Eur J Med Chem (1995) 30, 697–706

Antimalarial activity of new ethers and thioethers of dihydroartemisinin

B Venugopalan[1], PJ Karnik[1], CP Bapat[1], DK Chatterjee[2], N Iyer[2], D Lepcha[2]

[1]*Department of Chemistry, Research Centre, Hoechst India Ltd;*
[2]*Department of Chemotherapy, Research Centre, Hoechst India Ltd, Mulund, Bombay, 400 080, India*

(Received 5 January 1995; accepted 10 May 1995)

Summary — Various ethers and thioethers of dihydroartemisinin were prepared by treating dihydroartemisinin with hydroxy alkyl, substituted phenol, hydroxy aralkyl, hydroxy alkynyl and hydroxy heteroalkyl or thiols in the presence of BF_3Et_2O. The thioethers **64** and **65** were further oxidised to the respective sulfoxides. These derivatives were tested in the *Plasmodium berghei* K-173-infected mice and some active compounds were tested in chloroquine-resistant *P yoelii nigeriensis* (NS)-infected mice. Initially the compounds were administered subcutaneously and subsequently by the oral route. The antimalarial activity of the compounds **22**, **23**, **36**, **66** and **79** were found to be comparable to that of arteether when tested in the K-173-infected mice. These compounds also showed activity in the *P y nigeriensis* (NS)-infected mice.

antimalarial activity / artemisinin / dihydroartemisinin / ether / thioether

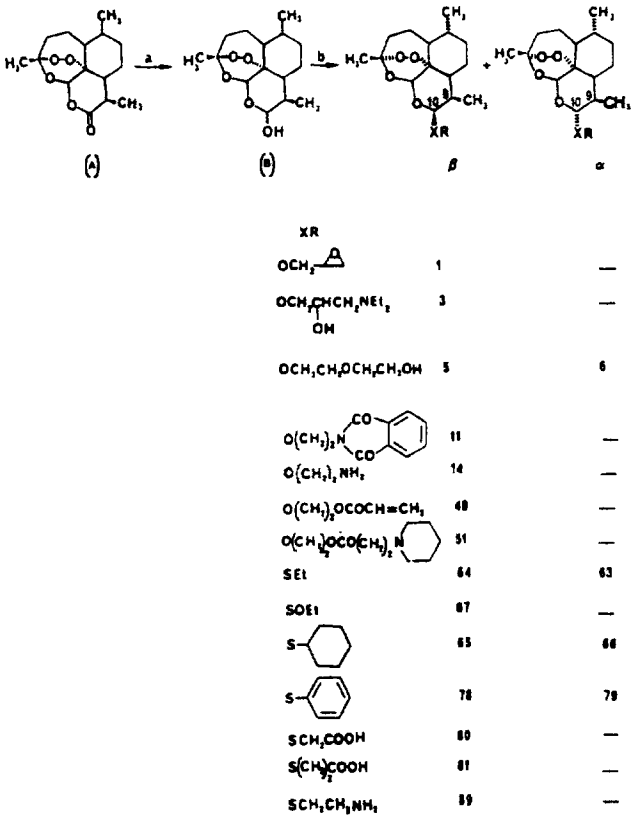
Introduction

Malaria has become epidemic because of the increasing numbers of chloroquine-resistant malaria cases [1]. Any newly discovered medicament should be active against chloroquine-resistant malaria and itself should not induce resistance. Although the first problem can be tackled, the second may be difficult to solve. However, it could be overcome with the use of different combinations of available antimalarial drugs [2, 3]. In this connection, the synthesis of new antimalarials with a novel mode of action becomes essential. Artemisinin [3–8], which is isolated from the leaves of the plant *Artemisia annua*, showed very potent activity especially in the case of cerebral malaria [9]. Unfortunately, its poor solubility in water or oil has hampered further development. A search for a new preparation with a better therapeutic index and good solubility and bioavailability has become the prime target of many laboratories around the world [10–15]. An extensive structural modification of artemisinin to prepare ether derivatives of dihydroartemisinin has been reported in the literature [11, 16, 17]. Herein we wish to report the preparation of a series of new ethers of dihydroartemisinin, some of which possess potent antimalarial activity on oral administration in mice. We also report a series of new thioethers of dihydroartemisinin, as there is no report on thioethers of dihydroartemisinin in the literature. The thioethers discussed here were prepared to study their efficacy against *Plasmodium berghei* K-173 in the mice model.

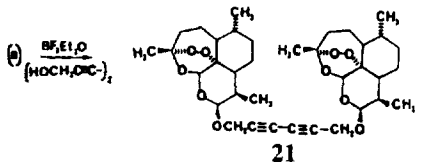
Chemistry

Reduction of artenisinin (**A**) with excess sodium borohydride in methanol at 0–5°C gave dihydroartemisimin [14] (**B**) in 80% yield as reported previously. Treatment of dihydroartemisinin (**B**) with a variety of oxygen or sulfur nucleophiles in the presence of BF_3Et_2O gave the corresponding ether or thioether derivatives (scheme 1). In all cases a mixture of two isomers were formed, as seen from their proton NMR spectra; they were separated using a flash silica column chromatography. The configuration at the C-10 position of the ethers and thioethers was assigned based on the vicinal couplings [12] $J_{10,9}$. The large coupling constant between protons at positions 10 and 9 in the case of the α series, J_{H10H9} = 9.6 Hz, indicates that the relative configuration at the positions 10 and 9 is *trans*. Similarly in the case of the β series the configuration at the positions 10 and 9 is *cis*. In the α-series the signals due to OCH_2 and H_{10} appear upfield compared with those in the β-series.

The aminoalcohols **2–4** were prepared from the epoxide **1** and the aminoethoxy derivative **14** was prepared from the phthalamido derivative **11**. Hexa-2,4-diyne-1,6-diol reacted with two moles of dihydro-



Scheme 1. a: $NaBH_4$/MeOH; b: BF_3·Et_2O, RXH.

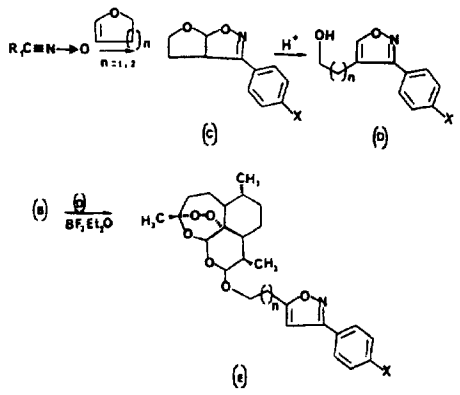


Scheme 2.

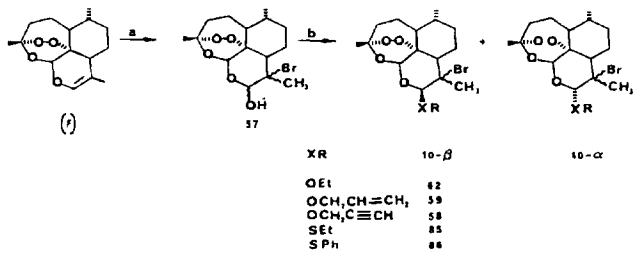
artemisinin (**B**) to give the *bis*-adduct **21** (scheme 2). A system of the type (**E**) (scheme 3), in which the artemisinin unit is attached to a liphophilic group through a pharmocophore and a spacer, is of interest. The pharmocophore could be a heterocycle, namely, isoxazole. In this regard, compounds **37–47** were prepared using the corresponding isoxazole derivatives, which in turn were prepared using dipolar cycloaddition rections.

Isoxazole (**D**) was prepared as shown in scheme 3. The cyclo adduct (**C**) underwent a ring-opening reaction to give 4-substituted isoxazole (**D**), thus providing an easy access to the synthesis of 4-functionalised isoxazoles [18]. Dihydroartemisinin (**B**) reacted with the isoxazole (**E**) to give the product **46** from which the acid derivative **44** was prepared by hydrolysing ester **46** using 0.1 N NaOH solution in methanol. Bromohydrin [19] **57** was prepared from the olefin (**F**) (scheme 4), which in the presence of BF_3Et_2O and either alcohol or thiol underwent nucleophilic substitution to give the derivatives **58**, **59**, **62**, **85** and **86**. In this connection bromoarteether **62** was also prepared. Refluxing the ethers **58** and **59** with $n$$Bu_3SnH$/AIBN in toluene gave the radical-initiated cyclic products **60** and **61** respectively [20] (scheme 5).

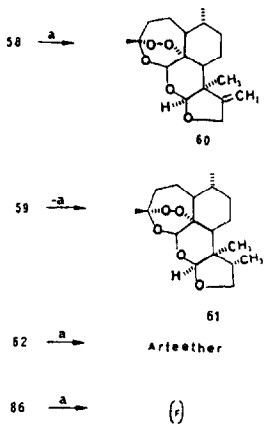
The thioethers **63–89** were prepared using the respective thiols in place of alcohols. In most of the cases the α- and β-diastereomers were separated using flash silica column chromatography. Sulfoxides **67** and **68** were prepared by the oxidation of the respective thioethers **64** and **66**. α,α'-Dimercapto-*m*-xylene reacted with two moles of dihydroartemisinin (**B**) to give the *bis*-adduct **77**. Compounds with an amino group were treated with either ethereal HCl or maleic acid to give the corresponding water-soluble salt.



Scheme 3. X = Cl, n = 2: **37**; X = NO_2, n = 2: **38**; X = CF_3, n = 1: **42**; X = COOH, n = 1: **44**; X = Cl, n = 1: **45**; X = COOEt, n = 2: **46**.



Scheme 4. a: Br_2/H_2O; b: BF_3Et_2O, RXH.



Scheme 5. a: n-Bu_3SnH_4/AIBN.

Compounds containing a free carboxylic acid were treated with aq $NaHCO_3$ to give the corresponding sodium salt of the free acid which were tested for antimalarial activity. Aromatic thiols, heterocyclic thiols were also used as nucleophiles to give the corresponding thioether derivatives. The physical data of the compounds described herein are given in the tables I and II.

Biological results and discussion

All the compounds were initially tested in mice infected with *P berghei* K-173 strain which is sensitive to chloroquine, at a dose of 5 mg/kg × 5 by the subcutaneous route. The active compounds were tested at a lower dose by the same route and also by the oral route at

Table I. Synthesis of 10-ether derivatives of dihydroartemisinin.

Compound	*10-R*	*Isomer*	*Mp (°C)*	*Solvent*[a]	*Yield (%)*	*MF*
	OCH_2CH_3	β	80		75	Ref [9]
1	$OCH_2CH\text{-}CH_2$ (O)	β	55–57		25	$C_{18}H_{28}O_6$
2	[1-(4-Methyl)piperazinyl]$CH_2CH(OH)CH_2O$	β	Oil		41	$C_{23}H_{40}NO_6H_2O$
3	$OCH_2CH(OH)CH_2NEt_2$	β	Oil		70	$C_{22}H_{39}NO_6{\cdot}HClH_2O$
4	(1-Piperidinyl)$CH_2CH(OH)CH_2O$	β	Oil		40	$C_{23}H_{39}NO_6{\cdot}1/2H_2O$
5	$OCH_2CH_2OCH_2CH_2OH$	β	95–97	B	38	$C_{19}H_{32}O_7$
6	$OCH_2CH_2OCH_2CH_2OH$	α	Oil		19	$C_{19}H_{32}O_7$
7	$OCH_2CH(OH)CH_3$	β	Oil		30	$C_{18}H_{30}O{\cdot}2H_2O$
8	$OCH_2CH(OH)CH_3$	α	Oil		11	$C_{18}H_{30}O_6$
9	$OCH_2CH_2CH_2OH$	β	74–75	B	21	$C_{18}H_{30}O_6$
10	$OCH_2CH_2CH_2OH$	α	Oil		10	$C_{18}H_{30}O_6$
11	OCH_2CH_2N(CO, CO)	β	139	C/E	25	$C_{25}H_{31}NO_7$
12	OCH_2CH_2N(CO, CO, Cl)	β	150–152	C/E	32	$C_{25}H_{30}ClNO_7$
13	OCH_2N(CO, CO)	α + β	Oil		27	$C_{24}H_{29}NO_7$
14	$OCH_2CH_2NH_2$	β	Oil		40	$C_{17}H_{29}NO_5$
15	$OCH_2CH_2CH_2NH_2$ maleate	β	138–140	D/E	22	$C_{22}H_{35}NO_9$
16	$OCH(CH_3)CH_2CH_2NH_2$ maleate	α + β	92–94	E	20	$C_{23}H_{37}NO_9H_2O$
17	(1-Piperidinyl)CH_2CH_2O	β	62–64	A	59	$C_{22}H_{37}NO_5$
18	$OCH_2CH(CH_2CH_3)NH_2$ maleate	β	Oil		18	$C_{23}H_{37}NO_9$
19	[1-(4-Methyl)piperazinyl]CH_2CH_2O	β	71–73	B	71	$C_{22}H_{38}N_2O_5$

Table I. *(Continued.)*

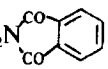
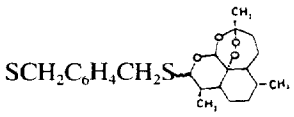
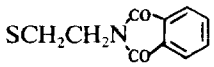
Compound	*10-R*	*Isomer*	*Mp (°C)*	*Solvent*[a]	*Yield (%)*	*MF*
20	$OC(CH_3)(C_2H_5)C{\equiv}CH$	β	Oil		40	$C_{21}H_{32}O_5$
21	$(OCH_2C{\equiv}C)_2C_{15}H_{23}O_4$	β	162–164	B	25	$C_{36}H_{50}O_{10}$
22	$OCH(CH_3)C{\equiv}CH$	β	91–93	B	35	$C_{19}H_{28}O_5$
23	$OC(CH_3)_2C{\equiv}CH{\cdot}1/2H_2O$	β	58–60	B	57	$C_{20}H_{30}O_5{\cdot}H_2O$
24	$[4\text{-}(HOOC)HC{=}CHC_6H_4]\text{-}O\text{-}$	β	146–148	C/B	79	$C_{24}H_{30}O_7{\cdot}1/2H_2O$
25	$[4\text{-}(EtOOC)CH{=}CHC_6H_4]\text{-}O\text{-}$	α + β	137–139	B	19	$C_{26}H_{34}O_7$
26	$OCH_2CH{=}CHC_6H_5$	β	Oil		57	$C_{24}H_{32}O_5$
27	$OCH_2NHCOCH_2C_6H_5$	α + β	163–164	B	12	$C_{24}H_{33}NO_6$
28	$(4\text{-}ClC_6H_4)CH_2CONHCH_2O\text{-}$	α + β	158–160	B	10	$C_{24}H_{32}ClNO_6$
29	OCH_2COCH_3	β	106–107	B	24	$C_{18}H_{29}O_6$
30	$OCH(CH_3)COCH_3$	β	Oil		25	$C_{19}H_{30}O_6$
31	$(4\text{-}ClC_6H_4)\text{-}O\text{-}$	β	58–60	B	35	$C_{21}H_{27}ClO_5$
32	$(4\text{-}COCH_3C_6H_4)\text{-}O\text{-}$	β	Oil		35	$C_{23}H_{30}O_6{\cdot}H_2O$
33	$(4\text{-}COOCH_3C_6H_4)\text{-}O\text{-}$	α + β	145–147	C/B	50	$C_{23}H_{30}O_7$
34	$(4\text{-}COOHC_6H_4)\text{-}O\text{-}$	α + β	167–171	C/B	76	$C_{22}H_{28}O_7$
35	$(4\text{-}NH_2C_6H_4)CH_2CH_2O\text{-}$	β	152–154	E	26	$C_{23}H_{33}NO_5$
36	$(4\text{-}OCH_2C{=}CHC_6H_4)\text{-}O\text{-}$	β	Oil		25	$C_{24}H_{30}O_6$
37	$(4\text{-}[3\text{-}(4\text{-}ClC_6H_4)]Isoxazolyl)CH_2CH_2CH_2O\text{-}$	α	Oil		63	$C_{27}H_{34}ClNO_6$
38	$(4\text{-}[3\text{-}(4\text{-}NO_2C_6H_4)]Isoxazolyl)CH_2CH_2CH_2O\text{-}$	α	Oil		35	$C_{27}H_{34}N_2O_8$
39	$(4\text{-}[3\text{-}(4\text{-}NO_2C_6H_4)]Isoxazolyl)CH_2CH_2CH_2O\text{-}$	β	Oil		40	$C_7H_{34}N_2O_8$
40	$(4\text{-}[3\text{-}Br]Isoxazolyl)CH_2O\text{-}$	β	Oil		63	$C_{19}H_{26}BrNO_6$
41	$(4\text{-}[3\text{-}Cl]Isoxazolyl)CH_2O\text{-}$	β	106	B	53	$C_{19}H_{26}BrNO_6{\cdot}1/2H_2O$
42	$(4\text{-}[3\text{-}(4\text{-}CF_3C_6H_4)]Isoxazolyl)CH_2O\text{-}$	β	117–118	C/B	37	$C_{26}H_{30}F_3NO_6$
43	$(3\text{-}[5\text{-}COOCH_3]Isoxazolyl)\text{-}O\text{-}$		96–98	A	50	$C_{20}H_{27}NO_8$
44	$(4\text{-}[3\text{-}(4\text{-}COOHC_6H_4)]Isoxazolyl)CH_2O\text{-}$	α + β	150–152	C/B	99	$C_{26}H_{31}NO_8$
45	$(4\text{-}[3\text{-}(4\text{-}ClC_6H_4)]Isoxazolyl)CH_2CH_2O\text{-}$	β	146–148	C/B	70	$C_{26}H_{32}ClNO_6$
46	$(4\text{-}[3\text{-}(4\text{-}COOEtC_6H_4)]Isoxazolyl)CH_2O\text{-}$	β	142–143	C/B	55	$C_{28}H_{35}NO_8$
47	$(4\text{-}[3\text{-}(4\text{-}ClC_6H_4)]Isoxazolyl)CH_2O\text{-}$	β	Oil		47	$C_{25}H_{30}ClNO_6$
48	$OCH_2CH_2OCOC(CH_3){=}CH_2$	β	Oil		40	$C_{21}H_{32}O_7$
49	$OCH_2CH_2OCOCH{=}CH_2$	β	Oil		50	$C_{20}H_{30}O_7$
50	$(1\text{-}Piperidinyl)CH_2CH(CH_3)COOCH_2CH_2O\text{-}$	β	Oil		40	$C_{26}H_{43}NO_7$
51	$(1\text{-}Piperidinyl)CH_2CH_2COOCH_2CH_2O\text{-}$	β	Oil		57	$C_{25}H_{41}NO_7$
52	$OCH_2CH_2H_2N$ 	β	47–49	B	50	$C_{26}H_{33}NO_7$
53	OCH_2CH_2CN	β	137–138	C/B	67	$C_{18}H_{27}NO_5$
54	$(3\text{-}[6\text{-}Cl\text{-}Pyridazinoxy])CH_2CH_2O\text{-}$	β	80–82	B	60	$C_{21}H_{29}ClN_2O_6$
55	$(3\text{-}[6\text{-}Cl\text{-}Pyridazinoxy])CH_2CH_2O\text{-}$	α	Oil		40	$C_{21}H_{29}ClN_2O_6$

Table I. *(Continued.)*

Compound	*10-R*	*Isomer*	*Mp (°C)*	*Solvent*[a]	*Yield (%)*	*MF*
56	$OCH[CH_2OCH(CH_3)_2]_2$	β	Oil		39	$C_{24}H_{42}O_7$
63	SC_2H_5	α	51–54	A	48	$C_{17}H_{28}O_4S$
64	SC_2H_5	β	95–97	A	35	$C_{17}H_{28}O_4S$
65	S-Cyclohexyl	β	107–109	B	10	$C_{21}H_{34}O_4S$
66	S-Cyclohexyl	α	98–100	B	62	$C_{21}H_{34}O_4S$
67	$S(O)CH_2CH_3$	β	112–114	A	85	$C_{17}H_{28}O_5S$
68	$S(O)C_6H_{10}$		126–128	C/B	57	$C_{21}H_{34}O_5S$
69	S-β-Naphthyl	β	113–115	A	20	$C_{25}H_{30}O_4S$
70	SCH_2-α-Naphthyl	β	153–155	C/B	20	$C_{26}H_{32}O_4S$
71	S-β-Naphthyl	α	144–145	B	20	$C_{25}H_{30}O_4S$
72	$(4\text{-}COOHC_6H_4)$-S-	β	63–65	C/B	25	$C_{22}H_{28}O_6S \cdot 1/2\ H_2O$
73	$(2\text{-}COOCH_3C_6H_4)$-S-	α	145–147	B	11	$C_{23}H_{30}O_6S$
74	$(2\text{-}COOCH_3C_6H_4)$-S-	β	152–153	B	35	$C_{23}H_{30}O_6S$
75	$(4\text{-}COOCH_3C_6H_4)$-S-	α	130–131	A	20	$C_{23}H_{30}O_6S$
76	$(2\text{-}COOCH_3C_6H_4)$-S-	β	135–137	A	42	$C_{23}H_{30}O_6S$
77		α + β	Oil		10	$C_{38}H_{54}S_2O_8 \cdot 1/2\ H_2O$
78	SC_6H_5	β	95–97	B	22	$C_{21}H_{28}O_4S \cdot 1/2\ H_2O$
79	SC_6H_5	α	85–87	B	30	$C_{21}H_{28}O_4S$
80	SCH_2COOH	β	103–105	B	47	$C_{17}H_{26}SO_6$
81	SCH_2CH_2COOH	β	Oil		43	$C_{18}H_{28}SO_6 \cdot H_2O$
82	SCH_2CH_2OH	α	Oil		41	$C_{17}H_{28}O_5S$
83		α + β	Oil		43	$C_{25}H_{31}NO_6S \cdot 1/2\ H_2O$
84	$(2\text{-Furyl})CH_2S$-	α + β	90–92	A	48	$C_{20}H_{23}O_5S$
87	$SCH_2CH{=}CHC_6H_5$		145–147	C/B	7	$C_{24}H_{32}O_4S$
88	$SCH(CH_3)_2$	α + β	Oil		75	$C_{18}H_{30}SO_4$
89	$SCH_2CH_2NH_2$ maleate		Oil		28	$C_{21}H_{33}NO_8S \cdot 1/2\ H_2O$

[a]A: *n*-pentane; B: petroleum ether; C: methylene chloride; D: ethyl acetate; E, diisopropyl ether.

20 mg/kg × 5 doses. Compounds **22**, **23**, **36**, **66** and **79** were tested against the chloroquine-resistant *P yoelii nigeriensis* (NS) strain for further evaluation. The antimalarial activity of the test compounds was compared with that of chloroquine and arteether. If the compound was found to be active 7d later (d + 7), the treated animals were observed for parasitaemia and mortality on d + 14, d + 21 and d + 28 as reported by Raether and Fink [21]. This method gives a clear idea of whether a particular compound has shown recrudescence. The antimalarial activities of the compounds showing good activity are given in table III. Among the 89 compounds tested, 24 showed total clearance of parasites from the blood smear on d + 7.

Table II. Synthesis of 9- and 10-disubstituted dihydroartemisinin derivatives.

Compound	*10-R*	*9-R_2*	*Mp (°C)*	*Solvent*[a]	*Yield (%)*	*MF*
57	OH	Br	124–125	A	79	$C_{15}H_{23}BrO_5$
58	$OCH_2C{\equiv}CH$ (β)	Br	114–115	A	72	$C_{18}H_{25}BrO_5$
59	$OCH_2CH{=}CH_2$ (β)	Br	107–108	A	61	$C_{18}H_{27}BrO_5$
60	(Scheme 5)		137	A	82	$C_{18}H_{26}O_5$
61	(Scheme 5)		125–128	A	75	$C_{18}H_{28}O_5$
62	OC_2H_5	Br	120–122	A	70	$C_{17}H_{27}BrO_5$
85	SC_2H_5	Br	120–121	A/B	20	$C_{17}H_{27}BrO_4S$
86	SC_6H_5	Br	130–132	A	56	$C_{21}H_{27}BrO_4S$

[a]A: petroleum ether; B: ethyl acetate.

Table III. Blood schizonticidal activity of 10-ether and 10-thioether derivatives against *P berghei* K-173 (strain sensitive to chloroquine) and *P y nigeriensis* NS (strain resistant to chloroquine) in Swiss mice.

Compound	*Dose (mg/kg × 5)*	*Route*	*K-173*		*NS*	
			Parasitaemia (%) on d + 7[a]	*Percentage cured on d + 28*[b]	*Parasitaemia (%) on d + 7*[a]	*Percentage cured on d + 28*[b]
Chloroquine	10	sc	0	29/36(81)	–	–
	5	sc	0	8/27(29)	–	–
	12.5	*po*	0	39/39(100)	–	–
	50	*po*	–	–	> 50	0/22(0)
Arteether	10	sc	–	–	0	5/6(83)
	5	sc	0	13/16(81)	10	0/6(0)
	2.5	sc	0	26/36(72)	–	–
	20	*po*	0	15/28(54)	–	–
12	10	sc	0	5/6(83)		
13	30	sc	0	4/6(67)		
20	5	sc	0	4/6(67)		
22	5	sc	0	6/6(100)	0	11/12(91)
	2.5	sc	0	10/11(91)		
	20	*po*	0	5/5(100)		
23	5	sc	0	10/10(100)	0	0/6(100)
	2.5	sc	0	10/10(100)		
	20	*po*	0	5/6(83)		
30	5	sc	0	6/6(100)		
32	5	sc	0	4/6(67)		
36	5	sc	0	10/10(100)	0	6/10(60)
	2.5	sc	0	1/6(17)		
	20	*po*	1	2/6(33)		
38	5	sc	0	4/4(100)		

Table III. *(Continued.)*

Compound	*Dose (mg/kg × 5)*	*Route*	*K-173*		*NS*	
			Parasitaemia (%) on d + 7[a]	*Percentage cured on d + 28*[b]	*Parasitaemia (%) on d + 7*[a]	*Percentage cured on d + 28*[b]
40	5	sc	0	5/6(83)		
41	5	sc	0	4/6(67)		
47	5	sc	0	3/6(50)		
	20	*po*	0	5/7(71)		
52	5	sc	1	1/6(17)		
56	5	sc	0	4/6(67)		
63	5	sc	0	8/12(67)		
	20	*po*	0	8/9(89)		
64	5	sc	0	5/6(83)		
	20	*po*	0	1/5(20)		
65	5	sc	0	1/6(17)		
66	5	sc	0	11/12(91)	0	16/18(89)
	20	*po*	0	6/18(33)		
76	5	sc	0	3/5(60)		
77	5	sc	5	2/6(33)		
78	5	sc	0	3/6(50)		
79	5	sc	0	11/11(100)	0	4/6(66)
	2.5	sc	0	19/21(90)		
	25	*po*	1	11/11(100)		
83	5	sc	0	9/11(81)		
84	5	sc	0	2/7(28)		

Infected untreated control mice died between d + 6 and d + 8. At that time the average parasitaemia was > 70%. [a]Average percentage parasitaemia. [b]No of mice cured/No of mice treated.

These 24 compounds were tested at lower doses of 2.5 mg/kg × 5 sc and the compounds **22**, **23**, **36**, **66** and **79** showed excellent antimalarial activity. The compounds **22**, **23**, **66** and **79** showed good antimalarial activity against the chloroquine-resistant NS strain. Two compounds, **22** and **23**, had even better activity than arteether against sensitive and resistant strains. The compounds **1–19**, **21**, **24–29**, **31**, **33–35**, **37**, **39**, **42**, **43**, **48–51**, **53–55**, **57–62**, **67–75**, and **80–82** were found to be inactive at 5 mg/kg × 5 sc on d + 7 and were excluded from further studies. In the thioether series, the α-isomers (compounds **66** and **79**) showed better activity than the corresponding β-isomers whereas in the ether series, the β-isomers (compounds **22**, **23** and **36**) showed better activity than the corresponding α-isomers. Further evaluation of the activity of compounds **22** and **23** is underway.

Experimental protocols

Chemistry

All melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were run on a Jeol FX90Q spectrophotometer using Me_4Si as an internal standard. Analysis were performed on a Heraeus microelemental analyser.

Isolation of artemisinin
Around 250 kg of dried leaves harvested from the plant *A annua* was extracted with petroleum ether (30–60°C). Purification using column chromatography over silica gel yielded around 40 g of the pure artemisinin [4–6] (mp 152°C, yield 0.006%), which was used for the preparation of the derivatives reported herein.

Preparation of dihydroartemisinin
Dihydroartemisinin was prepared according to a previously reported procedure [13].

Preparation of 10-[1-(2-hydroxyethoxy)ethoxydihydroartemisinin 5 and 6
To a solution of dihydroartemisinin (0.1 g, 35 mmol) and bis-hydroxy ethyler (1 ml) in 15 ml chloroform was added BF_3Et_2O (3 drops) at 0°C. After the addition, the mixture was stirred in an ice-bath for an additional 15 min. The reaction mixture was then diluted with water and extracted with chloroform. The organic layer was washed with water, dried and concentrated. The crude product was passed through a flash column of silica gel and elution with ethyl acetate/petroleum (1:1) initially gave the β-isomer **5** as a solid, mp 95–97°C; yield 49 mg, 38%. ^{1}H NMR ($CDCl_3$) δ 5.42 (s, 1H, H_{11a}), 4.8 (d, J = 4.5 Hz, 1H, H_{10}), 3.75 (m, 8H, CH_2). Anal ($C_{19}H_{32}O_7$) C, H.

Further elution with ethyl acetate/petroleum ether (1:1) gave the corresponding α-isomer **6** as an oil, yield 25 mg, 19%. ^{1}H NMR ($CDCl_3$) δ 5.24 (s, 1H, H_{11a}), 4.45 (d, J = 9 Hz, 1H, H_{10}), 3.6 (m, 8H, CH_2). Anal ($C_{19}H_{32}O_7$) C, H.

Preparation of 10-β-[(2,3-oxopropan-1)-oxy]dihydroartemisinin 1
The above reaction was carried out using 2,3-oxo-1-propanol in place of hydroxy ethylether. The crude product was passed through a flash column of silica gel to get pure β-isomer **1** as a solidp 55–57°C, Yield 30 mg, 25%. ^{1}H NMR ($CDCl_3$) δ 5.38 (s, 1H, H_{11a}), 4.8 (br d, 1H, H_{10}), 4.2 (dd, 1H, OCH_2), 3.72 (d, J = 3.8 Hz, 1H), 3.4 (m, 1H), 3.14 (m, 1H), 2.7 (m, 4H). Anal ($C_{18}H_{28}O_6$) C, H.

A solution of 10-β-propanepoxide **1** (0.1 g, 0.3 mmol) and diethylamine (0.5 ml) in chloroform (10 ml) was stirred at room temperature for 1 h and then heated at 50°C for 1 h. After the completion of the reaction, the mixture was poured onto ice and extracted with dichloromethane, washed with water, dried and concentrated. The crude product was passed through a flash column of silica gel and on elution with chloroform/methanol (95:5, gave the required product as an oil. The crude product was dissolved in dry ether and ethereal hydrochloric acid was added dropwise until the solution was acidic. Excess hydrochloric acid and the solvent were removed from the mixture and dried to give the product **3** as an oil, yield 96 mg, 70%. ^{1}H NMR ($CDCl_3$) δ 5.40 (s, 1H, H_{11a}), 4.80 (d, J = 3.6 Hz, 1H, H_{10}), 3.79 (m, 3H, OCH_2), 3.43 (m, 2H, CH_2), 2.2–2.8 (m, 12H, NEt_2, CHOH). Anal ($C_{22}H_{39}NO_6HClH_2O$) C, H, N, Cl.

Preparation of 10-β-aminoethoxydihydroartemisinin 14
To a solution of dihydroartemisinin (0.1 g, 0.3 mmol) with hydroxy phthalimide (250 mg, 1.25 mmol) in chloroform (5 ml) was added BF_3Et_3O (4 drops). The product **11** was isolated as described in the preparation of **6** as a solid, mp 139°C, yield 42 mg, 25%. ^{1}H NMR ($CDCl_3$) δ 7.6–7.72 (m, 4H, Ar), 5.24 (s, 1H, H_{11a}), 4.74 (d, J = 3.8 Hz, 1H, H_{10}), 3.98 (m, 4H, OCH_2). Anal ($C_{25}H_{32}NO_7$) C, H, N.

To a solution of phthalimido derivative **11** (45 mg,0.1 mmol) in absolute ethanol (3 ml) was added 99–100% hydrazine hydrate (0.2 ml) and the reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the reaction mixture was heated in diisopropyl ether and filtered. The filtrate was washed with water and concentrated. The crude product was passed through a column of silica gel and upon eluting with diisopropyl ether/isopropanol (85:15) gave the product **14** as an oil, yield 13 mg, 40%: ^{1}H NMR ($CDCl_3$) δ 5.38 (s, 1H, H_{11a}), 4.7 (d, J = 3.6 Hz, 1H, H_{10}), 3.8 (m, 2H, OCH_2), 3.4 (m, 2H, NCH_2). Anal ($C_{17}H_{29}NO_5$) C, H, N.

Preparation of hexa-2,4-diyne-1,6-dioxydihydroartemisinin 21
Following the procedure described in the preparation of **6**, using hexa-2,4-diyne-1,6-diol in place of bis-hydroxy ethylether, compound **21** was obtained as a solid, mp 162–164°C, yield 57 mg, 25%. ^{1}H NMR ($CDCl_3$) δ 5.34 (s, 1H, H_{11a}), 4.9 (d, J = 2.5 Hz, 1H, H_{10}), 4.36 (s, 4H, CH_2). Anal ($C_{36}H_{50}O_{10}$) C, H.

Preparation of isoxazoles (D)
Reaction of propargyl alcohol, dihydrofuran and dihydro pyran with nitrile oxide (prepared *in situ*) were carried out in the usual way. To a suspension of the tetrahydropyran (**C**) (X = Cl) (50 mg, 0.21 mmol) in methanol, conc HCl (1 ml) was added at 5°C. The reaction mixture was brought to room temperature and left for 1 h. The reaction mixture was poured in water and extracted with chloroform, washed with water and concentrated to give the crude product which was purified by passing through a column of silica gel and eluting with ethyl acetate/petroleum ether (1:4). An analytical sample of (**D**) (X + Cl) was prepared by crystallising the column product from ethyl acetate/petroleum ether, mp 57°C, yield 47 g, 94%. ^{1}H NMR ($CDCl_3$) δ 8.2 (s, 1H, H_5), 3.63 (dt, J = 9 Hz, 3.5 Hz, 2H, OCH_2), 2.6 (t, J = 10 Hz, 2H, CH_2), 1.8 (m, 2H, CH_2), 7.26 (d, J = 1 Hz, 2H, ArH), 7.46 (d, J = 12 Hz, 2H, ArH). Anal ($C_{12}H_{12}ClNO_2$) C, H, N, Cl

The procedure described for the preparation of **6** was followed using the hydroxyalkyl isoxazole (**D**) (X = Cl) in place of hydroxy ethylether. The crude product was passed through a column of silica gel and upon elution with ethyl acetate/petroleum ether gave the required compound **37** as an oil, yield 110 mg, 63%. ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H, isoxazole H), 7.48 (dd, J = 7.7 Hz, 4H, Ar), 5.26 (s, 1H, H_{11a}), 4.72 (d, J = 3.8 Hz, 1H, H_{10}), 3.86 (m, 1H, OCH), 3.41 (m, 1H, OCH), 2.62 (m,4H, CH_2). Anal ($C_{27}H_{34}ClNO_6$) C, H, N, Cl.

Similarly, product **46** was obtained as a solid, mp 142–143°C, yield 100 mg, 55%. ^{1}H NMR ($CDCl_3$) δ 7.94 (dd, 4H, Ar), 6.52 (s, 1H, isoxazole H), 5.42 (s, 1H, H_{11a}), 4.92 (d, J = 3.8 Hz, 1H, H_{10}), 4.8 (dd, 2H, OCH_2), 4.38 (q, 2H, CH_2CH_3), 1.4 (t, 3H, CH_3). Anal ($C_{28}H_{35}NO_8$) C, H, N.

Upon treatment with aqueous 0.1 N KOH, compound **46** gave the potassium salt of the corresponding acid **44** from which the free acid was isolated by neutralising the salt to pH 7 using dilute acetic acid. Treatment of the acid with aq sodium bicarbonate gave the sodium salt which was submitted for the biological testing.

Preparation of 10-β-acrylethoxydihydroartemisinin 49
Following the procedure described for the preparation of **6** using hydroxy ethylacrylate in the place of bis-hydroxy ethylether, compound **49** was obtained as an oil, yield 67 mg, 50%. ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 1H, H_{11a}), 4.78 (d, J = 5 Hz, H_{10}), 6.1 (m, 3H, olefinic H), 3.87 (m, 4H, OCH_2). Anal ($C_{20}H_{30}O_7$) C, H.

Though the corresponding α-diastereomer was detected by TLC and ^{1}H NMR, it was found to be negligible and was not isolated. A solution of 10-β-acrylate **49** (25 mg, 0.05 mmol) in piperidine (1 ml) was heated at 60°C for 3 h. After completion of the reaction, the mixture was poured onto ice and extracted with chloroform. The organic layer was washed with water, dried and concentrated. The crude product was passed through a column of silica gel and on elution with ethyl acetate/petroleum ether (1:3) gave product **51** as an oil, yield 12 mg, 37%. ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 1H, H_{11a}), 4.76 (d, J = 3 Hz, 1H, H_{10}), 3.89 (m, 6H, OCH_2), 2.48 (m, 4H, NCH_2), 2.06 (m, 2H, CH_2). Anal ($C_{25}H_{41}NO_7$) C, H.

Preparation of 10-ethoxy-9-bromodihydroartemisinin 62
To a solution of bromoacetal [19] **57** (20 mg, 0.06 mol) in chloroform (3 ml) and ethanol (4 drops), was added BF_3Et_2O (3 drops) at 0–5°C and the reaction mixture was heated at 50°C for 10 h. After the completion of the reaction, the mixture was extracted with chloroform, washed with water, dried and concentrated. The crude reaction mixture was passedthrough a flash column of silica gel and elution with ethyl acetate/petroleum ether (5:95) gave product **62** as a solid, mp 120–122°C, yield 15 mg, 70%. ^{1}H NMR ($CDCl_3$) δ 5.44 (s, 1H, H_{11a}), 4.80 (s, 1H, H_{10}), 3.75 (m, 2H, CH_2), 1.3 (t, 3H, CH_3). Anal ($C_{17}H_{27}BrO_5$). C, H, Br.

9-Bromo-thioether **86**, 9-bromo-10-allylether **59** and 9-bromo-10-propargylether **58** were prepared using the corresponding thiols or alcohols in place of ethanol in the above reaction. In some cases both 10-α- and 10-β-derivatives were isolated but the stereochemistry at the 9-position was not confirmed.

Radical cyclisation reactions [20] compounds 58, 59, 62 and 86
To a solution of 9-bromo-10-β-propargyloxydihydroartemisinin **58** (50m g, 0.13 mmol) in dry toluene (5 ml), were added AIBN (86 mg, 63 mmol) and tributyltin hydride (0.15 ml, 0.15 mmol). The reaction mixture was refluxed for 20 h. Usual work-up followed by passing the crude product through a flash column of silica gel gave the pure product **60** as a solid, mp 137°C, yield 33 mg, 82%. ^{1}H NMR ($CDCl_3$) δ 5.56 (s, 1H, H_{15}), 5.45 (s, 1H, H_{13}), 5.08 (t, 1H, olefinic H), 4.72 (t, 1H, olefinic H), 4.4 (two d, J = 7 Hz, 2H, H_{10}),1.42 (s, 3H, 3-CH_3), 1.4 (s, 3H, 9-CH_3), 1.0 (bd, 3H, 6-CH_3). Anal ($C_{18}H_{26}O_5$) C, H.

Under similar conditions the compound **59** gave compound **61**. In contrast, compounds **62** and **86** gave arteether and dihydroartemisinin (**F**) respectively under similar conditions.

Preparation of 10-α-ethanethiodihydroartemisinin 63 and 10-β-ethanethiodihydroartemisinin 64
To a solution of dihydroartemisinin (0.5 g,1.75 mmol) and ethanethiol (0.26 ml, 3 mmol) in 25 ml chloroform, was added BF_3Et_2O (8 drops) at 0°C. The mixture was then stirred in ice bath for an additional 15 min. The reaction mixture was then diluted with water and the organic layer that separated was washed thoroughly with water, dried and concentrated to obtain an oil which was passed through a column of silica gel. Elution with petroleum ether/ethyl acetate (9.5:0.5) gave the β-isomer **64** as a solid, mp 95–97°C, yield 200 mg, 35%. ^{1}H NMR ($CDCl_3$) δ 5.56 (s, 1H, H_{11a}), 5.24 (d, J = 3.8 Hz, 1H, H_{10}), 2.7 (q, J = 6.4 Hz, 2H, SCH_2), 1.3 (t, 3H, CH_3). Anal ($C_{17}H_{28}O_4S$) C, H, S.

Further elution of the column gave the α-isomer **63** as a solid, mp 51–54°C, yield 276 mg, 48%. ^{1}H NMR ($CDCl_3$) δ 5.2 (s, 1H, H_{11a}), 4.46 (d, J = 10.2 Hz, H_{10}), 2.73 (q, 2H, J = 6.4 Hz, CH_2), 1.3 (t, 3H, CH_3). Ana: ($C_{17}H_{28}O_4S$) C, H, S.

Preparation of 10-β-ethylsulfoxodihydroartemisinin 67
To a solution of 10-β-ethylthio derivative **64** (2 mg, 0.06 mmol) in methylene chloride (3 ml) was added *meta*-chloroperbenzoic acid (9 mg, 0.1 mol) and the reaction mixture was stirred at 0–5°C for 2 h. After completion of the reaction, the mixture was diluted with methylene chloride, washed with aq $FeSO_4$, washed with water, dried and concentrated. The crude product was passed through a column of silica gel and on elution with ethyl acetate/petroleum ether (1:1) gave product **67** as a solid, mp 112–114°C, yield 18 mg, 85%. ^{1}H NMR ($CDCl_3$) δ 5.30 (s, 1H, H_{11a}), 4.62 (d, J = 6.4 Hz, 1H, H_{10}). Anal ($C_{17}H_{28}O_5S$) C, H, S.

Biology

In vivo studies were carried out in Swiss mouse following the method of Peters *et al* [22]. Initially all the compounds were tested against *P berghei* K-173 strain, which is sensitive to chloroquine. The active compounds were tested in Swiss mice against *P y nigeriensis* (NS) strain, which is resistant to chloroquine. All the strains of malaria used in the present study were obtained from Medical Protozoology Department of London School of Hygiene and Tropical Medicine by the courtesy of W Peters.

Inoculum preparation
Infective inoculum was prepared from a previously infected donor mouse with rising parasitaemia (20%). Blood was drawn from the heart of the mouse under ether anesthesia in a sterile heparinized disposable syringe. It was then diluted with sterile RPMI 1640 medium such that 0.25 ml contains about 5×10^6 infected red blood corpuscles. Infection was given to the mice (18–22 g) by the intravenous route (iv) using the tail vein.

Preparation of the test compounds
The compounds were first dissolved in 200 μl of Saffola 'Kardi' oil (commercially available edible oil) followed by the addition of 200 μl of Tween 80 and finally reconstituted in 0.5% Tylose (carboxymethylcellulose) for oral administration. For subcutaneous application, the compounds were dissolved in 200 μl of 'Kardi' oil followed by 200 μl of Tween 80 and finally reconstituted in sterile distilled water. Each mouse received about 0.25 ml of the suspension or solution, according to the body weight, by the subcutaneous route.

Treatment regiment
A slightly modified test procedure of Peters *et al* [22] used. The infected mice were treated with the test compounds for five consecutive days. Treatment began at 2 h post-infection, followed by single daily dose for next four days and the parasitaemia was counted on d +7.

Untreated infected control
The untreated control mice (six per group) were either dead or moribund with high parasitaemia on d + 7. Animals transfer was performed each week to keep the virulence of the strain. When a compound displayed activity, the mice were observed for 28 d for recrudescence following the method of Raether and Fink [21]. In such cases blood smears were also prepared and examined for parasitaemia on d + 14, d + 21 and d + 28. Normally blood smears were drawn on d + 7, fixed in methanol and stained with Gimsa. The slides were scanned under oil immersion lens (100 ×) and parasitaemia was noted. If the mice were free from any malaria parasites it was considered as cured but they were kept under observation for 28 d. At least 250 fields were checked to record the mice as 'cured'.

Acknowledgments

Our thanks are due to PK Inamdar for the spectral and analytical data and to V Shah for the supply of plant extracts.

References

1 Bruce-Chwatt CJ (1987) *Ann Rev Pub Health* 8, 75–110
2 Naig UT, Win UH, Nwe DYY, Myint UPE, Shwe UT (1988) *Trans R Soc Trop Med Hyg* 82, 530–531
3 Kyaw W, Marlar T, Ye T (1992) *Bull WHO* 70, 777–782
4 Klayman DL (1985) *Science* 228, 1049–1055
5 Butler AR, Wu Y (1992) *Chem Soc Rev* 85–90
6 Woerdenbag HJ, Pras N, Uden W, Wallaart TE, Beekman AC, Lugt CB (1994) *Pharm World Sci* 16, 169–180
7 Luo XD, Shen CC (1987) *Med Res Rev* 7, 29–52
8 Acton N, Klayman DL, Rollman IJ, Novotny JF (1986) *J Chromatogr* 355, 448–450
9 Shen CC, Zhuang L (1984) *Med Res Rev* 4, 57–59
10 Gu H, Lu B, Qu Z (1980) *Chung-kuo Yao Li Hsueh Pao* 1, 48–50; *Chem Abstr* (1981) 94, 24954f
11 Cao MZ, Hu SC, Li MH, Zhang S (1982) *Nanjing Yaoueyuan Xuebao* 18, 53; *Chem Abstr* (1984) 100, 34720h
12 Brossi A, Venugopalan B, Dominguez Gerpe L *et al* (1988) *J Med Chem* 31, 645–650
13 Lin AJ, Klayman DL, Milhous WK (1987) *J Med Chem* 30, 2147–2150
14 Lin AJ, Lee M, Klayman DL (1989) *J Med Chem* 32, 1249–1252
15 Lin AJ, Liang-quan Li, Klayman DL, George CF, Flippen-Anderson JL (1990) *J Med Chem* 3, 2610–2614
16 Li Y, Yu P, Chen Y *et al* (1981) *Yaoxue Xuebao* 16, 429–439; *Chem Abstr* (1982) 97, 92245n
17 Yu P, Chen Y, Li Y, Ji R (1985) *Yaoxue Xuebao* 20, 357–365 (Ch); *Chem Abstr* (1986) 105, 24454p
18 Venugopalan B, Shinde SL, Karnik PJ (1994) *Indian J Chem* 33B, 767–768
19 Venugopalan B, Bapat CP, Karnik PJ *et al* (1991) *Eur Pat EP* 456, 149; *Chem Abstr* (1992) 116, P 83708z
20 Venugopalan B, Shinde SL, Karnik PJ (1993) *Tetrahedron Lett* 34, 39, 6305–6308
21 Raether W, Fink E (1979) *Ann Trop Med Parasitol* 73, 505–526
22 Peters W, Howells RE, Portus J, Robinson BL, Thomas S, Warhust DC (1977) *Ann Trop Med Parasitol* 71, 407–418