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Synthesis and Evaluation of Artesunate-Indoloquinoline Hybrids as Antimalarial Drug Candidates

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Abstract:

The hybrids of artesunate-indolo[2,3-*b*]quinoline, -indolo[3,2-*c*]quinoline, and -indolo[3,2-*b*]quinoline were synthesized and screened for their antiplasmodial activity against two different malaria strains (CQS and CQR) and their cytotoxic activities against normal cells were evaluated. All the synthesized hybrids showed a decreased cytotoxicity and increased antimalarial activity relative to the individual, non-hybridized compounds. Furthermore, these hybrids were stronger *β*-haematin inhibitors than the corresponding molecules from which they were derived. The most effective antimalarial hybrid showed

the IC_{50} value of 0.45 nM against the CQS strain. At the same time this hybrid also showed effective activity against the CQR strain with the IC_{50} value of 0.42 nM and the RI value of 0.93. Furtheremore, this hybrid showed an in vivo toxicity for a single intraperitoneal dose of 10 mg/kg once a day for four consecutive days with a significant reduction in parasitemia on day 4, with an activity of 89.6%, and a mean survival time of 7.7 days.

Introduction

Malaria, a deadly infectious parasitic disease, is a major issues of public health in the world today[1] and already produce serious economic constraints in the endemic countries.[2] Unfortunately, because of the complex, multi-stage life cycles of the *Plasmodium* parasites, a fully effective vaccine has yet to be developed.[3] Therefore, drug therapy has been the main tool used to control the disease since the first use of quinine in the 1630s.

Because of the spread of chloroquine resistance against and first signs of artemisinin resistance in southeast Asia against the *Plasmodium falciparum* strains,[4,5] the most dangerous protozoal parasite,[6] the World Health Organization (WHO) recommended the use of the artemisinin-based combination therapy (ACT),[7] that incorporates the fast acting antimalarial of the arteminisin family with other longer half-life antimalarials.[8]

Artemisinin and its derivatives, which are based on a plant-origin sesquiterpene containing a biologically important 1,2,4-trioxiane structure, are now the first-line treatment for multidrug-resistant malaria.[9] The artemisinin peroxide in the presence of the flat, achiral iron(II)-heme forms covalent heme drug adducts, and accumulation of non-polymerizable redox-active heme derivatives is considered to be toxic for the parasite. An alternative controversial suggestion is that artemisinin exerts its activity by inhibiting a calcium ATPase (PfATP6).[10] The artemisinins will rapidly kill the parasites but are also rapidly excreted.[11]

On the other hand, chloroquines are considered to act against the malaria parasites by blocking haemozoin formation through π-π stacking of the 4-aminoquinoline core to the heme ring system or by docking into grooves on the haemozoin crystal and preventing

further crystal growth. The toxic haematin is then left to cross from the digestive vacuole into the parasite cytosol where it induces oxidative membrane damage.[12]

Hence, various artemisinin-derivatives have been combined with quinine, 4-aminoquinolines, and mefloquine by C-O, C-C, and C-N covalent bonds for improvement of the antimalarial activities.[10,13,14]

Medicinal plants have long been used for the treatment of parasitic diseases, including malaria, and constitute an important source of new molecules for optimization programs.[15] The indoloquinolines isolated from the roots of the climbing shrub *Cryptolepis sanguinolenta*, i.e., neocryptolepine, isocryptolepine, and cryptolepine are promising as leads for novel antimalarials, since an aqueous macerate or decoction of this root is used for the treatment of endemic diseases such as malaria fever.[16–19]

In our search for new plant-based lead compounds for antimalarial agents, we investigated whether the indoloquinolines substituted with aminoalkylamino groups at the appropriate positions would further increase the activities or be comparable to the standard drugs, chloroquine and artemisinin, against the CQR (K1) and CQS (NF54) strains. Since these indoloquinolines showed a highly effective β-haematin inhibition activity,[20–23] the mechanism of action of these compounds are considered to be similar to that of chloroquine.

Incidentally, the bleomycin was demonstrated as an excellent example of hybrid molecule-based anticancer drug, which was a very popular research topic until 2007. The molecule of bleomycin includes three distinct structural domains with different biological roles. Based on this point, new design and strategy of antimalarial drugs was proposed by Meunier to prepare the hybrid molecules trioxaquines bearing a dual mode of action to increase activity, and antimalarial mechanism of hybrid molecules trioxaquines was proposed.[24,13]

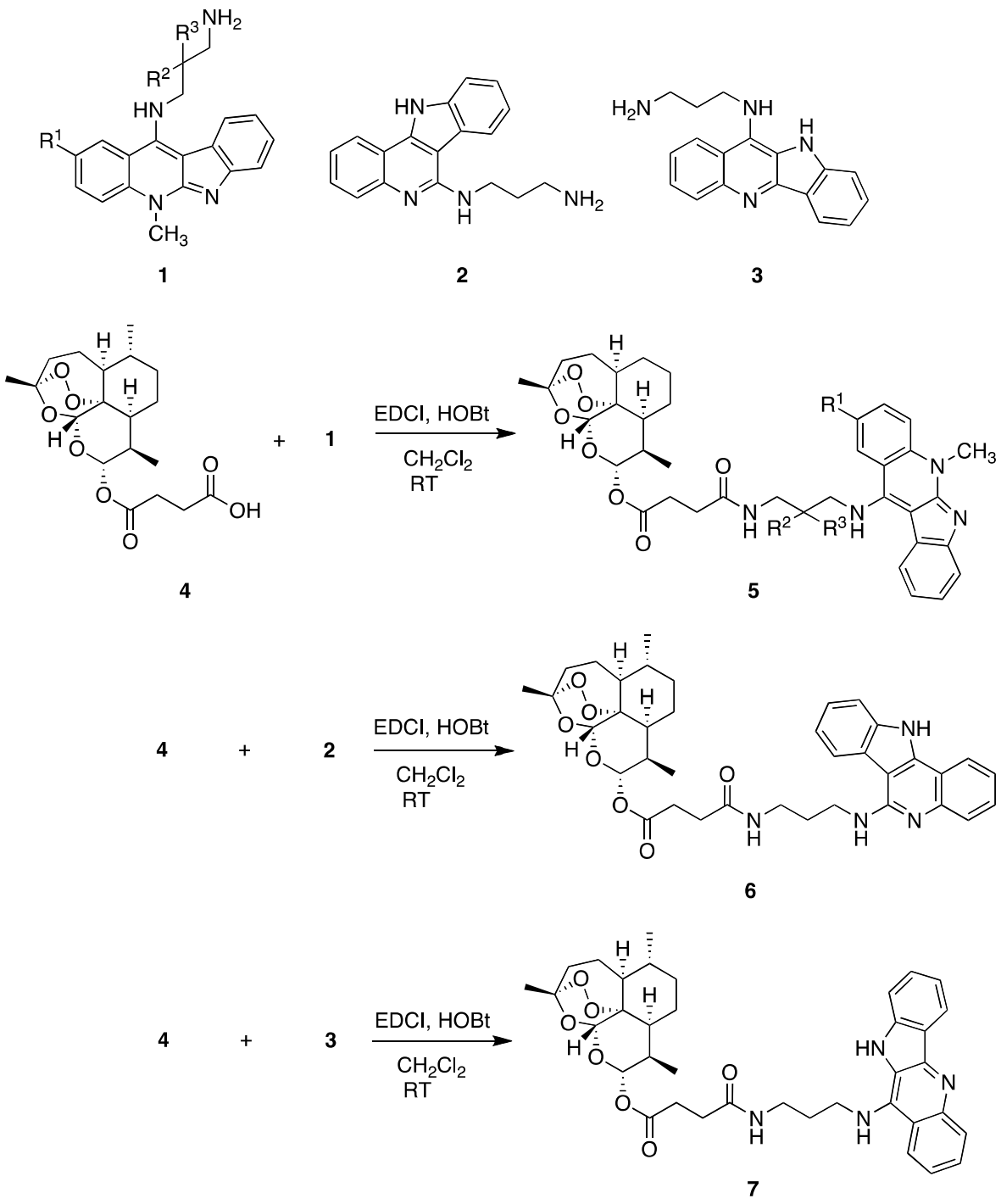
In our attempt to find antimalarial synergism by the linkage of artemisinin derivatives with quinine or its synthetic analogues, we examined the synthesis of covalently linked artesunate-indoloquinoline hybrids **5**–**7** in which the 3-aminopropylamino substituents of indoloquinolines were combined with the water soluble artesunate (**4**) through an amide

bond. In this study, we applied the derivatives of neocryptolepine, isocryptolepine, and cryptolepine as the counterpart and the activity was compared to that of artemisinin alone or indoloquinolines alone.

Results and discussion

Chemistry

The preparation of the 11-aminoalkylamino-5-methyl-5*H*-indolo[2,3-*b*]quinolines **1**, and 6-aminoalkylamino-11*H*-indolo[3,2-*c*]quinoline **2**, were carried out according to the method we previously described.[21–23] The 11-aminoalkylamino-10*H*-indolo[3,2-*b*]quinoline **3** was available according to the method reported by Lavrado *et al*.[25] The artesunate-indoloquinoline hybrids **5**–**7** were obtained using the synthetic process shown in Scheme 1. We utilized the acid function of artesunate (**4**) to link with the amino group of the compounds **1**–**3** in the presence of EDCI and HOBt at room temperature.



Scheme 1 Synthesis of the artesunate-indoloquinoline hybrids **5**, **6**, and **7**

Antiplasmodial activity and cytotoxicity

The results of the antiplasmodial activity of the hybrid compounds **5**–**7** against the NF54 (CQS) and K1 (CQR) strains of *P. falciparum*, cytotoxicity toward L6 cells, the SI that is used as the parameter of clinical significance of the test sample by comparing general toxins and the selective inhibitory effect on *P. falciparum*, as well as the RI which provides a quantitative measurement of the antiplasmodial activity against the CQR strains relative to that against CQS strains and reveals promising drug discovery leads,[26] are shown in Table 1. All the hybrids have a significant antiplasmodial activity compared to the monomer artemisinin and the (3-aminopropylamino)-substituted indoloquinolines **1**–**3**. All the hybrids' RI data were below 0.5 (artemisinin RI 0.7). Hybridization of the two moieties with potential antiplasmodial activities mutually improved the antiplasmodial activity. The hybrid compounds showed no significant difference in antiplasmodial activity regardless of the identity of the indoloquinoline moiety. The artesunate-indolo[2,3-*b*]quinoline hybrids **5** showed a significantly improved antimalarial activity against both the NF54 and K1 strains, and lower cytotoxicity (SI between 220 to 3720) compared to **2** (SI below 41). In particular, compound **5a** showed the highest antimalarial activity with an IC_{50} value of 0.45 against the CQS strain (NF54), and IC_{50} value of 0.42 against the CQR strain (K1). Furthermore, the hybrid **5a** showed 3 times higher activity than the mixture of monomer artesunate (**4**) and **1a**. Compound **5g** bearing a hydroxy group pendant on the ω-aminoalkylamine spacer at N6 showed a higher antimalarial activity than that of both **5e** without a pendant and **5f** with the geminal dimethyl group pendant. The chlorine atom at C2 of the indolo[2,3-*b*]quinoline moiety with the geminal dimethyl-substituted pendant (**5c**) was more effective than its analogues with a H, Br or COOMe group at C2 (**5b**, **5d** and **5f**). In comparison to the monomer indolo[3,2-*c*]quinoline **3**, the hybrid **6** showed an increased antimalarial activity, i.e. about 8 times higher against the NF54 strain and about 120 times higher against the K1 strain, and at the same time, the cytotoxicity decreased from 626 nM to 2201 nM.

Table 1 Antiplasmodial activity against *P. falciparum* (CQS, NF54; CQR, K1), cytotoxicity toward L6 cells and *β*-haematin inhibition

Compounds	R^1	R^2	R^3	L6 cells IC_{50} nM[c]	NF54 IC_{50} nM[c]	SI[a] L6/NF54	K1 IC_{50} nM[c]	SI[a] L6/K1	RI[b] K1/NF54	β-haematin inhibition IC_{50} μM
5a	H	H	H	1529.5	0.45	3398.9	0.42	3641.7	0.93	222.50
5b	H	CH_3	CH_3	2962.0	1.14	2598.2	0.93	3184.9	0.82	70.16
5c	Cl	CH_3	CH_3	1284.6	1.50	856.4	0.41	3133.2	0.27	37.78
5d	Br	CH_3	CH_3	991.3	3.21	308.8	0.95	1043.5	0.30	28.88
5e	COOMe	H	H	3718.3	2.88	1291.1	1.00	3718.3	0.35	29.24
5f	COOMe	CH_3	CH_3	695.0	3.17	219.2	1.15	604.3	0.36	25.79
5g	COOMe	H	OH	1223.1	1.48	826.4	0.40	3057.8	0.27	41.66
6				2201.0	1.67	1318.0	0.67	3285.1	0.40	15.69
7				2618.8	12.2	214.7	NT[d]			17.12
Artesunate (**4**) + **1a** (monomer mixture)				6155.5	1.16	5306.5	NT[d]			43.76
1a[22]	H	H	H	279.2	78.8	3.5	NT[d]			NT[d]
1e[21]	COOMe	H	H	337	8.28	40.7	22.1	15.2	2.67	104.4
2[23]				626.8	13.7	45.8	82.7	7.6	6.0	626.8
3				275.5	17.2	16.0	NT[d]			16.82
Artemisinin					4.3		2.8		0.7	
Chloroquine					9.4		209.5		22.3	30-33

[a]Selectivity Index is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/P.f.).

[b]Resistance index is the ratio of IC_{50} for the resistant versus the sensitive strain (K1/NF54).

[c]The IC_{50} values are the means of two independent assays; the individual values vary by less than a factor of 2.

[d]Not tested.

β-Haematin inhibition

Francoise *et al.* described that the artemisinin derivatives featuring an endoperoxide are active on the young erythrocytic stages of *P. falciparum*, and the chloroquine derivatives may only exert action on the late trophozoite stage.[24a] Although controversial, Meunier reported that haemozoin formation is the target for the artemisinin derivatives, which interacts with the haem to form covalent heme-artemisinin adducts, which can inhibit the formation of haemozoin.[24b] Furthermore, Egan suggested that haemozoin is also the target for the chloroquine derivatives, which can interact with the fastest-growing

face of the Fe(III)-ferriprotoporphyrin IX [Fe(III)PPIX].crystal to prevent further growth of heamozoin.[27] Based on these hypotheses, we examined he *β*-haematin (*β*H, synthetic haemozoin) inhibition of the synthesized hybrids.

The measured *β*H inhibition IC_{50} values, determined using the NP-40 detergent based assay method described by Cater *et al.*,[28–29] are shown in Table 1. Non-crystallized haem was detected using the pyridine-ferrochrome method developed by Egan and Nkokazi.[30] All of the hybrids showed *β*-haematin inhibition activity to some extent, in spite of compound **5a** showing the lowest *β*H inhibition activity, with IC_{50} value 7 times higher than the chloroquine control. In comparison to the monomer **1e** (IC_{50} value of 104.4), the artesunate-indolo[2,3-*b*]quinoline hybrid **5e** showed about a 4 times higher *β*H inhibition activity with the IC_{50} value of 29.2. The artesunate-indolo[3,2-*c*]quinoline hybrid **6** showed about a 40 times higher *β*H inhibition activity than that of the non-hybrid compounds **2**. Inspection of the *β*H activity data revealed that the two moieties of the hybrid molecule act on the individual biologic targets as distinct pharmacophores. Interestingly, hybrid **5a** with the lowest *β*H activity exhibited the highest antimalarial activity. This result further proves the disconnection between the IC_{50} values from the extracellular *β*H assay and intracellular parasite growth assay, as reported previously.[21] Reasons for this direct lack of correlation are factors such as solubility, lipophilicity and polarity as well as vacuolar accumulation which determine how efficiently the molecule crosses cellular membranes. Since these factors are not taken into account in the *β*H inhibition assay, the results serve to show a likely mechanism of antimalarial action rather than an absolute indication of antimalarial potency.

In vivo antimalarial activity

In vivo antimalarial activity was assessed basically as previously described by Peters.[31] In vivo efficacy studies in mice were conducted according to the rules and regulations for the protection of animal rights (“Tierschutzverordnung”) of the Swiss “Bundesamt für Veterinärwesen”. They were approved by the veterinary office of Canton Basel-Stadt, Switzerland.

The indol[2,3-*b*]quinoline derivative **5a** with strong antiplasmodial activity against NF54 and K1 strains *in vitro* was selected for an in vivo drug testing model against *Plasmodium berghei* in mice. Groups of three female NMRI mice (20–22 g) intravenously infected with 2 x 107 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain ANKA.[32] Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water and administered intraperitoneally or orally in a volume of 10 ml kg^{-1} on four consecutive days (4, 24, 48 and 72 h post infection). Parasitemia was determined on day 4 post infection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitemia for the control (n = 5 mice) and treated groups, expressed as a percentage relative to the control group. Compound **5a** showed in vivo toxicity at a single intraperitoneal dose of 50 mg/kg. After dosing at 10 mg/kg once a day for four consecutive days, **5a** showed a significant reduction in parasitemia on day 4, with an activity of 89.6%, and a mean survival time of 7.7 days.[33]

Compounds	Dose mg/kg	route	formulation	% of activity	MSD*
5a	4 x 10	i.p.	10% DMSO	89.6	7.7

*MSD = mean survival time (in days), untreated control mice showed a mean survival time of 6 days.

Conclusions

Syntheses of artesunate-indolo[2,3-*b*]quinoline hybrids **5**, -indolo[3,2-*c*]quinoline hybrid **6**, and -indolo[3,2-*b*]quinolin hybrid **7** were carried out successfully. The hybrids prepared showed increased antimalarial activity and *β*-haematin inhibition, as well as low cytotoxicity. The highest antimalarial activity among all synthesized hybrids was **5a** with IC_{50} value of 0.45 nM against CQS (NF 54) strains, IC_{50} value of 0.42 nM against CQR (K1) strains and RI value of 0.93. The hybrid compound **5a** showed a significant reduction in parasitemia on day 4, with an activity of 89.6%, and a mean survival time of 7.7 days.

Abbreviations

SAR Structure–activity relationship

CQS chloroquine-sensitive

CQR chloroquine-resistant

ACT artemisinin-based combination therapies

SI selectivity index

RI resistance index

L6 toxicity of the tested compounds assessed against a mammalian primary cell line derived from rat skeletal myoblasts

P. falciparum *Plasmodium falciparum*

EDCI 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

HOBt 1-hydroxybenzotriazole

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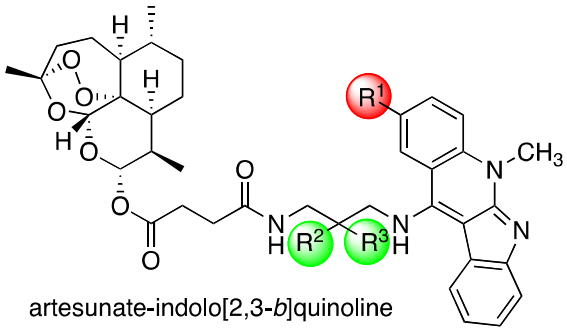
Graphical abstract



high antimalarial activity
$R^1 = R^2 = R^3 = H$
IC_{50} = 0.45 nM (NF54 strain in vitro)
IC_{50} = 0.42 nM (K1 strain in vitro)
in vivo activity of 89.6% on day 4;
more active than artesunate-indolo[3,2-*c*]quinoline, and -indolo[3,2-*b*]quinoline hybrids