Synthesis, reactions and biological activity of 11-azaartemisinin and derivatives

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Abstract

In this review, we will give an overview of the synthesis and reactions of 11-azaartemisinins and their derivatives, from the first report in 1995 till now. The desoxo as well as the 9-desmethyl analogues are included. The biological activity of the azaartemisinins is also briefly discussed, with an emphasis on their potency as antimalarials. Computational studies on this type of compounds are beyond the scope of this review and will not be discussed, nor referenced.

**Keywords:** Azaartemisinin, antimalarial, natural product, artemisinin

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1. Introduction

Malaria is still one of the most widespread parasitic diseases. Annually 1-2 million deaths are caused by malaria worldwide. Though 4 species of anopheles can infect man (*Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*), almost all the deaths are caused by *P. falciparum*, as a result of cerebral malaria.

Artemisinin **1** was identified in 1972 as the active ingredient of *Artemisia annua*, a Chinese herb that has been used since 341 B.C. for the treatment of severe fevers.[1] Despite several positive studies of both Chinese and Western researchers, the potential of artemisin was underestimated for years.[2] Currently, artemisinin derivatives are recommended by the World Health Organization (WHO) for the treatment of malaria, especially as part of a combination therapy, whereby artemisinin derivatives are administered together with other antimalarials. Because of the poor solubility of artemisinin in both water and oil, some simple more soluble derivatives were developed. The clinically used artemisinin derivatives are dihydroartemisinin **2** (DHA), artemether **3**, artemotil **4** and artesunate **5** (Structure block 1).
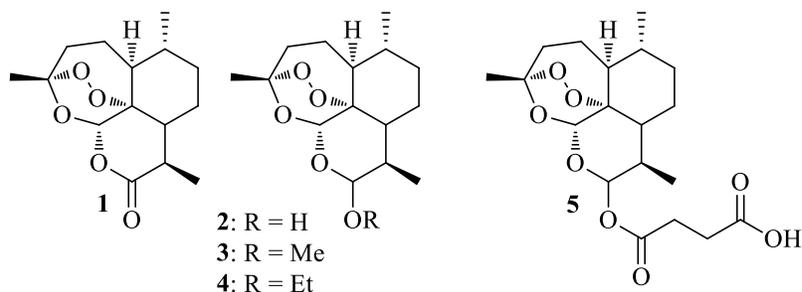
Structure block 1. Artemisinin and clinically used derivatives.
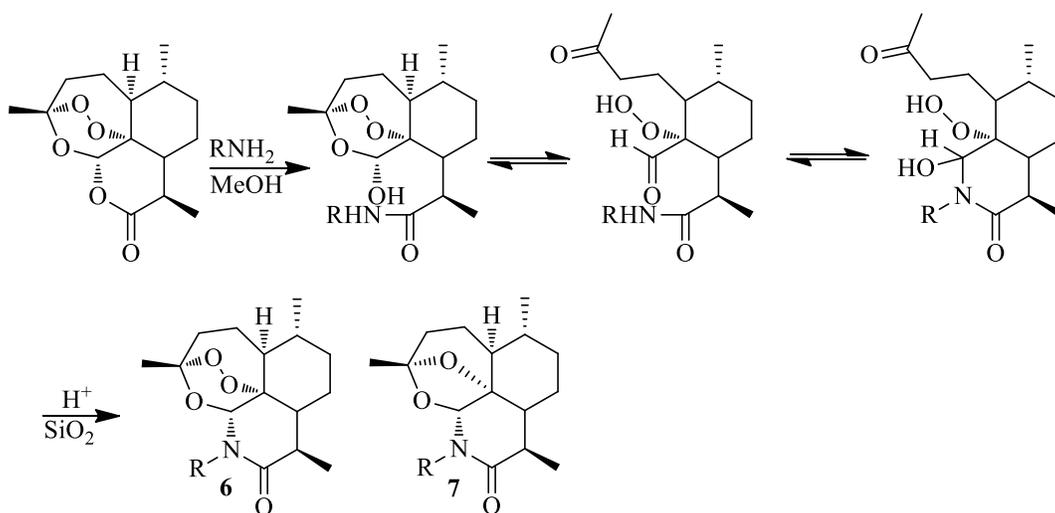
2. 11-Azaartemisinins

Oral use of artemisinin **1** and its derivatives is mostly restricted due to the labile nature of the peroxide and facile hydrolysis of the lactone by acid in the stomach. Therefore a more stable core was pursued. One of the possible solutions was substituting the lactone by the more stable lactam function.

2.1. Conversion of artemisinin to azaartemisinins
Until 1995, all of the reaction sequences for the decoration of the artemisinin scaffold left the tetracyclic ring system intact. Most of the artemisinin derivatives were limited to preparing the corresponding esters, ethers, carbonates and urethanes of dihydroartemisinin. The group of Ziffer was the first to communicate a novel reaction sequence, that started with the opening of the

lactone to produce an amide, whereby the tetracyclic ring system is reformed in the process of converting the amide into a lactam (Scheme 1).[3]

Scheme 1. Conversion of artemisinin to 11-azaartemisinin.

Reaction of artemisinin **1** with methanolic ammonia led to a complex mixture that was partially identified by ^{1}H NMR. This reaction mixture was subjected to reaction conditions developed by Avery *et al.* to synthesize artemisinin derivatives (silica gel, dilute sulfuric acid and BHT).[4] This yielded two compounds **6a** and **7a** in 45% and 9% isolated yield respectively. The structure of the major compound **6a** was characterized by mass spectrometry (CI-MS indicated that its molecular weight was 1 dalton less than artemisinin) and ^{1}H and ^{13}C NMR data. Employing [15]NH₃ yielded additional information, such as coupling between H-12 and [15]N ($J =$ 86.9 Hz) and coupling of ^{15}N with C-10 ($J = 11.9$ Hz) and C-12 ($J = 10.3$ Hz), as well as a two bond coupling to C-9 ($J = 5.9$ Hz). The second compound was identified as *N*-containing deoxy compound **7a**.

When Amberlyst was used instead of H_2SO_4/SiO_2, the yield of the desired compound increased to 65% and formation of the deoxy analogue was not observed.[5] An attempt to allylate the *N*-atom of **6a** with allyl bromide in the presence of silver oxide exclusively led to the undesired *O*-allyl derivative **8** (Scheme 2). Fortunately, the former approach is easily extended to prepare *N*-substituted 11-azaartemisinins using alkyl-, aromatic, or heteroaromatic amines instead of ammonia. It appears to be necessary to remove any unreacted amine present in the reaction before acid treatment, to prevent unwanted formation of the deoxy compounds, either by removal in vacuo (volatile amines) or extraction of a DCM solution of the reaction mixture with an aqueous citrate buffer (pH 4.5). Incorporating a lipophilic or hydrophilic substituent could provide a way to control the solubility.
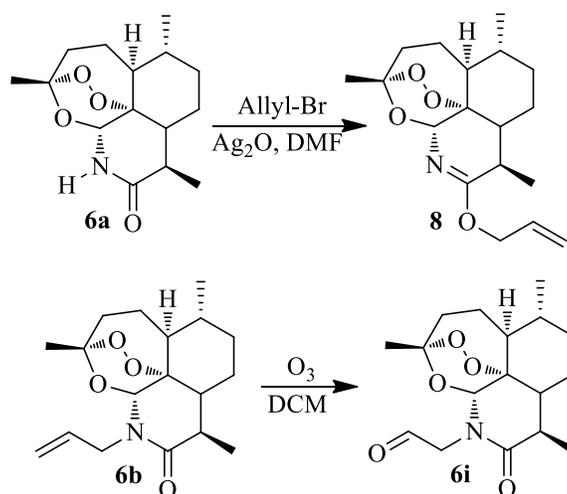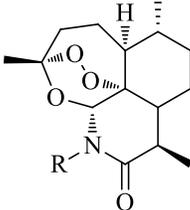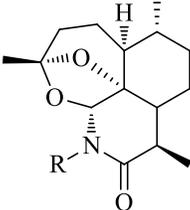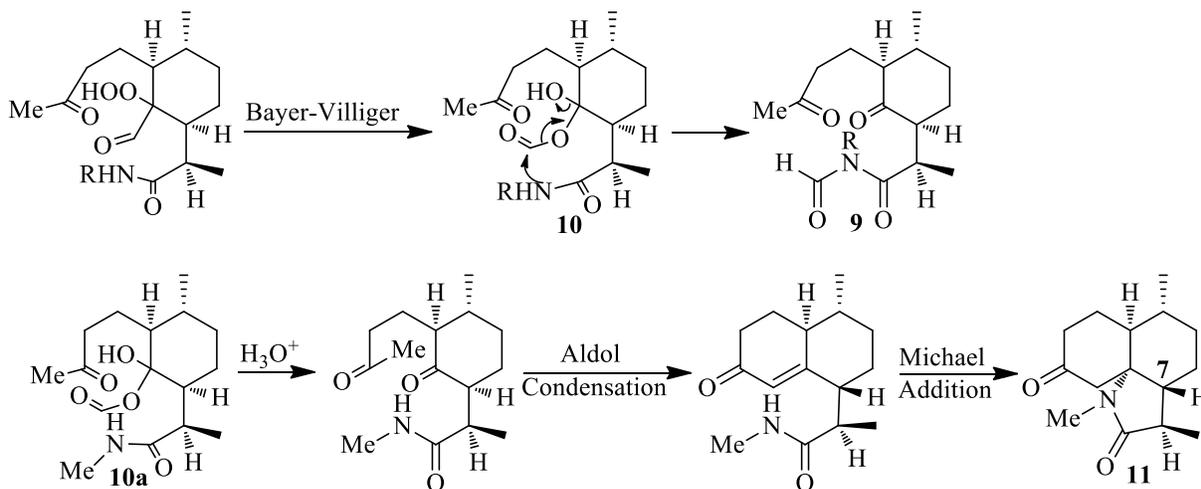
Scheme 2. *O*-Allylation of azaartemisinin and ozonolysis of *N*-allylated azaartemisinin.

The azaartemisinins **6** and **7** of Ziffer[5] were screened against a chloroquine-resistant strain (FCR3) of *P. falciparum*. As expected, the deoxy compounds **7** did not show any activity. The *in vitro* results showed that three of the compounds had antimalarial activities one order of magnitude larger than that of artemisinin (Table 1). Biological tests showed that compound **6i** (Scheme 2, Table 1) was 26 times more active *in vitro* and 4 times more active than artemisinin (= approximately as active as β-arteether) *in vivo* (against *P. berghei* in mice). This compound **6i** was obtained by reductive ozonolysis of the *N*-allyl analog **6b** (Scheme 2).

An attempt of the McPhail group to reproduce the results of Ziffer et al.,[5] surprisingly led to the formation of some by-products (Scheme 3).[6] Reaction of artemisinin with anhydrous ammonia, yielded the expected products **6a** and **7**, along with the isolation of 25% of **9**. Monocyclic **9** is postulated to arise from reaction of artemisinin with ammonia, followed by an internal Baeyer-Villiger oxidation to give the formate ester **10**, that intramolecularly rearranges to **9**. Substituting ammonia by allylamine gave analogous compounds. In the case of methylamine however, an additional product **11** was isolated in 32% yield. Remarkable is the inversion of the stereochemistry at C-7, relative to artemisinin, as was confirmed by single-crystal X-ray analysis. Formation of this product can be explained by a mechanism that involves acid hydrolysis of **10a**, followed by an aldol-type condensation. Subsequent Michael addition of the amide group to the α,β-unsaturated ketone in the intermediate leads to the observed product **11**.

**Table 1.** *In vitro* antimalarial activity of azaartemisinins **6** and desoxo azaartemisinins **7**

Compd	R	$IC_{50}(1)/IC_{50}$ (Compd)	Compd	R	$IC_{50}(1)/IC_{50}$ (Compd)
6a	H	1.0	**7a**	H	0.01
6b	$CH_2CH=CH_2$	0.8	**7b**	$CH_2CH=CH_2$	/
6c	$CH_2CH(CH_3)_2$	9.0	**7c**	$CH_2CH(CH_3)_2$	0.001
6d	CH_3	2.6	**7d**	CH_3	/
6e	CH_2Ph	/	**7e**	CH_2Ph	/
6f	2-pyridylmethyl	22.0	**7f**	2-pyridylmethyl	0.008
6g	2-furylmethyl	1.0	**7g**	2-furylmethyl	/
6h	2-thienylmethyl	1.1			
6i	CH_2CHO	26.0			

Scheme 3. Postulated reaction mechanisms for the by-products **9** and **11**.

When the work of Ziffer[3] was repeated by the group of Haynes,[7] they found that in their hands identical reaction conditions (reaction of **1** in MeOH with ammonia, benzylamine or other primary amines; followed by removal and treatment of the crude residue in DCM with silica gel/H_2SO_4 or *p*-TsOH) gave the deoxy compound **7** exclusively. Lowering the reaction temperature in DCM to 0-5 °C, followed by acid treatment, yielded 11-azaartemisinins **6** in

moderate yield, together with the desoxo compound **7** in about 40% yield. At higher temperatures, desoxo compound **7** was the major product.

Upon treatment of artemisinin (2eq) with a tertiary amine (*N*-methylmorpholine, 5eq), no reaction was observed. However, addition of benzylamine (1eq) to this reaction mixture gave the *N*-oxide **12** in 72% isolated yield, along with the desoxo compound **7j** (R = Bn) (Scheme 4). Optimal reaction conditions consisted of a two phase system (water/DCM) followed by extraction of the *N*-oxide into the water layer (60% yield of *N*-oxide after purification). The organic phase contained azadesoxoartemisinin **7j** as the major product, as well as up to 10% of recovered artemisinin and small quantities of desoxoartemisinin. Other tertiary amines underwent analogous reactions.
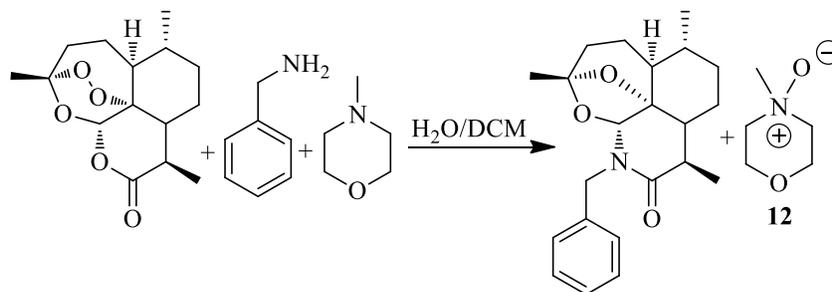
Scheme 4. Formation of deoxy artemisinin.

Haynes and co-workers later on optimized the reaction conditions for the synthesis of the unsubstituted lactam **6a**.[8] Artemisinin was treated with 33% aqueous ammonium hydroxide in a 10:3 THF:methanol mixture at -15 to -10 °C, followed by solvent removal under reduced pressure and treating the crude residue with *p*TsOH in DCM at ambient temperature. The desired product **6a** could be easily obtained in 70% yield by crystallization on multi-gram scale.
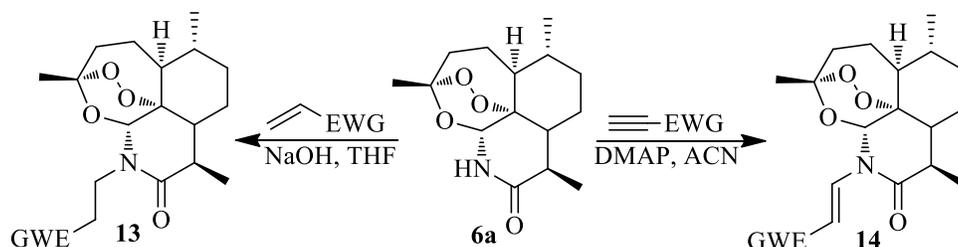
2.2. Michael additions to 11-azaartemisinins

To elaborate on their previous lead,[5] the group of Ziffer pursued an efficient and direct route to analogs of **6i**.[9] Considering that treatment with alkylation agents was not an option (as this approach leads to the *O*-alkyl derivatives), the Michael addition to **6a** was investigated as an alternative. Reaction of **6a** with catalytic amounts of sodium hydroxide, and ethyl acrylate in THF at room temperature, yielded the desired *N*-alkyl derivative **13** in 86 % yield (Scheme 5, left arrow). This procedure was easily extended towards other electron withdrawing groups (EWGs) on the double bond (e.g. nitriles, ketones, sulfonates, sulfones, and sulfoxides, succinic diesters) to prepare the desired adducts in 70-90% yields. However, a second substituent on the double bond (either in α- or β-position) completely prevented the addition of the amide moiety.

In a follow-up paper, Ziffer and co-workers investigated the addition of the lactam **6a** to terminal acetylenes conjugated to EWGs (Scheme 5, right arrow).[10] While reaction with methyl propiolate and DMAP in acetonitrile yielded the product **14** in high yield after short reaction

time, the additions of **6a** to 3-butyn-2-one and *N,N*-dimethyl propiolamide were much more sluggish and incomplete. NMR analysis of the adduct suggests *E*-stereochemistry of the double bond, based on the magnitude of the coupling constant $J_{13\text{-}14}$ (14-16 Hz).

However, when the EWG was a nitrile, both isomers were formed, with the major product (40% isolated yield) possessing the *Z*-geometry ($J_{13\text{-}14}$ = 11 Hz).[11] The minor *E*-adduct ($J_{13\text{-}14}$ = 15 Hz) was only formed in 4% yield. Changing the solvent from acetonitrile to DMF did not have any effect on the stereochemistry of the reaction.
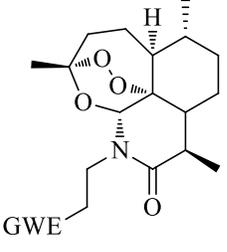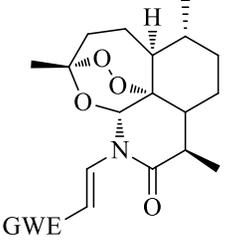

Scheme 5. Michael additions on azaartemisinin.

The noteworthy activity of **6i**, with an aldehyde function on the β-carbon of the nitrogen atom, prompted Ziffer et al. to further investigate whether other electronegative substituents at the γ-carbon of the substituent on the lactam nitrogen atom, could further increase the antimalarial activity.[11] Therefore, the adduct of 11-azaartemisinin with methyl acrylate **13g** was reduced with DIBAL to yield a mixture of alcohol **13h** and aldehyd **13i** (Scheme 6). The aldehyd was further reacted with methyl lithium or phenyl lithium respectively, after which the resulting alcohol was oxidized with Jones reagent to the corresponding ketone **13j,k**.
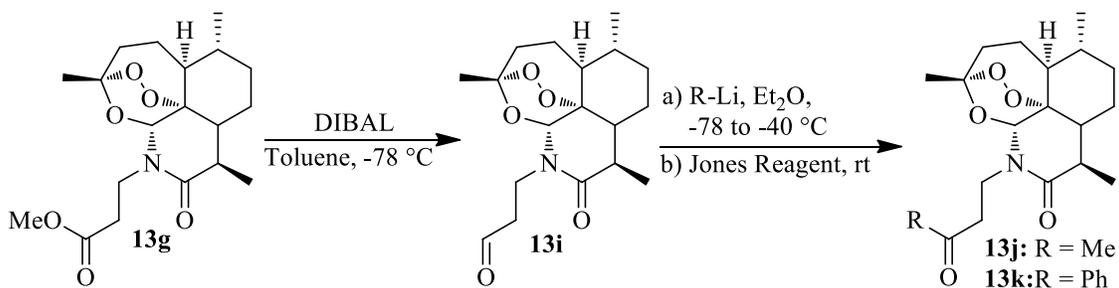
Scheme 6. Conversion of Michael adduct with ester function to ketone.

Of the fifteen azaartemisinins in Table 2, ten were more active than artemisinin against the D-6 clone and six were more active against the W-2 clone. The activity increased significantly on converting the aldehyd **13i** into ketones **13j** or **13k**. Introducing a double bond did not cause a significant change in the antimalarial activity. Neither was a large effect observed by replacing the aldehyde group with SO₃Ph or SO₂Ph moieties.

Table 2. *In vitro* antimalarial activity of Michael adducts of azaartemisinin

		In vitro antimalarial activity against the W-2 clone of *P. falciparum*	*In vitro* antimalarial activity against the D-6 clone of *P. falciparum*			*In vitro* antimalarial activity against the W-2 clone of *P. falciparum*	*In vitro* antimalarial activity against the D-6 clone of *P. falciparum*
Compd	EWG	$IC_{50}(1)/IC_{50}$ (Compd)	$IC_{50}(1)/IC_{50}$ (Compd)	Compd	EWG	$IC_{50}(1)/IC_{50}$ (Compd)	$IC_{50}(1)/IC_{50}$ (Compd)
13a	CO_2Et	0.41	1.5	**14a**	CO_2CH_3 (*E*)	1.2	2.0
13b	CN	0.43	1.3	**14b**	$COCH_3$ (*E*)	1.25	2.54
13c	SO_3Ph	0.96	1.7	**14c**	$CON(CH_3)_2$ (*E*)	0.35	0.39
13d	SO_2Ph	0.32	1.1	**14d**	CN (*Z*)	1.7	2.7
13e	$CO_2C(CH_3)_2$	0.0012	0.03				
13f	EtO_2CCH-CH_2CO_2Et	0.066	0.24				
13g	CO_2CH_3	1.0	1.77				
13h	CH_2OH	0.64	1.02				
13i	CHO	0.18	0.49				
13j	$COCH_3$	3.0	3.2				
13k	COPh	1.8	1.8				

2.3. *N*-Amino and *N*-hydroxy-11-azaartemisinins

In their pursuit to synthesize *N*-amino-11-azaartemisinins **15**, Singh[12] used a modification of the method developed by Ziffer.[3] They noticed that by switching the solvent from chloroform to a 7:3 methanol-chloroform mixture at 0 °C during the first step of the reaction, they could suppress formation of the deoxy analogue completely (Scheme 7). Reactions with hydroxylamine and 2-aminoethanol could easily be performed by using the same method, affording hydroxy derivatives **16** and **17**.
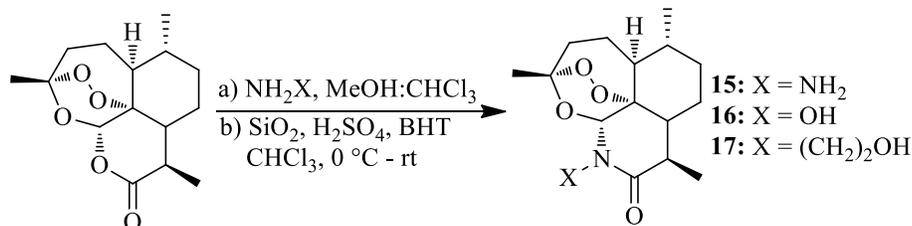
Scheme 7. Synthesis of *N*-amino and hydroxyl azaartemisinins.

The *N*-amino azaartemisinins **15** presented the perfect scaffold for derivatisation with different functionalities (Table 3). Amide derivatives **18** were obtained in average to good yields (60-93%) by reacting **15** with a series of acid chlorides in anhydrous benzene with triethylamine as base (Scheme 8). Also dimers were accessible, albeit in low yields (20—34%), by reacting 2 eq of **15** with terephthaloyl chloride and oxalyl chloride respectively, under the same conditions. Treating **15** with benzaldehyd derivatives led to the corresponding imines **19**, which are easily converted to amines **20** using conventional methods (sodium borohydride reduction).

Table 3. Amide, imine and amine derivatives

| R	Compd	Compd	Compd
C_6H_5	**18a**	**19a**	**20a**
4'-BrC_6H_4	**18b**	**19b**	**20b**
4'-$F_3CC_6H_4$	**18c**	**19c**	**20c**
4'-C_6H_5-C_6H_4	**18d**	**19d**	**20d**
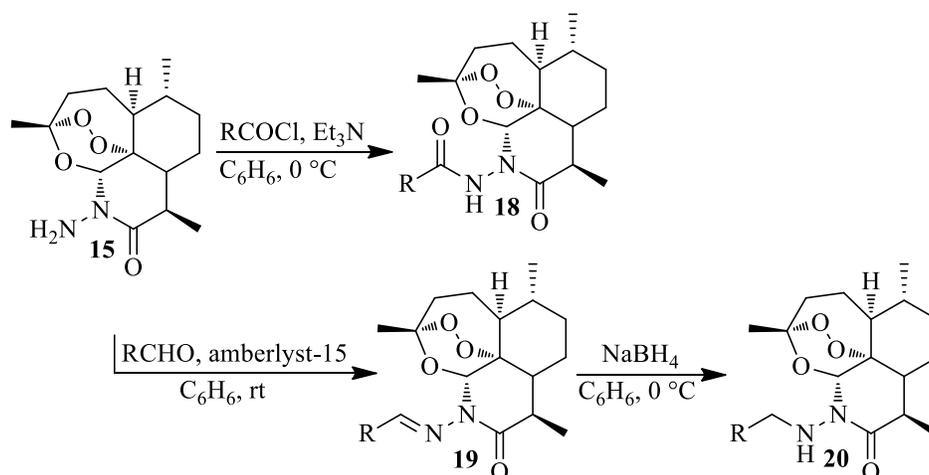
Scheme 8. Conversion of *N*-amino azaartemisinin to amide, imines and amines.

Reaction of the deprotonated hydroxy azaartemisinins **16** and **17** with a range of alkyl bromides, furnished the corresponding ethers **21** and **22** in acceptable yields (60-74%, Scheme 9, Table 4).

Table 4. Ether derivatives

R	Compound	Compound
$C_6H_5CH_2$	**21a**	**22a**
$C_6H_5(CH_2)_3$	**21b**	**22b**
4'-C_6H_5-$C_6H_4CH_2$	**21c**	**22c**
2'-$FC_6H_4CH_2$	**21d**	**22d**

Although **15**, **16** and **17** displayed poor antimalarial activity *in vivo* against *P. yoelii* in mice (Table 5; oral administration), some derivatives showed very promising results (most notably **18d**, **20d** and **21a**).
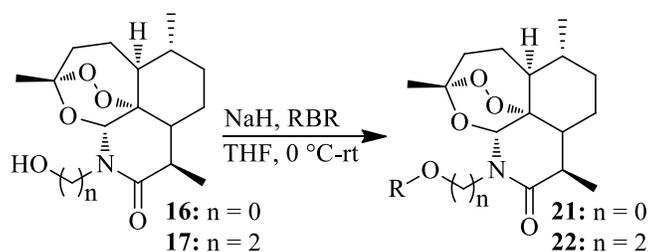
**Scheme 9.** Conversion of hydroxy azaartemisinin into ether derivatives.

Table 5. *In vivo* antimalarial activity of selected *N*-amino and *N*-hydroxy against *P. yoelii* in mice

Compd	Oral dose (mg/kg x 4 days)	% suppresion of parasitaemia on day 4	# mice survived
15	24	100	2/5
16	48	78.11	0/5
17	48	100	0/5
18d	12	100	5/5
	6	94.37	0/5
20d	12	100	5/5
	6	100	1/5
21a	24	100	5/5
	12	100	2/5
4	48	100	5/5
	24	100	1/5

2.4. *N*-sulfonyl and *N*-carbonyl-11-azaartemisinins

In an attempt to improve the thermal stability, and thus the shelf life storage in tropical countries where malaria is prevalent, *N*-sulfonyl and *N*-carbonyl azaartemisinins were recently prepared by the group of Haynes.[8]

Initial attempts to prepare the *N*-sulfonyl derivatives by treatment of the lactam **6a** with methanesulfonyl chloride or methanesulfonic anhydride in the presence of amine bases were unsuccessful. However, by switching to LDA in THF at -78 °C, **23a** could be obtained in a satisfactory 62% yield (Scheme 10). Sodium hydride in THF at 0 °C gave similar results (55% yield). The structure of **23a** was confirmed by single-crystal X-ray analysis. The same conditions could be adapted to build a small library. Aliphatic sulfonyl chlorides gave better yields than their aromatic counterparts. Moreover, Haynes *et al.* managed to obtain the dimer **23u** (through reaction with biphenyl-4,4'-disulfonyl chloride), albeit in low yield (7%). No product could be isolated from reaction with benzene-1,3-disulfonyl chloride though.
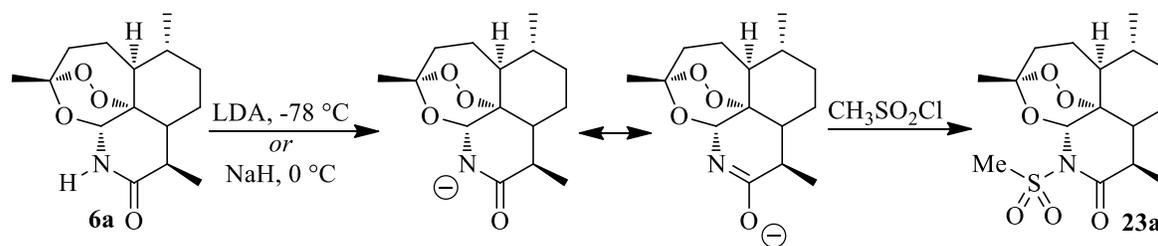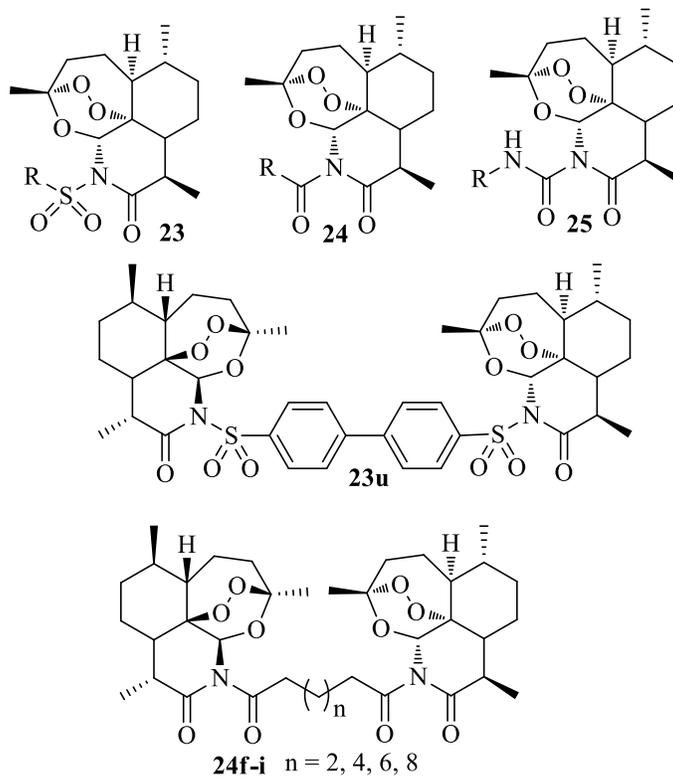
**Scheme 10.** Synthesis of *N*-sulfonyl azaartemisinins.

This methodology also proved successful in the synthesis of *N*-carbonyl derivatives **24**, by treating the deprotonated 11-azaartemisinin **6a** with acyl chlorides (Structure block 2). It is important to note that reaction exclusively took place at the nitrogen atom. Dimeric adducts **24f-i** were obtained from diacid chlorides in low to moderate yields (13-35%). Finally, even acylureas **25** were accessible by this method through reaction of the deprotonated azaartemisinin **6a** with aryl isocyanates. A summary of all the products can be found in Table 6. Especially the *N*-sulfonyl compounds **23** possessed some desirable properties: they were nicely crystalline and much more thermally stable than the clinically used artemisinins DHA, artemether and artesunate. Moreover, they showed no sign of degradation during storage at room temperature for periods of well over 3 years.

In a next step, Haynes *et al.* attempted to reduce the carbonyl group of azaartemisinin **6a** (Scheme 11). Test reactions using common reagents to reduce amides to amines, or to convert artemisinin into 10-deoxo derivatives were unsuccessful. Even for **23a** with an electron withdrawing group this reaction failed. Treatment of **23a** in DCM and an excess of trimethylsilyl chloride with lithium borohydride caused isomerization to **26** and **27**. Reduction of **23a** with excess diisobutyl aluminium hydride (DIBAL) in DCM yielded the crystalline alcohol **28** (22%). However, it was not possible to remove the hydroxy group. Compounds **27** and **28** were unambiguously characterized by X-ray analysis.

The compounds in this study possess relatively low cytotoxicity. Several of the arylsulfonyl derivatives have antimalarial activity comparable with artesunate against the drug-sensitive 3D7 clone of the NF54 isolate and the multidrug-resistant Thailand K1 strain of *P. falciparum* (Table 6). In general, the *N*-carbonyl derivatives **24** surpass the *N*-sulfonyls **23** as antimalarials. The activity of the *N*-nitroarylcarbonyl **24j,k** and arylurea **25b** derivatives were situated in the subnanogram/ml range. However, despite log P values below 3.5, most of the compounds were poorly soluble in water (in sharp contrast with **6a**, that has a solubility at pH 7 of >1000mg/L).

Structure block 2. *N*-sulfonyl and carbonyl azaartemisinins.
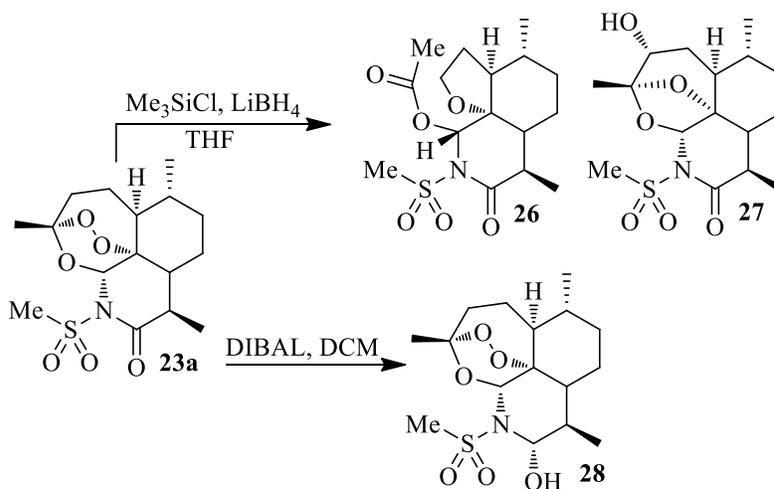
Scheme 11. Attempts at reduction of the lactam carbonyl group.

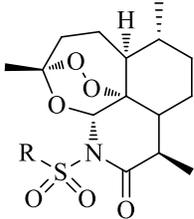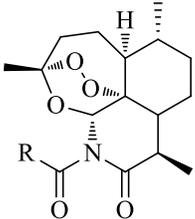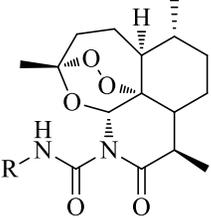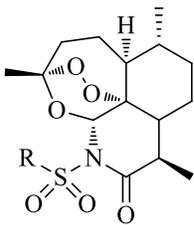**Table 6.** *In vitro* antimalarial activity of *N*-sulfonyl, carbonyl and acylurea derivatives

Compd	R	*In vitro* antimalarial activity against the 3D7 strain of *P. falciparum* IC$_{50}$ [ng/ml (nM)]	*In vitro* antimalarial activity against the K1 strain of *P. falciparum* IC$_{50}$ [ng/ml (nM)]	Compd	R	*In vitro* antimalarial activity against the 3D7 strain of *P. falciparum* IC$_{50}$ [ng/ml (nM)]	*In vitro* antimalarial activity against the K1 strain of *P. falciparum* IC$_{50}$ [ng/ml (nM)]
23a	CH_3	41.1 (11.4)	3.2 (8.9)	**24a**	CH_3	3.0 (9.3)	4.5 (13.9)
23b	CH_3CH_2	1.7 (4.6)	3.7 (9.9)	**24b**	CH_3CH_2	2.0 (5.9)	1.0 (3.0)
23c	$CH_3(CH_2)_2$	/	/	**24c**	$CH_3(CH_2)_2$	2.0 (5.7)	2.0 (5.7)
23d	$CH_3(CH_2)_7$	1.1 (2.4)	2.1 (4.6)	**24d**	$CH_3(CH_2)_5$	1.0 (2.6)	2.0 (5.3)
23e	$CH_3(CH_2)_{15}$	/	50	**24e**	$CH_3(CH_2)_{14}$	>50	>50
23f	$4'-FC_6H_4$	/	8.0 (18.2)	**24f**	$-(CH_2)_4-$	2.3 (3.4)	50 (74.3)
23g	$4'-ClC_6H_4$	>50	17	**24g**	$-(CH_2)_6-$	9.2 (13.1)	9.0 (12.8)
23h	$4'-BrC_6H_4$	>50	>50	**24h**	$-(CH_2)_8-$	13 (17.8)	2.0 (2.7)
23i	$4'-CH_3C_6H_4$	>50	37	**24i**	$-(CH_2)_{10}-$	>50	40
23j	$4'-O_2NC_6H_4$	17.1	9.1 (19.5)	**24j**	$4'-O_2NC_6H_4$	0.45 (1.0)	0.6 (1.4)
23k	$3'-O_2NC_6H_4$	2.1 (4.5)	2.2 (4.7)	**24k**	$3'-O_2NC_6H_4$	0.46 (1.1)	0.6 (1.4)
23l	$2'-O_2NC_6H_4$	/	10 (21.4)	**24l**	$3',5'-(O_2N)_2C_6H_3$	/	2.0 (4.2)
23m	$3''-NCC_6H_4$	/	>50				
23n	$4'-Cl-3'-O_2NC_6H_4$	1.5 (3.0)	3.8 (7.6)				
23o	$3',4'-(CH_3O)_2C_6H_3$	>50	40				
23p	$4'-CH_3SO_2C_6H_4$	/	13 (26)				
23q	$4'-C_6H_5-C_6H_4$	40	45	Compd	R		
23r	$5'-Cl-2'-$thienyl	/	40	**25a**	C_6H_5NH	/	/
23s	2-naphthyl	2.8 (5.9)	0.2 (0.42)	**25b**	$4-O_2NC_6H_4NH$	0.6 (1.3)	0.4 (0.9)

Table 6. Continued

| Compd	R	In vitro antimalarial activity against the 3D7 strain of *P. falciparum* IC$_{50}$ [ng/ml (nM)]	In vitro antimalarial activity against the K1 strain of *P. falciparum* IC$_{50}$ [ng/ml (nM)]
23t	5'-dansyl	/	/
23u	8'-quinolinyl	1.9 (4.0)	2.0 (4.2)
23v	bis-4',4"-biphenyl	/	/
5	/	1.5 (3.9)	2.2 (5.7)

2.5. *N*-Biotinylated azaartemisinin derivatives

As the exact mechanism of artemisin remains under debate till this date, Meng and Yao decided to couple artemisinin with biotin through a series of linkers, in order to develop a small molecular probe.[13] Indeed, the biotin-avidin system has often been used to identify the target proteins of a substrate. Building on the work of Singh and co-workers,[12] their group synthesized the azaartemisines **6j** and **15** (Structure block 3). In a next step the molecular probe could be assembled by coupling either D-biotin itself, or a biotinylated triethylene glycol spacer **29** with terminal carboxylic acid function, with the free amine function of the azaartemisin derivatives **6j** and **15** (Structure block 4). The condensation furnished the desired products **30** and **31** in good yield (55-65%) by using standard reagents for amide bond formation (EDCI, HOBT) in DMF as solvent and triethylamine as base.

The resulting biotinylated artemisinin derivatives showed poor *in vitro* anti-tumor activity against the HCT-116 human colon carcinoma cell line. Furthermore, solubility of the samples in the bioassay presented a serious problem at higher concentrations.
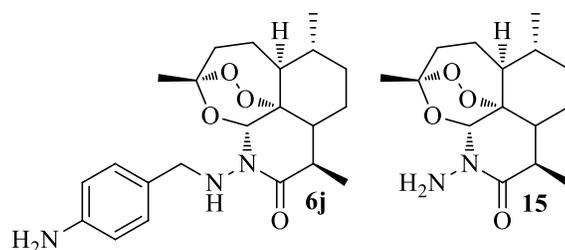
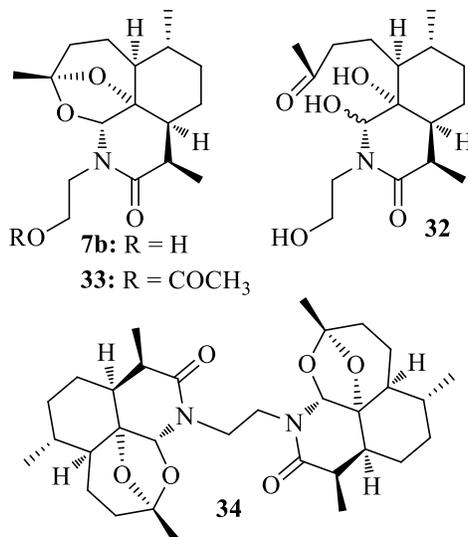**Structure block 3.** Azaartemisinins with free primary amine function.
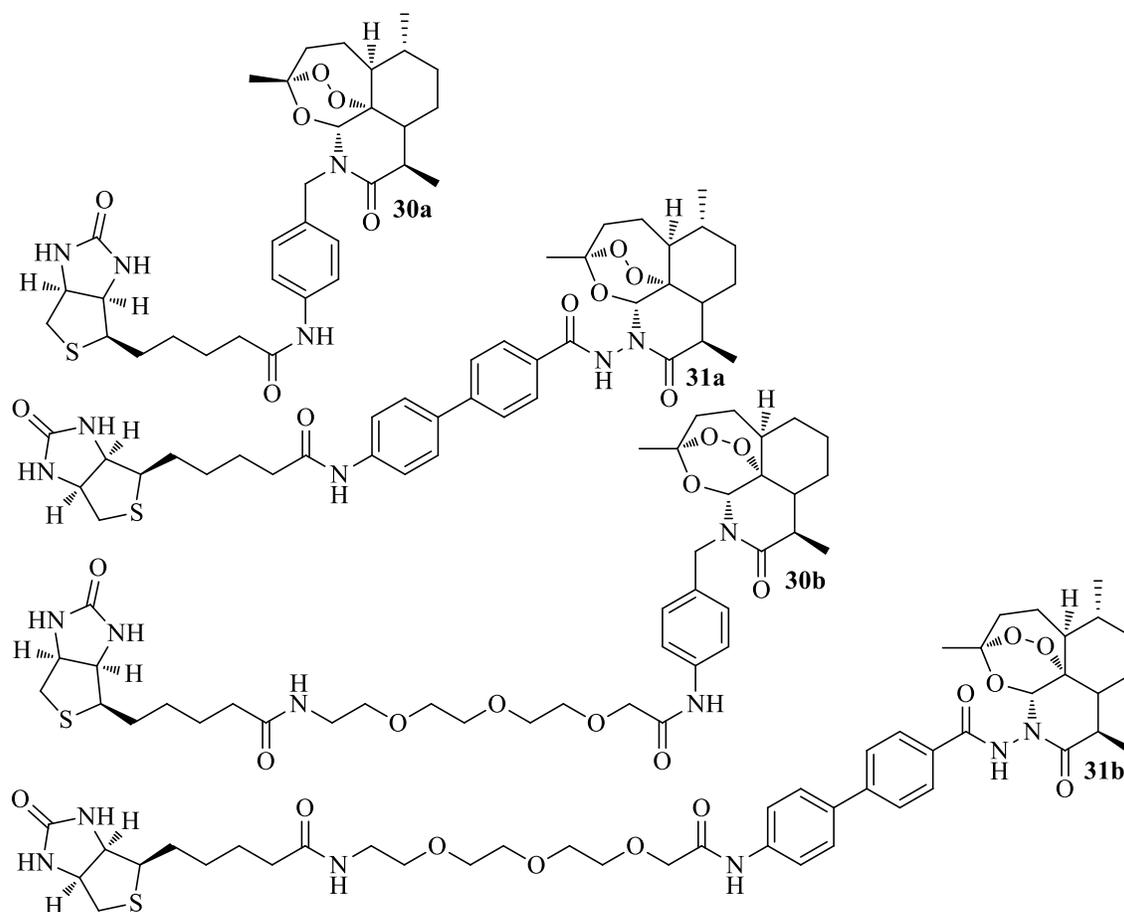
Structure block 4. Biotinylated artemisnin derivatives.

2.6. 11-Azaartemisinin desoxo derivatives

El-Feraly *et al.* investigated the reaction of artemisinin with ethanolamine.[14] After chromatography of the crude reaction mixture, the desoxo azaartemisinin **7k** was isolated (Structure block 5). However, when the crude residue was crystallized from a *n*-hexane/ether mixture instead of purifying with column chromatography, the intermediate **32** could be identified as the sole product. Though this intermediate had been postulated, it had never before been isolated or characterized. The primary alcohol group of the lactam tether could easily be acylated to **33**. Neither **7k** or **33** showed any activity in the antimalarial testing (Table 7); diol **32** however, was found to be cytotoxic to human breast ductal carcinoma.

Reaction of artemisinin in a methanolic solution of ethylenediamine afforded the symmetrical desoxodimer **34** in almost quantitative yield. It is noteworthy that this compound, though lacking the endoperoxide function, nevertheless surprisingly exhibited approximately the same antimalarial activity against the D-6 and W-2 strain of *P. falciparum* as artemisinin.

In 2005, the group of Galal tested the antifungal acivity of **7k** and **34** against *Cryptococcus neoformans* and *Candida albicans*.[15] Both compounds were completely inactive, which was attributed to the lack of the endoperoxide function.
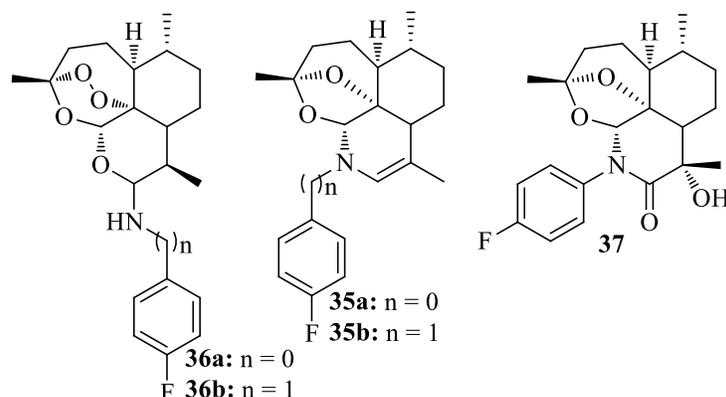

Structure block 5. Desoxo 11-azaartemisinins.

Table 7. *In vitro* antimalarial activity of desoxo 11-azaartemisinins

Compd	*In vitro* antimalarial activity against the D-6 clone of *P. falciparum*[a] IC$_{50}$ [ng/ml (nM)]	*In vitro* antimalarial activity against the W-2 clone of *P. falciparum*[b] IC$_{50}$ [ng/ml (nM)]
7k	/ (>400)	/ (>400)
32	/ (>400)	/ (>400)
34	12.0 (21.6)	8.7 (15.6)
1	6-10 (21.3-35.5)	6-10 (21.3-35.5)

In the course of their study on the Fe^{2+}-mediated decomposition of artemisinin derivatives, the group of Haynes isolated the desoxo enamines **35a** and **35b** as part of the reduction products of **36a** and **36b** respectively (Structure block 6).[16] Enamine **35a** could be converted to the corresponding hydroxy lactam **37** by treating it with ruthenium trichloride/sodium periodate.

Structure block 6. Desoxo enamines and azaartemisinin.

2.7. 11-Aza-9-desmethylartemisinins

The group of Avery synthesized a number of aza-desmethylartemisinins **38**,[17] as a late synthetic precursor **39** was readily available from their artemisinin total synthesis project (Scheme 12).[4] Carboxylic acid **39** was converted to the corresponding mixed anhydride with ethyl chloroformate. Subsequent condensation with the appropriate primary amines led to ketal amides that were converted into the desired products in 20-65% yield by exposure to ozone and acid-catalyzed cyclization. The analogs were tested *in vitro* in parasitized whole blood (humans) against two strains of *P. falciparum*, the chloroquine-resistant (and mefloquine-sensitive) Indochina W-2 clone and the chloroquine-sensitive (and mefloquine resistant) Sierra Leone D-6 clone respectively (Table 8). Most of these analogs were also tested *in vivo* against both strains. In order to gain a better understanding of the SAR involved, the log P values for the majority of these compounds were determined, using known procedures. No correlation was found between log P and the activity, whether *in vivo* or *in vitro*. Although removal of the C-9 methyl of artemisinin improves the activity about 6-fold,[18] the same trend was not observed for the azaartemisinins. In general, the 11-azaartemisinins proved to be approximately as active as artemisinin, although it is noteworthy to point out that the *N*-methyl analog **38a** (Table 8) was more than 5 times as potent as artemisinin in the resistant W-2 strain.

Dayan *et al.* investigated the phytotoxicity of a number of known artemisin analogues, including some aza derivatives (Table 9).[19] Derivative **38d** was the only aza compound more potent than artemisinin. It is worth mentioning that, although the other azaartemisinins were less active than artemisinin, they were the only products that showed some selectivity, being more toxic against *Lolium* than to *Lactuca* or *Arabidopsis*. The decreased activity of these derivatives could be explained by the fact that they bear large hydrophobic moieties, that may cause poor uptake by plants.
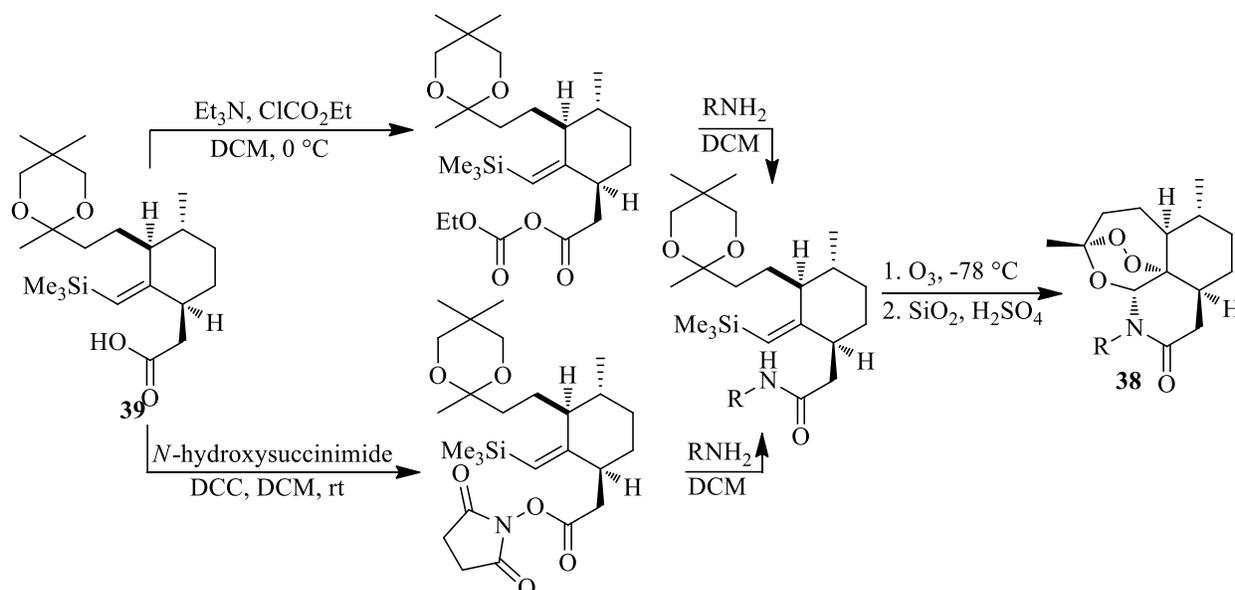
Scheme 12. 11-Aza-9-desmethylartemisinins.

Table 8. *In vitro* anti-malarial activity of 11-aza-9-desmethylartemisinins

Compd	R	*In vitro* antimalarial activity against the W-2 clone of *P. falciparum* $IC_{50}(1)/IC_{50}(Compd)$ x MW(Compd)/MW (1)	*In vitro* antimalarial activity against the D-6 clone of *P. falciparum* $IC_{50}(1)/IC_{50}(Compd)$ x MW(compd)/MW(1)
38a	CH_3	5.00	2.13
38b	$CH_3(CH_2)_2$	1.11	0.75
38c	$C_4H_9(i)$	1.05	1.45
38d	$C_5H_{11}(n)$	0.63	1.10
38e	$C_5H_{11}(i)$	0.92	1.49
38f	$(CH_3)_2NCH_2CH_2$	/	0.37
38g	HO_2CCH_2	/	0.06
38h	$HO_2C(CH_2)_5$	0.02	0.12
38i	$(CH_3)_3CO_2CCH_2$	/	/
38j	$C_6H_5CH_2$	2.17	1.89
38k	$p\text{-}ClC_6H_4CH_2$	0.69	1.11
38l	$C_6H_5(CH_2)_2$	1.43	1.97
38m	$C_6H_5(CH_2)_3$	1.05	2.05

Table 9. Phytotoxicity of selected 11-aza-9-desmethylartemisinins

Compd	Concentration		Root inhibition %			Seed germination score	Herbicidal activity		
	(mg/ml)	(μM)	*Lactuca*	*Lolium*	*Arabidopsis*	*Lactuca*[a]	*Lolium*[a]	*Arabidopsis*[b]	
	0.001	3	68	81	4.5	-	-	-	
38c	0.01	30	100	100	0.0	+	+	+	
	0.1	300	100	100	0.0	+	+	+	
	0.001	3	0	15	5.0	-	-	-	
38d	0.01	30	31	62	5.0	-	-	-	
	0.1	300	33	100	0.0	-	-	+	
	0.001	2.7	0	43	5.0	-	-	-	
38l	0.01	27	0	100	5.0	-	-	-	
	0.1	270	16	100	2.0	-	+	+-	
	0.001	2.6	0	0	5.0	-	-	-	
38m	0.01	26	0	32	4.0	-	-	-	
	0.1	260	0	90	0.0	-	+	+	

[a]Activity based upon *Lactuca* and *Lolium* root inhibition: high (+) 85-100%, moderate (+-) 70-85%, no activity (-) <70%; [b]Activity based upon *Arabidopsis* seed germination score: high (+) 0-1 (0-20% germination), moderate (+-) 1-2 (20-40% germination), no activity (-) 2-5 (40-100% germination).

3. Conclusions

In this review, we tried to give a comprehensive overview of the synthesis, reactions and biological activity of 11-azaartemisinins and their derivatives, including the desoxo analogues.

The acid labile lactone function of artemisinin can be transformed into a more stable lactam through a sequence that consists of opening the lactone with ammonia or primary amines (including alkyl-, allyl-, aromatic or heteroaromatic amines, hydrazine or hydroxylamine), followed by closing the resulting amide under acidic conditions to restore the tetracyclic core. These scaffolds can be further converted into the corresponding alkyl, carbonyl, imine , amine, ether, sulfonyl and acylurea derivatives, etc.

Besides the azaartemisinins originating from artemisinin, 9-desmethyl derivatives are synthetically accessible by elaborating on a precursor that was previously used for the total synthesis of artemisinin.

The biological activity (*in vitro* and *in vivo*) of the azaartemisinins indicates their importance as potential drug candidates, most notably as antimalarials.

Noteworthy is the report of a compound with aldehyd function in β-position of the nitrogen atom, which was 26 times more active *in vitro* (against *P. falciparum*) and 4 times more active *in vivo* (against *P. berghei* in mice) than artemisinin.

Therefore, it is really astonishing and incomprehensible that this group of compounds has received so little attention until now.

We can only hope that this review will contribute to give these 'neglected' artemisinin derivatives the appreciation they deserve. Certainly there remains a lot to be explored...

4. Acknowledgements

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