Enhanced Antimalarial Activity of Novel Synthetic Aculeatin Derivatives

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Abstract: We report the design, synthesis, and in vitro evaluation of novel polyspirocyclic structures, inspired by the antimalarial natural products, the aculeatins. A divergent synthetic strategy was conceived for the practical supply and has allowed the discovery of two novel and more potent analogues active on the *Plasmodium falciparum* 3D7 strain. Moreover, these compounds proved to be potent against *Toxoplasma gondii*. A number of features that govern these inhibitions were identified.

Malaria is the most widespread parasitic disease in tropical and subtropical regions, and its burden steadily increases, infecting 300−500 million people and killing 1−3 million people a year.[1,2] This major disease is caused by blood protozoan parasites of *Plasmodium* genus classified in the Apicomplexa phylum, of which the most deadly species is *Plasmodium falciparum*. The rise of malaria death toll is notably due to the spread of resistance to all currently used antimalarial drugs, artemisinin derivatives still remaining an exception, although forerunners of resistant forms have recently given cause for concern.[3] There is thus a constant need for the search for novel antimalarial molecules. Priority should be given to approaches with novel mechanisms of action and whose compounds are structurally unrelated to existing antimalarial agents. These new compounds would keep a therapeutic rampart in controlling malaria.[4]

Natural products extracted from traditional medicines (e.g., quinine and more recently artemisinin) have provided the starting point for the discovery of the most efficient antimalarial drugs.[4−8] In 2000, a novel type of polyspirocyclic natural products, the aculeatins (Figure 1), was isolated from the rhizome of *Amomun aculeatum* by Heilmann and co-workers.[9] This plant has been used in the folk medicine of Papua New Guinea to treat fever and malaria.[10] Very recently, Kinghorn and co-workers have isolated from the leaves and rachis of *Amomun aculeatum* new members of this family, the aculeatols, the amomols, and novel analogues of aculeatin having a shorter lipophilic side chain.[11,12] Some of these compounds showed interesting antimalarial activities with inhibitory effects in the submicromolar range, and especially against *P. falciparum* strain K1 (Figure 1), with a low cytotoxicity against human cell lines in vitro.[9,12] A selectivity for MCF-7 cells in an in vivo hollow fiber assays has been reported for aculeatin A.[12]

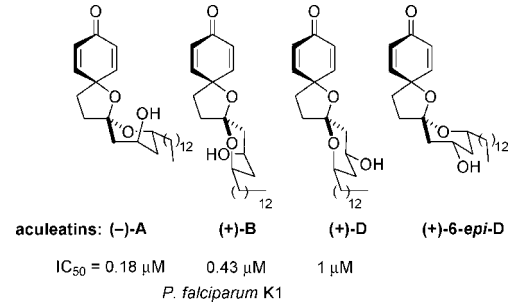
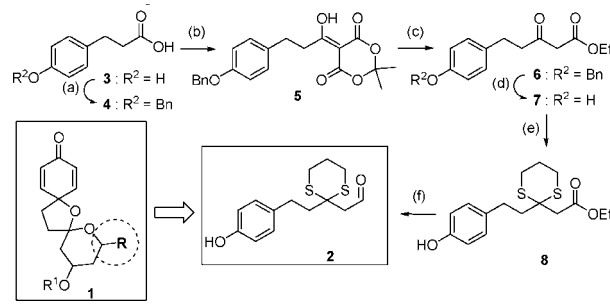


Figure 1. Antimalarial activity of aculeatins.

Scheme 1. Synthesis of **2** as Key Precursor for Accessing to New Analogues **1**[a]



[a] Reagents and conditions: (a) BnBr, NaOH, Bu_4NHSO_4 cat., THF, 99%; (b) $(COCl)_2$, CH_2Cl_2, 4 h, then Meldrum's acid, pyridine, CH_2Cl_2, 0°C; (c) EtOH, reflux (72% from **4**); (d) H_2, Pd/C, AcOEt, 99%; (e) 1,3-propanedithiol, $BF_3 \cdot Et_2O$, CH_2Cl_2, 79%; (f) DIBAL-H, toluene, −78°C, 73%.

A few years ago, we selected the aculeatins to start a program aiming to develop new antiprotozoal agents inspired by natural products. Interestingly, aculeatin A was described to be 5-fold more efficient than aculeatin D (Figure 1) based on in vitro proliferation assays of *P. falciparum* strain K1, suggesting that a biological selectivity could arise from a proper spatial arrangement of the polyspiro structure and their appendage orientations. Moreover, this unexplored biologically active scaffold represents a family of new probes, with the prospect that new antimalarial biological targets, directly or indirectly interacting with this scaffold, could be identified in the future.[13] We report here the evaluation of new aculeatin analogues and the discovery of improved antimalarial compounds, with the identification of features contributing to their biological activity. Recently, we were keen to develop novel diastereodivergent and tandem one-pot synthetic approaches giving rise to desired molecules within few steps.[14,15] Although quite complex polyspirocylic structures are targeted, the synthesis of new aculeatin analogues **1** (Scheme 1) is designed in a practical manner that allows a specific structural variability, mainly located at the R tail position. All these new derivatives are conveniently derived from a common precursor **2**.

An approach allowing large amounts of products to be handled was desired to synthesize the key precursor **2**. Starting from the acid **3** protected as a benzylated derivative **4**, the corresponding acyl chloride was condensed with Meldrum's acid to afford **5**, which was then refluxed in ethanol to give the

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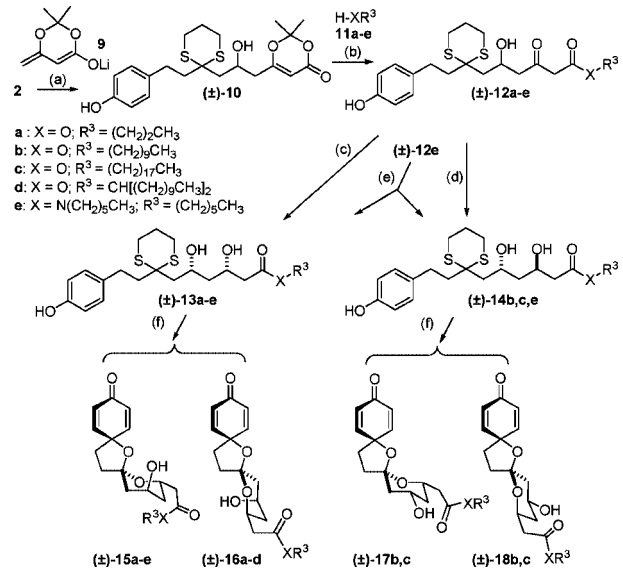
Scheme 2. Synthesis of the Precursor (±)-**10** from **2** and the Successive Conversions To Give the Range of New Aculeatin Analogues[a]



[a] Reagents and conditions: (a) THF, −78°C, 80%; (b) toluene, 110°C; (c) Et_3B, $NaBH_4$, THF/MeOH (4:1), −78°C; (d) $(CH_3)_4NBH(OAc)_3$, CH_3CN/AcOH (4:1), 0°C; (e) $NaBH_4$, MeOH/CH_2Cl_2, 0°C; (f) PIFA, acetone/H_2O, 15 min.

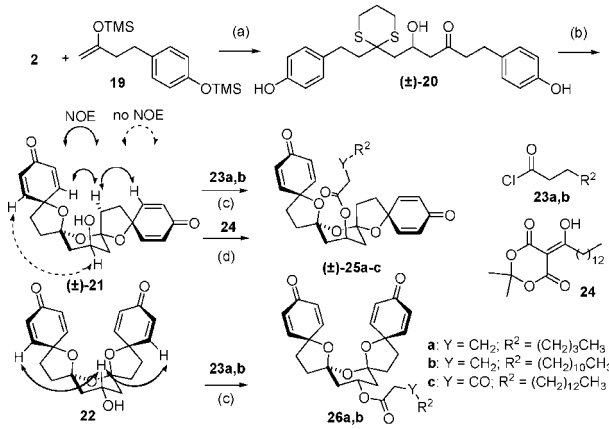
β-ketoester **6** (71% overall yield from **3**). Hydrogenation of **6** gave the phenol β-ketoester **7**, which was next transformed into the dithiane ester **8**. Finally, reduction with DIBAL-H gave the corresponding aldehyde **2** in good yield.

To introduce different lipophilic side chains, the dioxinone (±)-**10** was prepared by condensing the aldehyde **2** with the lithiated dioxanone dienol ether **9** (Scheme 2). The dioxinone functional group of (±)-**10** allows the facile addition of various nucleophiles **11a**−**e** by simply heating both reactants in toluene, giving rise to a series of β-ketoesters (±)-**12a**−**e**.[16] Although the yields range from 35% to 64%, it is noteworthy that neither the secondary alcohol nor the phenol group of (±)-**10** needs to be protected.[17]

Hydroxyl-directed selective reductions of (±)-**12a**−**d** were successfully applied to obtain syn diols (±)-**13a**−**d**[18] or anti diols (±)-**14b**,**c**.[19] Same conditions were applied to the β-ketoamide (±)-**12e** but failed to run the reactions until completion resulting in poor yields. The β-ketoamide (±)-**12e** was finally reduced by $NaBH_4$, yielding an inseparable mixture of diastereomers (±)-**13e**/**14e** (76%).

According to our previous work,[14] the diols (±)-**13a**−**d** and (±)-**14b**,**c** were treated by phenyliodine(III) bis(trifluoroacetate) reagent (PIFA) in acetone/H_2O to promote a tandem phenolic oxidation/dithiane deprotection sequence leading to the formation of aculeatins polyspirocyclic structures. Hence, the transformation of syn diols (±)-**13a**−**d** gave two separable aculeatin derivatives (±)-**15a**−**d** (12−19%) and (±)-**16a**−**d** (11−21%), as analogue of aculeatins A and B, respectively, whereas the anti diols (±)-**14b**,**c** led to two separable products (±)-**17b**,**c** (16%) and (±)-**18b**,**c** (16−17%), as analogues of aculeatins D and 6-*epi*-D, respectively. The mixture of syn/anti diols (±)-**13e**/**14e** gave with PIFA a mixture of stereoisomers of which only (±)-**15e** (9%) could be isolated from the other products by flash chromatography. Comparisons of the NMR data of (±)-**15a**−**e**, (±)-**16a**−**d**, (±)-**17b**,**c**, and (±)-**18b**,**c** with those arising from previous configurational NMR studies of natural products[12,14c,15b,c] allow the assignment of their structures.

Scheme 3. Synthesis of (±)-**20**, (±)-**21**, and **22** (with Representative NOE Signals) and Further Derivations To form (±)-**25a**−**c** and **26a**,**b**[a]



[a] Reagents and conditions: (a) (i) $BF_3.OEt_2$, −78°C, CH_2Cl_2; (ii) TBAF, THF; (b) PIFA, acetone/H_2O, 18 h; (c) DMAP, Et_3N, CH_2Cl_2; (d) THF, reflux, 6 h.

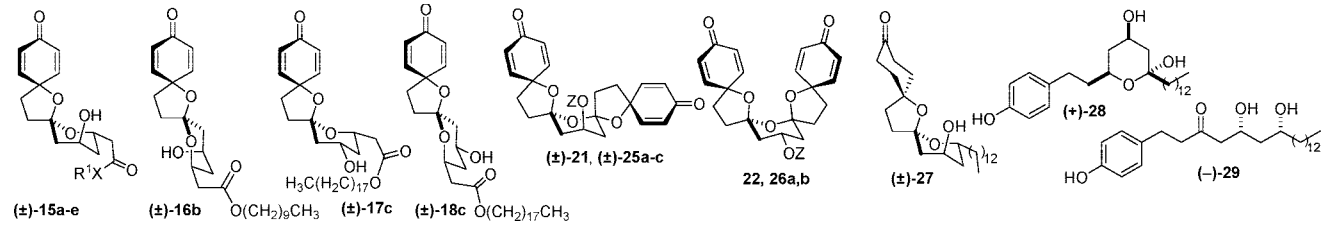
To explore the biological effect of cyclohexadienone ring, we suspected that introducing twice this moiety on the same molecule, while keeping a polyspirocyclic structure, would amplify the putative pharmacophoric feature. Within the context of our oxidative spiroannulation strategy, we envisioned that the diphenolic product **20** should be the precursor of choice (Scheme 3). Compound **20** in racemic form was easily obtained in good yield (84%) by the reaction of the aldehyde **2** with the enolsilane **19**[14b] according to the Mukaiyama coupling reaction, followed by a treatment with TBAF to remove all silyl groups. When treating this linear precursor (±)-**20** with PIFA in acetone/H_2O, we were pleased to mainly obtain in one step two novel and complex structures (±)-**21** (19%) and **22** (11%) having five successive spirocyclic rings in junction. As evidenced by the NMR spectroscopy, product **22** has a symmetric structure and NOE measurements assigned the configuration, notably with the NOE signals observed between the axial proton at geminal position of the hydroxyl group and the protons of both cyclohexadienone rings. For product (±)-**21**, representative NOE signals also confirm the configuration. No NOE signal between the proton at geminal position of the hydroxyl and the proton of cyclohexadienone ring has been observed, suggesting that the hydroxyl group is at axial position.

To restore the hydrophobic part of aculeatins, different lipophilic chains were grafted on the remaining hydroxyl group, either with the acyl chlorides **23a**,**b** or by heating with **24** to give either the polyspirocyclic esters (±)-**25a**,**b** and **26a**,**b** or the polyspirocyclic β-ketoester (±)-**25c**, respectively.

Finally, to verify the plausible role of the cyclohexadienone ring on the biological activity, (±)-aculeatin A[14a] was converted into the cyclohexanone derivative (±)-**27** by catalytic hydrogenation with palladium (Scheme 4). A dramatic decrease in activity should confirm that the cyclohexadienone ring plays a crucial role.

Considering that another pathogenic member of the Apicomplexa phylum includes *Toxoplasma* species, the series of natural and non-natural aculeatins A, B, D and 6-*epi* D[14b] and the new synthesized analogues were evaluated for their potency against both *P. falciparum* 3D7 and *Toxoplasma gondii* RH-β1 (Table 1). We also tested our compounds for their toxicity against mammalian cells using the human erythroblasts K562 to

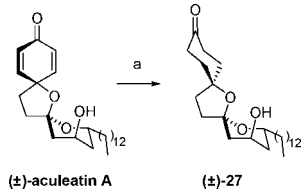
Table 1. In Vitro Activity of the Compounds against the Parasites *Plasmodium falciparum* (*P.f.*) and *Toxoplasma gondii* (*T.g.*) and against the Human Erythroblasts (h.e.) K-562[a]



compd	substituent	CC_{50} (μM), h.e. K-562	IC_{50} (μM) *P.f.* 3D7	IC_{50} (μM) *T.g.* RH-β1
chloroquine		9.7	0.008	
(−)-aculeatin A[b]		25.5	0.288	0.309
(+)-aculeatin B[b]		27.5	0.454	0.365
(+)-aculeatin D[b]		28.0	0.478	0.640
(+)-aculeatin 6-*epi*-D[c]		30.2	0.451	0.377
(+)-aculeatin A[c]		25.8	0.335	0.436
(−)-aculeatin B[c]		22.1	0.329	0.627
(−)-aculeatin D[c]		26.7	0.220	0.436
(−)-aculeatin 6-*epi*-D[c]		30.8	0.366	0.400
(±)-**15a**	X = O; $R^3 = (CH_2)_2CH_3$	109.3	1.840	0.500
(±)-**15b**	X = O; $R^3 = (CH_2)_9CH_3$	28.2	0.654	0.341
(±)-**15c**	X = O; $R^3 = (CH_2)_{17}CH_3$	13.9	0.770	2.40
(±)-**15d**	X = O; $R^3 = CH[(CH_2)_9]CH_3]_2$	22.1	1.151	4.45
(±)-**15e**	$X = N(CH_2)_5CH_3$; $R^3 = (CH_2)_5CH_3$	0.39	0.899	ND
(±)-**16b**		41.2	1.364	1.63
(±)-**17c**		17.5	1.031	3.54
(±)-**18c**		15.6	1.133	3.52
(±)-**21**	Z = H	0.39	0.414	0.173
(±)-**25a**	$Z = CO(CH_2)_5CH_3$	<0.38	0.343	ND
(±)-**25b**	$Z = CO(CH_2)_{12}CH_3$	8.75	0.081	0.346
(±)-**25c**	$Z = COCH_2CO(CH_2)_{12}CH_3$	<0.38	0.120	ND
22	Z = H	cytot.	0.584	1.50
26a	$Z = CO(CH_2)_5CH_3$	<0.38	0.331	ND
26b	$Z = CO(CH_2)_{12}CH_3$	11.3	0.092	0.636
(±)-**27**		25.6	>5	11
(+)-**28**		17.6	4.80	ND
(−)-**29**		34.5	>10	ND

[a] All IC_{50} values were calculated from experiment carried out in triplicate on *T.g.* and from two independent experiments carried out in duplicate on *P.f.* [b] Natural product. [c] Non-natural product.

Scheme 4. Reduction of (±)-Aculeatin A to the Cyclohexanone (±)-**27**[a]



[a] Reagents and conditions: (a) H_2/Pd, EtOH, 16 h, 71%.

establish an in vitro selectivity index (SI, the ratio of the IC_{50} for in vitro cytotoxicity on K-562 to the IC_{50} of inhibition on parasite).

First, we confirmed the submicromolar antimalarial activity of natural aculeatins, with their corresponding non-natural enantiomers displaying the same level of inhibition (IC_{50} = 0.22−0.48 μM). Interestingly, these compounds similarly inhibited the growth of *T. gondii* RH-β1 at submicromolar concentration (IC_{50} = 0.31−0.64 μM). Moreover, these molecules showed a good to excellent SI, which ranges from 35 to 121 for both parasites. For the new aculeatin A ester analogues (±)-**15a**−**d**, the SI was in general lower, mainly because of a less pronounced antiparasitic potency. A remarkable exception was the low cytotoxicity (IC_{50} = 109 μM) together with a good antitoxoplasmal activity (IC_{50} = 0.50 μM) of (±)-**15a** which gave a SI of 219. Furthermore, a comparison of the ester analogues (±)-**15** revealed that the length of the lipophilic chain can impact the selectivity between *P. falciparum* 3D7 and *T. gondii* RH-β1. When this alkyl chain is short ((±)-**15a**, $R^3 = (CH_2)_2CH_3$), a better inhibitory effect was observed for *T. gondii* (IC_{50} = 0.50 μM) compared with P. *falciparum* (IC_{50} = 1.84 μM). A much longer chain ((±)-**15c**, $R^3 = (CH_2)_{17}CH_3$) resulted in a reversal of affinity in favor of *P. falciparum* (0.77 μM versus 2.40 μM with *T. gondii*). An intermediate chain length ((±)-**15b**, $R^3 = (CH_2)_9CH_3$) in turn proved adequate to have a similar effect on both parasites at submicromolar concentrations (IC_{50} = 0.65 and 0.34 μM). To extend the study of the lipophilic effect, we tested the ester analogue (±)-**15d** ($R^3 = CH[(CH_2)_9CH_3]_2$) and the amide analogue (±)-**15e** ($X = N(CH_2)_5CH_3$, $R^3 = (CH_2)_5CH_3$), which have an extra side chain. We observed no improvement on the inhibition of *P. falciparum*, while the amide analogue (±)-15e was shown to be too toxic.

We next focused on the impact of aculeatin configurations on the biological activities. Considering the most potent ester analogue (±)-**15b** for *P. falciparum* and *T. gondii*, we tested the corresponding stereoisomer (±)-**16b** of aculeatin B type. This aculeatin B configurration proved to be less efficient over (±)-**15b** for both parasites (IC_{50} = 1.36 and 1.63 μM). We also evaluated the stereoisomers of aculeatins D and 6-*epi* D types, i.e., (±)-**17c** and (±)-**18c**, respectively, which hold the proper alkyl length to be selective for *P. falciparum* (R^3 =

$(CH_2)_{17}CH_3$). Although these tests again confirmed the selectivity observed for *P. falciparum* over *T. gondii*, the inhibition on *P. falciparum* was lower than with the stereoisomer (±)-**15c**. As a result, it seems from this series that the aculeatin A configuration (±)-**15** exhibits a higher antiparasitic potency over the other stereoisomers.

We also evaluated the compounds (±)-**21**, **22**, (±)-**25a**−**c**, and **26a**,**b**, obtained in only one or two steps from (±)-**20** (Scheme 3). Essentially, these molecules feature two cyclohexadienone rings and a more complex polyspirocyclic structure. The unsubstituted derivatives (±)-**21** and **22** (with the free secondary alcohol) and the ester-substituted derivatives (±)-**25a** and **26a** with a relatively short chain length (Z = $CO(CH_2)_5CH_3$) exhibited a high cytotoxicity against mammalian cell that resulted in a poor SI. However, when the lipophilic chain was longer (Z = $CO(CH_2)_{12}CH_3$), the corresponding products (±)-**25b** and **26b** showed a high antimalarial activity (81 and 92 nM, respectively) with a decreased cytotoxicity compared to the former products. The SI is about 109 and 123, respectively. This enhancement of antimalarial activity seems to be due to the presence of the two cyclohexadienones ring combined with a proper hydrophilic chain length. The role of the configuration from the products seems to be less important in this case.

Finally, tests using reduced analogues of aculeatins confirmed the crucial role of cyclohexadienone ring on the antimalarial activity. The cyclohexanone (±)-**27**, derived from aculeatin A, and (+)-**28** and (−)-**29**, the phenolic precursors of aculeatins,[14b] were weakly active against *P. falciparum*.

In conclusion, we have developed a divergent approach to obtain in practical manner novel aculeatins derived from a common precursor **2**. From this study, we have identified two novel polyspirocyclic products, (±)-**25b** and **26b**, whose antiparasitic activities against the two widespread Apicomplexa occur at very low concentration (e.g., IC_{50} = 81 and 92 nM, respectively, against *P. falciparum* 3D7 strain). Very interestingly, their toxicities against mammalian cells occur at much higher concentration, resulting in a SI higher than 100 for *P. falciparum*. The cyclohexadienone moiety, in association with a well-defined aliphatic chain length, has been characterized as important functions for antimalarial and antitoxoplasmal activities. As many other analogues can be easily produced, these valuable candidates clearly deserve further structural optimizations to improve their antiparasitic activities and to decrease their cytotoxicity. Future prospects include additional tests, synthetic efforts, and the complementary development of probes derived of the best analogues described here to fish out and study the still unknown biological target(s) in Apicomplexan parasites.

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Supporting Information Available: Details of biological assays and synthetic procedures of new compounds tested and precursors, product characterization, ^1H and ^{13}C NMR spectra, and HPLC purity results for (±)-**25b** and **26b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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