

# Functionalization and Antimicrobial Evaluation of New Linear Azo-Phenothiazine Derivatives

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**Received:** 1 December 2017; **Accepted:** 21 December 2017; **Published:** 12 February 2018

## Abstract:

Phenothiazine and its derivatives are very important compounds that have many biological and industrial applications. Azo-compounds on the other hand have also been identified to possess good dyeing and biological properties as well. This work is focused on the synthesis of new linear phenothiazine azo-dye compounds via diazotization reaction as well as the determination of their biological activity against some microorganisms. The above was achieved by the condensation reaction of 3-nitroaniline and phenol in presence of potassium hydroxide and DMF (solvent) to furnish 3-nitrodiphenylamine. Sulphonation of this compound in presence of molecular iodine gave 4-nitro-[10H]-phenothiazine. The conversion of nitro group in 4-nitro-[10H]-phenothiazine to an amino group was achieved by treating it with dilute hydrochloric acid and iron (III) chloride. The amino compound formed, (4-amino-[10H]-phenothiazine) was thereafter converted to an unstable diazonium ion in the presence of sodium nitrite and concentrated hydrochloric. The ion formed above was immediately coupled with the following compounds; 3-nitroaniline, 4-nitroaniline and phenol respectively to furnish four new azophenothiazine compounds namely; 4-azo-(4-amino-2-nitroanilino)-[10H]-phenothiazine, 4-azo-(2-amino-5-nitroanilino)-[10H]-phenothiazine and 4-azo-(4-hydroxyphenyl)-[10H]-phenothiazine with good percentage yields. The synthesized compounds were tested for activity against some microorganisms and they showed some level of inhibition.

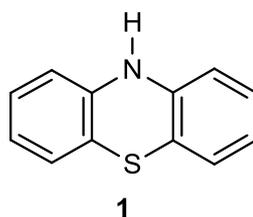
## Keywords:

Antimicrobial Screening, Microorganisms, Diazotization, Diazonium Ion, Inhibition, Sulphonation, Condensation

## 1. Introduction

Phenothiazine derivatives are known as tricyclic fused heterocyclic compounds of the dibenzo-1, 4-thiazine structure that exhibit important biological actions and

interesting chemical properties [1]. Right from the time, the parent compound **1** was prepared by Bernthsenin [2], a lot of structural modifications on the above have been done, resulting to the emergence of thousands of derivatives that have been synthesized and reported [3].



**Figure 1.** The structure of a parent phenothiazine compound.

These compounds play a very important role in the chemical industry. For instance, a good number of phenothiazine derivatives have wide applications in the pharmaceutical industry and this has stimulated more research on these compounds. Amongst these applications include their use in the production of anticancer and antitumor drugs [4] as well as active components in sedatives, tranquilizers, antibacterial, antipsychotic and anti-malaria drugs [5-10]. They are also useful in paints and plastic industries where they serve as pigments and thermal stabilizers for polymers [12-13]. These compounds have also been successfully employed as antioxidants that increase the life-span of lubricating oils and fuels in petroleum industry [14]. Some of the derivatives have also used in material science as electrophoric sensors, photography and photocopying inks [15]. Structural modification on phenothiazine compounds has produced very many useful derivatives of industrial and biological importance. Therefore, in this work, some modifications have been made on the phenothiazine nucleus via diazotization reaction [16] and we hereby report the synthesis of these new azo-phenothiazine derivatives.

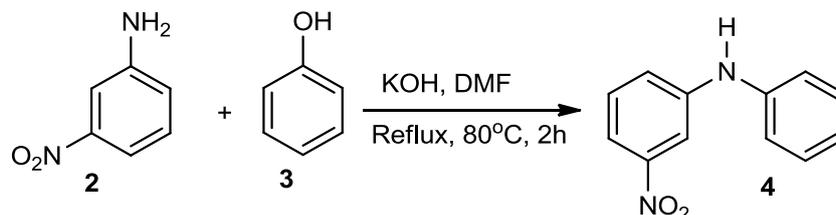
## 2. Materials and Methods

Most of the reagents used were sourced locally from commercial chemical shops and were obtained in sealed containers and were used without further purification. The melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. The UV-Vis spectra were recorded in DMF on a UV-2500PC series V2.30 spectrum version at NARICT, Zaria, Nigeria, using matched 1 cm quartz cells. Absorption maxima are given in nanometer (nm) while the numbers in parenthesis are  $\epsilon$ -values. Infrared Spectral data were obtained on FTIR-8400S (Fourier Transform Infrared Spectrophotometer), NARICT in Zaria, Nigeria using KBr disc and absorptions are given per centimeter ( $\text{cm}^{-1}$ ). The analytical samples were obtained by recrystallization from benzene.

### 2.1. 3-Nitrodiphenylamine **4**

A mixture of 3-nitroaniline **2** (27g, 0.2mole), phenol **3** (19g, 0.2mole), dimethylformamide (DMF) (70ml) and a solution of potassium hydroxide (6.0g, 0.1mole), (15ml) were placed in a two-neck round bottom flask and heated under reflux for two hours. Thereafter the mixture was cooled and filtered and a yellowish solid product was obtained. This was dried and recrystallized from methanol, a yellowish crystalline product, 3-nitrodiphenylamine **4** was obtained in 56% yield (27.0g), melting at 110°C. UV-Vis ( $\lambda_{\text{max}}$ ) ( $\epsilon$ ): 450nm (1.9908), 495nm (1.8098), 435nm (1.5940). IR 333.2cm N-H stretching, 2724.7cm<sup>-1</sup>(C-H stretching), 1580.4 cm<sup>-1</sup>

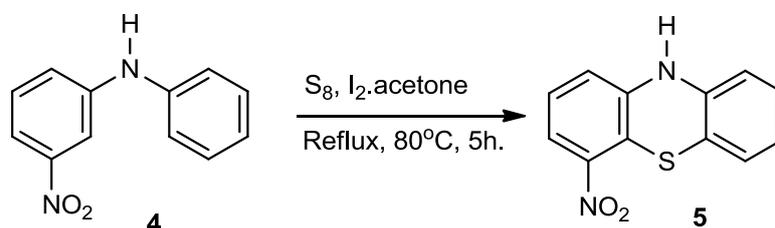
<sup>1</sup> (C=C stretching), 1263.6cm<sup>-1</sup>(C-N stretching), 1110.7 cm<sup>-1</sup>(C-H bending). Chemical Formula: C<sub>12</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>, Mol. Wt: 214.22, m/z: 214.07 (100.0%), 215.08 (13.2%), 216.08 (1.2%). Elemental Analysis: C, 67.28; H, 4.71; N, 13.08; O, 14.94.



**Figure 2.** Reaction scheme for the synthesis of 3-nitrodiphenylamine 4.

## 2.2. 4-Nitro-[10H]-phenothiazine 5

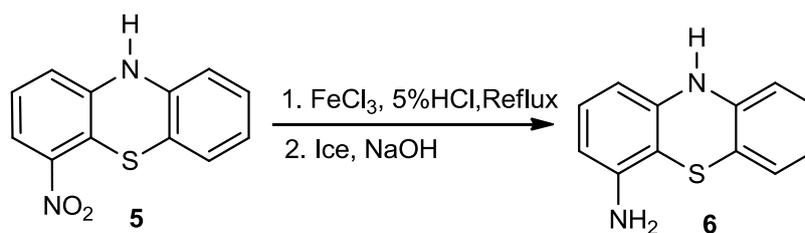
A mixture of 3-nitrodiphenylamine **4** (19g, 0.1mole), sulfur (6.4g, 0.2mole) and iodine (2g, 0.01mole) were dissolved in acetone (150ml) and poured into a three-neck round bottom flask. The mixture was heated under reflux for five hours. After the reaction, a yellowish product was obtained. It was filtered, dried and recrystallized from methanol and a brownish-yellow compound, 4-nitro-[10H]-phenothiazine **5**, was obtained with a yield of 68.2%, (18.7g), melting at 124°C. UV-Vis ( $\lambda_{max}$ ) ( $\epsilon$ ): 485nm --(2.1456), 460nm (2.0350), 430nm(1.9023), IR 3570.8 cm<sup>-1</sup> (N-H stretch), 3429.2 cm<sup>-1</sup> (C-H stretch), 1513.3 cm<sup>-1</sup> (C=C stretch), 1263.6 cm<sup>-1</sup> (C-N stretch), 1110.7 cm<sup>-1</sup> (C-S stretch), 667.2 cm<sup>-1</sup>(C-S). Chemical Formula: C<sub>12</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>S, Mol.Wt: 244.27, m/z: 244.03 (100.0%), 245.03 (14.6%), 246.03 (5.1%). Elemental Analysis: C, 59.00; H, 3.30; N, 11.47; O, 13.10; S, 13.13



**Figure 3.** Reaction scheme for the synthesis of 4-nitro-[10H]-phenothiazine 5.

## 2.3. 4-Amino-[10H]-phenothiazine 6

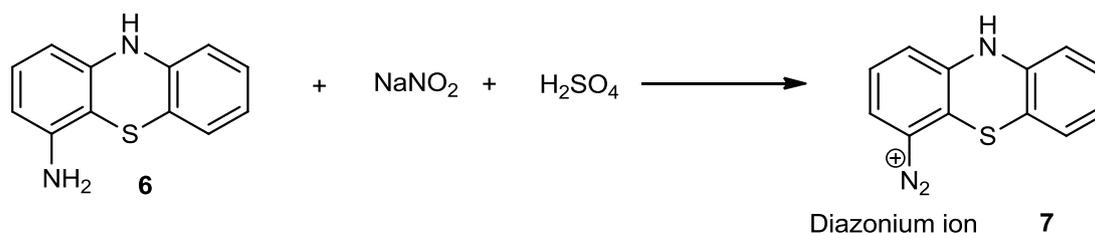
Iron (III) chloride (2g, 0.01mole), distilled water (50ml), 5% aqueous HCl (5.0ml) and 4-nitro[10H]phenothiazine **5** (9g, 0.02mole).were placed in a 250ml reaction flask. The mixture was refluxed for 45minutes on a water bath. After the reaction, the content of the flask was cooled in ice bath, and sodium hydroxide solution (50ml) was added and treated with crushed ice. The product was filtered and the residue was allowed to dry [14]. It was recrystallized from methanol and a greenish solid product, 4-amino phenothiazine **6**, was obtained with a yield of 72%, (8.0g), melting at 132°C. UV-Vis ( $\lambda_{max}$ ) ( $\epsilon$ ): 710(3.1409), 680(3.0083), 595(2.6322), IR: 3328.5cm<sup>-1</sup>(N-H), 2931.1cm<sup>-1</sup>(C-H stretching), 1617.7cm<sup>-1</sup> (C=C stretching), 1263.5cm<sup>-1</sup> (C-N stretching), 734.3 cm<sup>-1</sup> (C-H bending out plane of aromatic).Chemical Formula: C<sub>12</sub>H<sub>10</sub>N<sub>2</sub>S, Mol. Wt: 214.29, m/z: 214.06 (100.0%), 215.06 (13.9%), 216.05 (4.5%), Elemental Analysis: C, 67.26; H, 4.70; N, 13.07; S, 14.96.



**Figure 4.** Reaction scheme for the conversion of 4-nitro-[10H]-phenothiazine **5** to 4-amino-[10H]-phenothiazine **6**.

#### 2.4. Synthesis of an unstable diazonium ion **7**

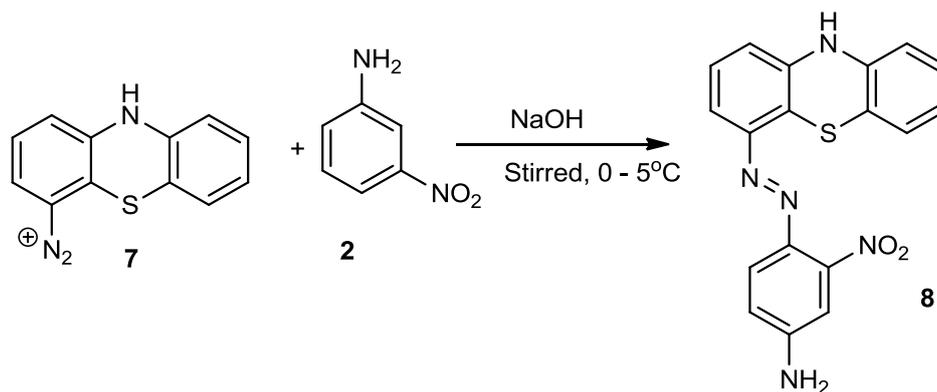
Concentrated tetraoxosulphate (VI) acid,  $\text{H}_2\text{SO}_4$  (5ml) was added to a solution of 4-amino-[10H]-phenothiazine **6**, (1.0g) (10ml) contained in a 500ml beaker. The content of the beaker was placed on an ice bath and stirred thoroughly at  $-5^\circ\text{C}$  for 30min and kept for subsequent use. In another 100ml beaker, sodium nitrite ( $\text{NaNO}_2$ ) (1.38gm, 0.02mole) (10ml) was placed and cooled on an ice bath and added drop wise to the mixture contained in former beaker with continuous stirring for about 5min at a temperature of  $0-5^\circ\text{C}$ . A foamy brownish intermediate was observed but could not be isolated due its unstable nature. It was immediately used for azo-coupling to avoid its decomposition [16 – 19].



**Figure 5.** Reaction scheme for the conversion of 4-amino-[10H]-phenothiazine **6** to an unstable diazonium ion **7**.

#### 2.5. 4-Diazo-(4-amino-6-nitroanilino)-[10H]-phenothiazine **8**

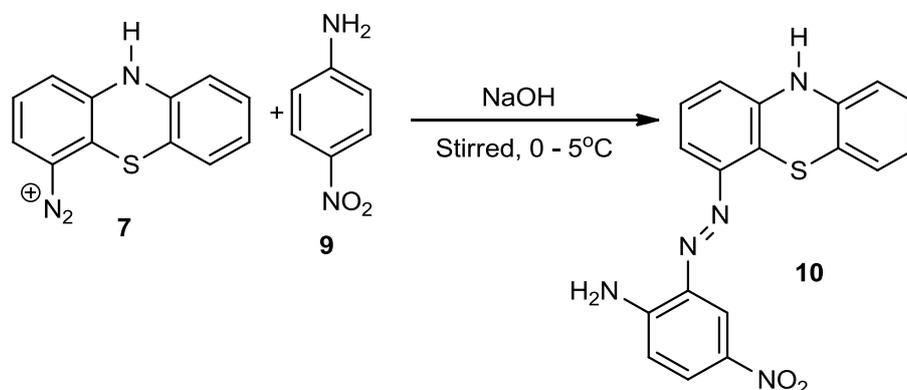
3-Nitroaniline (2.0g) **2** was placed in a 250ml beaker containing a solution of 10% NaOH (10ml) and diazonium ion **7** was slowly added to the above mixture and stirred for 5minutes while maintaining a temperature of  $0-5^\circ\text{C}$ . A dark brown foamy precipitate obtained was filtered and allowed to dry. The dried filtrate was then recrystallized from methanol and a light yellowish solid compound **8** was obtained with a % yield of 86.8%, (3.5g), melting at  $140^\circ\text{C}$ . UV-Vis ( $\lambda_{\text{max}}$ ) ( $\epsilon$ ): 460nm (2.0350), 4259nm (1.8801), 365nm (1.6147), IR  $3369.5 \text{ cm}^{-1}$  (N-H),  $3280.1 \text{ cm}^{-1}$  (C-H),  $1617.7 \text{ cm}^{-1}$  (C=C),  $1244.9 \text{ cm}^{-1}$  (C-N),  $670.7 \text{ cm}^{-1}$  (C-H bending). Chemical Formula:  $\text{C}_{18}\text{H}_{13}\text{N}_5\text{O}_2\text{S}$ , Mol. Wt: 363.39, m/z: 363.08 (100.0%), 364.08 (22.2%), 365.07 (4.5%), 365.09 (1.8%), Elemental Analysis: C, 59.49; H, 3.61; N, 19.27; O, 8.81; S, 8.82.



**Figure 6.** Reaction scheme for the synthesis of 4-diazo-(4-amino-6-nitroanilino)-[10H]-phenothiazine 8.

### 2.6. 4-Diazo-(2-amino-5-nitroaniline)-[10H]-phenothiazine 10

4-Nitroaniline (2.0g) **9** was placed in a 250ml beaker containing a solution of 10% NaOH (10ml) and diazonium ion **7** was slowly added to the above mixture and stirred for 5 minutes while maintaining a temperature of 0-5 °C. A dark brown foamy precipitate obtained was filtered and allowed to dry. The dried filtrate was then recrystallized from methanol and a dark yellowish solid compound **10** was obtained with a % yield of 79.9%, (3.5g), melting at 170°C. UV-Vis ( $\lambda_{max}$ ) ( $\epsilon$ ): 980nm (4.3354), 460nm (2.3509), 440nm (1.9465). IR: 3369.5 $cm^{-1}$ (N-H stretching), 3108.6 $cm^{-1}$ (C-H stretching), 1636.3  $cm^{-1}$ (C=C stretching), 1107.0  $cm^{-1}$ (C-N stretching), 689.6  $cm^{-1}$ (C-S stretching). Chemical Formula:  $C_{18}H_{13}N_5O_2S$ , Mol. Wt: 363.39, m/z: 363.08 (100.0%), 364.08 (22.2%), 365.07 (4.5%), 365.09 (1.8%), Elemental Analysis: C, 59.49; H, 3.61; N, 19.27; O, 8.81; S, 8.82.

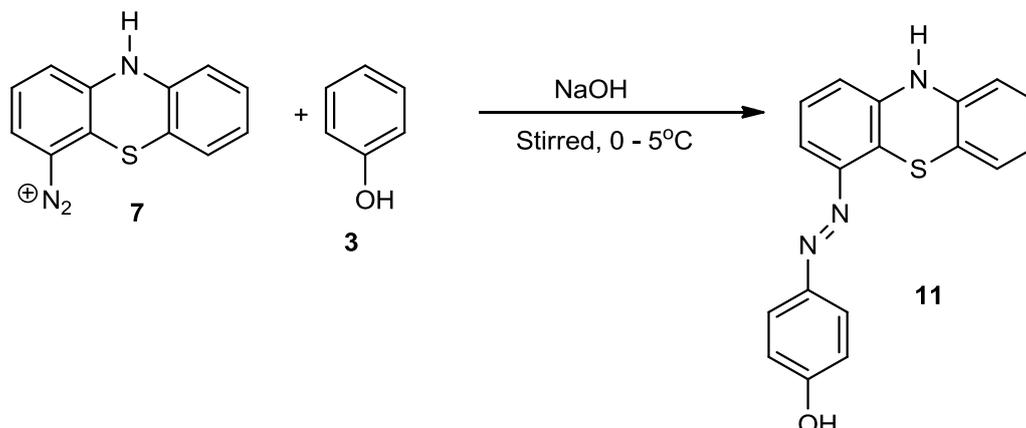


**Figure 7.** Reaction scheme for the synthesis of 4-diazo-(2-amino-5-nitroanilino)-[10H]-phenothiazine 10

### 2.7. 4-Diazo-(4-hydroxyphenyl)-[10H]-phenothiazine 11

Phenol (2.0g) **3** was placed in a 250ml beaker containing a solution of 10% NaOH (10ml) and diazonium ion **7** was slowly added to the above mixture and stirred for 5 minutes while maintaining a temperature of 0-5 °C. A dark brown foamy precipitate obtained was filtered and allowed to dry. The dried filtrate was then recrystallized from methanol and a deep brownish solid compound **11** was obtained with a % yield of 43.3%, (1.9g), melting at 160°C. UV-Vis ( $\lambda_{max}$ ) ( $\epsilon$ ): 490nm (2.1677), 425nm (1.8801), 415nm (1.8359). IR 3470.2 $cm^{-1}$  (O-H), 3397.7  $cm^{-1}$ (N-H stretching), 2855.1 $cm^{-1}$ (C-H stretching), 1271.0  $cm^{-1}$ (C-N stretching), 1138.8 $cm^{-1}$ (C-O stretching),

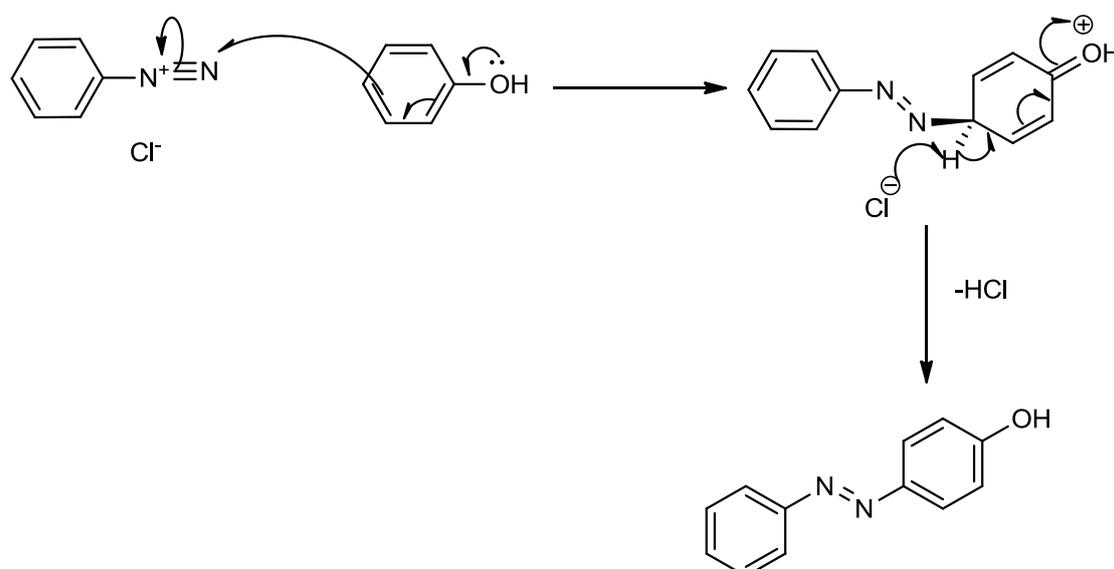
738.0cm (C-H bending of aromatic). Chemical Formula:  $C_{18}H_{13}N_3OS$ , Mol. Wt: 319.38, m/z: 319.08 (100.0%), 320.08 (20.5%), 321.07 (4.5%), 321.08 (2.4%), 320.07 (1.1%), Elemental Analysis: C, 67.69; H, 4.10; N, 13.16; O, 5.01; S, 10.04.



**Figure 8.** Reaction scheme for the synthesis of 4-diazo-(4-hydroxyphenyl)-[10H]-phenothiazine 11.

## 2.8. Mechanism for Azo Coupling

In practice, a solution of a benzene diazonium salt is added to an alkaline solution of a phenol or aromatic amine. The benzene diazoniumcation behaves as an electrophile, but it is a weak electrophile and so the aromatic ring which it attacks must have an activating group such as -OH or -NH<sub>2</sub> attached to it. An electrophilic substitution reaction occurs to form an azo-dye or compound. In the mechanism shown below, the electrophile, chloride ion activates the reaction and electrophilic substitution then takes place on phenol. Substitution reaction takes place always in *para* position except when the position is already occupied, in which case *ortho* position is favoured. The *pH* of solution is quite important; it must be mildly acidic or neutral, since no reaction takes place if the *pH* is too low [20].



**Figure 9.** Reaction mechanism for azo coupling of a benzene diazonium intermediate and phenol.

## 3. Anti microbial Analysis

### 3.1. Preparation of Potato Dextrose Agar

Potato dextrose agar powder (3.9g) was weighed into a 250ml conical flask and then dissolved in distilled water (150ml). The media was homogenized by agitation and then sterilized by autoclaving at 121°C for 15minutes, after which it was aseptically poured into sterile Petri dishes and allowed to gel.

### **3.2. Preparation of Nutrient Broth**

0.637g of the potato nutrient broth powder was weighed into a 200ml conical flask and then dissolved distilled water (50ml). The media was homogenized by agitating and then dispensed into different test tubes, sterilized by autoclaving at 121°C for 15minutes.

### **3.3. Preparation of Sabouraud Dextrose Agar**

Sabouraud dextrose agar powder (33.5g) was weighed into a 500ml conical flask and then dissolved in distilled water (500ml). The media was homogenized by agitating and then sterilized by autoclaving at 121°C for 15 minutes, after which it was aseptically poured into sterile Petri dishes and allowed to gel.

### **3.4. Preparation of Mueller Hinton Agar**

Mueller Hinton agar powder (38.0g) was weighed into a 1000ml conical flask and then dissolved in distilled water (1000ml). The media was homogenized by agitating and then sterilized by autoclaving at 121°C for 15 minutes, after which it was aseptically poured into sterile Petri dishes and allowed to gel.

### **3.5. Test Organisms**

The test organisms used were obtained from the microbiology laboratory section and mycology laboratory section of the University of Nigeria Teaching Hospital Ituku- ozala Enugu State, Nigeria. The test organisms used were four in number and they include one gram positive bacteria (*Staphylococcus aureus*), one gram negative bacteria (*Escherichia coli*) and two fungi *Aspergillus* and *Penicillium*.

### **3.6. Growing and Subculture of Microorganisms**

From the pure culture in a bijou bottle, a loopful of each test organism was transferred into different test tubes containing an already sterilized nutrient broth. After the inoculation, the test tubes were put in an incubator at a temperature of 35-37 °C for the bacteria and 25-27 °C for the fungi. The bacteria culture was incubated for eighteen (18) hours while the fungi were incubated for about twenty-four (24) hours.

### **3.7. Antibacterial and Antifungal Assay**

The agar well diffusion method with modification was used to evaluate the antimicrobial and antifungal activity against the test microorganisms [21].

The test organisms were spread aseptically using a cotton swab on the surface of the already prepared Mueller Hinton agar for the bacteria and the fungi was aseptically inoculated on the already prepared Sabouraud dextrose agar.

All culture plates were allowed to dry for about five (6) minutes and six wells were made on the agar using a 6mm sterile cork borer. Four wells were filled with 200µl of 200mg/ml, 150mg/ml 100mg/ml, 50mg/ml concentration of each synthesized

compound respectively, the remaining two wells were filled with 200 $\mu$ l of 50mg/ml concentration of the positive control and 200 $\mu$ l of the negative control. The plates were kept on the work bench to allow the agents diffuse into the agar and incubated accordingly.

Ciprofloxacin and fluconazole were used as the positive controls for the antibacterial and antifungal evaluation respectively while distilled water was used as the negative control.

The Mueller Hinton agar plates were incubated at 37 °C for twenty-four (24) hours and the Sabouraud dextrose agar plates were incubated at 27 °C for two (2) days.

The antimicrobial activities were evaluated by measuring the diameter of the inhibition zones in millimeters and the readings were recorded accordingly as wells as the minimum inhibition concentration (MIC) of the synthesized compounds. . The experiment was replicated twice and an average of two independent reading for each microorganism was used.

#### 4. Results and Discussion

Four new linear phenothiazine azo-dye derivatives were synthesized. These are 4-nitro-[10H]-phenothiazine, 4-amino-[10H]-phenothiazine, 4-azo-(4-amino-2-nitroanilino)-[10H]-phenothiazine, 4-azo-(2-amino-5-nitroanilino)-[10H]-phenothiazine and 4-azo-(4-hydroxyphenyl)-[10H]-phenothiazine. The starting materials, 3-nitroaniline and phenol were reacted in presence of potassium hydroxide and DMF (solvent) to give 3-nitrodiphenylamine. Thereafter, 3-nitrodiphenylamine was sulphonated in presence of iodine to give the parent compound, 4-nitro-[10H]-phenothiazine with a of 56% yield (27.0g), melting at 110°C. The UV-visible spectrum of the parent compound showed the following absorption band 485nm -- (2.1456), 460nm (2.0350), 430nm(1.9023). This indicated a bathochromic shift which is a shift to the right (longer wavelength) while the IR-spectrum revealed absorption band at 3570.8  $\text{cm}^{-1}$  (N-H stretch), 3429.2  $\text{cm}^{-1}$  (C-H stretch) for aromatic systems, 1513.3  $\text{cm}^{-1}$  (C=C stretch), 1263.6  $\text{cm}^{-1}$  (C-N stretch), 1110.7  $\text{cm}^{-1}$  (C-S stretch), 667.2  $\text{cm}^{-1}$ (C-S). The conversion of the nitro group in 4-nitro-[10H]-phenothiazine to amino functionality was achieved by treating the compound with iron (III) chloride in the presence of hydrochloric acid to give 4-amino-[10H]-phenothiazine. The compound obtained was then subjected to diazotization using sodium nitrite and concentrated hydrochloric acid to yield diazonium ion which was subsequently coupled with the following aromatic compounds compounds; 3-nitroaniline, 4-nitroaniline and phenol to furnish 4-azo-(4-amino-2-nitroanilino)-[10H]-phenothiazine, 4-azo-(2-amino-5-nitroanilino)-[10H]-phenothiazine and 4-azo-(4-hydroxyphenyl)-[10H]-phenothiazine respectively in vary % yields. The U-visible spectra of these compounds also exhibited bathochromic shifts (longer wavelength) due the presence of extended conjugation. Both aliphatic and aromatic primary amines can form diazonium salts. They do this by reacting with nitric (III) acid (nitrous acid),  $\text{HNO}_2$ , at a temperature of 0-5 °C. Due to the instability of nitric (III) acid, it is always generated during a reaction, by the action of dilute tetraoxosulphate (VI) acid or hydrochloric acid on sodium nitrate (III) (sodium nitrite),  $\text{NaNO}_2$ . The acid used to generate nitric (III) acid provides the anion of the diazonium salt. However, alkyl diazonium salts are extremely unstable and always decompose to evolve the colourless unreactive nitrogen gas, amongst other products. The diazonium cations of aromatic diazonium salts are somewhat more stable than their aliphatic counterparts. With phenylamine the benzene diazonium ion is formed. Although benzene diazonium salts can be isolated in the crystalline form, they are usually retained in solution and used immediately as they decompose on standing even in the cold. In the solid state the salts are explosive and can be easily detonated by a slight shock or on mild warming [20]. Antimicrobial screening of synthesized compounds was also carried out and the compounds showed good inhibition on the tested organisms.

All the synthesized compounds were screened for their antimicrobial activity at concentration of 200mg/mol, 150mg/mol, 100mgmol<sup>-1</sup> and 50mg/mol using agar well diffusion method [21].

Ciprofloxacin was used as positive control for bacteria while Fluconazole was used as the positive control for antifungal activity. Some of the compounds were found to be moderately active against *Escherichia coli* (gram negative bacteria) and *Stapylococcus aureus* (gram positive bacteria) as well as against *Aspergillus fumigates* and *Penicillium chrysogenum* (fungi). A close look at the activity index reveals that the compound 20 is better antimicrobial agent than other synthesized compounds. Although the activity index of compound 10 was greater in the antibacterial region. The results of the all activities (antibacterial and antifungal) are summarized in the tables below.

**Table 1.** Zones of Inhibition (mm) observed when the synthesized compounds were used.

Compound	Conc.(mg/ml)	<i>E. coli</i>	<i>S. aureus</i>	<i>Aspergillus</i>	<i>Penicillium</i>
6	200	0.0	9.0	10.0	4.5
“	150	0.0	7.5	8.5	2.8
“	100	0.0	6.0	7.0	2.0
“	50	0.0	3.5	3.6	1.0
8	200	0.0	0.0	9.5	9.5
“	150	0.0	0.0	7.8	7.7
“	100	0.0	0.0	6.5	6.5
“	50	0.0	0.0	2.5	3.0
10	200	0.0	7.9	15.3	16.0
“	150	0.0	6.7	12.3	13.0
“	100	0.0	5.0	10.0	12.0
“	50	0.0	2.1	6.5	8.5
11	200	6.5	0.0	10.2	5.2
“	150	5.5	0.0	8.6	4.0
“	100	4.0	0.0	7.0	2.0
“	50	2.0	0.0	4.5	1.5

Key: 0mm = no inhibition, 0-10mm = moderate sensitivity, 10-20mm = very sensitive

The minimum Inhibitory Concentration (MIC) of the compounds was also determined. For both bacteria and fungi, various concentrations of compounds (200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml) were used to inhibit the test organisms using broth dilution methods. Compounds **6**, **8**, and **10**, compound **11** showed no inhibition at all against *E. coli*, while compounds **8** and **11** showed no inhibition against *Staphylococcus aureus*. The lowest MIC (100mg/ml) against the bacterial strains was found to be in compounds **6**, **10**, and **11**. This implies that these compounds are not good antibacterial agents. For fungi, the lowest MIC (50.0mg/ml) was found in compounds **6**, **8**, **10** and **11** against *Aspergillus fumigates* and in compounds **8** and **10** against *Penicillium chrysogenum* respectively.

**Table 2.** Minimum inhibitory concentration of the synthesized compounds and micro-organisms turbidity at different concentrations (mg/ml).

Compound	Conc.(mg/ml)	<i>E. coli</i>	<i>S. aureus</i>	<i>A. fumigates</i>	<i>P. chrysogenum</i>
6	200	+++	–	–	–
“	150	+++	–	–	+
“	100	+++	+	+	++
“	50	+++	++	+	+++
8	200	+++	+++	–	–

“	150	+++	+++	–	-
“	100	+++	+++	–	-
“	50	+++	+++	+	+
				–	–
10	200	+++	–	–	–
“	150	+++	–	–	–
“	100	+++	++	–	–
“	50	+++	+++	+	+
11	200	–	+++	–	-
“	150	–	+++	–	+
“	100	+	+++	–	+
“	50	++	+++	+	++

Key: - = no growth, + = slight turbidity, ++ = moderate turbidity, +++ = very turbid.

## 5. Conclusions

The synthesis of the phenothiazine derivatives discussed above was carried out using simple commercially available starting materials. The methods employed are straight forward and stereo-selective products were obtained. These newly synthesized compounds are novel and will be useful in pharmaceutical, textile, petroleum, agricultural industries etc. The high melting points exhibited by these compounds suggest that they can be used as thermal stabilizers. Also, due to their highly coloured nature, they are suitable to be used as vat dyes. Since these compounds also exhibited some level of activity against the tested microorganism, they can be employed as starting materials for drug synthesis.

## Conflicts of Interest

The authors declare they have no conflicts of interest.

## Acknowledgments

The authors are very grateful to the Vice Chancellor of Godfrey Okoye University, Enugu, Nigeria, Professor Christian Anieke, for providing an enabling environment for academic work and research. We also wish to extend our profound appreciation to the staff of the Chemistry Laboratory, Chemical Sciences Department and those of Biological Sciences Department of Godfrey Okoye University, Enugu, Nigeria for their technical assistance.

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