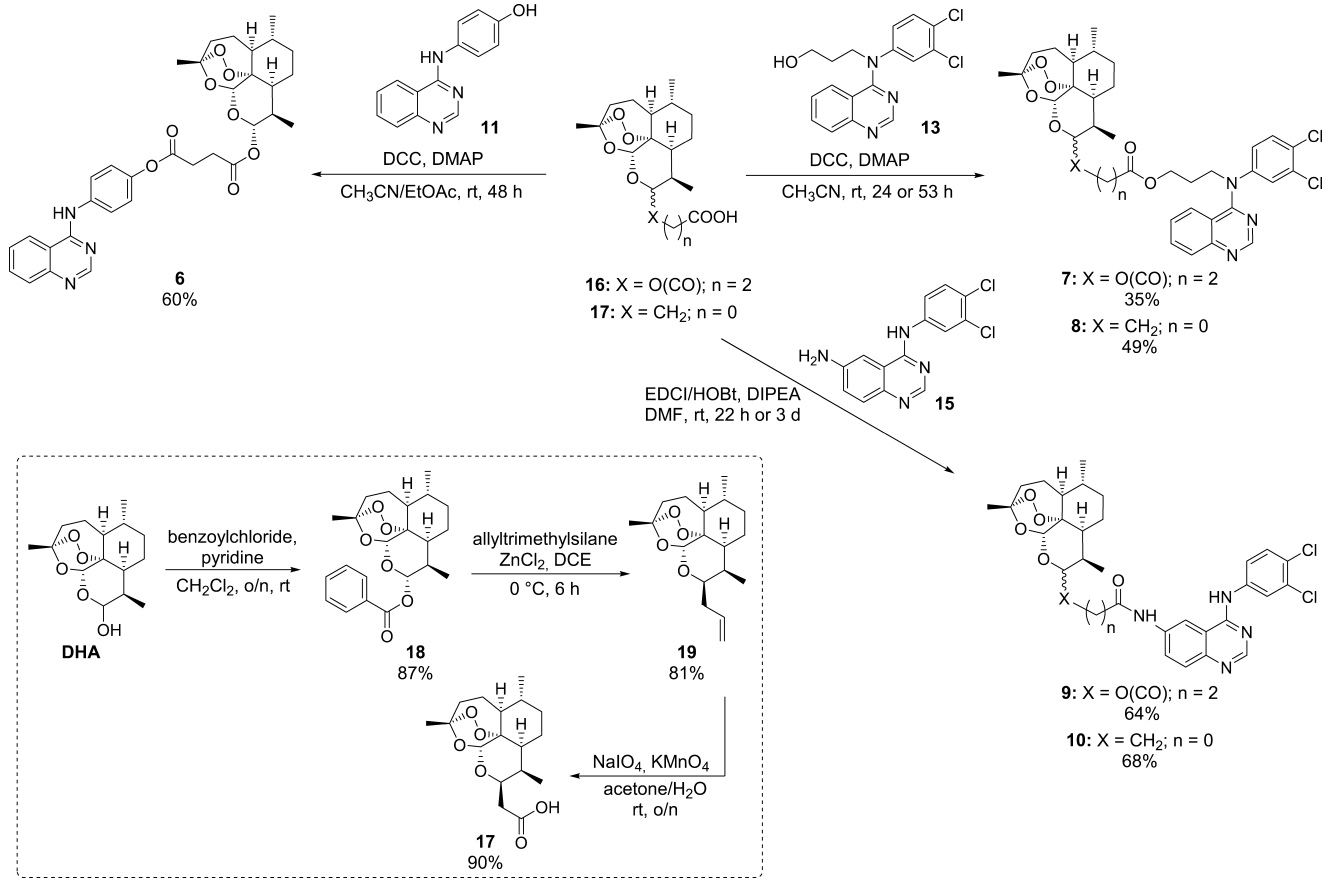
Scheme 1. Synthesis of Quinazoline-Derived Precursors 13 and 15



Scheme 2. Synthesis Route for Hybrids 6−10



misinin (DHA) according to the literature (Scheme 2)[47] and was chosen as the starting material for the hybrid synthesis, as it belongs to the group of C-10 nonacetals, which are known to be more hydrolytically stable than common artemisinin derivatives, such as artesunic acid (**16**).[48]

The stability of all our new target compounds **6−10** was tested by heating them for 20 h at 60 °C. After utilizing these conditions, less than 5% decomposition was observed via ^{1}H NMR spectroscopy, indicating that all synthesized hybrids are sufficiently stable.

2.2. Biological Evaluation and Discussion. *2.2.1. Cytotoxicity toward P. falciparum 3D7.* The antimalarial activity of all synthesized hybrids **6−10**, as well as their parent compounds artesunic acid (**16**) and quinazoline derivatives **12** and **14**, was evaluated in vitro against *P. falciparum* 3D7 (Table 1).

In general, it can be said that all hybrids exhibit excellent antimalarial efficacy with EC_{50} values within the nanomolar range (1.4−39.9 nM). This is quite remarkable because their corresponding quinazoline precursors **12** and **14** only exhibit antimalarial activities in the low micromolar and moderate nanomolar ranges (EC_{50} = 148−3177 nM). All hybrids except for hybrid **10** (EC_{50} = 39.9 nM) had a similar or even higher activity than that of the tested reference compound, artesunic acid (**16**) (EC_{50} = 9.7 nM), and clinically used chloroquine

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Table 1. EC_{50} Values for Chloroquine, Dihydrartemisinin, Artesunic Acid (16), Quinazoline Derivatives 12 and 14, and Hybrids 6−10 Tested against *P. falciparum* 3D7 Parasites

compound	molecular weight (g/mol)	3D7 EC_{50} (nM)
chloroquine[a]	319.87	9.8 ± 2.8
DHA[a]	284.25	2.4 ± 0.4
artesunic acid (**16**)	384.42	9.7
12	290.15	3177 ± 443
14	335.14	148 ± 8.2
6	603.67	3.8 ± 1
7	714.64	14.4 ± 0.3
8	656.60	15.3 ± 1.9
9	671.57	1.4 ± 0.4
10	613.54	39.9 ± 0.8

[a]EC_{50} values have been previously reported.[44]

(EC_{50} = 9.8 nM). The most active compounds in this study concerning antimalarial efficacy were hybrids **6** (EC_{50} = 3.8 nM) and **9** (EC_{50} = 1.4 nM), which were comparable to or even more active than the clinically used drug DHA (EC_{50} = 2.4 nM). On analyzing the structure−activity relationship of the hybrids, interesting observations can be made. C-10 acetal hybrid **9** is 30 times more active than hybrid **10**, which contains a C-10 nonacetal-linked artemisinin subunit. In consequence, a C-10 acetal linkage seems to be beneficial for antimalarial activity in the case of quinazoline−artemisinin hybrids.

A contrary effect was observed in connection with artemisin-derived dimers,[49] which supports the assumption that the underlying mechanism of action might be different. The free secondary amine group of 4-anilinoquinazoline-derived hybrids seems to play an important role with regard to antimalarial efficacy, as hybrids **7** and **8**, in which the artemisinin moiety is attached to the N-atom of the aniline subunit, are approximately up to 10 times less active than hybrids **6** and **9**. Therefore, the functionalized aromatic subunits in 4-anilinoquinazoline derivatives should be used for hybridization, and the secondary amine should be left untouched.

2.2.2. Inhibitory Activity against HCMV in Primary Human Fibroblasts. The antiviral activity of compounds was investigated for HCMV, strain AD169-GFP. This recombinant virus expresses the green fluorescent protein (GFP) that can be reliably quantitated as a reporter of viral replication (Table 2). Infection experiments were carried out with cultures of primary human foreskin fibroblasts (HFFs), and measurements of antiviral activity were performed according to an already published protocol.[50−52] Used as two reference compounds, artesunic acid (**16**) and ganciclovir both exerted strong anti-HCMV activity at EC_{50} levels of 3.80 and 2.60 μM, respectively, and were compared to that of the novel quinazoline−artemisinin hybrids. Four of five hybrids analyzed showed anti-HCMV activity in the submicromolar range with EC_{50} values of 0.86 μM (hybrid **7**), 0.65 μM (hybrid **8**), 0.21 μM (hybrid **9**), and 0.15 μM (hybrid **10**; the fifth compound, hybrid **6**, was less active but still showed anti-HCMV activity in the low micromolar range, with EC_{50} = 5.89 μM). Thus, these hybrids were up to 17-fold more effective than the reference drug ganciclovir.

We concluded from these data that a similar structure−activity trend refers to the anti-HCMV activity as already previously stated for antimalarial efficacy. Hybrids **7** and **8**, in which the secondary amine group of the 4-anilinoquinazoline derivatives were used for hybridization, were less active than

Table 2. EC_{50} Values of Anti-HCMV Activity (AD169-GFP) Displayed in Virus-Infected HFFs: Artesunic Acid (16) Compared with Five Chemically Different Quinazoline−Artemisinin Hybrids 6−10 and Their Precursors 12−14

compound	molecular weight (g/mol)	HCMV EC_{50} (μM)
ganciclovir[a]	255.23	2.60 ± 0.50
artemisinin (**5**)[b]	282.42	>10
DHA[b]	284.35	>10
artesunic acid (**16**)[a]	384.42	3.80 ± 0.40
12	290.15	2.01 ± 0.10
13	348.23	>10
14	335.14	0.05 ± 0.02
6	603.67	5.89 ± 1.07
7	714.64	0.86 ±0.20
8	656.60	0.65 ± 0.03
9	671.57	0.21 ± 0.12
10	613.54	0.15 ± 0.05

[a]EC_{50} values have been previously reported.[53] [b]EC_{50} values have been previously reported.[54]

hybrids **9** and **10** with the free secondary amine group. This effect was even more pronounced for quinazoline precursors **12** and **13**: quinazoline derivative **12** possessed an efficacy comparable to that of ganciclovir (EC_{50} = 2.01 μM), whereas compound **13** was completely inactive (EC_{50} > 10 μM). However, unlike antimalarial efficacy, C-10 nonacetal-linked artemisinin-derived hybrids **8** and **10** were more potent against HCMV than their corresponding C-10 acetal-linked counterparts (hybrids **7** and **9**).

The highest antiviral efficacy in vitro among the tested hybrids was noted for hybrid **10**; therefore, it is tempting to speculate whether the specific chemical nature of this hybrid compound may combine in an optimized manner the antiviral mechanisms displayed by artesunic acid and quinazolines. Previous reports demonstrated that the antiviral mode of action of artesunic acid is based on its signaling-inhibitory potential, particularly its interference with the canonical NF-κB (RelA/p65) pathway,[55−57] whereas quinazolines typically act on the basis of a direct catalytic inhibition of viral and cellular protein kinases.[57−60] For these compounds, no or only modest signs of cytotoxicity were detectable on HFFs up to a concentration of 10 μM, as routinely monitored by light and fluorescence microscopy, thus strongly arguing for a specific antiviral effect. Combined, these results indicate a strong anti-HCMV potential of quinazoline−artemisinin hybrids.

Surprisingly, the most active compound tested in this study against HCMV was new quinazoline **14** with an EC_{50} value in the nanomolar range (50 nM). The compound exerted a 50-fold higher activity than the standard drug ganciclovir and also 40-fold higher activity than other tested quinazoline derivatives **12** and **13**.

2.2.3. Cytotoxicity toward Sensitive CCRF-CEM and Multidrug-Resistant CEM/ADR5000 Leukemia Cells. The most promising hybrids in this study, **9** and **10**, and its precursor, **14**, were tested for a potential in vitro antileukemia effect in sensitive wild-type CCRF-CEM and multidrug-resistant P-glycoprotein-overexpressing CEM/ADR5000 cells (Table 3). Hybrid **10** and precursor **14** showed no promising antileukemia behavior. In contarst, hybrid **9** with EC_{50} values of 2.8 μM (CCRF-CEM) and 0.5 μM (CEM/ADR5000) exhibited an antileukemia effect similar to that of artesunic acid. Especially, the activity against the multidrug-resistant

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Table 3. EC_{50} Values for Quinazoline−Artemisinin Hybrids 9/10 and Its Precursor 14 in Sensitive Wild-Type CCRF-CEM and Multidrug-Resistant P-Glycoprotein-Overexpressing CEM/ADR5000 Cells

compound	molecular weight (g/mol)	CCRF-CEM EC_{50} (μM)	CEM/ADR5000 EC_{50} (μM)	degree of cross-resistance
doxorubicin[a]	579.98	0.009	23.27	2585
artemisinin (**5**)[b]	282.14	36.90 ± 6.90	26.90 ± 4.40	0.73
artesunic acid (**16**)	384.42	0.4 ± 0.1	0.1 ± 0.1	0.3
9	671.57	2.8 ± 0.2	0.6 ± 0.2	0.2

[a]EC_{50} values for both cell lines have been previously reported.[61] [b]EC_{50} values for both cell lines have been previously reported.[42]

CEM/ADR5000 cells is quite promising, as it is 45 times higher than that of doxorubicin (EC_{50} = 23.27 μM). This is important as drug resistance is one of the biggest challenges to be overcome in current cancer treatment.

3. CONCLUSIONS

In conclusion, five quinazoline−artemisinin-derived hybrids, **6**−**10**, were successfully synthesized and investigated for the first time for their antimalarial, antiviral, and antileukemia activity. All novel quinazoline−artemisinin hybrids showed high antimalarial efficacy against the *P. falciparum* 3D7 strain (EC_{50} = 1.4−39.9 nM), which was better or comparable to that of artesunic acid (EC_{50} = 9.7 nM). Remarkably, hybrid **9** (EC_{50} = 1.4 nM) was even more active than the clinically used drugs DHA (EC_{50} = 2.4 nM) and chloroquine (EC_{50} = 9.8 nM). With EC_{50} values of 0.15−0.21 μM, compounds **9** and **10** were most potent in the inhibition of HCMV replication in primary cell cultures, surpassing the antiviral activity of ganciclovir by factors 12−17. In the course of this study, we also characterized a novel, highly active new quinazoline **14** that was most active with an EC_{50} in the nanomolar range (HCMV EC_{50} = 50 nM). Moreover, hybrid **9** showed an antileukemia effect similar to that of artesunic acid with EC_{50} values in the low micromolar range and was 45 times more active toward the multidrug-resistant CEM/ADR5000 cells (EC_{50} = 0.6 μM) than the standard drug doxorubicin. These promising results further underline the high potential of the hybridization concept for further investigations and hybrid-based drug design.

4. EXPERIMENTAL SECTION

4.1. Chemistry. *4.1.1. Synthesis of Hybrid Molecules: General.* All reactions were performed in flame-dried glassware under a nitrogen atmosphere. If necessary, the synthesized hybrids were further purified after column chromatography via reprecipitation from CH_2Cl_2 in *n*-hexane to yield a pure compound for elemental analysis and biological tests. EtOAc was purchased as an anhydrous solvent. CH_2Cl_2 was dried initially over $CaCl_2$ and then distilled from P_2O_5. All other solvents were purified by distillation using rotary evaporation or were purchased in high-performance liquid chromatography-quality. Reagents obtained from commercial sources were used without further purification. Thin-layer chromatography was performed on precoated aluminum silica gel SIL G/UV254 plates (Macherey-Nagel & Co.). The detection occurred via fluorescence quenching or development in a phosphomolybdic acid solution (10% in EtOH). All products were dried under high vacuum (10^{-3} mbar). ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature (rt) either on a Bruker Avance spectrometer operating at 300 and 400 MHz, respectively, or on a JEOL JNM GX 400 spectrometer operating at 400 MHz. ESI mass spectra were recorded on a Bruker micrOTOF II focus time-of-flight mass spectrometer (TOF MS) or a Shimadzu Axima Confidence MALDI-TOF MS without a matrix. IR spectra were recorded on a Varian IR-660 apparatus. The absorption is indicated in wave numbers [cm^{-1}]. Elemental analysis (C, H, N), carried out with a Euro EA 3000 (Euro Vector) machine and an EA 1119 CHNS, CE machine, is within ±0.50% of the calculated values, confirming a purity of >95%. Artesunic acid (**16**) and DHA were obtained from ABCR (Karlsruhe, Germany).

*4.1.2. General Procedure for Hybrids **6**−**8**.* To the corresponding quinazoline alcohol, **11** or **13**, in CH_3CN (and anhydrous EtOAc) with 4 Å molecular sieves, DMAP and artesunic acid (**16**) or artemisinin-derived acid **17** (prepared according to the literature procedures,[47] see Scheme 2) were added consecutively, and the reaction mixture was cooled to 0 °C. Subsequently, DCC was added, and the reaction mixture was warmed to rt and stirred for 24, 48, or 53 h. Subsequently, DCU was removed by filtration, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography, and hybrids **6** and **7** were obtained as pale yellow solids and hybrid **8** as a pale yellow oil.

*4.1.3. Quinazoline−Artesunic Acid Hybrid **6**.* Quinazoline alcohol **11** (177 mg, 0.74 mmol, 1.0 equiv), CH_3CN (13.0 mL), EtOAc (2.5 mL), DMAP (45.4 mg, 0.37 mmol, 50 mol %), artesunic acid (**16**) (373 mg, 0.97 mmol, 1.3 equiv), DCC (200 mg, 0.97 mmol, 1.3 equiv). Column conditions: CH_2Cl_2/MeOH 80:1 → CH_2Cl_2/MeOH 40:1. Yield: 269 mg, 0.45 mmol, 60%, off-white solid. R_f = 0.49 (CH_2Cl_2/MeOH 20:1, UV and phosphomolybdic acid). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, J = 7.1 Hz, 3H), 0.93 (d, J = 5.8 Hz, 3H), 0.96−1.05 (m, 1H), 1.19−1.51 (m, 7H), 1.56−1.63 (m, 1H), 1.63−1.77 (m, 2H), 1.82−1.91 (m, 1H), 1.96−2.04 (m, 1H), 2.28−2.40 (m, 1H), 2.51−2.61 (m, 1H), 2.72−2.97 (m, 4H), 5.41 (s, 1H), 5.80 (d, J = 9.9 Hz, 1H), 7.05−7.11 (m, 2H), 7.52−7.59 (m, 1H), 7.68−7.74 (m, 2H), 7.75−7.81 (m, 1H), 7.87−7.97 (m, 2H), 7.98−8.04 (m, 1H), 8.70 (s, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.0, 20.2, 21.9, 24.5, 25.9, 29.0, 29.2, 31.8, 34.0, 36.2, 37.2, 45.2, 51.5, 80.1, 91.5, 92.4, 104.5, 115.0, 120.9, 122.0, 123.1, 126.8, 128.3, 133.1, 135.7, 147.1, 149.2, 154.4, 157.7, 170.9, 171.0 ppm. MS (ESI): m/z = 604 ([M + H]$^+$); HRMS (ESI): calcd for [$C_{33}H_{38}N_3O_8$]$^+$ 604.2653, found 604.2656. FT-IR (ATR): $\tilde{\nu}$ = 3386 (w), 2924 (w), 2878 (w), 2358 (w), 2350 (w), 2020 (w), 1747 (s), 1620 (w), 1572 (s), 1528 (s), 1507 (s), 1494 (s), 1416 (m), 1405 (m), 1358 (m), 1308 (w), 1249 (w), 1195 (s), 1165 (m), 1135 (s), 1099 (m), 1033 (s), 1008 (s), 920 (w), 875 (m), 842 (w), 766 (m), 732 (w), 679 (w), 595 (w), 515 (w) cm^{-1}. Anal. Calcd for $C_{33}H_{37}N_3O_8 \cdot 0.3CH_2Cl_2$: C, 63.57; H, 6.02; N, 6.68; found: C, 63.32; H, 5.98; N, 6.75.

*4.1.4. Quinazoline−Artesunic Acid Hybrid **7**.* Quinazoline alcohol **13** (56.0 mg, 0.16 mmol, 1.0 equiv), CH_3CN (5.0 mL), DMAP (9.50 mg, 0.08 mmol, 48 mol %), artesunic acid (**16**) (80.5 mg, 0.21 mmol, 1.3 equiv), DCC (43.1 mg, 0.21 mmol, 1.3 equiv). Column conditions: hexanes/EtOAc 3:1 → hexanes/EtOAc 1:2. Yield: 40.0 mg, 0.06 mmol, 35%, yellow

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solid. R_f = 0.20 (hexanes/EtOAc 1:4, UV and phosphomolybdic acid). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, J = 7.1 Hz, 3H), 0.92 (d, J = 5.5 Hz, 3H), 0.94−1.08 (m, 1H), 1.17−1.44 (m, 7H), 1.53−1.77 (m, 5H), 1.78−2.00 (m, 2H), 2.07−2.14 (m, 1H), 2.31 (td, J = 13.8, 3.8 Hz, 1H), 2.46−2.59 (m, 1H), 2.61−2.83 (m, 4H), 3.94−4.4.08 (m, 2H), 4.25−4.36 (m, 1H), 5.29 (s, 1H), 5.73 (d, J = 9.9 Hz, 1H), 6.94 (dd, J = 8.4, 2.2 Hz, 1H), 7.14−7.21 (m, 1H), 7.23 (d, J = 2.2 Hz, 1H), 7.31−7.43 (m, 2H), 7.55−7.64 (m, 1H), 7.75 (s, 1H), 8.39 (d, J = 8.1 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.0, 20.2, 21.9, 24.5, 25.8, 27.7, 29.0, 29.2, 29.7, 31.7, 34.0, 36.1, 37.2, 45.1, 46.4, 51.5, 60.9, 80.1, 91.4, 92.3, 104.4, 113.8, 120.8, 122.3, 124.3, 125.5, 126.2, 127.6, 130.1, 132.0, 132.3, 137.4, 150.2, 153.2, 171.4, 171.7 ppm. MS (ESI): m/z = 714 ([M + H]$^+$); HRMS (ESI): calcd for $[C_{36}H_{42}Cl_2N_3O_8]^+$ 714.2344, found 714.2362. FT-IR (ATR): $\tilde{\nu}$ = 2953 (w), 2924 (m), 2872 (w), 2358 (w), 2249 (w), 2013 (w), 1736 (s), 1614 (s), 1578 (m), 1546 (s), 1462 (s), 1401 (m), 1376 (m), 1312 (w), 1243 (m), 1155 (m), 1129 (m), 1098 (m), 1011 (s), 908 (w), 875 (m), 843 (w), 804 (m), 755 (s), 729 (s), 667 (m) 545 (w) cm^{-1}. Anal. Calcd for $C_{36}H_{41}Cl_2N_3O_8 \cdot 1.1H_2O$: C, 58.87; H, 5.93; N, 5.72; found: C, 58.96; H, 5.57; N, 5.67.

4.1.5. Quinazoline−Artemisinin Hybrid ***8***. Quinazoline alcohol **13** (50.0 mg, 0.14 mmol, 1.0 equiv), CH_3CN (4.5 mL), DMAP (8.77 mg, 0.07 mmol, 50 mol %), artemisinin-derived acid **17** (61.0 mg, 0.19 mmol, 1.3 equiv), DCC (38.6 mg, 0.19 mmol, 1.3 equiv). Column conditions: hexanes/EtOAc 1:19. Yield: 46.0 mg, 0.07 mmol, 49%, pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (d, J = 7.5 Hz, 3H), 0.93 (d, J = 5.9 Hz, 3H), 1.20−1.29 (m, 6H), 1.58−1.68 (m, 4H), 1.76−1.91 (m, 3H), 1.94−2.02 (m, 3H), 2.24−2.34 (m, 1H), 2.38−2.45 (m, 1H), 2.55−2.77 (m, 2H), 4.00−4.37 (m, 4H), 4.70−4.77 (m, 1H), 5.23 (s, 1H), 6.96 (d, J = 6.7 Hz, 1H), 7.19 (d, J = 8.3 Hz, 1H), 7.25 (s, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.81 (s, 1H), 8.41 (bs, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.1, 14.2, 20.1, 21.2, 24.6, 24.7, 25.9, 27.7, 29.7, 34.3, 36.1, 36.4, 37.4, 44.1, 46.2, 52.2, 60.4, 60.7, 72.3, 80.8, 89.0, 92.2, 103.4, 113.9, 122.5, 124.4, 126.3, 127.7, 130.1, 131.9, 132.5, 137.3, 150.3, 171.7 ppm. MS (ESI): m/z = 656 ([M + H]$^+$); HRMS (ESI): calcd for $[C_{34}H_{40}Cl_2N_3O_6]^+$ 656.2289, found: 656.2304. FT-IR (ATR): $\tilde{\nu}$ = 2925 (w), 2873 (w), 2333 (w), 2201 (w), 1969 (w), 1733 (s), 1613 (s), 1577 (m), 1545 (s), 1462 (s), 1401 (m), 1377 (m), 1311 (w), 1271 (w), 1243 (w), 1219 (w), 1169 (m), 1124 (m), 1092 (m), 1047 (s), 1011 (s), 940 (w), 874 (m), 821 (w), 756 (s), 689 (w), 669 (w), 617 (w), 528 (w), 479 (w), 442 (w), 421 (w) cm^{-1}. Anal. Calcd for $C_{34}H_{39}Cl_2N_3O_6 \cdot 2.5H_2O$: C, 58.20, H, 6.32, N, 5.99; found: C, 57.78, H, 5.87, N, 5.72.

4.1.6. General Procedure for Hybrids ***9*** *and* ***10***. A solution of artesunic acid (**16**) or artemisinin-derived acid **17** and HOBt in dry DMF (2.0 mL) was cooled to 0 °C. EDCI·HCl was added to the reaction mixture at 0 °C under N_2. After stirring the reaction mixture for 10 min, a solution of quinazoline amine **15** in dry DMF (5.0 mL) and DIPEA were added subsequently at 0 °C under N_2. The resulting reaction mixture was slowly warmed to rt and stirred overnight for 22 h or 3 days. After this time period, EtOAc (30 mL) and H_2O (30 mL) were added. The two phases were separated, and the water phase was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with H_2O (3 × 15 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a yellow oil. The crude product was purified by gradient column chromatography to obtain hybrids **9** and **10** as off-white solids.

4.1.7. Quinazoline−Artesunic Acid Hybrid ***9***. Artesunic acid (**16**) (94.5 mg, 0.25 mmol, 1.0 equiv) HOBt (33.2 mg, 0.25 mmol, 1.0 equiv), EDCI·HCl (47.2 mg, 0.25 mmol, 1.0 equiv), quinazoline amine **15** (75.0 mg, 0.25 mmol, 1.0 equiv), DIPEA (40.2 μL, 31.7 mg, 0.25 mmol, 1.0 equiv). Column conditions: CH_2Cl_2/MeOH 9.8:0.2 → CH_2Cl_2/MeOH 9.5:0.5. Yield: 112 mg, 0.17 mmol, 68%, off-white solid. R_f = 0.24 (CH_2Cl_2/MeOH 9.5:0.5, UV and phosphomolybdic acid). ^{1}H NMR (MeOD + drops of $CDCl_3$, 300 MHz): δ = 0.83 (d, 3H, J = 6.9 Hz), 0.93 (d, 3H, J = 6.0 Hz), 0.99−1.09 (m, 1H), 1.18−1.52 (m, 8H), 1.53−1.64 (m, 1H), 1.65−1.81 (m, 2H), 1.82−1.95 (m, 1H), 1.96−2.10 (m, 1H), 2.32 (td, 1H, J = 13.5 Hz, 2.4 Hz), 2.45−2.58 (m, 1H), 2.73−2.90 (m, 4H), 5.44 (s, 1H), 5.75 (d, 1H, J = 9.9 Hz), 7.42 (d, 1H, J = 8.7 Hz), 7.62−7.78 (m, 3H), 8.06 (d, 1H, J = 2.4 Hz), 8.54 (s, 1H), 8.61 (d, 1H, J = 1.8 Hz) ppm. ^{13}C NMR (MeOD + drops of $CDCl_3$, 75.5 MHz): δ = 11.2, 19.3, 21.3, 24.0, 24.7, 28.7, 30.4, 31.2, 33.4, 35.6, 36.7, 44.7, 60.0, 79.7, 91.1, 91.9, 104.1, 110.0, 115.1, 121.1, 123.3, 126.2, 127.4, 129.7, 131.7, 136.4, 138.1, 145.5, 152.6, 157.4, 170.8, 171.4 ppm. MS (ESI): m/z = 671 ([M + H]$^+$), 693 ([M + Na]$^+$); HRMS (ESI): calcd for $[C_{33}H_{36}Cl_2N_4O_7Na]^+$ 693.1853, found 693.1862. FT-IR (ATR): $\tilde{\nu}$ = 3337 (w), 2929 (w), 2873 (w), 2479 (w), 2196 (w), 2033 (w), 1745 (m), 1692 (m), 1567 (m), 1533 (s), 1517 (s), 1475 (s), 1450 (m), 1415 (s), 1377 (s), 1318 (m), 1284 (m), 1230 (w), 1153 (s), 1132 (s), 1096 (w), 1053 (m), 1007 (s), 926 (m), 900 (w), 875 (m), 844 (m), 800 (m), 680 (m), 636 (w), 574 (w), 532 (m), 502 (m), 434 (m) cm^{-1}. Anal. Calcd for $C_{33}H_{36}Cl_2N_4O_7$: C, 59.02; H, 5.40; N, 8.34; found: C, 58.48; H, 5.58; N, 8.10.

4.1.8. Quinazoline−Artemisinin Hybrid ***10***. Artemisinin-derived acid **17** (104 mg, 0.32 mmol, 1.2 equiv) HOBt (43.1 mg, 0.32 mmol, 1.2 equiv), EDCI·HCl (61.2 mg, 0.32 mmol, 1.2 equiv), quinazoline amine **15** (81.0 mg, 0.27 mmol, 1.0 equiv), DIPEA (90.0 μL, 68.4 mg, 0.53 mmol, 2.0 equiv). Column conditions: CH_2Cl_2/MeOH 9.9:0.1 → CH_2Cl_2/MeOH 9.7:0.3. Yield: 104 mg, 0.17 mmol, 64%, off-white solid. R_f = 0.40 (CH_2Cl_2/MeOH 9.5:0.5, UV and phosphomolybdic acid). ^{1}H NMR (MeOD + drops of $CDCl_3$, 300 MHz): δ = 0.91−0.98 (m, 7H), 1.20−1.49 (m, 8H), 1.62−1.76 (m, 2H), 1.77−1.88 (m, 1H), 1.89−2.08 (m, 2H), 2.29 (td, 1H, J = 14.0 Hz, 3.0 Hz), 2.50 (dd, 1H, J = 15.2 Hz, 2.1 Hz), 2.70−2.84 (m, 2H), 4.82−4.92 (m, 1H), 5.53 (s, 1H), 7.39 (d, 1H, J = 8.7 Hz), 7.48−7.53 (m, 2H), 7.62 (d, 1H, J = 9.0 Hz), 7.69 (dd, 1H, J = 8.9 Hz, 2.7 Hz), 8.09 (d, 1H, J = 2.4 Hz), 8.50 (s, 1H), 8.61 (d, 1H, J = 2.1 Hz) ppm. ^{13}C NMR (MeOD + drops of $CDCl_3$, 75.5 MHz): δ = 13.1, 20.4, 25.2, 25.3, 26.1, 30.7, 34.9, 36.9, 38.0, 38.6, 44.5, 52.7, 72.4, 81.6, 90.3, 104.1, 111.1, 116.1, 122.0, 124.2, 127.1, 127.5, 128.4, 130.7, 132.7, 137.0, 139.2, 146.6, 153.8, 158.3, 171.8 ppm. MS (ESI): m/z = 635 ([M + Na]$^+$); HRMS (ESI): calcd for $[C_{31}H_{34}Cl_2N_4O_5Na]^+$ 635.1799, found 635.1800. FT-IR (ATR): $\tilde{\nu}$ = 3321 (w), 2928 (w), 2874 (w), 2175 (w), 2004 (w), 1673 (m), 1597 (m), 1572 (m), 1527 (s), 1474 (s), 1416 (s), 1380 (s), 1318 (m), 1287 (m), 1264 (m), 1224 (m), 1192 (m), 1126 (m), 1091 (m), 1041 (m), 1010 (m), 938 (m), 918 (m), 873 (m), 838 (m), 681 (m), 645 (w), 614 (w), 575 (w), 531 (m), 503 (m), 438 (m) cm^{-1}. Anal. Calcd for $C_{33}H_{36}Cl_2N_4O_7$: C, 60.69; H, 5.59; N, 9.13; found: C, 60.19; H, 5.55; N, 9.03.

4.2. Cytotoxicity Studies against *P. falciparum* 3D7 Strains. *4.2.1. P. falciparum culture. P. falciparum* 3D7

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parasites were cultured in type A-positive human erythrocytes at a hematocrit of 5% in RPMI 1640 supplemented with 25 mM HEPES, 0.1 mM hypoxanthine, 50 μg/mL gentamycin, and 0.5% albumax I. Cultures were incubated at 37 °C under controlled atmospheric conditions of 5% O_2, 3% CO_2, and 92% N_2 at 95% relative humidity.

4.2.2. In Vitro Antimalarial Activity Assay. Cultures used in cell proliferation assays were synchronized by sorbitol treatment.[62] Concentrations to inhibit parasite growth by 50% (EC_{50}) were determined using the SYBR Green I malaria drug sensitivity assay.[63] Aliquots of 100 μL of a cell suspension containing ring stages at a parasitemia of 0.2% and a hematocrit of 2% were added to the wells of 96-well microtiter plates. Plates were incubated for 72 h in the presence of different drug concentrations. Subsequently, cells of each well were lysed with 100 μL of lysis buffer (40 mM Tris, pH 7.5, 10 mM ethylenediaminetetraacetate, 0.02% saponin, 0.08% Triton X-100) containing 8.3 μM SYBR Green. Plates were incubated for 1 h in the dark at rt under constant mixing before fluorescence (excitation wavelength 485 nm; emission wavelength >520 nm) was determined using a microtiter plate fluorescence reader (Victor X4; Perkin Elmer). Drugs were serially diluted to 1:3 with the initial drug concentrations being 243 nM for chloroquine, artesunic acid (**16**), and its derivates and 81 nM for DHA and its derivates. Each drug concentration was examined in triplicate and repeated at least three times. Uninfected erythrocytes (hematocrit 2%) and infected erythrocytes without the drug served as controls and were investigated in parallel. Percent growth was calculated as described by Beez.[64] Data were analyzed using the SigmaPlot (version 12.0; Hill function, three parameters) and Sigma Stat programs.

4.3. HCMV GFP-Based Replication Assay. The HCMV GFP-based replication assay was carried out over a duration of 7 days (multiround HCMV infection) with primary HFFs infected with a GFP-expressing recombinant HCMV (AD169-GFP), as described before.[50,52,54,57] 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 were performed by the use of Excel (means and standard deviations).

4.4. Cytotoxicity Studies against Leukemia Cells. *4.4.1. Cell Culture.* Human leukemic CCRF-CEM and the P-glycoprotein expressing CEM/ADR5000 cells were obtained from the University of Jena (Department for Pediatrics, University of Jena, Germany) and were cultivated in the RPMI 1640 medium supplemented with 10% (v/v) inactivated fetal calf serum and 1% penicillin/streptomycin at 37 °C with 5% CO_2 in a humidified atmosphere (95% relative humidity). CEM/ADR5000 cells were treated with 5000 ng/mL doxorubicin once per week to keep them resistant.[65] The multidrug resistance profile of CEM/ADR5000 has been reported.[66,67] Cells were passaged twice a week and used for experiments in the logarithmic phase.

4.4.2. Cell Viability Assay. CCRF-CEM or CEM/ADR5000 cells were seeded at an appropriate density (10 000 cells/well) in a 96-well plate with a total volume of 200 μL. Compounds were added in varying concentrations [0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 100 μM]. Each concentration was tested six times within each experiment, and each experiment was repeated three times. In addition, CEM/ADR5000 cells were tested with doxorubicin alone and in combination with the three derivatives (10 μM) and verapamil [0.1, 0.3, 1, 3, 10, and 100 μM]. After 72 h at 37 °C and 5% CO_2, 20 μL of resazurin 0.01% w/v in ddH_2O was added to each well, and there was further incubation of the plates for 4 h. The plates were measured using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. The test compound concentrations required to inhibit 50% of cell proliferation were represented by EC_{50} values, calculated from the dose–response curves.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00310.

Experimental conditions and procedures for quinazoline precursors **11**–**15** and artemisinin derivatives **17**–**19**; spectral data of quinazoline precursors **11**–**15** and artemisinin derivatives **17**–**19**; recorded spectra of target compounds **6**–**10**, quinazoline precursors **11**–**15**, and artemisinin derivatives **17**–**19** (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCE, 1,2-dichloroethane; DCU, *N*,*N*′-dicyclohexylurea; DHA, dihydroartemisinin; DIEA, *N*,*N*-diisopropylethylamine; DMAP, 4-(dimethylamino)-pyridine; equiv, equivalent; EtOAc, ethyl acetate; GFP, green fluorescent protein; HCMV, human cytomegalovirus; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HFF, human foreskin fibroblasts; HOBt, 1-hydroxybenzotriazole; TBAI, tetrabutylammonium iodide

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