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Synthesis of Some Novel and Potent Anti-Plasmodial Aminoalkyl Chalcone Derivatives

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Abstract: Malaria remains to be a health and an economic burden to many people living in Sub-Sahara and Africa. According to World health Organization (WHO) in 2017, 219 million cases of malaria worldwide were documented. Its increase by 2 million from the year 2016 resulted in 435 thousand deaths every day among 1190 deaths of young children more than cases 5 per day have been reported. Among the different species of plasmodium parasite, *Plasmodium falciparum* is mainly responsible for causing malaria in Africa whereas *Plasmodium vivax* is the most prevalent in countries outside Africa. Africa suffers from malaria a lot. Almost half of the world's population is at risk of contracting malaria and approximately 90% of the death cases of Malaria in the world appear from Africa. In our research group in past, we have established that chalcones with an aminoalkyl moiety on one of the aromatic rings have promising *in vitro* antimalarial activity. We successively enhanced the bioavailability from 3% to 25% in mice model with the derivatization of the potential leads with the help of substitutions of the function groups at 4- position from -F to -CF_3.

Keywords: Synthesis; Characterizations; Chalcones; Bioavailability.

1. Introduction

Malaria remains to be a health and an economic burden to many people living in the world; particularly in Asia, Sub-Sahara and Africa [1-5]. Due to drug resistance against many current treatments, including the first-line treatment based on the plant derived compound, artemisinin, there is a dire need to identify new antimalarial medicines [6-10]. We have established that chalcones with an aminoalkyl moiety (Scheme 1) on one of the aromatic rings have promising *in vitro* antimalarial activity.

The Mannich reaction was used to introduce the aminoalkyl moiety. This reaction requires an aromatic OH at the ortho position. We have established that chalcones with an aminoalkyl moiety on one of the aromatic rings have promising in vitro antimalarial. This finding supports our hypothesis that nitrogen containing flavonoids will have enhanced biological activity as compared to naturally occurring non nitrogen containing flavonoids. We synthesize a total of 60 compounds in an effort to enhance bioactivity, reduce toxicity and increase oral bioavailability. Some of our compounds have similar or lower IC 50 values than chloroquine against chloroquine sensitive malaria strains (D10, NF54).

There is little difference in activity against chloroquine sensitive and chloroquine resistant strains (Dd2, K1) as is evident from the small differences in IC50 values.

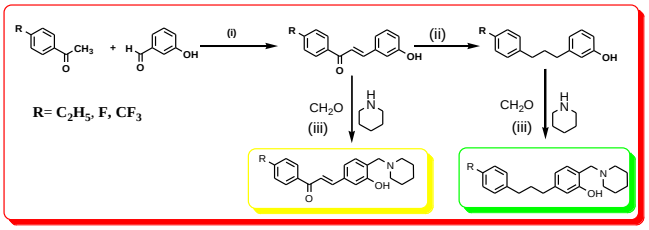
This activity against drug resistant malaria strains is probably to be expected as our compounds are novel with structures totally unrelated to the currently used antimalarial drug. Our reported antimalarial compounds are relatively non-complicated and inexpensive to manufacture and promise cheap antimalarial drugs [11-12]. Toxicity test (in vitro CHO cell assays) suggests that our compounds are relatively nontoxic with high SI indices. This is supported by initial in vivo tests on mice which heaved no adverse effects after dosage with selected analogs. Initial *in vivo* bioavailability determinations afforded poor results (3%). Efforts to enhance bioavailability via protecting the phenolic OH group against first pass metabolism (prodrug strategy) failed because the OH group resisted ether or ester formation. We attribute this to hydrogen bonding of the ortho OH to the ortho aminoalkyl group. Further investigations indicated that bulky groups on the A-ring increase bioavailability. Our best candidate with large moieties on the A-ring so far has a bioavailability of 25%. Further work is in progress to increase the bioavailability of our compounds to a standard that is attractive to pharmaceutical companies in the near future.

2. Materials and Methods

All chemicals were purchased from Sigma-Aldrich and used as received. Melting points were recorded in open capillaries.^{1}H NMR spectra were recorded in $CDCl_3$on a Bruker Bio-Spin spectrometer at 600 MHz using TMS as an internal standard. Mass spectra ESIMS were recorded and IR spectra were recorded on a Shimadzu FTIR spectrometer in KBr pallets.

2.1. General Procedure for the synthesis of Aminoalkylated Chalcones via the Mannich reaction.

A mixture of the appropriate chalcone (1 eq.), paraformaldehyde (1.5 eq.), and the appropriate amine (2 eq.) was dissolved in EtOH (2 mL) and conc. HCl (5 drops). The reaction mixture was refluxed for 9 hours until TLC (Toluene: Acetone 7:3) showed the disappearance of the starting material. The reaction mixture was quenched with solid $NaHCO_3$ and extracted with EtOAc (2 x 50 mL) and water (2 x 50 mL). The organic layers were combined, dried by using anhydrous Na_2SO_4 and concentrated under reduced pressure to afford crude product. The crude product was further purified by column chromatography. During this study, several substituents on ring A such as -H, -CH_3,-C_2H_5, -F and -CF_3 were employed.



Scheme 1. Reagents and conditions: (i) 50% KOH-solution, EtOH, rt; (ii) Pd $(OH)_2$/C, H_2, EtOAc:H_2O, (iii) EtOH & HCl reflux (9 h).

2.2. Bioassay.

Antimalarial activity screening of the synthesized compounds was used to determine the potential of these compounds as sources of antimalarial compounds [13]. A good source of antimalarial compounds should inhibit parasites selectively and be harmless towards other cells. Cytotoxicity experiments were used to determine general toxic properties of compounds against living cells. Antimalarial activity and cytotoxicity information are used to determine

selectivity indexes, which are used as a guide to determine the potential of a compound for further investigation. An active antimalarial compound should be at least tenfold more active against the targeted organism than against mammalian cells to be considered for further testing. Cytotoxicity of the synthesized compounds was tested against Chinese hamster ovarian (CHO) cells which were cultured according to a standard operating procedure prepared by the collaborators. The MTT assay as described by Mosmann (with minor modifications) was used to determine cell viability [14].

2.3. Experimental procedure.

2.3.1. Synthesis of 5-(3-(4-fluorophenyl)propyl)-2-(piperidin-1-ylmethyl)phenol (A).

Compound (A) was synthesized according to the general procedure using 3-(3-(4-fluorophenyl)propyl)phenol (0.200 g; 0.87 mmol), paraformaldehyde (0.052 g; 1.73 mmol), and piperidine (0.18 mL; 1.84 mmol) as starting materials. The crude reaction mixture was chromatographed (TLC, Toluene: Acetone 7:3). The synthesized compounds were well characterized in our previous studies [11, 15]. The fraction R_f 0.50 yielded 5-(3-(4-fluorophenyl)propyl)-2-(piperidin-1-ylmethyl)phenol (A) as a yellow oil (0.269 g, 95%) (Figure 1).

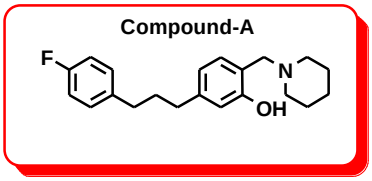


Figure 1. Structure of 5-(3-(4-fluorophenyl)propyl)-2-(piperidin-1-ylmethyl)phenol.

^{1}H NMR (600 MHz, $CDCl_3$, Me_4Si) δ ppm: 1.64 - 1.32 (6H, H-3''', H-4''', H-5'''), 1.87-1.78 (2H, m, H-2), 2.41 - 2.04 (4H, H-2''', H-6'''), 2.50 - 2.46 (2H, m, H-2), 2.55 - 2.51, (2H, m, H-3), 3.55 (2H, s, CH_2), 6.50 (1H, dd, J = 7.6, 1.3 Hz, H-6''), 6.57 (1H, d, J = 1.3 Hz, H-2''), 6.78 (1H, d, J = 7.6 Hz, H-5''), 6.87 (2H, t, 3JH-H = 8.6 Hz; 4JH-F = 8.6 Hz, H-3', H-5'), 7.05 (2H, dd, 3JH-H = 8.6 Hz; 4JH-F = 5.5 Hz, H-2', H-6'); ^{13}C APT NMR (150 MHz, $CDCl_3$, Me_4Si) δ ppm 24.0 (C-4'''), 25.9 (C-3''', C-5'''), 32.9 (C-2), 34.6 (C-1), 35.1 (C-3), 53.9 (C-2''', C-6'''), 61.9 (CH_2), 115.0 (2C, d, $^2J_{C-F}$ = 20.9 Hz, C-3', C-5'), 116.0 (C-2''), 119.0 (C-6''), 119.1 (C-4''), 128.3 (C-2''), 129.7 (2C, d, $^3J_{C-F}$ = 7.7 Hz, C-2', C-6'), 138.0 (1C, d, $^4J_{C-F}$ = 3.2 Hz, C-1'), 142.8 (C-1''), 158.0 (C-3''), 161.2 (1C, d, $^1J_{C-F}$ = 243.1 Hz, C-4') ; ^{19}F NMR δ (282.4 MHz, $CDCl_3$, C_6F_6) -118.1 (s, F); IR (neat): ν_{max} cm^{-1} = 819.83, 1218.86, 1508.34, 2934.84; Found (TOF MS ES) $[M+H]^+$ 328.2073, ($C_{21}H_{26}FNO + H^+$) : *m/z* 328.2077; HPLC purity 96.9%, t_R = 1.62 min.

2.3.2. Synthesis of 5-(3-(4-ethylphenyl)propyl)-2-(piperidin-1-ylmethyl)phenol (B).

Compound (B) was synthesized using 3-(3-(4-ethylphenyl)propyl)phenol (0.100 g; 0.42 mmol), paraformaldehyde (0.076 g; 2.53 mmol) and piperidine (0.10 mL; 1.00 mmol) according to the general procedure. The crude reaction mixture was chromatographed (TLC, Toluene: Acetone 7:3). The fraction R_f 0.50 yielded 5-(3-(4-ethylphenyl)propyl)-2-(piperidin-1-ylmethyl)phenol (B) as a yellow oil (0.087 g, 62%) (Figure 2). ^{1}H NMR (600 MHz, $CDCl_3$, Me_4Si) δ ppm 1.14 (3H, t, J = 7.6 Hz, CH_3), 1.38 (2H, broadend s, 1 x N-CH_2), 1.58 – 1.51 (4H, m, 2 x N-CH_2), 1.89 – 1.80 (2H, m, H-2), 2.26 – 2.02 (2H, m, CH_3-CH_2), 2.57 – 2.29 (8H,

m, H-1, H-3, 2 x N-CH_2), 3.54 (2H, s, CH_2), 6.51 (1H, dd, J = 7.6, 1.4 Hz, H-6''), 6.58 (1H, d, J = 1.4 Hz, H-2''), 6.77 (1H, d, J = 7.6 Hz, H-5''), 7.03 (4H, s, H-2', H-6', H-3', H-5'); ^{13}C NMR (150 MHz, $CDCl_3$, Me_4Si) δ ppm : 15.7 (CH_3), 24.1 (C-4'''), 25.9 (C-3''', C-5'''), 28.5 (CH_3-CH_2), 32.8 (C-2), 35.0 (C-1), 35.2 (C-3), 53.9 (C-2''', C-6'''), 62.0 (CH_2), 116.0 (C-2''), 119.0 (C-4''), 119.1 (C-6''), 127.8 (C-3', C-5'), 128.3 (C-5''), 128.4 (C-2', C-6'), 139.6 (C-1'), 141.5 (C-1''), 143.1 (C-4'), 158.0 (C-3''); IR (neat): ν_{max} cm^{-1} = 782.72, 1390.20, 1452.40, 2919.13; Found (TOF MS ES) $[M+H]^+$ 338.2486, ($C_{23}H_{31}NO + H^+$) : *m/z* 338.2484. HPLC purity 99.4%, t_R = 1.75 min.

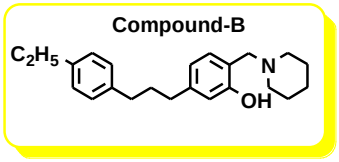


Figure 2. Structure of 5-(3-(4-ethylphenyl)propyl)-2-(piperidin-1-ylmethyl)phenol.

2.3.3. Synthesis of 2-(piperidin-1-ylmethyl)-4-(3-(4-(trifluoromethyl)phenyl)propyl)phenol (C).

Compound (C) was synthesized according to the general procedure using 4-(3-(4-(trifluoromethyl)phenyl)propyl)phenol (116)(0.050 g; 0.18 mmol), paraformaldehyde (0.052 g; 1.73 mmol), and piperidine (0.18 mL; 1.84 mmol) as starting materials. The crude reaction mixture was separated by flash column chromatography (H: EtOAc 6:4, 1.5 cm x 15 cm). The fraction R_f 0.52 yielded 2-(piperidin-1-ylmethyl)-4-(3-(4-(trifluoromethyl)phenyl)propyl)phenol (C) as a yellow oil (0.058 g, 86%) (Figure 3).

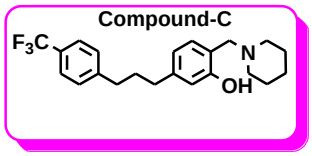


Figure 3. Structure of 2-(piperidin-1-ylmethyl)-4-(3-(4-(trifluoromethyl)phenyl)propyl)phenol.

^{1}H NMR (600 MHz, $CDCl_3$, Me_4Si) δ ppm: 1.40 (2H, s, H-4'''), 1.61 – 1.51 (4H, m, H-3''', H-5'''), 1.90 - 1.75 (2H, m, H-2), 2.49 - 2.42 (2H, m, H-3), 2.63 - 2.56 (2H, m, H-1), 3.16 - 2.02 (4H, H-2''', H-6'''), 3.55 (2H, s, CH_2), 6.66 (1H, d, J = 8.2 Hz, H-5''), 6.67 (1H, d, J = 2.2 Hz, H-2''), 6.88 (1H, dd, J = 8.2, 2.2 Hz, H-6''), 7.20 (2H, d, J = 8.0 Hz, H-2', H-6'), 7.45 (2H, d, J = 8.0 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $CDCl_3$, Me_4Si) δ ppm: 24.0 (C-4''') (Plate 45b and 45c), 25.9 (C-3''', C-5'''), 33.0 (C-2), 34.5 (C-1), 35.2 (C-3), 53.9 (C-2''', C-6'''), 62.2 (CH_2), 115.8 (C-2'', C-5''), 121.4 (C-6''), 124.4 (1C, q, $^1J_{C-F}$ = 272.2 Hz, CF_3), 125.2 (2C, q, $^3J_{C-F}$ = 3.9 Hz, C-3', C-5'), 128.1 (1C, q, $^2J_{C-F}$ = 32.3 Hz, C-4'), 128.3 (C-4''), 128.4 (C-6''), 128.7 (C-2'), 132.1 (C-1'), 146.6 (C-1''), 156.1 (C-3''); ^{19}F NMR δ (282.4 MHz, $CDCl_3$, C_6F_6) -118.1 (s, CF_3); IR (neat): ν_{max} cm^{-1} = 1067.03, 1116.64, 1323.65, 1497.96, 2936.14; Found (TOF MS ES) $[M+H]^+$ 378.2043, ($C_{22}H_{26}F_3NO + H^+$) : m/z 378.2045; HPLC purity 87.3%, t_R = 1.73 min.

3. Results and Discussion

The collection of blood samples was done by tail bleeding at predetermined time intervals. The compounds having substituents 4-C_2H_5 and 4-CF_3 were incubated *invitro* with human and mouse liver microsomes. In addition, the compounds were subjected to a parallel artificial membrane permeation assay. The results of *in vitro* studies revealed rapid

absorption and hepatic metabolism of both the compounds B and C. The maximum concentration of drug (C_{max}) for the compounds B and C were observed to be 0.2 ±0.4 µM and 0.7± 0.3 µM, respectively and the elimination half-life of both compounds was 6.1 h. Thus, we could successfully enhance the bioavailability from 3% to 25%.

Pharmacokinetic studies were conducted for the compounds B & C in C57/BL6 mice using reported method [7]. Doses of the compounds used are summarized in Table 1. The collection of blood samples was done by means of tail bleeding at pre-determined time intervals and the drug concentrations were determined with a LC/MS/MS method. During our study we found that the bioavailability of the compound was enhanced after the replacement of bulky groups at aromatic group. The *in vitro* studies were found to be more promising against *Plasmodium falciparum.* Fluorine at the 4th position exhibited showed low bioavailability.

We synthesized different aminoalkylated diarylpropanes possessing 4-CH_3 group and 4-CF_3 group substitutions as an attempt to enhance the bioavailability. Pharmacokinetic studies were screened in C57BL/6 mice having with 15 mg/kg 4-CH_3 or 4-CF_3 administered orally and the 5 mg/kg 4-CH_3 or 4-CF_3 administered intravenously.

Table 1. Pharmacokinetic parameters for the synthesized compounds B and C following oral and i.v. administration in mice.

Compound B				
Pharmacokinetic parameter	**IV administration (5 mg/kg)**		**Oral administration (15 mg/kg)**	
	Average	**SEM**	**Average**	**SEM**
C_{max} (µM)	n/a	n/a	0.180	0.010
T_{max} (min)	n/a	n/a	0.500	0.00
Apparent half-life (h)	6.033	0.338	n/a	n/a
AUC (min.µmol/L)	142	17	31	9
BA (%)	n/a	n/a	7	2

n/a = not applicable

Compound C				
Pharmacokinetic parameter	**IV administration (5 mg/kg)**		**Oral administration (15 mg/kg)**	
	Average	**SEM**	**Average**	**SEM**
C_{max} (µM)	n/a	n/a	0.727	0.118
T_{max} (min)	n/a	n/a	0.500	0.00
Apparent half-life (h)	6.173	0.570	n/a	n/a
AUC (min.µmol/L)	239	32	178	7
BA (%)	n/a	n/a	25	1

n/a = not applicable

4. Conclusions

In summary, herein we report synthesis, characterization and anti-plasmodial activity of some aminoalkyl derivatives via the Mannich reaction. The *in vitro* absorption, metabolism and *in vivo* pharmacokinetics studies confirm the study revealed better absorption of both the compounds B and C as compared to A. The in vitro and in vivo clearance values were found to correlate well. This has a triggered structure-activity relationship in an attempt to block metabolic sites without compromising the anti-malaria activity which will be a future guide of this series.

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Conflicts of Interest

The authors declare no conflict of interest.

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