



## Synthesis of Benzothiazinophenothiazine Derivatives and their Antimicrobial Screening

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### ABSTRACT

The synthesis of benzothiazinophenothiazine derivatives from simple heterocyclic compounds was thoroughly investigated. The intermediate, 6-chloro-5H-benzo[a]phenothiazin-5-one was afforded by the condensation of 2-aminothiophenol with 2,3-dichloro-1,4-naphthoquinone in an alkaline medium. Further condensation of the intermediate with 2,4-diamino-6-hydroxypyrimidine-5-thiol obtained by alkaline hydrolysis of 2,4-diamino-6-hydroxy-5-thiacyanatopyrimidine gave 7-amino-9-hydroxy-6,8-diazabenzothiazino[3,2-c]phenothiazine. However, facile acid-catalyzed synthesis of four other benzothiazinophenothiazine ring systems was accomplished with improved yield and lesser reaction time. The Structural confirmation was done using UV-Visible spectroscopy, FT-IR, <sup>1</sup>H and <sup>13</sup>C-NMR and elemental analysis. The synthesized compounds were screened against some micro-organisms and the results showed that the complex derivatives were significantly active against the microorganisms.

**Key words:** Benzothiazinophenothiazine derivatives, antimicrobial screening, Sensitivity test, minimum inhibition concentration and inhibition zone diameter.

### INTRODUCTION

Phenothiazines are amongst the most frequently encountered bioactive heterocycles in compound of biological interest.<sup>1</sup> Phenothiazine derivatives have been shown to possess a broad spectrum of pharmacological activity depending on their particular structure like antiparkinsonian<sup>2-3</sup>,

tranquilizer<sup>4</sup>, anti-inflammatory<sup>5-7</sup>, antimalarial<sup>8-9</sup>, antipsychotic,<sup>10-12</sup> antimicrobial<sup>13-15</sup>, anti-tubercular<sup>16-18</sup>, antitumor<sup>19-20</sup>, antihistaminic<sup>21-22</sup>, analgesic<sup>23</sup>, prion disease drug<sup>24</sup>. In textile, paint and plastic industries, they are used as dyes and pigments<sup>25-26</sup> and in agricultural industries as insecticides.<sup>27</sup> In petroleum industries, they are found useful as antioxidants in lubricants and fuels.<sup>28</sup> It has been

observed that some phenothiazines inhibit intracellular replication of viruses including human Immunodeficiency viruses (HIV) <sup>29</sup>. On the other hand, some have been reported to exhibit significant anticancer activity<sup>30-31</sup>. Owing to the wide range of applications of phenothiazine, intensive research has been in progress for more derivatives with highly improved pharmacological and biological activities. Hence, several papers describing the successful synthesis of these derivatives had been reported especially on the angular derivatives including the non-aza and the congeneric aza-analogues, but there are still limited literatures on the complex derivatives of this phenothiazine ring system. The past work done was based on their dye and pigment properties, not much is known of their antimicrobial properties. Therefore, the authors describe the synthesis of complex aza derivatives of benzothiazinophenothiazine and their antimicrobial screening.

### EXPERIMENTAL

All chemicals used were of laboratory grade (Sigma Aldrich). The melting points were determined with a Fischer John's apparatus and were uncorrected. UV/Visible spectra were recorded on UV-25500PC series spectrophotometer using matched 1 cm quartz cells. The IR spectra were recorded on 8400S FT-IR spectrometer using KBr discs (at NARICT, ZARIA). The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR were scanned at university of stretchclyde, Scotland on a Jeol FX 90Q spectrometer using TMS as internal standard (chemical shift in  $\delta$ ). Elemental analysis was carried on CHN rapid analyzer and the antimicrobial screening was done at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

#### Synthesis of 6-chloro-5H-benzo[a]phenothiazin-5-one (1)

To a mixture of 2-aminothiophenol (4.0 g, 32 mmol) and anhydrous sodium trioxocarbonate (IV) (3.3 g, 31 mmol) in a 250 mL two-necked flask equipped with magnetic stirrer, thermometer and reflux condenser, was added a solution of benzene (100 mL) and DMF (10 mL). The mixture was boiled for 1 h and thereafter, 2,3-dichloro-1,4-naphthoquinone (7.26 g, 32 mmol) was added and the entire solution refluxed with continuous stirring

for more 7 h at 78-80 °C. Then, the solvent was distilled off and the slurry poured into water and stirred for 20 min to dissolve the inorganic materials. It was left overnight, filtered and recrystallized from methanol-acetone mixture to obtain a purple microcrystalline powder of **1** (8.5 g, 85% yield). m.p 234 °C; Uv-V (MeOH)  $\lambda_{\max}$  (nm) log( $\epsilon$ ): 210 (1.978), 221 (1.4779), 228 (1.2517), 234 (1.3543), 247 (0.2255), 255 (0.5149), 261 (1.4326), 266 (1.2138), 289 (2.3308), 314 (2.3640), 379 (2.3041), 491 (2.2271); FT-IR (KBr):  $\nu_{\max}$  3063 cm<sup>-1</sup> (aromatic C-H str.), 1631 cm<sup>-1</sup> (C=O), 1502 (aromatic C=N), 1467 (aromatic C=C) 780 (C-Cl), 1292, 1228, 1155, 1082, 1039, 902, 680, 567 and 462 cm<sup>-1</sup>.

#### Synthesis of 7-amino-9-hydroxy-6, 8-diazabenz[a][1,4]benzothiazino[3,2-c]phenothiazine (2)

The same procedure as in compound **1** above was employed using 2,4-diamino-6-hydroxypyrimidine-5-thiol (1.0 g, 6 mmol) and anhydrous sodium trioxocarbonate (IV) (1.5 g, 14 mmol) but 6-chloro-5H-benzo[a] phenothiazin-5-one (1.8 g, 6 mmol) in the second stage instead of 2,3-dichloro-1,4-naphthoquinone to give **2** (2.46 g 84 % yield) as purple-red crystals; m.p. > 300 °C; Uv-V (MeOH) (nm) log: 780 (1.7000), 745 (1.7204), 688 (1.7704), 486 (2.5758), 380 (2.6022), 319 (2.6437), 265 (2.8636), 254 (2.7968), 247 (2.7875); IR (KBr):  $\nu_{\max}$  3761 (OH), 3400, 3331 (>N-H str) 3063, (aromatic C-H str.), 1608 (arom. C=N) 1494, 1452 (arom. C=C), 1300, 1242, 1151, 1087, 1024, 877, 850, 758, 673, 642, 538 and 455 cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO:  $d_6$ )  $\delta$ : 8.87 (2H, dd,  $J_1 = 7.87$ ,  $J_2 = 1.36$  Hz), 8.24 (2H, dd,  $J_1 = 7.55$ ,  $J_2 = 1.61$  Hz). 8.08 (4H, m, Ar-H), 7.92-7.67 (4H, m, 1-H, 2-H, 3-H), 5.46 (1H, s, 9-OH); <sup>13</sup>C-NMR (DMSO)  $\delta$ : Few peaks, due to the insolubility of the compound. Absence of chemical shift at 150 ppm and above indicates the absence of C=O. Analysis calculated for C<sub>20</sub>H<sub>11</sub>N<sub>5</sub>OS<sub>2</sub>: C, 59.84, H, 2.76, N, 17.44 and S, 15.97. Found: C, 59.88, H, 2.81, N, 17.45 and S, 15.99.

#### Synthesis of 7,14-diethylthio-9,12-dihydroxy-6,8,13,15-tetraazabenz[a][1,4] benzothiazino [3,2-c]phenothiazine. (3)

To 4-amino-2-ethylthio-6-hydroxypyrimidine-5-thiol (2 g; 10 mmol) in 250 mL two-necked flask equipped with magnetic stirrer, thermometer and a reflux condenser, was added absolute

ethanol (100 mL) and 15 % HCl (10 mL). The mixture was warmed to dissolve the solid, 2,3-dichloro-1,4-naphthoquinone (1.1 g; 5 mmol) was added and the mixture refluxed on a water bath for 6 h at 78 °C. On refluxing, an immediate appearance of orange brown ppt was observed which turned blue and persisted. Thereafter, the mixture was poured in cold water, stirred, filtered and the residue crystallized from acetone-methanol mixture to obtain **3** (3.2 g; 91 % yield) as blue powder. m.p > 310 °C; Uv-V (MeOH) (nm) log(): 748 (1.2533), 539 (2.4100), 328 (3.2316), 322 (3.2249), 305 (3.2634), 276 (1.5901), 266 (1.7812), 257 (1.0315), 239 (1.0895), 233 (0.9881), 288 (0.8554), 222 (1.3721); IR (KBr)  $\nu_{\max}$  3755, 3400 (OH), 3184 (aromatic C-H str.), 2937 (C-H str of CH<sub>3</sub> or CH<sub>2</sub>), 1626, 1556, (aromatic C=N), 1518, 1446 (aromatic C=C), 1325, 1276, 1224, 1130, 1095, 964, 885, 823, 779, 700, 648, 561, 447 cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO: d<sub>6</sub>)  $\delta$ : 8.08 (4H, m, Ar-H), 7.91 (4H, td, J<sub>1</sub> = 6.72, J<sub>2</sub> = 5.89, J<sub>3</sub> = 3.44 Hz, Ar-H), 3.3 (2H, q, J<sub>1</sub> = 8.16, J<sub>2</sub> = 7.3 Hz, -SCH<sub>2</sub>CH<sub>3</sub>), 3.06 (3H, t, -SCH<sub>2</sub>CH<sub>3</sub>), 1.33 (3H, dt, J<sub>2</sub> = 54.74, J<sub>2</sub> = 7.41 Hz); <sup>13</sup>C-NMR (DMSO)  $\delta$ : 142.98-127.63 (C<sub>1</sub> – C<sub>9</sub>, Ar-C), 15.19 (aliphatic carbon). Analysis: Calculated for C<sub>19</sub>H<sub>11</sub>N<sub>7</sub>O<sub>2</sub>S<sub>2</sub>: C, 52.65, H, 2.56, N, 22.62, S, 14.79. Found: C, 52.72, H, 2.59, N, 22.60, S, 14.90.

#### Synthesis of 8,3-dimethoxy-9,12-diazabenz[a][1,4]benzothiazino[3,2-c]phenothiazine (4)

3-Amino-6-methoxy-pyridin-2-thiol (2 g; 13 mmol) in absolute ethanol (100 mL) was placed in 2-necked reaction flask containing 10 mL of 15 % HCl and warmed. 2,3-dichloro-1,4-naphthoquinone (1.45 g, 6 mmol) was then added and the mixture refluxed on a water bath for 6.5 h at 78 °C. The initially formed yellow precipitate turned dark-brown which persisted throughout the reflux period. The mixture was then poured into a beaker and heated for 15 min to evaporate the remaining solvent. It was diluted twice with water and cooled. The dark-brown residue was collected after filtration, and crystallization from acetone-methanol mixture gave a brown microcrystalline powder of **4** (3.40 g; 94 % yield). m.p > 310 °C; Uv-V (MeOH) (nm) log(): 749 (1.6377), 478 (2.5529), 334 (3.0278), 296 (2.8135), 275 (1.2669), 269 (1.5525), 257 (1.0313), 246 (1.1897), 239 (1.4659), 228 (1.3409), 222 (1.5827), 212 (1.2566), 203 (1.6419); IR (KBr) 3088 (aromatic C-H str.), 1587 (C=N aromatic), 1504 (aromatic

C=C), 1346, 1278, 1147, 1087, 1022, 889, 831, 781, 692, 648, 555 and 462 cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO: d<sub>6</sub>)  $\delta$ : 10.57 (2H, d), 10.31 (2H, d, J = 7.51 Hz), 7.66 (4H, s, Het. Ar-H), 7.31 (4H, s, Ar-H), 4.28 (3H, d, -OCH<sub>3</sub>). <sup>13</sup>C-NMR (DMSO)  $\delta$ : There were few peaks due to difficulty in the solubility of the compound; 24.63-15.05 ppm (aliphatic carbon). Analysis: Calculated for C<sub>22</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>: C, 61.38, H, 3.28, N, 13.01, S, 14.90. Found: C, 61.45, H, 3.33, N, 13.21, S, 15.10.

#### Synthesis of 6,13-dichloro-3, 10-diethylthio-1,8-dihydroxy-2,4,9,11-tetrazatriphenodithiazine (5)

A mixture of 4-amino-2-ethylthio-6-hydroxypyrimidine-5-thiol (4 g; 19 mmol), absolute ethanol (150 mL) and 10 mL of 15 % HCl was warmed and tetrachloro-1,4-benzoquinone (2 g; 8 mmol) was then added. The mixture was refluxed on a water bath with continuous stirring for 6 h. An immediate formation of a yellow clear solution and then observation of yellowish-red precipitate occurs. At the end of the reflux period, the mixture was poured into a beaker, filtered and recrystallized from aqueous acetone to obtain a greenish yellow powder, **5** ((5.3,73 % yield g). m.p > 310 °C; Uv-V (MeOH) (nm) log(): 753 (1.1864), 671 (1.2357), 612 (1.5859), 531 (1.5920), 491 (1.6538), 322 (3.2634), 305 (3.2993), 274 (1.1819), 257 (1.4759), 251 (1.7954), 246 (1.2910); IR (KBr): 3751, 3375 (OH), 3196, (C-H str.), 1626 (aromatic C=N), 1548, 1437 (aromatic C=C), 1319, 1282, 1215, 1089, 962, 881, 773, 698, 545 and 451 cm<sup>-1</sup> Analysis: Calculated for C<sub>16</sub>H<sub>8</sub>Cl<sub>2</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub>: C, 42.58, H, 1.79, Cl, 15.70, N, 18.62, S, 14.21. Found: C, 42.67, H, 1.92, Cl, 15.73, N, 18.60, S, 14.26.

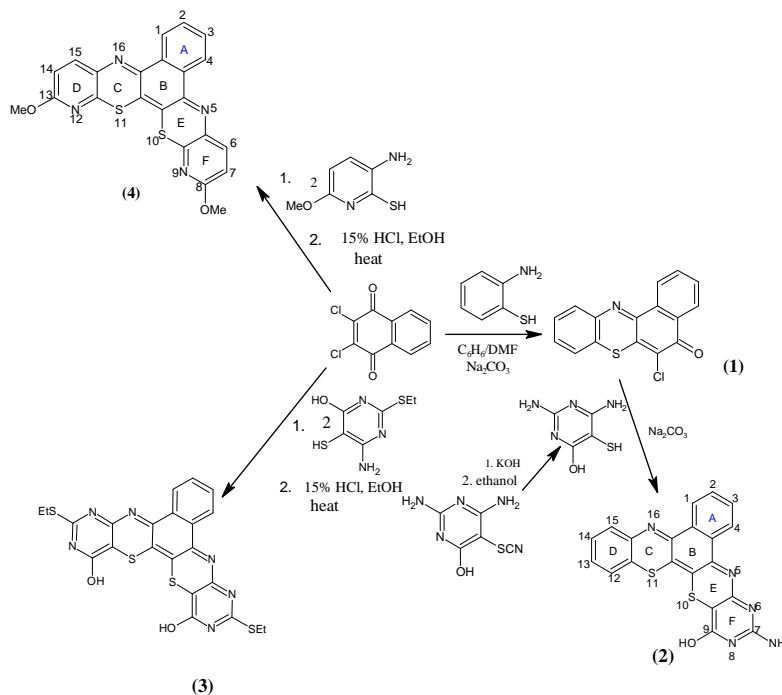
#### Synthesis of 6,13-dichloro-3-methyl-9-methoxy-1-hydroxy-2,4,8-triazatriphenodithiazine (6)

Mixture of 3-amino-6-methoxy-pyridine-2-thiol (2 g, 12 mmol), 4-amino-2-methyl-6-hydroxypyrimidine-5-thiol (2 g; 10 mmol) was placed in a reaction flask equipped with magnetic stirrer, thermometer and reflux condenser. Absolute ethanol (120 mL) and 10 mL of 15 % HCl were then added and solution was warmed to dissolve. Tetrachloro-1,4-benzoquinone (3 g, 12 mmol) was later added and the mixture refluxed with continuous stirring for 7 h at 78 °C. At the end of the reflux period, the mixture was poured into a clean beaker and 100 mL of water was added, it was

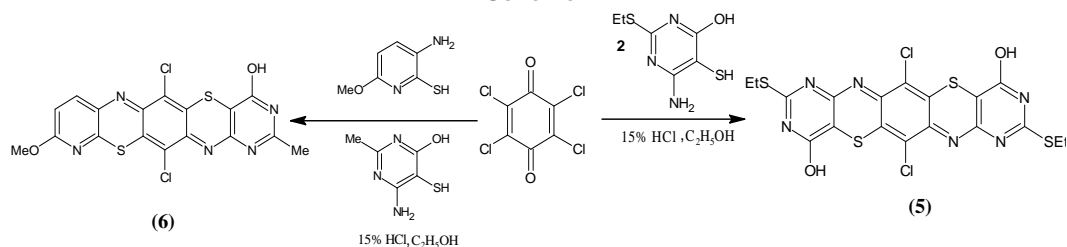
heated for 15 min and cooled. The crude product was collected by filtration, dried in an oven and crystallized from acetone-methanol mixture to give **6** as purple powder (4.79 g, 86 % yield). m.p > 300 °C; Uv-V (MeOH) (nm) log(ε): 750 (1.5670), 583 (2.1691), 343 (2.6682), 257 (3.0207), 228 (2.3856), 223 (1.2553); IR (KBr) : 3751, 3346 (OH), 3119 (aromatic C-H-stretch), 2918, 2852 (C-H stretch of CH<sub>3</sub>, CH<sub>2</sub>), 1641, 1595 (aromatic C=N), 1546, 1444 (aromatic C=C) 1354, 1273, 1155, 1085, 1012, 912, 833, 777, 723, 677, 608, 553, and 509cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) δ: 11.64 (1H, s), 7.01 (2H, d, J = 144.25 Hz Het. Ar-H), 2.4 (3H, s, -OCH<sub>3</sub>), 1.45 (3H, s, -CH<sub>3</sub>); <sup>13</sup>C-NMR (DMSO) δ: 165.96, 162.37, 159.79 (Ar-C), 88.53 (C=C), 21.43 (-CH<sub>3</sub>, aliphatic carbon); Analysis: Calculated for C<sub>17</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>2</sub>S<sub>2</sub>: C, 45.34, H, 2.01, Cl, 15.75, N, 15.55, S, 14.24. Found: C, 45.42, H, 2.30, Cl, 15.86, N, 15.53, S, 14.33.

## RESULTS AND DISCUSSION

2,3-Dichloro-1,4-naphthoquinone was refluxed with 2-aminothiophenol to yield 6-chloro-5*H*-benzo[*a*]phenothiazin-5-one **1**. Compound **1** on treatment with 2,4-diamino-6-hydroxypyrimidine-5-thiol, obtained from alkaline hydrolysis of 2,4-diamino-6-hydroxy-5-thiocyanatopyrimidine, in the presence of anhydrous Na<sub>2</sub>CO<sub>3</sub> under reflux yielded 7-amino-9-hydroxy-6,8-diazabenzobenzothiazino[3,2-*c*]phenothiazine **2**. The second derivative 7,14-diethylthio-9,12-dihydroxy-6,8,13,15-tetrazabenzobenzothiazino[3,2-*c*]phenothiazine **3** was synthesized as a blue powder by condensation of 2 moles of 4-amino-2-ethylthio-6-hydroxypyrimidine-5-thiol with a mole of 2,3-dichloro-1,4-naphthoquinone in an acid medium. 3-Amino-6-methoxypyridine-2-thiol also



Scheme 1:



Scheme 2:

condensed with the 2,3-dichloro-1,4-naphthoquinone in the presence of 15 % HCl and Ethanol to furnished a good yield of 8,13-dimethoxy-9,12-diazabenz[*a*][1,4]benzothiazino[3,2-*c*]phenothiazine 4 as a brown crystalline powder Scheme 1.

However, condensation of 2 moles of the 4-amino-2-ethylthio-6-hydroxypyrimidine-5-thiol with 1 mole of tetrachloro-1,4-benzoquinone in an acid medium gave 6,13-dichloro-3,10-diethylthio-1,8-dihydroxy-2,4,9,11-tetraazatriphenodithiazine 5 as greenish yellow powder. Another complex

azatriphenodithiazine ring system (6,13-dichloro-3-methyl-9-methoxy-1-hydroxy-2,4,8-triazatriphenodithiazine) 6 was synthesized as purple crystalline powder by condensing 3-amino-6-methoxypyridine-2-thiol, tetrachloro-1,4-benzoquinone and 4-amino-2-methyl-6-hydroxy-5-thiopyrimidine Scheme 2.

The IR spectra of compound 1 showed >NH stretching vibration as weak band in the region 3125- 3063  $\text{cm}^{-1}$  and the >C=O as strong band at 1631  $\text{cm}^{-1}$ . Bands at 1502 $\text{cm}^{-1}$  and 1608  $\text{cm}^{-1}$  were assigned to C=C and C=N stretching vibrations in

**Table 1: Results of Antimicrobial Sensitivity Test of the Synthesized Compounds**

Compound	Gram-positive bacteria			Gram-negative bacteria			Fungi Organisms	
	<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>C. albican</i>	<i>A. niger</i>
1	+	+	+	+	-	+	-	+
2	+	++	+	+	-	-	+	+
3	+++	+++	+++	+	-	+	+++	+++
4	+	+	-	-	+	-	++	+
5	+	++	-	-	+	-	-	++
6	-	+	-	+	-	+	+	-
Rf 1	++	++	+++	++	+	++	-	-
Rf 2	-	-	-	-	-	-	++	+++

+ = sensitive

++ = moderately sensitive

- = resistance

Rf 1, Rf 2 (reference drugs).

Rf 1 (Ciprofloxacin, antibacteria)

Rf 2 (Ketoconazole, antifungi)

**Table 2: Results of the Inhibition Zones Diameter (mm)**

Compound	Gram-positive bacteria			Gram-negative bacteria			Fungi Organisms	
	<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>C. albican</i>	<i>A. niger</i>
1	10	9	9	11	4	10	7	10
2	12	15	11	9	5	-	10	9
3	22	21	18	8	-	12	22	21
4	8	12	-	-	9	6	16	12
5	11	17	-	-	8	-	5	18
6	5	10	-	10	-	8	11	-
Rf 1	17	17	20	13	8	15	-	-
Rf 2	-	-	-	-	-	-	16	21

The compounds with the IZD > 8 were considered to be sensitive & active against the microorganisms, and upon serial dilution, gave the MIC. The higher the IZD values, the higher the activity.

both compound **1** and **2** respectively. The disappearance of a band due to C=O and the appearance of new band due to C=N at 1608 cm<sup>-1</sup> coupled with a shift in the absorption maxima from 491 nm in compound **1** to 780 nm in compound **2**, because of double bond extension, indicated the formation of **2** from **1**. The formations of **3** and **4** were also implicated by the appearance of bands 1556-1626 cm<sup>-1</sup> and 1587 cm<sup>-1</sup> (C=N), 561-648 cm<sup>-1</sup> and 648-692 cm<sup>-1</sup> (C-S-C) for compounds **3** and **4** respectively and disappearance of the band for the -NH<sub>2</sub> group in the precursor. Furthermore, the bands at 3375 cm<sup>-1</sup> (OH), 3196 cm<sup>-1</sup> (C-H str. aromatic), 1626 (aromatic C=N), 1548-1437 cm<sup>-1</sup> (aromatic C=C) and 698, 545 (C-S-C in the thiazine ring) were in agreement with the assigned structure of compound **5** likewise those at 3751-3346 cm<sup>-1</sup> (OH), 3119 (aromatic C-H str), 2918-2852 cm<sup>-1</sup> (C-H str of CH<sub>2</sub>, CH<sub>3</sub>), 1641- 1595 cm<sup>-1</sup> (aromatic C=N), 1546-1444 cm<sup>-1</sup> (aromatic C=C), 912-833 cm<sup>-1</sup> (CH-bend in substd benzene ring), 777 cm<sup>-1</sup> (C-Cl) and 677-609 cm<sup>-1</sup> (C-S-C in the thiazine ring) were consistent with the structure of compound **6**.

In the <sup>1</sup>H-NMR spectra of compounds **2- 4**, the multiplet due to aromatic protons appeared in the region δ 8.87- 7.91. The protons of the methine group and methyl group in -SCH<sub>2</sub>CH<sub>3</sub> appeared at δ 3.30 and 3.06 respectively in compound **3**. The peaks due to the four protons in pyridine rings of compounds **4** and **6** occurred between δ 7.66-7.01. The -OCH<sub>3</sub> protons in these two compounds showed a singlet in the region δ 2.40 – 4.28 likewise

the -CH<sub>3</sub> protons in compound **6** at δ 1.45. The structural confirmation of the synthesized compounds was achieved with elemental analysis. With the exception of compounds **4** and **5** which showed few peaks due to the insolubility, the <sup>13</sup>C-NMR of others led credence to the establishment of their structures.

#### Antimicrobial activity

All the synthesized compounds were screened for their antimicrobial activity at concentration 20 mg/disc in agar media following the method of Bauer *et al.*<sup>32</sup> Using Ciprofloxacin, an antibacterial and Ketoconazole, an antifungal activity as reference drugs, the compounds were screened against eight (8) micro-organisms, viz: *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albican* and *Aspergillus niger*. This was carried under sensitivity test and minimum inhibitory concentration (MIC).

#### Sensitivity Test

This assay was conducted by applying agar-well diffusion method<sup>33</sup> using a concentration of 20 mg/mL of each compound. From the result (**Table 1**), the compounds showed significant activity against the test organisms except *E. coli* which was only sensitive to compounds **4** and **5**. *Bacillus cereus* was sensitive to all the compounds. *S.aureus*, *P. aeruginosa* and *K. pneumonia* were resistant to compounds **4** and **5**, but were sensitive to other compounds.

**Table 3: Minimum Inhibitory Concentration of the Compounds (mg/ml)**

Compound	Gram-positive bacteria			Gram-negative bacteria			Fungi Organisms	
	<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>C. albican</i>	<i>A. niger</i>
1	0.1738	0.0871	0.1202	0.1514	-	0.1819	-	0.0912
2	0.1254	0.1047	0.0794	0.1202	-	-	0.1819	0.0912
3	0.0633	0.0505	0.0398	0.585	-	0.1514	0.1659	0.0603
4	0.1819	0.1738	-	-	0.1445	-	0.0724	0.1738
5	0.1445	0.1905	-	-	0.1689	-	-	0.1380
6	-	0.1521	-	0.1933	-	0.1831	0.1162	-
Rf 1	0.0212	0.0315	0.0213	0.0323	0.1677	0.0567	-	-
Rf 2	-	-	-	-	-	-	0.0622	0.1356

### Minimum Inhibitory Concentration (MIC) Determination

This was carried out using agar dilution following the procedure outlined by chemical laboratory Standards Institute (CLSI)<sup>34</sup> using the following serial dilutions 1, 0.5, 0.25, 0.125 and 0.0625. Almost all the synthesized phenothiazine derivatives were active against the microorganisms even at very low concentrations following from the fact that the lower the MIC values, the higher the activity. Compound **3** has the highest MIC values against bacteria which ranged from 0.0398- 0.1585 mg/mL (**Table 3**). There was no MIC for compound **4** and **5** against *S. aureus* and *P. aeruginosa*

respectively likewise for compounds **1, 2 & 3** against *E. coli* which indicated that the listed microorganisms were highly resistant to the respective compounds. The entire compounds were very active against *B.cereus* and *B.substilis* at lower MIC values. All the compounds were highly active against the fungi with an exception of *C. albican* which showed resistance to compounds **1** and **5**. All the compounds were very active against the fungi, *A. niger* except compound **6**. The reference drugs used were all active against the microorganisms with MIC lower than the synthesized compounds except in *A.niger* (**see table 3**).

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