Journal of Pharmacognosy and Phytotherapy Vol. 3(11), pp. 160-163, December 2011
Available online at http://www.academicjournals.org/jpp
DOI: 10.5897/JPP10.027
ISSN 2141-2502 ©2011 Academic Journals

Full Length Research Paper

4-(ethyl-p-chlorophenylglycinylamino)-7-chloroquinoline: Synthesis and *in vivo* antimalaria evaluation

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Accepted 21 September, 2011

4-amino-7-chloroquinoline derivative was synthesized and characterized using both1D and 2D nuclear magnetic resonance (NMR) techniques, with the application of correlation spectroscopy (COSY), nuclear overhauser enhancement spectroscopy (NOESY), heteronuclear multiple bond correlation (HMBC), heteronuclear single quantum coherence (HSQC), liquid chromatography-mass spectroscopy (LC-MS) and infra-red (IR) spectroscopy. The *in vivo* antimalaria activity of the compound against *Plasmodium berghei berghei* (Pbb) was assessed within 5 days (D5) of treatment. The antimalaria effect of JH7E evaluated at D30 shows a mean inhibition of parasitemia 46.32, 60.40 and 85.71% recorded on Pbb effected mice at 25, 50 and 100 mgKg^{-1}body weight dosages respectively as compared to 97.04 and 94.40% curative rate obtained for artemisinin and chloroquine (Standard drugs) used as positive control. The results of the investigation showed that JH7E is a possible lead candidate for the development of antimalarial drug, because of its strong antimalarial activity against Pbb.

Key words: 4-amino-7-chloroquinoline, antimalaria, *Plasmodium bergei bergei*. Spectroscopy

INTRODUCTION

Malaria is a serious, relapsing infection in humans, characterized by periodic attacks of chills and fever, *anemia,* enlargement of the spleen and often fatal complications. It is caused by one-celled parasites of the genus *plasmodium* that are transmitted to humans by the bite of *Anopheles* mosquitoes (Cooper and Magwere, 2008). The spread and distribution of malarial continue to be one of the greatest health problems facing Africa. The disease has become a global issue, over three billion people are living under the threat of malaria and it has been estimated that, there are 300 to 500 million new cases resulting in about 2 million deaths annually. The disease is endemic in about 100 developing countries, accounting for about 40 to 45 million disability adjusted life years (DALYs) and kills an estimated 1.2 million people each year in Africa. Making it one of the top killer diseases among communicable diseases in Africa (WHO, 2009) in Sub-Saharan Africa, one in five children will die before they are five and 75% of those deaths are attributed to malaria (Nwaka, 2005). Pregnant women and children under five years of age are the most vulnerable. The socioeconomic consequences of this disease are particularly dramatic in rural areas where poverty and malnutrition are more pronounced. In the absence of an effective vaccine, the fight against malaria depends on chemotherapy and the reduction and prevention of human/*Anopheles* mosquito contacts through the use of insecticides treated bed nets, insecticides and environmental care. The problem is further compounded by the emergence of drug resistant strains and the limited chemotherapeutic drugs available for treatment (Stepmiewska and White, 2008; Winstanley, 2000; Plowe et al., 1995). In this paper we report our investigation on the *in vivo* antimalaria evaluation of a synthesized compound based on the 4-amino-7-chloroquinoline nucleus a metabolic by-product of chloroquine.

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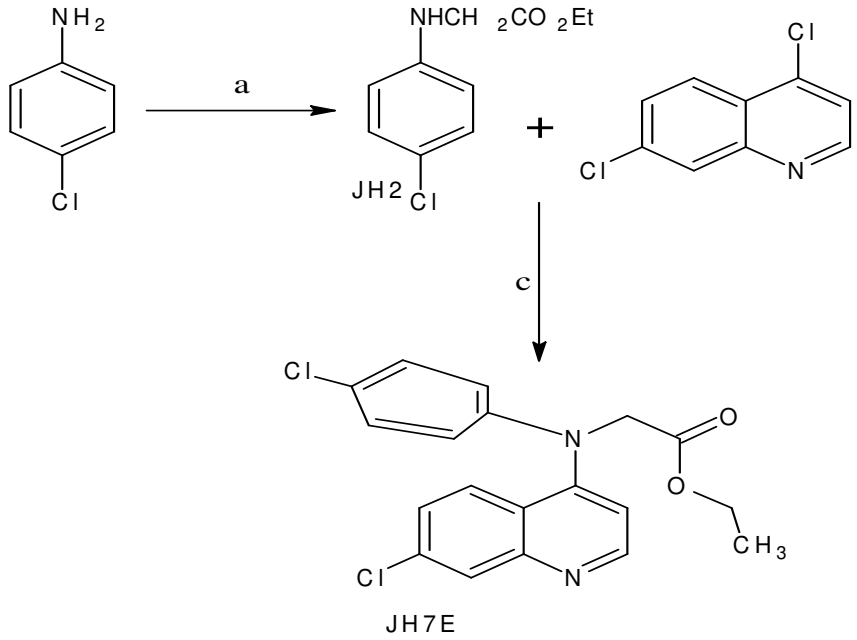

Figure 1. Synthesis of 4-(ethyl -p-chlorophenylglycinylamino)-7-chloroquinoline (a) $ClCH_2CO_2Et$, CH_3COONa, reflux 6hrs (b) PheOH, N_2 reflux 7 h.

MATERIALS AND METHODS

All commercially available reagents were used without further purification unless otherwise stated. All reactions were performed in pre-dried apparatus. The progress of the reaction was monitored by analytical thin layer chromatography (TLC) performed on Merck silica gel 60 F_{254} plates. Visualization was performed under UV-light at 254 nm and using 10% Sulphuric acid. The melting point (uncorrected) was determined on a Stuart Scientific SMP1 apparatus. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectrum (1D and 2D) were recorded on a Bruker-Advance 400 MHz FT-NMR Spectrometer operating at 400 MHz, using the residual solvent ($CDCl_3$) peaks as internal standard. The Electron Impact Mass Spectroscopy (LC-MS) was performed on Agilent Technologies 1200 Series Binary SL, the Infra-red spectroscopy were run on Perkin Elmer Spectrum 100 FTIR Spectrometer.

Preparation of 4-(ethyl-4-chlorophenyl glycinylamino)-7-Chloroquine (JH7)

This was done according to a modified method described elsewhere (Burnett et al. 20076, Elderfield et al. 1946). A mixture of 4,7-Dichloroquinoline (28 g), Phenol (85 g) and 4-chlorophenyl glycine ethyl ester (15 g) in a three neck round bottomed flask fitted with a magnetic stirrer. The mixture was heated under reflux condition at 180°C in Nitrogen gas atmosphere for 7 h. Afterwards it was allowed to cool to room temperature and NaOH (2M, 8%) solution was then added until the mixture was strongly alkaline. Diethyl ether (100 ml × 2) was then added and the two layers were separated using a separatory funnel. The organic layer was basified with NaOH (2M, 8%) separated and washed with distilled water then acidified with acetic acid (5%) until the solution was slightly acidic. The two layers were separated and to the aqueous layer was added 5% ammonia solution the precipitate formed was then filtered and washed thoroughly with water. It was re-crystallized from ethanol-water mixture Figure 1.

In-vivo antimalaria test

Male swiss albino mice (18 to 25 g) obtained from Department of Biochemistry Ahmadu Bello University, Zaria-Nigeria. Were acclimatized for a period of 10 days at National Research Institute For Chemical Technology (NARICT) Basawa, Zaria Nigeria. The mice were infected with 0.2 ml of infected blood containing about 1 × 10^7 dose of *Plasmodium berghei berghei* (NK65) from a donor mouse. Each mouse was inoculated on day one, intraperitoneally (Odetola and Bassir, 1980).

Drug administration

The drugs, chloroquine and artemisinin (Gumede et al., 2003) were used as positive control, while dextrose Normal saline was used as negative control and the compound JH7E was used as the treatment drug, all were administered intraperitoneally.

Acute toxicity (LD_{50}) test

A modified method of determining LD_{50} was used to determine the toxicity level of the compound in mice (Lorke, 1983; Odetola and Bassir, 1980). Two groups (A, B) containing four mice each were subjected to treatment intraperitoneally with 10% DMSO dextrose normal saline and solution of the compound (in 10% DMSO dextrose normal saline) at 100 mg/kg body weight, the maximum test concentration to be used. They were kept in check for ten days and no mortality was recorded from each group. This implies that the maximum test concentration was not a lethal dose.

Curative test

This was carried out according to method described elsewhere (Peters, 1970). Briefly, 20 mice were weighed, labeled and grouped

Table 1. ^{13}C-NMR ($CDCl_3$) δ (ppm).

Carbon position	^{13}C *calc	^{13}C expt	CHn	^{1}H	Hn
2	152.85	153.00	CH	8.71 d	1
3	108.43	114.09	CH	6.56 d	1
4	153.86	156.09	C	-	-
5	127.10	127.41	CH	8.38 d	1
6	122.35	122.85	CH	7.58 d	1
7	130.21	129.60	C	-	-
8	119.87	120.35	CH	8.18 d	1
9	136.68	136.66	C	-	-
10	133.88	138.68	C	-	-
1’	52.91	45.87	CH_2	3.90 s	2
2’	170.70	171.05	C=O	-	-
3’	61.86	61.49	CH_2	4.28 q	2
4’	14.19	14.19	CH_3	1.35 t	3
1”	142.09	145.61	C	-	-
2”, 6”	115.82	115.39	CH	6.88 d	1
3”, 5”	130.16	130.45	CH	7.61 d	1
4”	124.10	123.51	C	-	-

*ACD/CNMR DB Demo Application File; Version 3.0.3.0; Advanced Chemistry Development Inc.

Table 2. *In vivo* antimalaria evaluation of JH7.

Concentration (mgkg^{-1})	D_6	D_{18}	D_{30}
25	18.22±2.5	27.71±1.8	46.32±0.5
50	38.53±0.5	45.15±2.0	60.40±1.0
100	57.14±1.5	74.30±0.6	85.71±2.5

Table 3. *In vivo* antimalaria evaluation of the positive and negative control.

Concentration (5 mgkg^{-1})	D_6	D_{18}	D_{30}
Dihydroarteminisinin	78.47±0.5	95.16±1.5	97.04±2.0
Chloroquine	76±0.2	90.20±1.5	94.40±2.5
Normal saline	Dd	Dd	Dd

D_6 = day 6, D_{18} = day 18, D_{30} = day 30, Dd = died.

into four different groups of five mice each, these were infected with 0.2 ml of the standard inoculums. Doses of 25, 50 and 100 mg/kg body weight of the compound and 5 mg/kg body weight of chloroquine, arteminisinin (standard group) and 5 ml/kg body weight normal saline (control group) were administered for five days (D_1-D_5). At D_6 thin blood films collected from the tail region were prepared for parasitaemia determination. The blood films were examined using a light microscope X400 and the parasitized erythrocytes on each slide counted (Tables 2 and 3).

RESULTS AND DISCUSSION

The compound JH7 was synthesized from 4,7-dichloroquinoline and 4-chlorophenylglycine ethyl ester the nucleophile, to give a silvery plate-like crystals which was recrystalized from ethanol-water mixture. The melting point was recorded as 86 to 89°C; the structure was determined by the application of 1D (Table 1) and

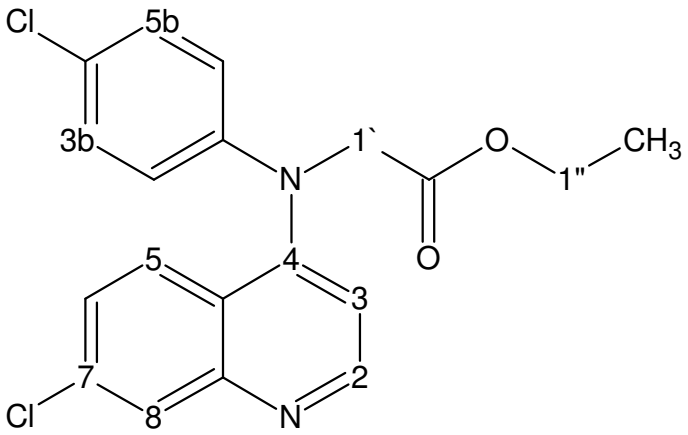

Figure 2. Compound JH7.

2D NMR technique, including correlation spectroscopy (COSY), nuclear overhauser enhancement spectroscopy (NOESY), heteronuclear multiple bond correlation (HMBC) and heteronuclear single quantum coherence (HSQC) experiments. The ^{13}C-NMR signal δ_c 114.09, 156.09, 127.41, 122.85, 129.60 and 120.35 were assigned to the quinoline ring while the signals δ_c 145.61, 115.39, 130.45 and 123.51 were assigned to the benzene ring (Table 1). The Distortionless Enhancement Polarization Transfer (DEPT) experiment shows one methyl (CH_3), two methylene (CH_2) Carbon atoms, sevenmethine (CH) Carbon atoms and seven quaternary Carbon atoms. The molecular formula $C_{19}H_{16}Cl_2N_2O_2Na$ was assigned by electron impact mass spectroscopy (EI-MS) the molecular ion $[M+H]^+$ at m/z 374.8 is in agreement with the formula mass 375.2 gmol^{-1}. The Infra red ($IR_{\square max}$ cm^{-1}) spectrum indicate the presence of N-H stretch signal at 3375 cm^{-1}, the signal at 2920 cm^{-1} is attributed to C-H stretching vibration from alkane (CH_3, CH_2). The band at 1719 cm^{-1} is characteristic of carbonyl carbon (C=O, ester), the absorption at 1606 cm^{-1} is due to (C=C aromatic), the vibration at 1488 cm^{-1} is characteristics of CH_3- and CH_2- stretch. The *in vivo* antimalaria study (Tables 2 and 3) of the compound shows a dose dependence percentage inhibition at day six (D_6), day eighteen (D_{18}) and day thirty (D_{30}) for the three concentration used (25, 50 and 100 mgkg^{-1}body weight). The mice in group D which served as positive control (PC) had no parasitemia inhibition and all the mice died between day 8 and day 14. The 10% DMSO used as a diluents (DC) for JH7E had no parasitemia inhibition therefore indicating that the observed activity against Pbb was that of JH7E. The antimalaria effect of the compound (JH7) evaluated after day thirty (D_{30}) shows a mean inhibition of 46.32, 60.40 and 85.71% recorded at 25, 50 and 100 mgkg^{-1}body weight dosages, respectively as compared to 97.04 and 94.40% curative rate obtained for artemisinin and chloroquine (standard drugs) used as positive control. The result of the investigation shows that the compound JH7 is a potent antimalaria agent, worth further investigation Figure 2.

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