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# Synthesis and Antimalarial Properties of Three Substituted Trinitro-Tribenzylamine

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## Abstract

Malaria infection is a major health burdens in Tropical Africa and this is made worst with the emergence of *Plasmodium falciparum* resistance to the commonly used antimalarial drugs. There is therefore the need for new potent antimalarial drugs. Syntheses of o-trinitro tribenzylamine (2TBN), m-trinitro tribenzylamine (3TBN) and p-trinitro tribenzylamine (4TBN) were carried and yields of 38.49%, 41.01% and 39.67% respectively were obtained on reacting the respective o-, m-, and p-nitro benzaldehyde with formamide. The three synthesized trinitro-tribenzylamine derivatives were tested in vitro against Plasmodium falciparum isolates (3D7 strain) using parasite lactate dehydrogenase assay (pLDH). The result showed significant activity with  $IC_{50}$  ranging from 18 to 30 nM. The meta derivative ( $IC_{50}$ : 18.823 nM) showed better antiplasmodial activity than ortho ( $IC_{50}$ : 30.287 nM) and para ( $IC_{50}$ : 22.297 nM) derivatives, and even chloroquine phosphate ( $IC_{50}$ : 22.777 nM).

**Keywords:** Antimalarial drugs; Malaria infection; Plasmodium falciparum; Trinitro tribenzylamine

**Abbreviations:** 2TBN: Ortho Trinitro-Tribenzylamine; 3TBN: Meta Trinitro-Tribenzylamine; 4TBN: Para Trinitro-Tribenzylamine; DHA: Dihydroartemisinin; CQ: Chloroquine phosphate; PIP-DHA: Piperaquine Dihydroartemisinin

## Introduction

Malaria remains one of the major causes of death in several parts of the world. Among the species of plasmodium that commonly infect humans, *Plasmodium falciparum* and *plasmodium vivax* are the most prevalent and plasmodium falciparum is the most dangerous [1-3]. The emergence of parasite resistance to commonly used antimalarial drugs, particularly in Plasmodium falciparum case, has increased the burden of malaria [4]. According to WHO, the burden is heaviest in African Region, where an estimated 90% of all malaria deaths occur [5]. The need for new antimalarial drug candidates is therefore very urgent.

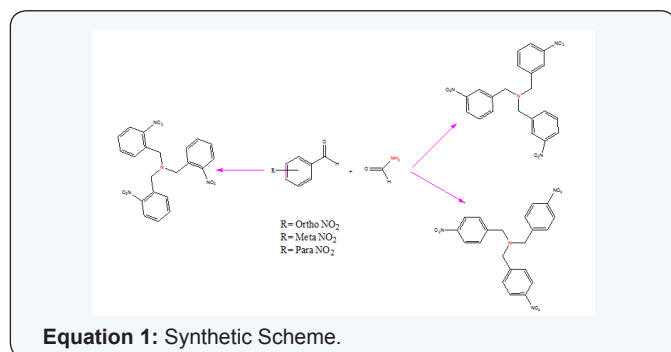
Amine compounds are recognized for their polarity and basicity; they are usually incorporated into several drugs to enhance binding to different drug targets as well as to facilitate their absorption. Many antimalarial drugs such as quinine, chloroquine, mefloquine, amodiaquine, etc. are alkaloid, and their mechanism

of action has been shown to also depend on the amine functional group present in their structures. The molecules are transported into the acidic food vacuole (pH 4.7) down the pH gradient and the amine gets protonated prior to inhibition of haem polymerization. Accumulation of the haem generated from the degradation of haemoglobin is lethal to the parasite due to the high toxicity of this waste product [6].

Despite decades of effort to eradicate malaria, it remains endemic in several parts of the world. The challenges associated with the treatment of malaria have led Medicinal chemists to keep working to discover potent antimalarial agents. This work aimed to synthesize, characterize and screen the antimalarial activity of some trinitro-tribenzylamine analogues. Also, to evaluate the effect of varying the position of the nitro group between the ortho, meta and para positions of the tribenzylamine moiety on the antimalarial activity.

## Materials and Methods

### Synthesis of compounds



Three trinitro-tribenzylamine analogues were obtained in full synthesis using Leuckart reaction (Equation 1). In a 250 ml-round bottom flask fitted with reflux condenser is placed 0.05 Moles of nitro benzaldehyde derivative (o-, m-, and p-nitro benzaldehyde) and 0.5 Moles of formamide. The contents of the flask were heated and stirred to reflux in acetic acid; after eight hours of refluxing and stirring, the solution was cooled to room temperature. The solution was then neutralized with sodium bicarbonate and extracted with diethyl ether; the ether extracts were concentrated in vacuo to give a product. The structures of synthesized compounds were determined by nuclear magnetic resonance (<sup>1</sup>H-NMR and <sup>13</sup>C-NMR), and FTIR techniques.

### Plasmodium falciparum culture and maintenance

The human parasite *Plasmodium falciparum* strain used in this study was Chloroquine sensitive (3D7), it was obtained from Hematology Department of Jos University Teaching Hospital, Plateau State-Nigeria. The O+ infected erythrocyte (at 4% of hematocrit and 0.5% of parasitemia) was cultured *in vitro* according to the method described by Trager and Jensen with some modifications [7], in RPMI 1640 medium (Catalog #: ABI-111-02), containing 2g/L of glucose, 1mg/L of L-glutamine, 2g/L of NaHCO<sub>3</sub>, Amino Acids, Vitamins, Inorganic Salts, Phenol Red and supplemented with 0.01 mg/mL of gentamicin (Sigma-aldrich), 25 of mMN-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer (Sigma-aldrich), and 5% of lipid-enriched bovine albumin preparation (Albumax®).

### Determination of *in vitro* antiplasmodial activity

The synthesized compounds (o-, m-, and p-nitro tribenzylamine) and standards drug: Dihydroartemisinin (Sigma-Aldrich), chloroquine phosphate (Sigma-Aldrich) and Piperaquine-dihydroartemisinin were dissolved in DMSO 0.5% to produce a stock solution of 100 nM. The infected erythrocyte was exposed to synthesized compounds and standards drug in various concentrations (from 0.2 to 47 nM for the synthesized compounds (2TBN, 3TBN and 4TBN), 0.1 to 24 nM for piperaquine-dihydroartemisinin (PIP-DHA), 0.2 to 48 nM for chloroquine phosphate (CQ), and 0.3 to 70 nM

for dihydroartemisinin (DHA) in triplicate in 96-well culture plates and incubated for 72 h at 37°C in an atmosphere of 92% N<sub>2</sub>, 5% CO<sub>2</sub> and 3% O<sub>2</sub>. For negative control wells, the infected Erythrocytes were devoid of trinitro-tribenzylamine analogues or standards drug. Synthesized compounds and standard drugs were assessed for antiplasmodial activity *in vitro* using parasite lactate dehydrogenase assay (pLDH).

### Parasite lactate dehydrogenase assay

After 72 h of incubation, 20 µL were removed and added to 100 µL of the Malstat reagent (Prepared by dissolving 0.11% of Triton X-100, 115.7mM of L-lactate, 30.27mM of Tris buffer, and 0.62mM of 3-acetyl pyridine adenine dinucleotide in deionized water, the pH was adjusted to 9.2 with hydrochloric acid) present in a new flat-bottomed 96-well micro titer plate in triplicate. 25 µL of NTB/PES (prepared by dissolving 1.96 mM of Nitro Blue Tetrazolium and 0.24mM of Phenazine Ethosulfate in deionized water) were then added to each well, thereby initiating the lactate dehydrogenase reaction. Absorbance was then measured at 630nm using an ELISA plate reader.

### Analysis of test results from the pLDH assay

The optical density values from negative control wells were referred to as having 100% pLDH activity. The IC<sub>50</sub>-values of each tribenzylamine analogues or standard drugs were obtained by regression analysis, using a non-linear dose-response curve-fitting equation in Graph Pad Prism 7.0 software.

## Results

### Characterization of synthesized compounds

As described above, 2TBN, 3TBN and 4TBN, were obtained by full synthesis. The structures of synthesized compounds were established through FTIR, <sup>1</sup>H-NMR, and <sup>13</sup>C-NMR analysis.

**O-trinitro tribenzylamine (2TBN):** Yield 38.49%, the Infra-Red spectrum (KBr) of this compound showed characteristic peaks at 2924.18, 1527.67, 1381.08 and at 756.12 cm<sup>-1</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm: 7.99 (d, J = 7.2 Hz, 3H), 7.66 (t, J = 6.4 Hz, 3H), 7.29 (t, J = 7.6 Hz, 3H), 6.76 (d, J = 10.4 Hz, 3H), 1.79 (s, 6H). <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>) δ ppm: 152.52, 148.16, 134.68, 131.80, 128.74, 124.59 and 23.10.

**M-trinitro tribenzylamine (3TBN):** Yield 41.01%, the Infra-Red spectrum (KBr) of this compound showed characteristic peaks at 2924.18, 2800.73, 2877.89, 1527.67, 1388.79, 671.25, 725.26, and at 810.13 cm<sup>-1</sup>. <sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>) δ ppm: 8.71 (d, J = 15.4 Hz, 3H), 8.44 (s, 3H), 7.88 (t, J = 8.2 Hz, 3H), 7.46 (d, J = 4.4 Hz, 3H), 1.76 (s, 6H). <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>) δ ppm: 148.45, 145.71, 140.09, 137.18, 128.86, 125.13 and 22.56.

**P-trinitro tribenzylamine (4TBN):** Yield 39.67%, the Infra-Red spectrum (KBr) of this compound showed characteristic peaks at 2924.18, 2854.74, 1527.67, 1388.79 and at 802.41

cm-1.  $^1\text{H-NMR}$  (600 MHz,  $\text{DMSO-d}_6$ )  $\delta$  ppm: 8.34 (d,  $J = 8.2$  Hz, 6H), 7.74 (d,  $J = 11.6$  Hz, 6H), 1.77 (s, 6H).  $^{13}\text{C-NMR}$  (101 MHz,  $\text{DMSO-d}_6$ )  $\delta$  ppm: 144.82, 141.60, 129.45, 127.48 and 22.95.

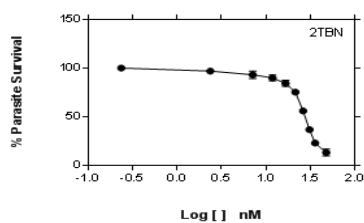
## Biological Screening

### *In vitro* anti-malarial activity

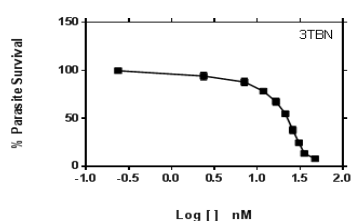
Parasite % survivals were plotted versus the decimal logarithm of concentration in Graph Pad Prism 7.0 software to determine the concentration inhibiting 50% of parasite growth ( $\text{IC}_{50}$  value), and are presented in (Figures 1- 6). The  $\text{IC}_{50}$  presented in Table 1, were obtained from three independent triplicate experiments and were expressed as mean  $\pm$  SEM.

## Discussion

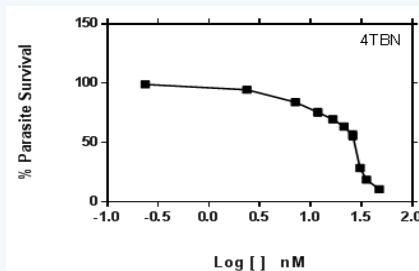
A series of trinitro-tribenzylamine derivatives (**ortho**-, **meta**- and **para**) were prepared in good yields. The structures of the synthesized compounds were confirmed based on the data from IR,  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectral analysis and the signals obtained were all in a good agreement with the proposed structures. From the graph presented to the (Figures 1-6), showed the decrease in parasite survival as signify by the negative curve in all the synthesise compounds and standard drugs. The results of this study showed that the three synthesized compounds possess *Plasmodium falciparum* suppressive effect, with  $\text{IC}_{50}$  between 18 to 30 nM. From literature, a drug is potentially interesting as antimalarial if its  $\text{IC}_{50}$  is below  $10\mu\text{M}$ ,  $5\mu\text{M}$  is used as a cut-off level to reduce the number of false positives [8]. Therefore the trinitro-tribenzylamine derivatives synthesized in this study can be added to the growing list of compounds with antiplasmodial activity against *plasmodium falciparum*, and hence serves as new lead compounds for the development of new antimalarial drugs.



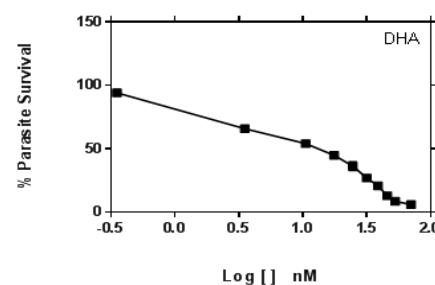
**Figure 1:** Dose response curve of o-trinitro tribenzylamine against *Plasmodium falciparum*.



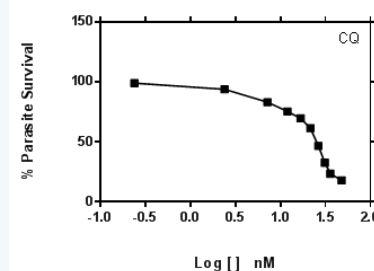
**Figure 2:** Dose response curve of m-trinitro tribenzylamine against *Plasmodium falciparum*.



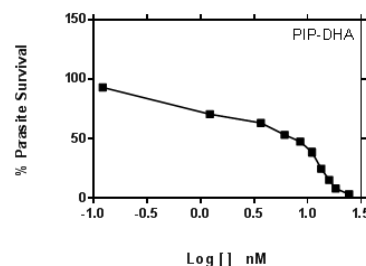
**Figure 3:** Dose response curve of p-trinitro tribenzylamine against *Plasmodium falciparum*.



**Figure 4:** Dose response curve of dihydroartemisinin against *Plasmodium falciparum*.



**Figure 5:** Dose response curve of chloroquine phosphate against *Plasmodium falciparum*.



**Figure 6:** Dose response curve of piperazine-dihydroartemisinin against *Plasmodium falciparum*.

The meta-trinitro tribenzylamine had a lower  $\text{IC}_{50}$  than chloroquine phosphate indicating a better antimalarial activity; the para substituted analogue is comparable to chloroquine phosphate, while the ortho has lesser activity to chloroquine phosphate. All the synthesized compounds showed lesser activity compared to **DHA**, and dihydroartemisinin-piperazine.

**Table 1:** In vitro susceptibility of Plasmodium falciparum isolates to tri nitro-tri benzyl amine derivatives and to standards drugs.

Synthetic Compounds/Standards Drugs	IC <sub>50</sub> (nM)
o-trinitro tribenzylamine (2TBN)	30.287 ± 1.707
m-trinitro tribenzylamine (3TBN)	18.823 ± 0.480
p-trinitro tribenzylamine (4TBN)	22.297 ± 0.846
Dihydroartemisinin (DHA)	10.449 ± 0.378
Chloroquine phosphate (CQ)	22.777 ± 0.067
Piperaquine- dihydroartemisinin (PIP-DHA)	4.931 ± 0.301

The IC<sub>50</sub> results, summarized in (Table 1), indicated that the introduction of nitro group into tribenzylamine skeletal in meta-position enhanced bioactivity against Plasmodium falciparum isolates by almost one point six-fold factor compared to the ortho-derivative and one point two-fold factor compared to the para-derivative.

## Conclusion

Ortho-, meta- and para-trinitro tribenzylamine derivatives have been synthesized successfully through a simple one-step reaction (**Leuckart reaction**). All the three compounds showed good antiplasmodial activity against chloroquine sensitive strain of Plasmodium falciparum (3D7). The substitution of nitro group in meta-position into tribenzylamine moiety has shown to be the most potent derivatives. These derivatives can represent a new template for antimalarial agent development.

## Acknowledgments

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