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Syntheses and antimalarial screening of some tris-benzylamine analogues

Ezekiel O. Afolabi[1*], Francis M. Agwom[1], Usman O. Quadri[1], David Arome[2] and Taiwo E. Alemika[1]

[1]Department of Pharmaceutical Chemistry; [2]Department of Science Laboratory Technology (Physiology–Pharmacology Section), University of Jos, Jos. 930001, Plateau State. Nigeria.

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Abstract
A number of literature reports have implicated some tris-benzylamines isolated from plant origin to be responsible for their anti-malaria activity. In view of these claims, we synthesized six analogues of tris-benzylamines and screen them for in-vivo curative anti-malaria activity. All the compounds synthesized showed good curative activities against infected Mice with *Plasmodium berghei* with dihydroartemisinin as positive control. The lethal dose [LD_{50} (mg/Kg)] in Mice of the compounds were predicted with ACD/iLab computer software. The structures of the compounds were confirmed with their mass spectra fragmentation patterns.

Keywords: Tris-benzylamine; Antimalarial; Dihydroartemisinin; *Plasmodium berghei berghei*

INTRODUCTION

Malaria is an infectious disease that is caused by Plasmodium spp, which is transmitted by the female Anopheles mosquito. The World Health Organisation (WHO) , has and is still making efforts to combat malaria, hence, forms part of the Millenium Development Goal (MDG). However, malaria is still a major health problem in the world especially in Africa and Asia. In addition resistance to antimalarial drugs is also on the increase even with the popular WHO recommended artemisinin combination therapy (ACT).

Over the years there had been indigenous therapy for treating malaria, one of such is found in the use of the stem bark of Locust bean plant (*Cerotonia siliqua*). Builders *et al.* 2012 had identified N,N-bis(3,4,5-trihydroxybenzyl)-1-(3,4,5-trihydro xyphenyl) methanamine (a tribenzylamine) as the chemical agent responsible for its anti-malaria activity.

Tribenzylamines are tertiary amines in which the three hydrogens in ammonia are replaced by benzyl group. They were first synthesised by Leuckart, in 1885. The Leuckart reaction is a reductive ammination, in which aldehydes and ketones are reacted with formamide or ammonium formate (Webers and Bruce, 1948; Lejon and Helland, 1999). In order to increase the yield of tribenzylamine, the reaction has been modified by the addition of acetic acid or formic acid.

* Corresponding author. *E-mail*: afolabie@yahoo.co.uk *Tel*: +234 (0) 8035889579
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Tribenzylamine have also been recently reported to be contained naturally in the Hop plant. One major biological activity of the tribenzylamines is their activity as Tyrosine kinase enzyme inhibitors, which make them potential antiproliferative agents.

Antimalarial test can be carried out either by *in vivo* or *in vitro* method. The *in vivo* method can be curative where the infection is first established in the experimental animal before treatment with the test drug or a prophylactic method where treatment with test drug before infecting the experimental animals with Plasmodium parasite to see if the drug will be able to prevent the development of infection.

In addition to the antimalarial screening, the effect of one of the synthesised compounds on urine output, electrolyte and creatinine level were also determined in order to assess any possible toxicity and drug disposition.

EXPERIMENTAL

Synthesis of tribenzylamines. A 5mMol of the aldehydes were refluxed with slightly excess formamide and acetic acid at 180°C exhaustively. The compounds were then extracted with diethyl ether, the aqueous portion neutralised with sodium carbonate and further extracted with double portion of diethyl ether. The combined ethereal extract was then washed with saturated sodium bicarbonate solution and brine. The same procedure was repeated on the aqueous portion using ethyl acetate.

Physicochemical data determination. The melting points of the synthesized compounds were determined using melting point apparatus. ^{13}C NMR and ^{1}H NMR spectral data were determined using the ACD/LAB software. GC/MS data: retention time and the corresponding mass spectra of the compounds were obtained on Agilent QP 2010 from NARICT lab, Zaria. Thin Layer Chromatography (TLC) profile was determined with ethyl acetate/ methanol/ ammonium hydroxide solvent system.

Antimalarial screening. The mice were weighed and grouped in threes in metabolic cages, they were then inoculated with *Plasmodium berghei berghei* parasite for four days. The mice were then treated orally with the compounds for another four days using dihydroartemisinin and no treatment as positive and negative control respectively. At the end of the four days treatment, blood smears were made from the tail of the mice on slides. The slides were then stained appropriately with Giem-Stain. The parasite count was done in two field microscopic view.

RESULTS AND DISCUSSION

The thin layer chromatography (TLC) of the synthesized and purified compounds showed one spot with no significance difference observed in their retardation factor (R_f) values which indicates the compounds are more likely to be chemically related while two compounds, D5 and D6, showed a lower R_f value which could be attributed to the presence of hydroxyl group in their structure. For all the compounds identification of spot using the UV light at 254nm because of the presence of the three benzene rings chromatophores present in their structures.

The highest yield was obtained with compound D2 (40.9%) which agrees with that documented in literature where it is said that 100g of aldehyde will yield 40g of tribenzylamine (Vasca-Lanza, 1982) and which has been explained by Vincent *et al.* (2008) to be due to the fact that three molecules of aldehyde will be needed to form one molecule of a tribenzylamine coupled with CO_2 and NH_3 gas losses especially in an exhaustive reaction. The other compounds whose yields are lower than 40% could arise from losses through spills during purification.

The HNMR and CNMR spectra are quite simple with no much multiplicities

because base on the chemical structure of the compounds there are no much coupling occurring but equivalent protons and carbon atoms are observed. The only differences in the spectra that exist are due to the different functional groups. This simplicity in spectra also existed with that in the GC-MS fragmentation patterns obtained.

HNMR DATA (Frequency=400MHz)

D1: δ3.66 (S), δ5.94(S), δ6.63(S), δ6.83(S), δ6 .95(S)
D2:δ3.61(S), δ3.66(S), δ3.74(S), δ6.82(S)
D3:δ3.66(S), δ7.33(S), δ7.50(S), δ7.98(S), δ8.13(S)
D4:δ2.89(S), δ3.58(S), δ6.68(S), δ7.19(S)
D5:δ3.66(S), δ3.88(S), δ5.82(S), δ6.80(S), δ7.18(S)

CNMR DATA

D1: δ=56.37, 100.89, 108.23, 121.82, 134.78, 146.49, 147.58
D2: δ=56.32, 56.37, 60.20, 110.47, 138.30, 142.70, 153.18
D3: δ=56.37, 121.96, 122.58, 128.32, 129.74, 139.46, 148.49
D4: δ=40.30, 56.37, 112.30, 128.77, 138.58, 150.00
D5: δ=56.20, 56.37, 111.47, 114.74, 120.88, 134.78, 145.45, 147.04
D6: δ=56.37, 113.04, 113.60, 121.57, 129.04, 140.65, 155.23

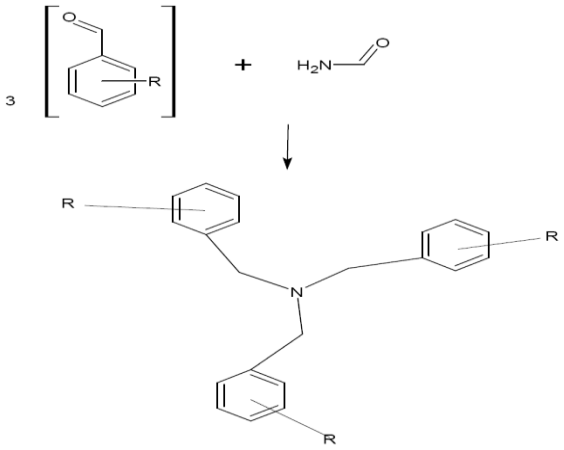
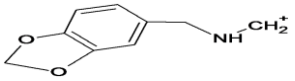
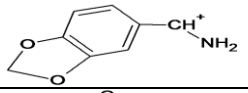
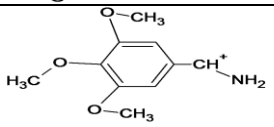
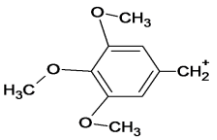
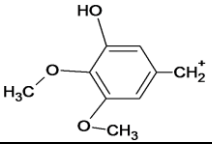
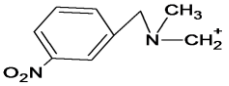
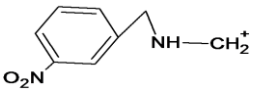
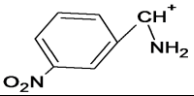
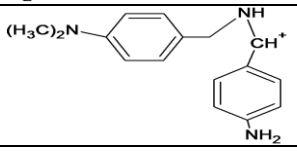
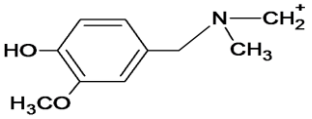
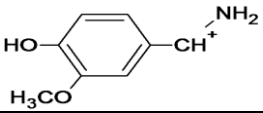
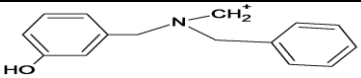
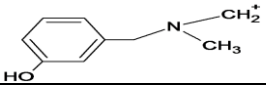


Figure 1: Scheme for synthesis

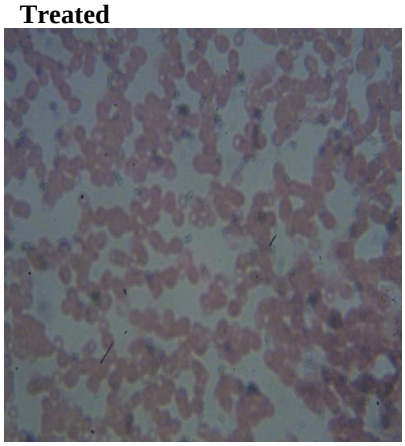
Table 1: The different compounds and their IUPAC names

Compound code	R (substituent)	IUPAC Name	Yield (%)	Melting point (^oC)	R_f
D1 3,4-dioxol	3,4-O-CH_2-O	1-(1,3-benzodioxol-5-yl)-N,N-bis(1,3-benzodioxol-5-yl-methyl) methanamine	32.7	80	0.74
D2 3,4,5-trimethoxy	tri -3,4,5-OCH_3	N,N-bis(3,4,5-trimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl) methanamine	40.9	50	0.85
D3 3-nitro	3-NO_2	N,N-bis(3-nitrobenzyl)-1-(3-nitrophenyl)methanamine	30.5	Semi-solid	0.85
D4 4-dimethylamino	4-N(CH_3)$_2$	4,4',4"-(nitrilotrimethanediyl) tris(N,N-dimethylaniline)	16.5	Semi-solid	0.84
D5 4-hydroxy-3-methoxy	4-OH,3-OCH_3	4,4', 4"-(nitrilotrimethanediyl) tris(2-methoxyphenol)	37.5	Semi-solid	0.43
D6 3-hydroxy	3-OH	3,3',3"-(nitrilomethanediyl)triphenol	41.2	Semi-solid	0.69

Table 2: Mass spectral fragmentations and retention times

Compound	Retention time (s)	GC peak #	m/z	Structure of fragment
D1	22.494	6	163	
		6	149	
D2	18.223	3	195	
		3	181	
		3	166	
D3	24.176	8	178	
		8	164	
		8	150	
D4	27.251	9	254	
D5	20.774	4	179	
		4	151	
D6	23.734	7	225	
		7	149	

Treated **Untreated(-ve control)**



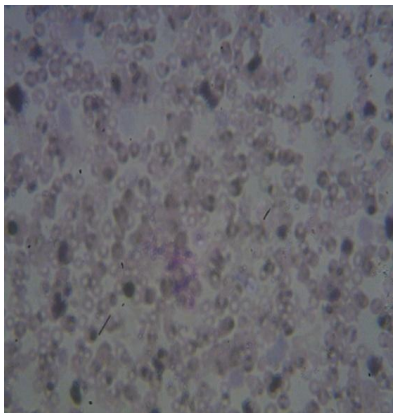


Table 3: Antimalarial screening result

Treatment	Mean parasite count	% suppression	Ld50(mg/kg)
Negative control (5ml soya)	19.50±1.50	-	-
Standard positive control(DHA)	2.50±0.50	87.18	-
D1	4.25±0.25	78.21	460
D2	2.75±0.25	85.90	1100
D3	2.00±0.50	94.87	590
D4	0.75±0.75	96.15	550
D5	5.00±1.00	74.36	1100
D6	14.00±1.25	28.20	1000

After treatment of mice having symptoms of already established plasmodium infection, with the synthesised compounds and the dihydroartemisinin as a positive control, it was observed that two of the compounds, D3 and D4 had a higher suppressive on *Plasmodium berghei* than the dihydroartemisinin indicating a better antimalarial property. The other compounds showed good antiplasmodial activity though less than the positive control. The compounds exhibiting the best antimalarial property have nitrogen containing functional groups containing nitrogen i.e. dimethylamine and nitro. This could possibly arise due to their similarity to other antimalarial drugs which also contain nitrogen groups like the aminoquinolones. Compounds D5 and D6 have the least activity and both compounds have hydroxyl functional groups in their structures but the D5 have a higher activity which might be an indication that the presence of a methoxy group increases activity.

Conclusion. Six tribenzylamines have been synthesised successfully using the Leuckart reaction with all compounds showing good antiplasmodial activity against *Plasmodium berghei* at a single dose level of 3mg/kg.

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