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Synthesis, and anti-malarial screening, of 1-diethylamino-4-(dihydroartemisinin-10-yl)amino pentane

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Abstract
Artemisinin and its derivatives have become antimalarial drugs of choice because they are effective against most stages in the life cycle of plasmodium and are safe for all, including pregnant women. World Health Organisation (WHO) has nevertheless recommended artemisinin based combination therapy (ACT) to guard against possible development of resistance, as observed with chloroquine. In this study we coupled dihydroartemisinin with 2-amino-5-diethyl aminopentane (the chloroquine handle) by using the Mitsunobu coupling (an S_N2 reaction). This involves the use of diisopropylazodicarboxylate (DIAD), triphenyl phosphine (Ph_3P), 2-amino-5-diethylaminopentane and dihydroartemisinin (DHA) at room temperature to synthesize the target compound, 1-diethylamino-4-(dihydroartemisinin-10-yl)amino pentane {coded: DHA-CQ; IUPAC Name: N^1,N^1-diethyl-N^4-(3,6,9-trimethyldecahydro-3,12-epoxy[1,2]dioxepino[4,3-*i*] isochromen-10-yl) pentane-1,4-diamine}. The structure of DHA-CQ was determined from spectroscopic data (IR, ^{1}H & ^{13}C NMR, MS). The compound was screened at three dose levels of 3 mg/kg, 10 mg/kg and 30 mg/kg, for *in vivo* curative antimalarial activity against mice infected with *Plasmodium berghei berghei.* The target compound also had an LD_{50} of 330 mg/kg in mice by the oral route. A single dose kinetics study was carried out and three different metabolites were identified.

Keywords: Artemisinin; Chloroquine; Dihydroartemisinin; *Plasmodium berghei berghei*; Mitsunobu coupling

INTRODUCTION

Artemisinin and its derivatives have become antimalarial drugs of choice because they are effective against most stages in the life cycle of plasmodium and are safe for all, including pregnant women (All-China Association of Traditional Chinese Medicine, 1982; Yang *et al.*, 1985). World Health Organisation (WHO) has nevertheless recommended artemisinin based combination therapy (ACT) to guard against possible development of resistance, as observed with chloroquine (Ashutosh, 2007; Berman *et* al., 2001; Gupta *et al.*, 2001). In this study we coupled dihydroartemisinin with 2-amino-5-diethyl aminopentane (the chloroquine handle) by using the Mitsunobu coupling (an S_N2 reaction). The Mitsunobu coupling method (Mitsunobu and Yamada, 1967), involves reacting diisopropyl azodicarboxylate (DIAD), triphenyl phosphine (Ph_3P), 2-amino, 5-diethyl amino pentane and dihydroartemisinin (DHA) at room temperature to synthesis our target compound, 1-diethylamino-4-(dihydroartemisinin-10-yl)amino pentane {coded: DHA-CQ; IUPAC Name: N^1,N^1-diethyl-N^4-(3,6,9-

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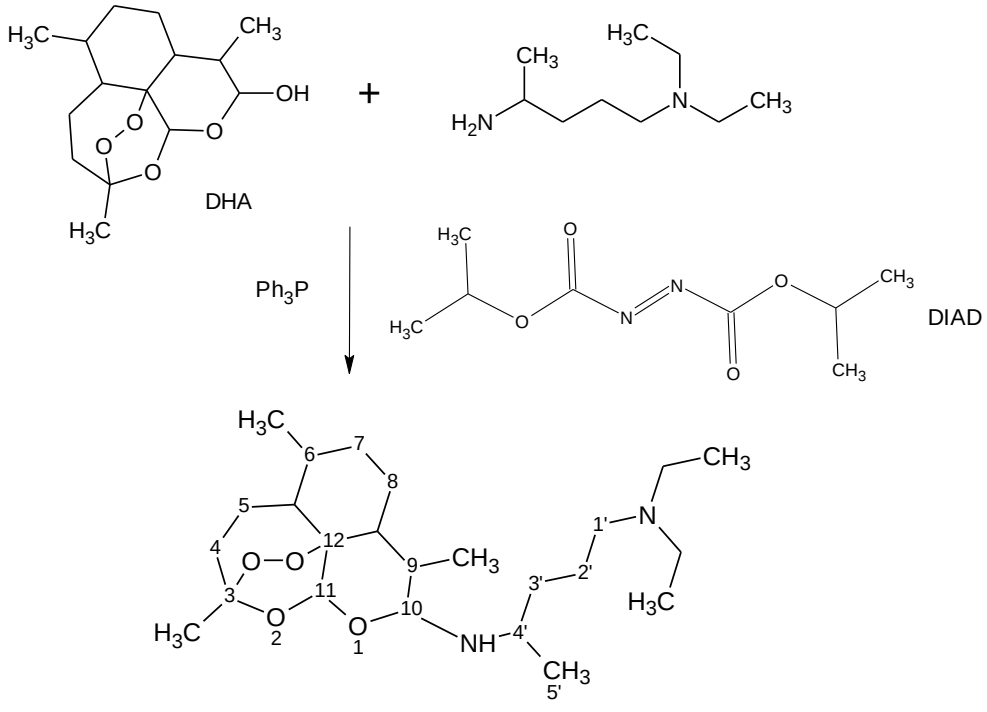
trimethyldecahydro-3,12-epoxy[1,2]dioxepino [4,3-*i*] isochromen-10-yl) pentane-1,4-diamine}.

EXPERIMENTAL

Preparation of DHA-CQ: Dihydroartemisinin (10 mM) and aminopentane (10 mM) were weighed and placed in a flask submerged in ice bath and stirred in 30 mL of dichloromethane (DCM) to form a solution. Equivalent weights of Ph_3P and DIAD were also placed in a conical flask containing 30 mL of DCM submerged in an ice bath. The two resultant solutions were mixed together and stirred for some more time in an ice bath, which was allowed to warm up to room temperature for about an hour (Mitsunobu and Yamada, 1967). The resulting residue was purified by flash column chromatography (50:1 hexane/ EtOAc) to afford the desired product. The product was isolated using AGC and TLC (paper), then subjected to spectroscopic analysis such as IR, NMR and GC-mass spectroscopy.

Antimalarial screening: The compound DHA-CQ was screened at three dose levels of 3 mg/kg, 10 mg/kg and 30 mg/kg, for *in vivo* curative antimalarial activity in mice infected with *Plasmodium berghei berghei*. DHA-CQ had an LD_{50} of 330 mg/kg in mouse by oral route.

Mice weighing (between 20 and 30 g) were grouped in six in five metabolic cages; they were inoculated with *Plasmodium berghei berghei* parasite. After the fourth day, the mice in cages marked I to III were treated orally with DHA-CQ at 3 mg/kg, 10 mg/kg and 30 mg/kg while the ones marked IV and V were treated with 3 mg/kg dihydroartemisinin and 5 mg/kg soya bean oil as positive and negative controls respectively (Table 1). At the end of another four days of treatment, blood smears were made from the tail of the mice on slides. The slides were stained appropriately with Giemsa Stain. The parasite count was done in two fields of view (Yan *et al.*, 2008).



Synthetic scheme for 1-diethylamino-4-(dihydroartemisinin-10-yl)amino pentane (DHA-CQ)

Spectroscopic data for DHA-CQ:

^{1}H NMR: δ 1.71, 1.28, 1.82, 1.35, 1.51, 1.24, 1.51, 1.24, 1.79, 4.39, 1.49, 1.87, 2.01, 2.36

^{13}C NMR: δ 11.54, 14.00, 19.70, 21.99, 23.20, 23.55, 24.55, 24.80, 25.30, 33.60, 34.71, 35.95, 37.40, 40.43, 41.70, 46.46

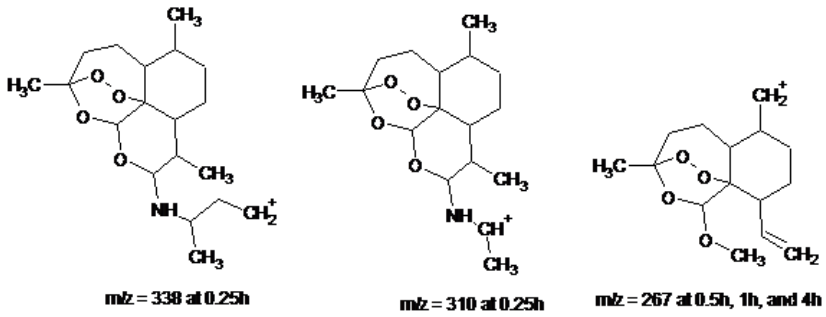
IR: (cm^{-1}): 3337.93, 2893.32, 1924.06, 1403.26, 1057.99, 881.50, 440.75

MS ($^{m}/_{z}$): 424, 338, 310, 267

Table1: Curative effect of DHA-CQ against *Plasmodium berghei berghei* infected mice

Treatment	Mean parasite count	% Suppression
Soya bean oil (5 mg/kg)	19.50 + 1.50	-
DHA (3 mg/kg)	2.50 + 0.50	87.17
DHA-CQ (3 mg/kg)	1.50 + 0.00	92.31
DHA-CQ (10 mg/kg	1.50 + 0.00	92.31
DHA-CQ (30 mg/kg)	0.75 + 0.25	96.15

Values expressed as mean± SEM, n=5, **(P<0.01), ***(P<0.0001)



Metabolites identified by GC-MS at various time intervals after administration of DHA-CQ

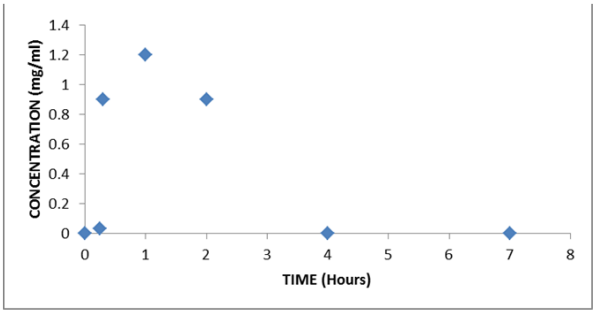


Figure 1: DHA-CQ concentration against time

Table 2: Showing the concentration, % intensity and retention time of DHA-CQ with respect to time

Time (h)	Conc (mg/mL)	% Intensity	RT (min)
0	0	0	26.583
0.25	0.03	1	27.1
0.3	0.9	30	26.9
1	1.2	40	26.9
2	0.9	30	26.9
4	0	0	26.9
7	0	0	27.1

DISCUSSION

The percentage suppression obtained clearly indicates that the synthesized compound possesses antimalarial activity against *Plasmodium berghei berghei*. A better activity was observed for the test compound as compared to dihydroartemisinin indicating a better antimalarial activity. The negative control which received no treatment but only soya beans had zero percentage suppression indicating that the antimalarial activity is attributed to the test drug (DHA-CQ) and not to any other factor.

The better antimalarial activity observed could be as a result of the chloroquine coupled to the dihydroartemisinin. This arm could act as both a pharmacophoric region as well as aid its penetration to diseased red blood cells. A dose dependent increase was observed in activity was observed with the test drug as the dose increased from 3 mg/kg to 30 mg/kg. No significant difference in activity was observed at the dose of 3 mg/kg and 10 mg/kg.

The use of software such as ACD/Lab to predict the median lethal dose [LD_{50}] which is the dose of the drug required to kill 50% of the test animal is actually a new trend in research (Aarons, 1999; Anari and Baillie, 2005). It obviates the use of animals in the determination of LD_{50} as well as saving both time and cost. It improves the efficiency of the researcher and reduces the overall time taken. However, its major shortcoming is that its idiosyncratic reactions and individual variation cannot be predicted using this software. Also *in vitro* models are not always the same with what is observed *in vivo* (Kaira *et al*., 2005; Steimer *et al.,* 1993).

The GC-MS results, alongside the fragmentation pattern of DHA-CQ, suggest that the compound was synthesized. GC-MS analysis was also used for the analysis of the compound's metabolites in mice after a single oral dose (Hop *et al.*, 2002; Hop and Prakash, 2005; Hopfgartner and Zell, 2005). The high sensitivity and selectivity of mass spectrometry detects low concentration compounds in complex biological matrices (Hsieh *et al*., 2006). It is expected that amino compounds would typically undergo Phase I N-dealkylation metabolism when used *in-vivo*. Structure-activity relationship studies has shown that dealkylated amines are good substrates for Phase II acetylation reaction (Franklin, 1995; Hurst *et al*., 2007; Lindeke *et al*., 1979), making the drug more readily excreted and hence reducing the chances of toxicity. However such metabolites were not detected in this study.

In conclusion, the synthesized compound, DHA-CQ [1-diethylamino-4-(dihydroartemisinin-10-yl)amino pentane], in this study, has proved to be more effective than dihydroartemisinin in the treatment of *Plasmodium berghei berghei* induced malaria in mice. This is evidenced by its large % suppression of *Plasmodium berghei* compared to DHA. It suffices to say that this compound is a promising antimalarial drug that can eliminate malaria parasite in man if further toxicological and structure-activity relationship studies are carried out.

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