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Synthesis, Molecular Structure, Anti-Plasmodial, Antimicrobial and Anti-Oxidant Screening of (E)-1-(Phthalazin-1-yl)-1-[(Pyridin-2-yl)Ethylidene]Hydralazine and 1-[2-(1-(pyridine-3-yl)ethylidene)hydrazinyl]phthalazine

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Abstract

Two new hydralazine hydrochloride-derived Schiff bases: (E)-1-(Phthalazin-1-yl)-1-[(Pyridin-2-yl)Ethylidene]Hydralazine (PPEH), and 1-[2-(1-(pyridine-3-yl)ethylidene)hydrazinyl]phthalazine (PEHP), were synthesized and partially characterized by spectroscopic and crystallographic methods including IR and X-ray. The single-crystal X-ray diffraction (SCXRD) analysis of PEHP indicates that the hydralazine moiety of both ligands possesses the exocyclic C=N bond. Both, PPEH and PEHP were tested as antimicrobials and antiparasites. Just PEHP could be considered as slightly antiplasmodial and antibacterial agent. In effect, PPEH showed low antimicrobial activity against one bacterial strain with Minimum Inhibitory Concentration (MIC) value of 250 µg/ml while PEHP showed very interesting activity against 18 out of 19 bacterial strains with MIC of 31.25 - 250 µg/ml compared to the standard drug, amoxicillin. PPEH and PEHP showed higher reducing activity on ferric ions compared to Vitamin C. On the other hand, both hidrazaline synthetized derivatives showed as better reducing agents than Vitamin C on ferric ions, while again, only the PEHP showed slightly high inhibition of lipid peroxidation using Vitamin C as standard. Regarding their catalase activity, both compounds showed concentration dependent effect, but Vitamin C

continued showing a higher stimulatory effect on the enzyme activity. Additionally, while PPEH showed less than 80% inhibition in the preliminary antiplasmodial assay and so was not considered for the dose-response studies, PEHP displayed an inhibition percentage of 83.60% and 50% Inhibitory Concentration (IC_{50}) value of 44.13 μg/mL compared to the standard drug, artemisinin and was classified as slightly active.

Keywords

Schiff Base, Hydralazine Hydrochloride, Anti-Plasmodial Activity, Antimicrobial Activity, Anti-Oxidant Activity

1. Introduction

The number of therapeutics used worldwide to treat or manage diseases and ailments has been on a steady rise thanks to research in the domain of health. However, the need for continued research and the discovery of new therapeutics is imminent as microorganisms develop resistance to existing therapeutics when the usage is recurrent [1].

Schiff bases are compounds that contain the azomethine or imine (-C=N-) functional group. First reported by Hugo Schiff, they are condensation products of primary amines with carbonyl compounds [2] [3] [4]. Hydralazine (hydrazone) based Schiff bases display significant medicinal properties including antifungal activities [5] [6], antimicrobial properties [7], antihypertensive agents [8], carbonyl scavenger and antiapoptotic activities [9] and luminescence properties [10]. They are reportedly less toxic than hydrazines from which they are derived owing to the blockage of the amino ($-NH_2$) group [11] [12]. The molecule hydralazine (common name: apresoline) is an efficient arterial vasodilator that has frequently been used in the treatment of patients with congestive heart failure and particularly for the treatment of hypertensive disorders especially in pregnant women [8].

Infectious microorganisms have recently developed resistance to existing therapeutics and this situation constitutes a global health threat [13]. There is therefore a dire need for new antimicrobials that if introduced, will fight against these pathogens and reverse this tendency [13]. The discovery of new strategies to manage drug resistance, prevent the proliferation of resistant bacteria, identify new antimicrobials/new drug combinations is an effort to alleviate suffering and should be the way forward for researchers [14].

Malaria and bacterial infections are infectious diseases that have been identified as the most recurrent and highest cause of mortality in Cameroon and Sub-Saharan Africa [15]. In fact, malaria continues to be the leading cause of mortality among children and especially those below 5 years of age with an estimated 303,000 (165,000 - 450,000) deaths in 2015; the parasite being transmitted by *Plasmodium falciparum* in most cases. Although there has been a 60% reduc-

tion in malaria-induced deaths since 2000 (owing to the institution of control measures such as the use of Artemisinin-based combination therapies (ACTs)), the elimination of malaria has so far been impossible because of the recent emergence and spread or ACT-resistant and insecticide resistant *Plasmodium falciparum* parasites [16] [17].

Antioxidants are molecules that delay, prevent or remove oxidative damage to target cells. Otherwise referred to as oxidative stress, oxidative damage generates free radicals; a veritable source of danger to the body. Such free radical species as hydrogen peroxides, nitric acid radical, singlet oxygen, superoxide anion radical, hydroxyl radical and various peroxides belong to a class of highly reactive molecules or highly reactive oxygen species (ROS) [18]. Sometimes, oxidative forces can surpass the antioxidant systems. When this happens, the cell suffers from oxidative stress which is one of the leading causes of many chronic and degenerative diseases like atherosclerosis, cancer, diabetes mellitus, neurodegenerative disorders, ischemic heart attack, aging, imuno suppression, infectious diseases and male infertility [19] [20].

Cognisant of the aforementioned, development of new technologies and drugs/leads for the treatment of malaria and bacterial infections as well as new antioxidant chemotypes is an urgent priority. As our own contribution towards achieving this objective, we embarked on the syntheses of new hydralazine-derived Schiff bases and evaluated them for their antiplasmodial, antibacterial and antioxidant activity *in vitro*.

2. Experimental

2.1. Materials

Hydralazine hydrochloride, 2-acetylpyridine, 2-acetylpyrazine and other solvents were purchased from commercial sources and used without further purification. All chemicals used were of reagent grade. Methanol was used as solvent all through the synthesis. Glass apparatus with standard interchangeable joint was used after being washed with concentrated sulphuric acid, distilled water and methanol. All the synthesis was carried out in a 250 mL round-bottomed flask fitted with a quick Liebig condenser where it involved refluxing. All reactions were performed on a hot plate equipped with a magnetic stirrer. Elemental analysis was performed on a VARIO EL (Heraeus) analyzer. IR spectra were obtained from a Perkin-Elmer spectrum 100-FT-IR spectrometer. ^{1}H-NMR spectra were obtained on a Varian unity plus 400 MHz instrument. ^{13}C-NMR spectra were recorded on a Bruker AV 100 MHz instrument.

2.2. Synthesis of (E)-1-(Phthalazin-1-yl)-1-[(Pyridin-2-yl)Ethylidene]Hydralazine (PPEH)

The Schiff base was prepared by mixing equimolar amounts of reagents; 1-(pyrazin-2-yl)ethan-1-one (or 2-acetylpyrazine) (3.20 g, 0.02 mmol) and hydralazine hydrochloride (2.20 g, 0.02 mmol) with sodium acetate as a buffer in

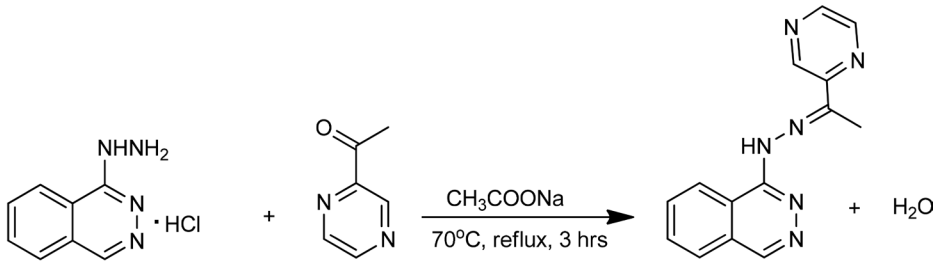
100 mL ethanol. A reddish-brown colour was observed upon addition of 2-acetylpyrazine to the hydralazine hydrochloride after which a bright yellow precipitate was immediately formed upon addition of sodium acetate (yield = 77%). The reaction was refluxed for three hours. The synthetic route for PPEH is shown in Scheme 1.

IR (KBr, cm^{-1}): ν 3250, 3000, 1600. ^{1}H NMR (DMSO-d_6, 500 MHz): 10.3 (s, 1H, N-H), 1.80 (s, 3H, H-8'), 6.94 and 8.38 (benzene hydrogens of the hydralazine moiety) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): 156.4 (C-1), 17.3 (C-8') ppm.

2.3. Synthesis of 1-[2-(1-(pyridine-3-yl)ethylidene)hydrazinyl]phthalazine (PEHP)

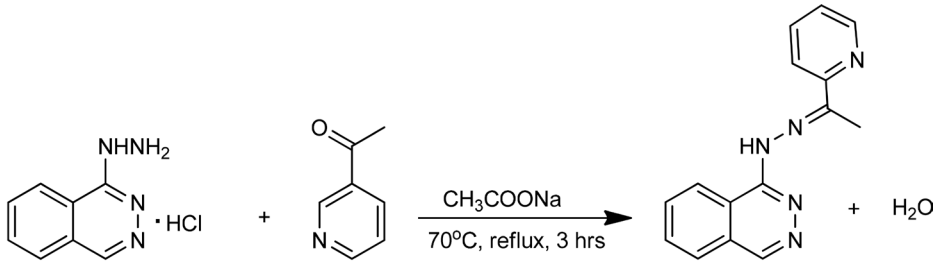
PEHP was prepared by mixing equimolar amounts of reagents; 1-(pyridin-3-yl)-ethan-1-one (or 2-acetylpyridine) (3.20 g, 0.02 mmol) and hydralazine hydrochloride (2.20 g, 0.02 mmol) (2.20 g, 0.02 mmol) with sodium acetate as a buffer in 100 mL ethanol. Upon addition of 2-acetylpyridine to the hydralazine hydrochloride, a reddish-brown colour was observed after which a bright yellow precipitate was immediately formed upon addition of sodium acetate (yield = 68%). The reaction was refluxed for three hours. Scheme 2 shows the synthetic route for PEHP.

IR (KBr, cm^{-1}): ν 3317, 1605.6, 1591.5, 1569.2, 1532.5. ^{1}H NMR (DMSO-d_6, 500 MHz): 10.25 (s, 1H, N-H), 1.83 (s, 3H, H-8'), 6.86 and 8. (benzene hydrogens of the hydralazine moiety) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): 156.7 (C-1), 17.0 (C-8') ppm.



Hydralazine hydrochloride 1-(pyrazin-2-yl)ethan-1-one

Scheme 1. Synthesis of PPEH.



Hydralazine hydrochloride 1-(pyridin-3-yl)ethan-1-one

Scheme 2. Synthesis of PEHP.

2.4. Single Crystal X-Ray Diffraction Analysis and Structure Determination of PEHP

Crystallographic data of PEHP were taken on a Gemini diffractometer (Agilent Technologies) using Mo-Kα radiation (λ = 71.073 pm), ω-scan rotation. CrysAlis Pro [21] was used for data including the program SCALE3 ABSPACK [22] for empirical absorption correction. The refinement of all non-hydrogen atoms was performed with SHELXL-97 [23] and structure was solved by direct methods with SHELXS-97. All non-hydrogen atoms were refined with anisotropic thermal parameters and a difference-density Fourier map was used to locate all hydrogen atoms. CCDC 1007670 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.uk). The molecular graphics were done with ORTEP-3 [24] and Mercury (version 3) [25].

2.5. Antimicrobial Assay

2.5.1. Organisms and Growth Conditions

Microorganisms were obtained from the culture collections of the Antimicrobial and biocontrol Agents Unit at the Department of Biochemistry, University of Yaounde 1, Cameroon. Organisms were as follows: *Streptococcus pneumoniae (Klein) Chester*, *ATCC*49619, *Staphylococcus aureus BAA* 917, *Staphylococcus aureus subsp. aureus Rosenbach*, *ATCC* 43300, *Staphylococcus aureus NR*45003, *Staphylococcus aureus NR*46003, *Staphylococcus aureus* CP7625, *Shigella flexineri* NR518, *Salmonella enterica subsp. enterica serovar Anatum*NR4294, *Salmonella enterica subsp. enterica serovar Muenchen*NR4311, *Salmonella enterica subsp. enterica*, *strain A*36 *is serovar Typhimurium*NR-13555, *Pseudomonas aeruginosa NMC*592, *Klebsiella pneumoniae subsp. pneumoniae* (Schroeter) Trevisan ATCC 13883, *Klebsiella pneumoniae subsp. pneumoniae* (Schroeter) Trevisan ATCC 700603, *Klebsiella pneumonae* NR41916, *Escherishia coli* ATCC25922, *Escherishia coli* ATCC35218, *Enterococcus feacalis* ATCC51219, *Staphylococcus aureus* NR46374, and *Hemophyllus influenza* ATCC49247. Organisms were maintained on Muller Hinton agar (MHA) (Oxoid). at 37˚C. Inocula were prepared by diluting overnight cultures in saline to 0.5 McFarland, approximately 10^8 cfu·ml^{-1}. These suspensions were further diluted with medium as required.

2.5.2. Drug Susceptibility Testing Procedure

1) Stock solution preparation

Stock solution of each sample was prepared at 100 mg/mL in DMSO.

2) Antimicrobial assays

For the estimation of the antimicrobial activities of the synthesized compounds, a broth dilution method was employed for minimum inhibitory concentration (MIC) following the Clinical and Laboratory Standards Institute (CLSI) guidelines M07-A10. Each compound was tested in duplicate for MIC

determination. More specifically, 50 µL of Mueller Hinton broth (MHB) were introduced in a 96-well microplate respectively. 50 µL of compound were added to wells of the first line. A serial twofold dilution was made by transferring 50 µL of the mixture of the first wells to the next one up to the last, final concentrations varying from 250 to 7.81 µg/mL. Then, 50 µL of an inoculum of 1×10^6 cells/mL were introduced in all the wells except those of the sterility control. The final concentration of DMSO was <1%. Each plate also contained a positive control, Amoxicillin, a negative control and a blank. Plates were incubated at 37˚C for 24 hours. The lowest concentration of compound that inhibited the visible growth of a microorganism was defined as minimum inhibitory concentration (MIC).

2.6. Antioxidant Assay

2.6.1. Organ Homogenates

A 2.5-month-old male Wistar albino rat of 150 g was obtained from the Biochemistry animal house of the Faculty of Science of The University of Bamenda. They were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory and were given food and water *ad libitum*. After sacrifice, the liver and testes were excised out, weighed and used to prepare 20% (W/V) homogenates using phosphate buffer (pH 7.4, 50 mM).

2.6.2. Chemicals

Ascorbic acid and Thiobarbituric, trichloroacetic acids were gotten from Griffin and George (Wembly Middlesex, England). Methanol was purchased from Loba Chemie Pvt. Ltd. 107, Woodehouse (Mumbai, India). Other chemicals were of high quality grade.

2.6.3. Evaluation of the Antioxidant Reducing Power

The antioxidant reducing power of the synthesized compounds PPEH and PEHP was evaluated using the method described by Kamtekar and collaborators in 2014 [26]. The standard ascorbic acid or synthesized compounds (50, 100, 150, 200, 250 and 300 µg/mL) was introduced in the tube, then 0.4 mL of phosphate buffer (pH 6.6, 0.2 M) and 0.4 mL of 1% $KFe(CN)_6$ were successively added. After homogenization, the mixture was incubated at 50˚C for 20 min, then cooled, and centrifuged (3500 rpm, 10 min, 25˚C). To 1 mL of supernatant was added 1 mL of 10% trichloroacetic acid (TCA), 1 mL of distilled water and 0.2 mL of 0.1% $FeCl_3$. After homogenization, the absorbance was measured at 593 nm using a UV-Vis spectrophotometer.

2.6.4. Inhibition of Lipid Peroxidation

A volume (0.8 mL) of phosphate buffer (pH 7.4, 50 mM) and 0.1 mL of liver or testis homogenate were added to standard ascorbic acid or synthesized compounds PPEH and PEHP (50, 100, 150, 200, 250 and 300 µg/mL), followed by 0.1 mL of $FeSO_4$. After homogenization, the mixture was incubated at 37˚C for 15 min, then 1 mL of 20% TCA and 1 mL of 0.67% thiobarbituric acid (TBA), were

added. The mixture was incubated at 90˚C for 10 min, cooled and centrifuged (3000 rpm, 15 min, 4˚C). The supernatant was collected and optical density read at 530 nm [27] and percentage inhibition (%I) of the standard and synthesized compounds computed.

2.6.5. Catalase Activity

Ascorbic acid or the synthesized compounds PPEH and PEHP (50, 100, 150, 200, 250 and 300 µg/mL) were introduced into test tubes containing 0.4 mL of 9% H_2O_2 and 2.3 mL of phosphate buffer (pH 7.2, 0.1 M) and 0.4 mL of the liver or testis homogenate were added. The absorbance was recorded at 240 nm using a UV-Vis spectrophotometer at 30, 60 and 90 seconds. Catalase activity was expressed as IU/mg protein [28].

2.6.6. Statistical Analyses

For ferric reducing power and lipid peroxidation, the fifty percent efficient (EC_{50}) and inhibitory (IC_{50}) concentration of the tested compound were determined. Differences between treatments were assessed by one factor ANOVA followed by the Student-Newman-Keuls test, and P values less than 0.05 were considered statistically significant. All analyses were performed using Graphpad Instat software Version 3.0.

2.7. Antiplasmodial Assay

SYBR Green I Based Fluorescence Assay

Drug sensitivity assay was carried out in 96-well microtitration plates using SYBR green I based fluorescence assay. Sorbitol-synchronized ring stage parasites, *Plasmodium falciparum* Dd2 (haematocrit: 3%, parasitaemia: 0.5%, 90 µl) under normal culture conditions were incubated in the presence of prediluted synthesized compounds and reference drug. The final concentration in the test plate was range from 0.781 to 100 µg/mL for synthesized compounds and 0.0078 to 1 µM for artemisinin and chloroquine, in the total volume of 100 µL of culture. After 72 h of incubation, SYBR Green I was added and the fluorescence was measured using a Fluoroskan Ascent multi-well plate reader with excitation and emission at 485 and 538 nm, respectively. The fluorescence counts were plotted against the logarithm of sample concentration and the 50% inhibitory concentration (IC_{50}) was determined by analysis of dose-response curves using GraphPad Prism 5. Experiments were done in duplicate. Cut off point for antiplasmodial activity of synthesized compounds is as follows: highly active ($IC_{50} \leq 5$ µg/mL), promisingly active ($5 > IC_{50} \geq 10$ µg/mL), good activity $10 > IC_{50} \leq 20$ µg/mL, moderately active ($20 < IC_{50} \leq 40$ µg/mL), marginally active ($40 > IC_{50} \leq 70$ µg/mL), poorly active ($70 > IC_{50} \leq 100$ µg/mL) [29] [30].

3. Results and Discussion

The condensation reaction between hydralazine hydrochloride and 1-(pyridin-3-yl) ethan-1-one afforded PPEH in moderate yield (**Figure 1**) and that between hy-

dralazine hydrochloride and 1-(pyrazin-3-yl)ethan-1-one afforded PEHP in moderate yield (Figure 2).

3.1. Infrared Spectra

The IR spectra of PPEH and PEHP (Figure 3 and Figure 4 respectively) was taken between 4000 - 400 cm^{-1} region. For PPEH the bands at 3250 cm^{-1}, 3000

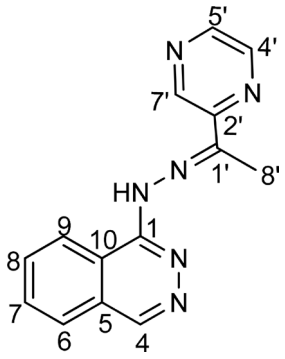


Figure 1. Structural features of PPEH.

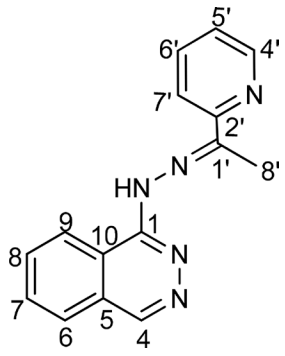


Figure 2. Structural features of PEHP.

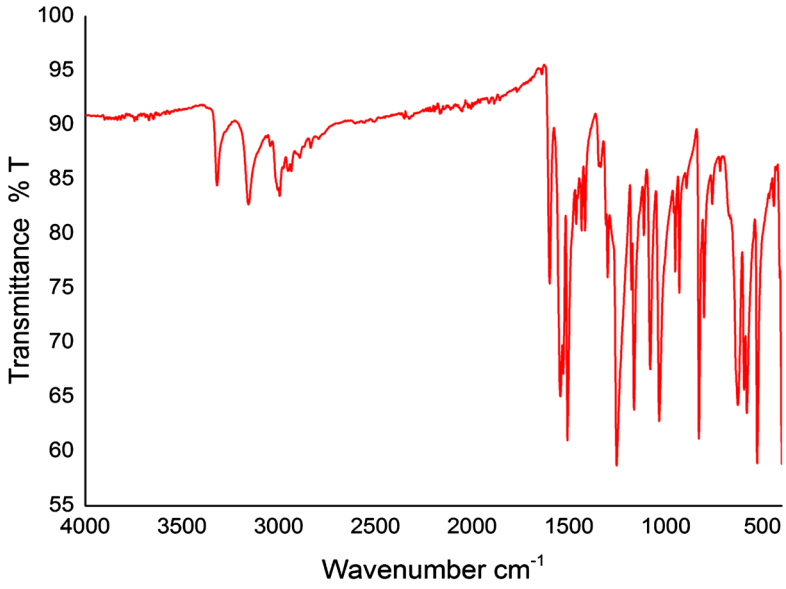


Figure 3. Infrared spectrum of PPEH.

cm^{-1} and 1600 cm^{-1} correspond to the hydrogen bonded N-H stretch [31], =C-H and C=N [5] respectively while PEHP the strong band seen at 3317 cm^{-1} correspond to the N-H stretch [31]. The strong bands that appeared at 1605.6 cm^{-1}, 1591.5 cm^{-1}, 1569.2 cm^{-1} and 1532.5 cm^{-1} are due to C=C, C=N and C=N-N=C stretches [5] of hydrazone. This proves the presence of these functional groups in PPEH and PEHP.

3.2. Single Crystal X-Ray Diffraction of PEHP

Single crystal X-ray diffraction analysis of PEHP (**Figure 5**) showed that it crystallizes in a new triclinic system, in the space group P-1. The crystal structure of PEHP indicated the migration of the proton from N(3) to N(4) as the ligand is formed from its reactants. This tautomeric behaviour had earlier been reported [5].

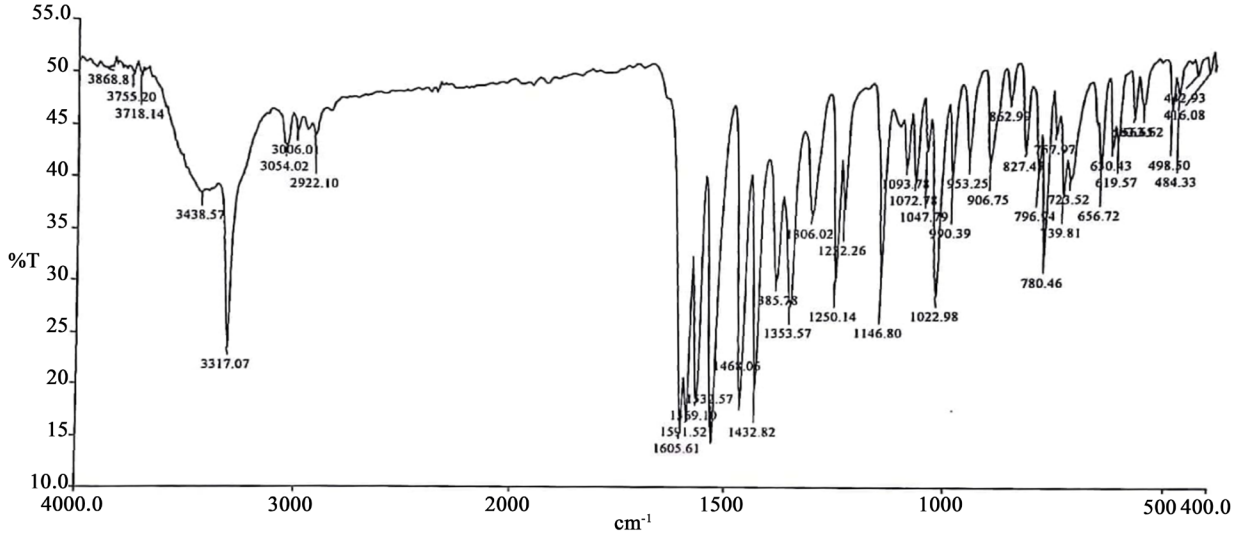


Figure 4. Infrared spectrum of PEHP.

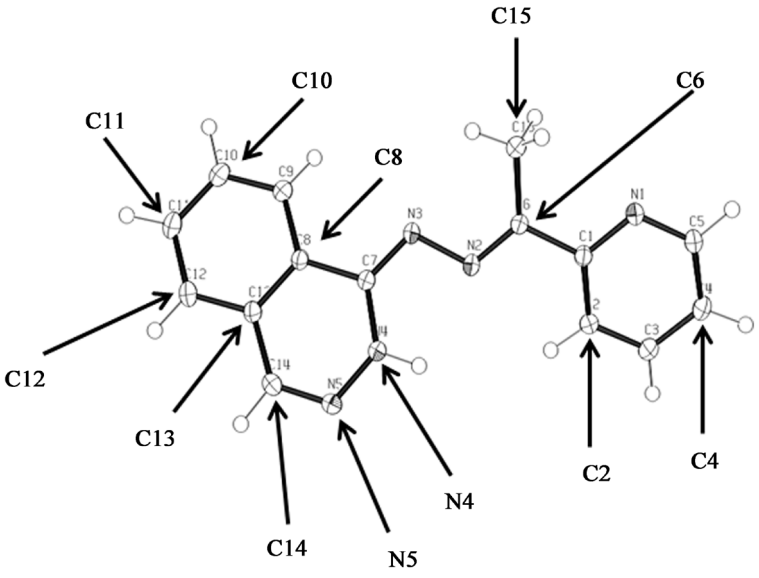


Figure 5. Molecular structure of PEHP.

3.3. Anti-Plasmodial Activity

PPEH and PEHP were tested for anti-plasmodial activity using the SYBR green I based fluorescence assay and the results are presented in Table 1. PPEH inhibited the growth of the malaria parasites with a percentage inhibition of 78.42%, lower than the 80.41% obtained with the standard treatment of malaria, Artemisinin. Likewise, PEHP inhibited the growth of the malaria parasites with a percentage inhibition of 83.60%, higher than the 80.41% obtained with Artemisinin. Considering that the percentage inhibition of PPEH in the preliminary assay was less than 80%, it was not considered for the Dose-Response studies. Meanwhile, PEHP which displayed an inhibition percentage of 83.60% and IC_{50} value of 44.13 µg/mL was classified as slightly active compared to Artemisinin. In addition, PEHP was more sensitive than the reference drug; chloroquine. This is normal as the strain used in the assay was the chloroquine resistant strain (Dd2) of *Plasmodium falciparum*.

3.4. Antimicrobial Activity

PPEH and PEHP were tested for their antimicrobial activity on 19 bacterial strains and the results are presented in Table 2.

PPEH showed activity only on one bacterial strain with MIC value of 250 µg/ml against *Streptococcus pneumoniae*. Antimicrobial activity of a compound is very interesting if the MIC is below to 10 µg/ml, interesting or moderate if 10 < MIC ≤ 100 µg/ml and low if MIC > 100 µg/ml [15] [30]. Based on above-mentioned criteria, PPEH had low antimicrobial activity. PEHP was effective (interesting activity) against 18 bacterial strains out of 19 with MIC of 31.25 - 250 µg/ml except for *Staphylococcus aureus* NR46003. The results obtained can however serve as starting point for lead optimisation in order to generate more active lead compounds that can be developed into new antibacterial drugs.

3.5. Antioxidant Activity

3.5.1. Ferric Reducing Powder and Lipid Peroxidation

The effects of different compounds and Vit C on reduction of ferric ions and peroxidation of lipids are presented in Table 3. PPEH and PEHP showed higher reducing activity ($P < 0.05$) on ferric ions compared to Vitamin C. On lipid peroxidation, PPEH showed lower inhibition ($P < 0.05$) and PEHP showed higher inhibition compared to Vitamin C.

Table 1. Antiplasmodial activity of synthetic compounds.

Compound	% Inhibition	IC_{50} (µg/mL)
PPEH	78.42	N.D.
PEHP	83.60	44.13
ART	80.41	18.18 nM
CQ	N.D.	449.3 nM

ART = artemisinine; CQ = Chloroquine; N.D. = Not determined.

Table 2. Antibacterial activities of PPEH and PEHP.

Antimicrobial activity (MIC in µg/mL)			
Bacterial strains, Synthetic compounds	**PPEH**	**PEHP**	**Amoxicilin**
Streptococcus pneumoniae (Klein) Chester, ATCC49619	250	31.25	0.5
Staphylococcus aureus BAA 917;	>250	62.5	8
Staphylococcus aureus subsp. Aureus Rosenbach, ATCC 43300	>250	62.5	>64
Staphylococcus aureus NR45003	>250	31.25	>64
Staphylococcus aureus NR46003	>250	>250	>64
Staphylococcus aureus CP7625	>250	62.5	4
Shigella flexineri NR518	>250	62.5	2
Salmonella enterica subsp. enterica serovar Anatum, *NR*4294	>250	125	16
Salmonella enterica subsp. enterica serovar Muenchen, NR4311	>250	62.5	0.5
Salmonella enterica subsp. enterica, strain A36 is serovar Typhimurium, NR-13555	>250	125	64
Pseudomonas aeruginosa NMC592	>250	125	0.5
Klebsiella pneumoniae subsp. pneumoniae (Schroeter) Trevisan ATCC 13883	>250	62.5	2
Klebsiella pneumoniaesubsp. pneumoniae (Schroeter) Trevisan ATCC 700603	>250	125	4
Klebsiella pneumonae NR41916	>250	125	2
Escherishia coli ATCC25922	>250	62.5	2
Escherishia coli ATCC35218	>250	125	>
Enterococcus feacalis ATCC51219	>250	250	4
Staphylococcus aureus NR46374	>250	31.25	1
Hemophyllus influenza ATCC49247	>250	31.25	0.5

Table 3. Effects of compounds and Vit C on reduction of ferric ions and peroxidation of lipids.

	Ferric reducing power EC_{50} (µg/mL)	**Lipid peroxidation IC_{50} (µg/mL)**
Vit C	1.749 ± 0.001	1.45 ± 0.01
PPEH	1.515 ± 0.015*	1.59 ± 0.01*
PEHP	1.221 ± 0.207*	0.99 ± 0.32*

*$P < 0.05$ compared to the reference Vit C, Student-Newman-Keuls test.

3.5.2. Catalase Activity

The catalase activity in the presence of PPEH, PEHP and Vitamin C is depicted in **Figure 6**. Catalase activity showed concentration dependent effect in the presence of both compounds. In general, Vitamin C presented higher stimulatory effect on the enzyme activity than PPEH and PEHP.

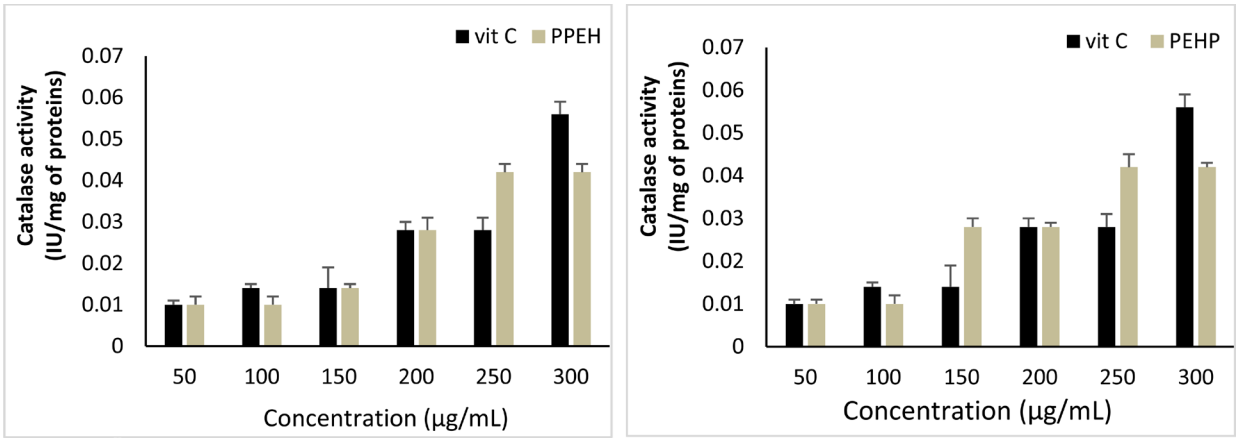


Figure 6. Effects of compounds and Vitamin C on catalase activity.

4. Conclusions

PPEH and PEHP were synthesized from hydralazine hydrochloride and characterized by spectroscopic methods. X-ray crystallographic data shows PEHP as possessing an exocyclic carbon-nitrogen double bond on the hydralazine moiety.

PPEH showed activity only on one bacterial strain with MIC value of 250 µg/ml against *Streptococcus pneumoniae*. PEHP was effective (interesting activity) against 18 bacterial strains out of 19 with MIC of 31.25 - 250 µg/ml except for *Staphylococcus aureus* NR46003.

For antioxidant activity, PPEH and PEHP showed higher reducing activity on ferric ions compared to Vitamin C. On lipid peroxidation, PPEH showed lower inhibition and PEHP showed higher inhibition compared to Vitamin C. Catalase activity showed concentration dependent effect in the presence of both compounds. In general, Vit C presented higher stimulatory effect on the enzyme activity than PPEH and PEHP.

PPEH inhibited the growth of the malaria parasites with a percentage inhibition of 78.42%, lower than the 80.41% obtained with the standard treatment of malaria, Artemisinin. Likewise, PEHP inhibited the growth of the malaria parasites with a percentage inhibition of 83.60%, higher than the 80.41% obtained with the standard treatment of malaria, Artemisinin. Considering that the percentage inhibition of PPEH in the preliminary assay was less than 80%, PPEH was not considered for the Dose-Response studies. PEHP that was considered for the Dose-Response studies displaying an inhibition percentage of 83.60% gave an IC_{50} value of 44.13 µg/mL.

The anti-microbial, anti-oxidant and anti-plasmodial activities of these compounds are reported for the first time and the results indicate that these novel ligands can be exploited as anti-microbial, anti-plasmodial and anti-oxidant agents.

In the continuing search for therapeutics or leads with good activity, PPEH and PEHP will be subjected to other biological activity tests with the aim of giving more value to these synthetic ligands.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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