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SYNTHESIS AND ANTIMALARIAL EVALUATION OF 1-(2,4-DIMETHOXYPHENYL)-3-(4-TRIFLUORO METHYL-PHENYL) PROPAN-2-ENE-1-ONE

[1]*A. N HAMZA, [1]A.Y IDRIS, [1]A.M MUSA and [2]A.B OLORUKOOBA

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria

[2]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria

Corresponding Author: hamza.kyauta.asmau@gmail.com

ABSTRACT

Malaria is one of the most serious health problems worldwide and Its treatment has been compromised by drug resistance. Chalcones represent an important chemical scaffold with promising antimalarial activity. A chalcone derivative; 1-(2,4dimethoxyphenyl)-3-(4-trifluoro methyl-phenyl) propan-2-ene-1-one (Compound P21) was synthesized by modified Claisen-Schmidt condensation reaction. The structure of compound P21 was established using Fourier transform infrared (FT-IR), proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy, and also mass spectrometry (MS). The compound was screened for *in-vivo* antimalarial activity in mice infected with *P berghii* parasite, using curative model. Compound P21 displayed appreciable activity (66% parasitaemia suppression) comparable to that of chloroquine (5 mg kg^{-1}) at a dose of 175mg kg^{-1} in the curative test ($p<0.05$). The present finding suggests that compound P21 is a promising antimalarial compound and is a candidate for further optimization and evaluation.

Keywords: Malaria, Chalcones, Synthesis.

INTRODUCTION

Malaria is a devastating infectious disease caused by a parasitic protozoan; *Plasmodium* and, it is prevalent in many tropical and subtropical areas (WHO, 2017). In Nigeria, malaria is the most significant public health problem and a risk for 97% of Nigeria's population (WHO, 2017). To date, resistance have emerged, towards all classes of antimalarial drugs except for the artemisinins. However, prominent reports have recently noted delayed parasite clearance suggestive of decreased drug sensitivity in Southeast Asia (WHO, 2017). Hence, this has motivated scientists to look for alternate effective and cost-effective medicines for the treatment of malaria.

In this context, chalcones (Figure 1) have attracted much attention due to their wide range biological profiles. Chalcones belongs to flavonoid family with discriminating suppression against *P. falciparum*, first reported for licochalcone A from a Chinese liquor rice (Chen *et al*., 1994). Chalcones were also identified through molecular modelling studies as potent inhibitors of plasmodial aspartate proteases or cysteine proteases (Li *et al*, 1995). Thereafter, so many derivatives were synthesized and found to have promising antimalarial activity (Liu *et al*, 2001; Liu *et al.,* 2003; Kumar *et al.,* 2010).

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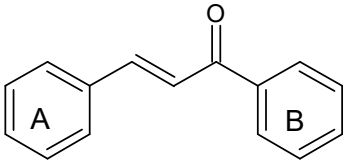

(2*E*)-1,3-diphenylprop-2-en-1-one

Figure 1: A chalcone

Previous studies showed the strong dependence of antimalarial activity on the specific substitution of rings **A** (Benzaldehyde ring) and **B** (Acetophenone ring) (Li *et al,* 1995; Liu *et al*, 2001; Liu *et al.,* 2003; Kumar *et al.,* 2010). Therefore, Li *et al,* 1995 revealed the importance of electron withdrawing substituents on ring **A** (decreased electron density) and electron donating substituents on ring **B** (increased electron density) for good antimalarial activity of chalcones (Li *et al,* 1995).

The current study, synthesized and studied the *in vivo* antimalarial activity of 1-(2,4dimethoxyphenyl)-3-(4-trifluoro methyl-phenyl) propan-2-ene-1-one (referred to Compound P21) (Figure 2) based on Li *et al,* 1995 proposal, to study the influence of substitutions on the two aromatic rings to add to the library of potential antimalarial chalcones.

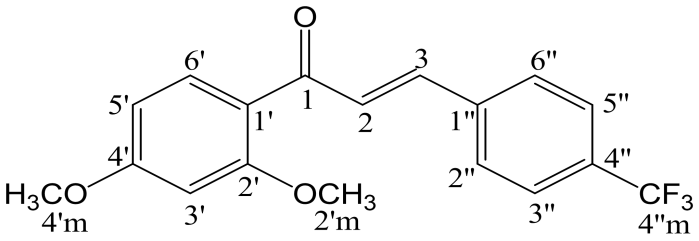

Figure 2: Compound P21

MATERIALS AND METHODS

Experimental Animals

Adult Swiss albino mice (18-22g) of both sexes, locally bred in the Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU), Zaria were used for the experiment. The animals were housed in clean polypropylene cages under standard laboratory conditions and allowed free access to a standard rodent pellet diet and water *ad libitum*. All experimental protocol on animals was in accordance with Ahmadu Bello University Research Policy and guides for the use and care of laboratory animals as accepted internationally.

Malarial Parasite

Chloroquine-sensitive malaria parasites (*Plasmodium berghei* NK 65) were obtained from the Department of Microbiology-National Medical Research Institute (NIMR), Lagos. The parasites were maintained viable in new groups of mice by continuous intraperitoneal injection of 0.2 ml of infected erythrocytes every four days (Adzu *et al*, 2007).

Evaluation of Theoretical Bioavailability

Prior to synthesis, the bioavailability of the chalcone was predicted theoretically using Lipinski ‘s rule of five, with SWISS ADME software (http://www.swissadme.ch).

Synthesis of Compound P21

The compound was synthesized via base catalyzed condensation of equimolar amounts of 2,4-dimethoxy acetophenone and 4-trifloromethyl benzaldehyde using NaOH as a catalyst (Figure 2). Silica gel thin layer

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chromatography (TLC) was used to monitor the progress of the reaction with ethyl acetate: n-hexane (7:3) as developing solvent (Kumar *et al*, 2010). The appearance of a single new spot and disappearance of the reactants spot indicate the formation of the product, which were visualized under 254nm ultraviolet light and iodine vapor.

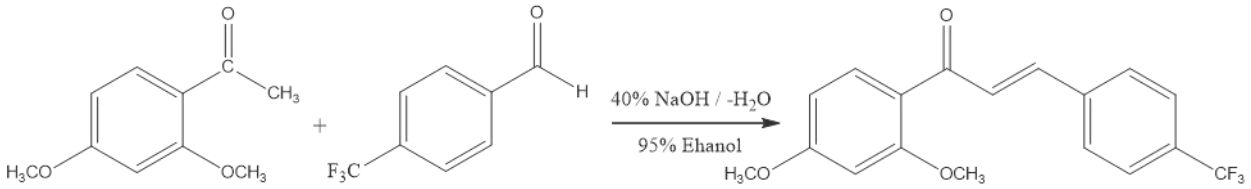

Figure 3: Scheme for the synthesis of compound P21.

Characterization

Melting points were recorded in open capillaries with gallenklamp melting point apparatus and were uncorrected. Detailed structural analysis of the synthesized compounds was performed using Fourier Transformed Infrared Spectroscopy (FTIR), Mass Spectrometry (MS) as well as, proton (^{1}H) NMR and, carbon-13 (^{13}C) NMR, Spectroscopy.

FTIR data were reported in terms of frequency of absorption ν cm-^{1}. Data for ^{1}H NMR were reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, dd = doublet of a doublet, m = multiplet), integration (J in Hz). Data for ^{13}C NMR reported in terms of chemical shift (δ ppm).

Evaluation of *In vivo* Antiplasmodial Activity

Blood samples were collected in a heparinized capillary tube via the tail vein from donor mouse with *P berghii* Parasitemia level of about 20-25 % and then transferred into a sterile plain bottle. About 2ml of the blood was diluted with 10 ml normal saline such that 0.2 ml contained approximately $1x10^7$ infected red blood cells. Each mouse was then inoculated intraperitoneally with 0.2 ml of blood suspension (Kalra *et al.,* 2006).

Evaluation of the curative potential of compound P21 against established plasmodia infection was carried out following the method of Ryley and Peters, 1970 (Rane 's test). Briefly, the mice were inoculated with parasites as described above and left untreated until the fourth-day post-inoculation. After parasitemia was established, the mice were randomly divided into five groups of five mice each. Groups 1-3 were intraperitoneally administered with 25, 50 and, 100 mg/kg body weight of test compound respectively. Group 4 and 5 were administered 25 mg/kg of chloroquine and 0.2 ml of 1%w/v acacia suspension intraperitoneally respectively. The treatment was given for four consecutive days. On the fifth day, blood was collected from the tail vein of each mouse and smeared on a slide to make a thin film for parasitemia determination (Saidu *et al,* 2000).

Average percentage parasite suppression relative to the control was calculated for each treatment group using the formula below (Iwalewa *et al,* 2008);

$$\%\ \textbf{Parasitemia} = \frac{\text{mean parasitemia}(-\text{ve control}) - \text{mean parasitemia}(+\text{ve control})}{\text{mean parasitemia}\ (-\text{ve control})} \text{x}100$$

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Data Analysis

SPSS statistical software (IBM SPSS statistic 25) was used to determine the mean parasitemia level and standard error of the mean. The mean values of the control group were compared with the mean values of the groups treated with the test compounds using one-way analysis of variance (ANOVA) test, followed by Bonferroni post-hoc test for statistical significance and P values <0.05 were considered significant. % parasitemia suppression for each group was computed.

RESULTS

Table 1: Analysis of theoretical bioavailability of compound P21

Parameters	Compound P21	Accepted values	Inference
Molecular weight	336g/mol	≤ 500g/mol	Pass
Log P	4.75±0.59	≤5	Pass
H-bond donors	Nil	≤ 5	Nil
H-bond acceptors	Two	≤10	Pass

Table 2: Percentage Yield and Some Physical Properties of compound P21

Physical properties	**Compound P21**
Percentage yield (%)	58.63
Melting point (^{0}C)	78-80
Rf value [a]	0.45
Appearance/Colour	Light yellow crystals

(a) Silica Thin layer chromatography plate was used with n-hexane: ethyl acetate (7:3) as development solvent. Rf is a retention factor.

Characterization

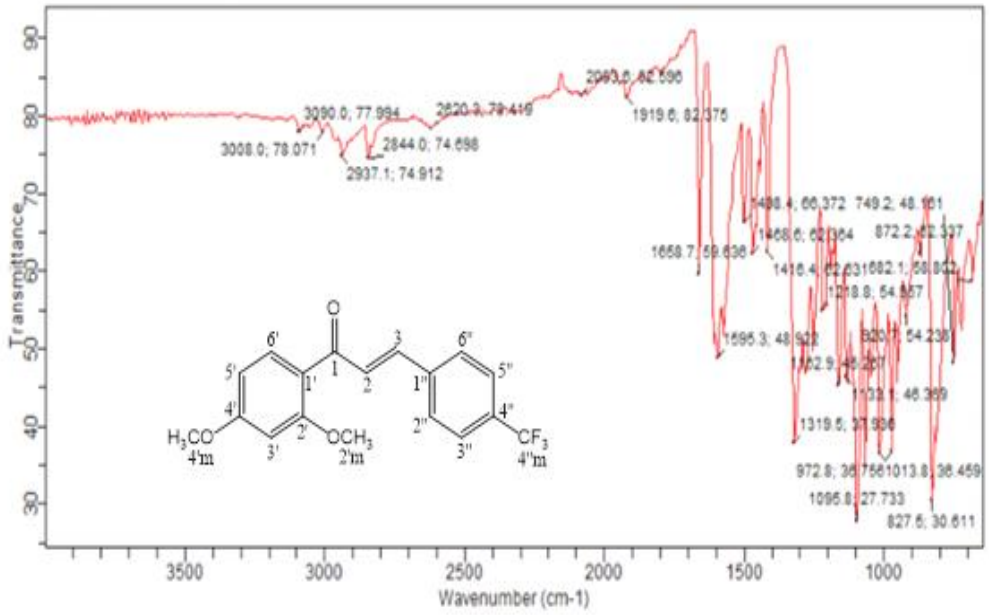

Figure 4: FT-IR Spectrum of compound P21

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Table 3: FT-IR Analysis of Compound P21

Wave Number (cm^{-1})	Assignment
1595	C=O stretch
1658	C=C stretch
1133	C-F stretch (aromatic)
2937	-C-H stretch (aliphatic)
3008, 3090	=C-H stretch(aromatic)
1319, 1095	C-O stretch(aromatic)

Table 4: ^{1}H and ^{13}C NMR Assignment of compound P21

Carbon Position	P21	
	^{13}C δ(ppm)	^{1}H δ(ppm)
1	189.76	-
2	124.75	7.60(d, J=16Hz,1H)
3	139.60	7.80(d, J=17.0Hz,1H)
1’	125.71	-
2’	160.60	-
3’	98.52	6.53(d, J=2.8Hz,1H)
4’	164.57	-
5’	105.35	6.57(dd, J=8.0Hz;2.8Hz,1H)
6’	133.13	7.82(d, J=9.0Hz, 1H)
1”	138.93	-
2”	128.57	7.68(d, J=9.0Hz, 1H)
3”	128.16	6.59(d, J=9.3Hz, 1H)
4”	129.33	-
5”	127.98	6.59(d, J=9.3Hz, 1H)
6”	128.30	7.68(d, J=9.0Hz, 1H)
2’m	55.73	4.04 (3H)
4’m	55.57	3.89 (3H)
4”m	121.66	-

In-vivo Antimalarial Studies (Curative test)

The curative potential of compound P21 is represented in Table 4, with appreciable paracetemia suppression.

Table 5: Effect of compound P21 on the parasitemia level of *P. bergheii* infected mice

Treatment groups	Dose (mg/kg)	Mean parasitemia	% Parasitemia Suppression
Normal saline	0.2ml	3.00 ± 0.00^{a}	0%
Group I	175	1.00 ± 0.00^{b}	66.7%
Group II	350	1.20 ± 0.20^{b}	60%
Group III	700	1.40 ± 0.25^{b}	53.3%
Chloroquine	5	1.00 ± 0.00^{b}	66.7%

Values are presented as Mean ± SEM; Data analyzed by one-way ANOVA followed by Bonferroni ‘s post-hoc test; n=5 a=$p<0.05$, b=$p<0.01$ versus control; Route of administration=ip

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DISCUSSION

Compound P21 (Figure 2) have passed the criteria put forward by Lipinski *et al.* (2004) for a chemical compound to be orally bioavailable (Table 1), and is likely to be drug candidate. Prediction of theoretical oral bioavailability is an important step in the drug design process (Drews, 2000). This is to reduce the chances of drug failure at a later stage of the process due to poor pharmacokinetics. The procedure applied for the synthesis of compound 21 furnishes a good yield of 58.63% (Table 2). The sharp melting point (Table 2) observed with the compound suggest that the compounds have high degree of purity (Vogel, 1956).

The FT-IR spectra (Figure 3) showed characteristic chalcone carbonyl peak C=O appearing at 1595 cm^{-1} and olefinic =C-H vibration at 3008 cm^{-1} is an indication of the formation of chalcone. The ^{1}H NMR and, ^{13}C NMR spectra confirmed the structures of compound P21 (Table 4). The characteristics α, β- unsaturated ketone linker protons as doublets were observed in the region of 7.60 ppm (H- α) and 7.80 ppm (H- β) with coupling constants of 16 and 17 Hz respectively (*trans*-isomers) and the methoxy protons appeared as singlet in the region of 3.89 and 4.04 ppm, the rest of the protons in the structure appeared in their expected regions with their usual coupling constants. ^{13}C NMR spectra of compound P21 (Table 4) showed the carbonyl carbon (C=O) of the α, β-unsaturated ketone linker in the characteristic region of 189.76. Also, the α and β- carbon atoms with respect to the carbonyl group give rise to characteristic signals of δ 124.25 - 139.60 ppm respectively. The positions of all carbon atoms in compound P21 have been assigned as shown on Table 4. The NMR spectra agree with the literature (Liu *et al.,* 2001).

The mass spectral data of compound P21 (Supplementary information) indicated the molecular ion peak (m/z) corresponding to its molecular weight (Table 1). The molecular ion peak $(M+1)^{+}$ is 337.

Rane 's test evaluates the curative capability of test compounds on established infection (Ryley and Peters, 1970). Compound P21 did not eradicate *P. falciparum* parasite completely on four days treatment but showed significant parasite suppressive effects compared to the negative control. Compound P21 at dose of 175 mg/kg has the same % suppression as chloroquine that is 66.7 % (Table 5). Additionally, at the dose of 350 and 700 mg/kg compound P21 has % suppression of 60 and 53.3 % respectively (Table 5). The response was dose dependent, with the smallest dose given higher suppression of the parasite. This study agrees with the findings of previous researches on the importance of electron withdrawing groups on ring A and electron donating group on ring B for good antimalarial activity (Li *et al.,* 1995; Liu *et al.,* 2001; Liu *et al.,* 2003).

CONCLUSION

1-(2,4dimethoxyphenyl)-3-(4-trifluoro methyl-phenyl) propan-2-ene-1-one (referred to Compound 21) have shown promising *in-vivo* antimalarial activity as compared to control. Theoretical bioavailability evaluation of the compounds revealed the potential of this compound as a drug candidate. The results of this study present compound P21 as a potential lead for optimization toward the development of a new therapeutic agent to fight malaria parasite.

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