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Convenient Syntheses and Antimicrobial Screening of Some Derivatives of Complex Benzoxazinophenothiazines

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Abstract: Four angular precursors for the synthesis of the complex derivatives were prepared by one step condensation reactions of 2,3-dichloronaphthalene-1,4-dione with 2-amino-4-nitrophenol, 2-amino-4-chlorophenol, 5-amino-4,6-dihydroxylpyrimidine and 5,6-diamino-4-hydroxylpyrimidine correspondingly in base catalyzed medium. The angular precursors on further condensation with aromatic thiols gave the complex derivatives. The Structural confirmation was done using UV-Visible spectroscopy, FT-IR, ^{1}H- and ^{13}C-NMR and elemental analysis. The synthesized compounds were screened for their anti-microbial activities and the results showed that the complex derivatives were significantly active against the microorganisms.

Keywords: Condensation reactions; 2,3-dichloronaphthalene-1,4-dione; 2-amino-4-nitrophenol; 2-amino-4-chlorophenol; 5-amino-4,6-dihydroxylpyrimidine; 5,6-diamino-4-hydroxylpyrimidine; base catalyzed medium.

1.0. Introduction

The chemistry of phenothiazines has generated intensive scientific interest due to their biological properties [**1]. S**ome phenothiazines are found to be worming agents for livestocks, their pesticidal action results from the fact that they affect the nervous system of insects by inhibiting the breakdown of acetylcholine [**2-3].** Structural modifications of phenothiazine have been successfully utilized in the design of variety of pharmaceuticals that are clinically used for antitubercular activity [**4],** cholinesterase inhibitor [**5],** histamine antagonist**6** and multiple drug resistance reverting agents [**7**]. Side substituted phenothiazine derivatives are also of great interest because of their photophysical and optoelectrochemical properties [**8-10].** A lot of Structural modifications of the phenothiazine ring have been reported as well as the synthesis of its complex structural analogues. Although some successful preparations of the angular system and three-branched system have been reported **[11-14]** there are still few reports on the anti-microbial properties. The authors here report the successful screening of some synthesized derivatives of complex benzoxazino-phenothiazines.

Experimental

All the starting materials and reagents were obtained from commercial sources and were used without further purification. All the reactions were performed in a functional fume chamber and the reactions completion were monitored with TLC. The purity of the compounds was also ascertained by TLC. The melting points were determined with Fischer John's melting point apparatus and were uncorrected. IR spectra were recorded on 8400S Fourier Transform Infrared (FTIR) spectrophotometer and were reported in wave number (cm^{-1}). UV spectra were also recorded on UV-2500 PC series spectrometer using matched 1cm quartz cells,

absorption maxima were given in nanometers (nm). The ^{1}H-NMR was scanned at University of Newcastle, United Kingdom on a JEOL FX-90Q spectrometer using TMS as internal standard (chemical shift in δ). Elemental analysis was carried on Heraeus Elemental Analyzer. Biological activities tests were carried out in the Laboratories of the Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka

2.0. General Procedure for the Preparation of Angular Phenoxazine Derivatives

2.1. 6-Chloro-10-nitrobenzo[a]phenoxazin-5-one (3)

2-Amino-4-nitrophenol (2.0 g, 13 mmole) **2** was poured in a reaction flask containing chloroform (100 mL), DMF (10 mL) and anhydrous sodium carbonate (1.40 g, 13 mmole). The mixture was warmed in water bath until complete dissolution. 2,3-dichloronaphthalene-1,4-dione (2.96 g, 13 mmole) **1** was later added and the mixture was refluxed for 5 h. At the end of the reaction period, chloroform solvent was distilled off in vacuum and the slurry poured into water and stirred to dissolve the inorganic materials. It was left to stand overnight, filtered, air dried and recrystallized twice from methanol-acetone mixture to give 6-chloro-10-nitrobenzo[a]phenothiazin-5-one (mp > 280 ^{0}C). UV-Visible (MeOH), λ_{max} (nm): 490, 443, 350, 314, 253, 220; IR (KBr) V_{max}: 3078 (=C-H), 1710 (C=O), 1630 (C=N of aromatic), 1584, 1451 (C=C of aromatic) cm^{-1}; ^{1}NMR (400 MHz, $CDCl_3$): 7.90 (2H, d), 7.75 (2H, d), 7.20 (2H, d), 7.05 (1H, d); Anal. Calcd (found) for $C_{16}H_7ClN_2O_4$: C, 58.82 (58.87); H, 2.16 (2.19); Cl, 10.85 (10.80); N 8.57 (8.63).

2.2. 6,10-Dichlorobenzo[a]phenoxazin-5-one (5)

2-Amino-4-chlorophenol (2.0 g, 13 mmole) **4** reacted with 2,3-dichloronaphthalene-1,4-dione (3.20 g, 13 mmole) **1** as described above for **(3)** to give 6,10-dichlorobenzo[a]phenoxazine-5-one **5** (mp > 300 ^{o}C). UV-Visible (MeOH), λ_{max} (nm) are: 483, 375, 334, 253 and 221; IR (KBr) V_{max}: 3070 (=C-H), 1690 (C=O), 1620 (C=N), 1560, 1470 (C=C of aromatic) cm^{-1}; ^{1}NMR (400 MHz, $CDCl_3$): 7.75 (2H, d), 7.20 (2H, d), 7.10 (2H, d), 7.10 (2H, d), 6.75 (1H, d); Anal. Calcd (found) for $C_{16}H_7Cl_2NO_2$: C, 60.79 (60.85); H, 2.23 (2.28); Cl, 22.43 (22.38); N4.43 (4.45).

2.3 11-Amino-6-chlorobenzo[a]-8,10-diazaphenoxazin-5-one (7)

5,6-Diaminopyrimidine-4-ol (1.5 g, 12 mmole) **5** coupled with 2,3-dichloronaphthalene-1,4-dione (3.6 g, 15 mmole) **1** to give 11-amino-6-chlorobenzo[a]-8,10-diazaphenoxazin-5-one **7** (mp > 280 ^{0}C). UV- Visible (MeOH), λ_{max} (nm) are: 450, 410, 350, 254 and 233; IR (KBr) V_{max}: 3328 (N-H), 1695 (C=O), 1630 (C=N), 1574, 1470 (C=C of aromatic) cm^{-1}; ^{1}NMR (400 MHz, $CDCl_3$): 7.75 (2H, d), 7.55 (3H, m), 5.70 (2H, s, (NH_2); Anal. Calcd (found) for $C_{14}H_7ClN_4O_2$: C, 56.30 (56.33); H, 2.36 (2.30); Cl, 10.87 (10.94); N, 18.76 (18.82).

2.4. 6-Chloro-11-hydroxylbenzo[a]8,10-diazaphenoxazin-5-one (9)

5-Aminopyrimidine-4,6-diol (2.0 g, 16 mmole) **6** reacted with 2,3-dichloronaphthalene-1,4-dione (2.7 g, 15 mmole) to give 6-chloro-11-hydroxylbenzo[a]-8,10-diazaphenoxazin-5-one **9** (mp > 300 ^{0}C). UV- Visible (MeOH), λ_{max} (nm) are: 520, 490, 420, 327, 304, 253 and 210; IR (KBr) V_{max}: 3428-3120 (br, O-H), 1701 (C=O), 1640, (C=N of aromatic), 1590, 1480 (C=C of aromatic) cm^{-1}; ^{1}NMR (400 MHz, $CDCl_3$): 7.75 (2H, d), 7.55 (3H, m), 5.90 (1H, OH); Anal. Calcd (found) for $C_{14}H_6ClN_3O_3$: C, 56.11 (56.15); H, 2.02 (2.02); Cl, 11.83 (11.85); N, 14.02 (14.08)

2.5 11-Amino-6-chloro-9-thiobenzo[a]8,10-diaphenoxazine-5-one (11)

5,6-Diamino-2-thiopyrimidin-4-ol (2.0 g, 13 mmole) **10** reacted with 2,3-dichloronaphthal ene-1,4-dione (2.83 g, 13 mmole) to give 11-amino-6-chloro-9-thiobenzo[a]8,10-diazaphenoxazin-5-one **11** (mp > 290 ^{0}C). UV- Visible (MeOH), λ_{max} (nm) are: 495, 443, 358, 334, 253 and 210; IR (KBr) V_{max}: 3328 (N-H), 1680 (C=O), 1610, (C=N), 1564, 1471 (C=C of aromatic) cm^{-1}; ^{1}NMR (400 MHz, $CDCl_3$): 7.75 (2H, d), 7.55 (2H, m), 5.70 (2H, s, (NH_2)., 2.80 (1H, s); Anal. Calcd (found) for $C_{14}H_7ClN_4O_2S$: C, 50.84 (50.93); H, 2.13 (2.17); Cl, 10.72 (10.65); N, 16.94 (17.05); S, 9.64 (9.60).

General Procedure for the Preparation of Complex Derivatives

2.6 9-Hydroxyl-7-methyl-14-nitro-6,8-diazabenzo[a][1,4]benzoxazino[3,2-c]phenothia-zine (13)

6-Amino-2-methyl-5-thiopyrimidin-4-ol (2.0 g, 13 mmole) **12** was weighed into a reaction flask. Chloroform (100 mL), DMF (20 mL) and sodium carbonate (1.4 g, 13 mmole) were added and the mixture was refluxed for 1 h. Thereafter, 6-Chloro-10-nitrobenzo[a]phenothiazin-5-one (4.2 g, 13 mmole) **3** was added and

the mixture was refluxed for further 7-8 h. The completion of the reaction was monitored by TLC. Chloroform solvent was distilled off, the slurry poured into water and stirred to dissolve the inorganic materials. It was allowed to stand overnight, filtered, air-dried and recrystallized twice from methanol-acetone-DMF mixture. The resulting compound 14-nitro-7-methyl-9-hydroxyl-6,8-diazabenzo[a] [1,4]benzoxazino[3,2-c]phenothiazine **13** was obtained (mp > 320 ^{0}C). UV- Visible (MeOH), λ_{max} (nm) are: 780, 741, 557, 498, 493, 357, 354, 353, 351 and 250; IR (KBr) V_{max}: 3328 (OH), 1630, (C=N), 1584, 1451 (C=C of aromatic) cm^{-1}, ^{1}NMR: 7.90 (2H, d), 7.75 (2H, d), 7.20 (2H, d), 7.05 (1H, d), 5.50 (1H, OH), 2.25 (3H, s, CH_3). Anal. Calcd (found) for $C_{21}H_{11}N_5O_4S$: C, 58.74 (58.80); H, 2.58 (2.63); N, 16.31 (16.27); S, 7.47 (7.50)

2.7 14-Chloro--9-hydroxyl-7-methyl-6,8-diazabenzo[a][1,4]benzoxazino[3,2-c] phenothiazine (14).

6-Amino-2-methyl-5-thiopyrimidin-4-ol (2.0 g, 13 mmole) **12** condensed with 6,10-dichloro-benzo[a]phenoxazine-5-one (4.0 g, 13 mmole) **5** to give 14-chloro-9-hydroxyl-7-methyl-6,8-diazabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine **14** (mp > 320 ^{0}C). UV- Visible (MeOH) λ_{max} (nm): 536, 439, 417, 413, 344, 342, 339, 338, 335, 278; IR (KBr) V_{max}: 3418 (OH), 1625 (C=N of aromatic), 1586, 1464 (C=C of aromatic) cm^{-1}; ^{1}NMR: 7.75 (2H, d), 7.20 (2H, d), 7.05 (2H, d), 5.50 (1H, OH), 2.25 (3H, s, CH_3); Anal. Calcd (found) for $C_{21}H_{11}ClN_4O_2S$: C, 60.22 (60.28); H, 2.65 (2.60); Cl, l8.46 (18.49); N, 13.38 (13.35); S, 7.66 (7.70).

2.8 14-Nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (16)

2-Aminothiophenol (2.0 mL, 16 mmole) **15** condensed with 6-chloro-10-nitrobenzo [a]phenoxazine-5-one (5.2 g, 16 mmole) **3** to give 12-nitrobenzo[a][1,4]benzoxazino[3,2-c] phenothiazine (mp > 350 ^{0}C). UV-Visible (MeOH) λmax (nm): 743, 731, 704, 691, 684, 459, 365, 314, 251; IR (KBr) Vmax: 3064 cm^{-1}(CH, aromatic), 1631 (C=N), 1502, 1452 cm^{-1}(C=C of aromatic) cm^{-1}. ^{1}NMR: 7.90 (1H, d), 7.80 (1H, d), 7.72 (1H, d), 7.45 (2H, d), 7.20-7.15 (5H, m); Anal. Calcd (found) for $C_{22}H_{11}N_3O_3S$: C, 66.49 (66.56); H, 2.79 (2.84); N, 10.79 (10.70); S, 8.07 (8.13).

2.9 14-chlorobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (17)

2-Aminothiophenol (1.0 mL, 8 mmole) **15** condensed with 6,10-dichloro-5[1H]benzo[a] phenoxazine-5-one (2.5 g, 8 mmole) **5** to give 12-chloro-benzo[a][1,4]benzoxazino[3,2-c] phenothiazine (mp > 350 ^{0}C). UV-Visible (MeOH): λ_{max} (nm): 743, 731, 704, 691, 684, 459, 365, 314 and 240; IR (KBr) Vmax: 3064 (=C-H of aromatic), 1631, 1589, (C=N), 1502, 1452 (C=C of aromatic), cm^{-1}; ^{1}NMR: 7.90 (1H, d), 7.80 (1H, d), 7.45 (2H, d), 7.20-7.10 (6H, m); Anal. Calcd (found) for $C_{22}H_{11}ClN_2OS$: C, 68.30 (68.25); H, 2.87 (2.83); Cl, 9.16 (9.20); N, 7.24 (7.28); S, 8.29 (8.24).

2.10 15-hydroxyl-12,14-diazabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (18).

2-Aminothiophenol (1.0 mL, 8 mmole) of **15** condensed with 6-chloro-11-hydroxylbenzo [a]-8,10-diazaphenoxazin-5-one (2.4 g, 8 mmole) **9** to furnish 15-hydroxyl-12,14-diazabenzo [a][1,4]benzoxazino[3,2-c]phenothiazine **18** (mp > 300 ^{0}C). UV-Visible (MeOH) λmax (nm): 650, 530, 481, 381, 317, 255; IR (KBr) Vmax: 3392 (O-H), 3057 (=C-H), 1631 (C=N), 1502, 1450 (C=C of aromatic) cm^{-1}; ^{1}NMR: 7.80 (2H, d), 7.35 (3H, m), 7.15-7.12 (4H, m), 5.60 (1H, OH); Anal. Calcd (found) for $C_{20}H_{10}N_4O_2S$. C 64.86; H 2.72; N 15.13; S 8.66

2.11 14-nitro-8-methoxyl-9-azabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (20).

6-Chloro-10-nitrobenzo[a]phenoxazine-5-one (2.1 g, 6 mmole) **3** condensed with 3-amino-6-methoxypyridine-2-thiol (1.0 g, 6 mmole) **19** of to give 14-nitro-8-methoxyl-9-azabenzo[a] [1,4]benzothiazino[3,2-c]phenoxazine (mp > 360 ^{0}C). UV-Visible (MeOH) λ_{max} (nm): 749, 580, 480, 334 and 234. IR (KBr) Vmax: 3086 (=C-H), 2951 (C-H) 1631 (C=N), 1560, 1475 (C=C of aromatic) cm^{-1}. ^{1}NMR: 7.90 (2H, d), 7.85 (1H, d), 7.75 (1H, d), 7.45-7.42 (4H, m), 3.65 (3H, s). Anal. Calcd (found) for $C_{22}H_{12}N_4O4S$, C 61.68; H 2.82; N 13.08; S 7.48

2.12. 15-Amino-8-methoxyl-9-12-14-triazabenzo[a][1,4]benzoxazino[3,2-c] phenothiazine (21).

11-Amino-6-chlorobenzo[a]-8,10-diazaphenoxazin-5-one (2 g, 6 mmole) **7** condensed with 3-amino-6-methoxypyridine-2-thiol (1.0 g, 6 mmole) of **19** to give 15-amino-8-methoxyl-9-12-14-triazabenzo [a][1,4] benzoxazino[3,2-c]phenothiazine **21** (mp > 360 ^{0}C). UV- Visible (MeOH) λ_{max} (nm): 749, 620, 560,480, 330 and 235; IR (KBr) Vmax: 3444 (N-H), 3086 (=C-H), 2951 (C-H of CH_3) 1631 (C=N), 1504 (C=C of aromatic)

cm^{-1}; ^{1}NMR: 7.80 (2H, d), 7.55 (1H, d), 7.45 (3H, s), 5.75 (2H, NH_2), 3.65 (3H, s); Anal. Calcd (found) for $C_{20}H_{12}N_6O_2S$, C 59.99; H 3.02; N 20.99; S 8.01.

2.13. 14-chloro-8-methoxyl-9-azabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (22).

6,10-Dichlorobenzo[a]phenoxazine-5-one (2.0 g, 8 mmole) **5** condensed with 3-amino-6-methoxypyridine-2-thiol (1.0 g, 6 mmole) **19** to give 14-chloro-8-methoxyl-9-azabenzo[a] [1,4]benzoxazino [3,2-c]phenothiazine (mp > 360 ^{0}C). UV-Visible (MeOH) λ_{max} (nm): 749, 670, 580, 520, 480, 337 and 250; IR (KBr) Vmax: 3066 (=C-H), 2951, (C-H, CH_3) 1631 (C=N), 1554 cm^{-1}(C=C of aromatic) cm^{-1}; ^{1}NMR: 7.90 (1H, d), 7.80 (1H, d), 7.35 (3H, s), 7.05-7.01 (4H, m), 3.65 (3H, s); Anal. Calcd (found) for $C_{22}H_{12}ClN_3O_2S$, C 63.23; H 2.89; C l8.48; N 10.06; S 7.67

3.0. Antimicrobial Activity.

The antimicrobial properties of the compounds were investigated in the form of the general sensitivity testing and minimum inhibitory concentration (MIC) with respect to freshly cultured targeted organisms. The eight organisms used in the present study were *Bacillus subtilis, Bacillus cereus,* and *Staphylococcus aureus* as gram-positive bacteria, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiellapneumoniae* as gram-negative bacteria and *Asperigellus niger, Candida albicans* as fungi organisms.

3.1. Sensitivity Testing of Compounds.

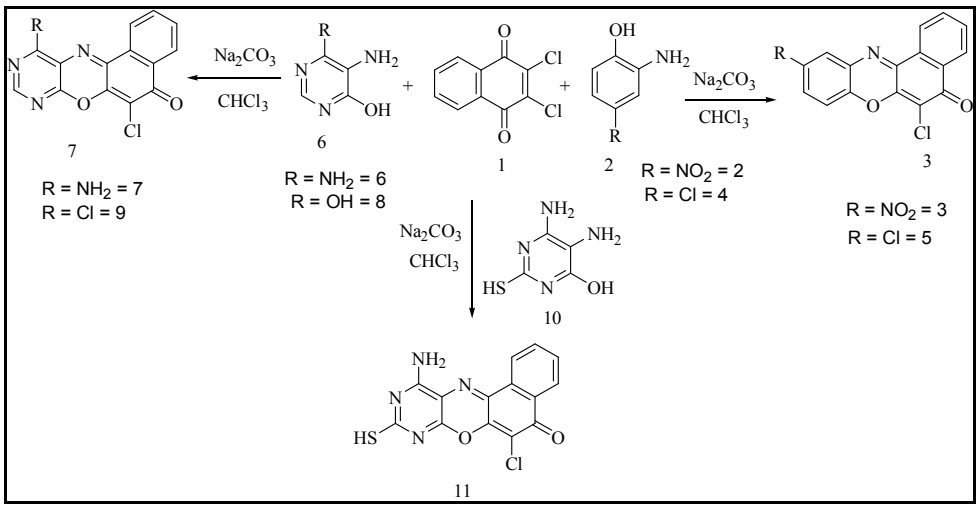
Agar diffusion technique [**15],** was used to determine the antimicrobial activities of the synthesized compounds. 20 mg/mL concentration of each compound was constituted by dissolving 0.04 g of each in 2 mL of dimethyl sulfoxide (DMSO).

3.2. Minimum Inhibitory Concentration (MIC) Testing of the Synthesized Derivatives.

This was carried out using agar dilution following the procedure outlined by chemical laboratory Standards Institute (CLSI) [**16**]. Sterile test tubes were arranged on a test tube rack and 1 mL of DMSO was dispensed into each of them.

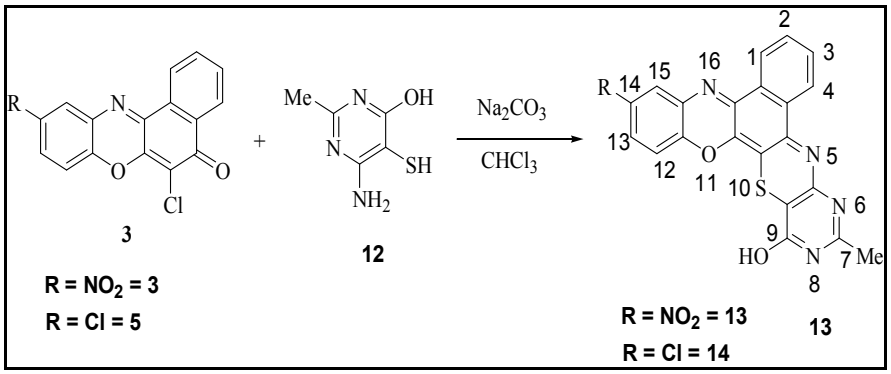
4.0. Results and Discussion.

The angular phenoxazines: 6-chloro-10-nitrobenzo[a]phenoxazin-5-one **3;** 6,10-dichloro-benzo[a] phenoxazin-5-one **5;** 11-amino-6-chloro-8,10-diazabenzo[a]phenoxazin-5-one **7;** 6-chloro-11-hydroxy-8,10-diazabenzo[a]phenoxazine-5-one **9** and 11-amino-6-chloro-9-thio-8,10-diazabenzo[a]phenoxazine-5-one **11** were obtained by alkaline condensation of 2,3-dichloronaphthalene-1,4-dione **1** with 2-amino-4-nitrophenol **2,** 2-amino-4-chlorophenol **4,** 5,6-diaminopyrimidin-4-ol **6,** 5-aminopyrimidine-4,6-diol **8** and 5,6-diamino-2-thiopyrimidin -4-ol **10** correspondingly in chloroform-DMF for 5 h. Elemental analysis agreed with their molecular formulas and structures were further supported by spectral data **(Scheme 1).**



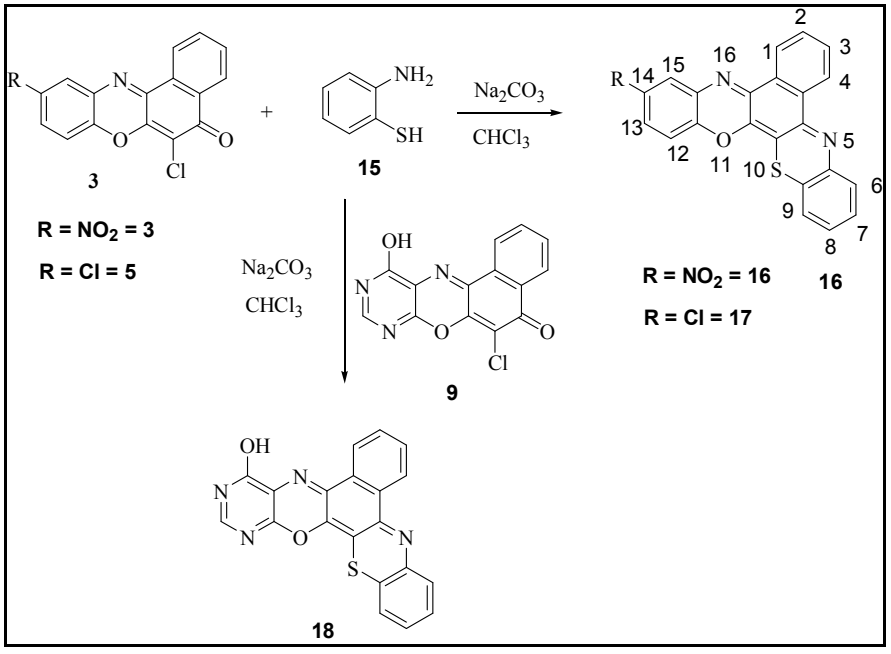
Scheme 1

Further condensation of 6-chloro-10-nitrobenzo[a]phenoxazin-5-one **3** with 6-amino-5-thio-2-methylpyrimidin-4-ol **12** and 6,10-dichlorobenzo[a]phenoxazin-5-one **5** with 6-amino-5-thio-2-methyl pyrimidin-4-ol **12** gave complex derivatives 9-hydroxyl-7-methyl-14-nitro-6,8-diazabenzo[a][1,4] benzoxazino [3,2-c]phenothiazine **13** and 14-chloro-9-hydroxyl-7-methyl-6,8-diazabenzo[a][1,4] benzoxazino [3,2-c] phenothiazine **14.** Elemental analysis agreed with their molecular formulas and structures were further supported by the spectral data **(Scheme 2).**



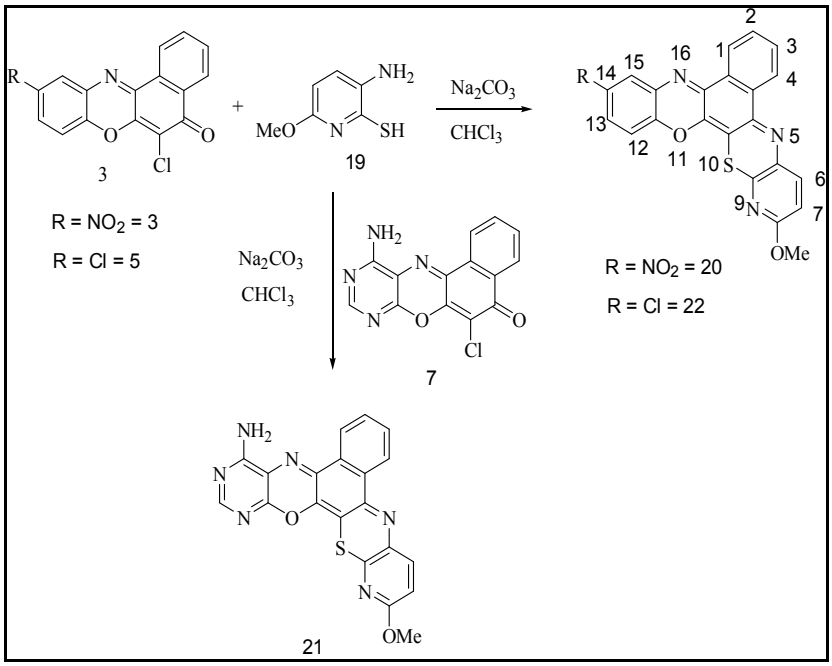
Scheme 2

Again further condensation of 6-chloro-10-nitrobenzo[a] phenoxazin-5-one **3**, 6,10-dichlorobenzo[a] phenoxazin-5-one **5** and 6-chloro-11-hydroxy-8,10-diazabenzo[a] phenoxazine-5-one **9** each with 2-aminobenzenethiol in alkaline medium gave more complex derivatives; 14-nitrobenzo[a][1,4] benzoxazino[3,2-c]phenothiazine **16,** 14-chlorobenzo[a][1,4] benzoxazino[3,2-c]phenothiazine **17** and 15-hydroxyl-12,14-diazabenzo[a][1,4] benzoxazino[3,2-c] phenothiazine **18** (**scheme 3**).



Scheme 3

Again condensation of 3-amino-6-methoxyppyridin-3-thiol **19** with 6-chloro-10-nitrobenzo[a] phenoxazin-5-one **3**, 6,10-dichlorobenzo[a]phenoxazin-5-one **5** and 11-amino-6-chloro-8,10-diazabenzo[a] phenoxazin-5-one **7,** gave other complex derivatives **20, 21** and **22** respectively **(Scheme 4).**



Scheme 4

Antimicrobial Activity Evaluation

The compounds were screened *in vitro* for their antibacterial activities against gram-positive bacteria *(B. subtilis, B. cereus and S. aureus)*, gram-negative bacteria (*P. aeruginosa, E. coli and K. pneumoniae)* and antifungal activities (*C. albicans and Asp. niger)* using the agar diffusion techniques. The choice of gram-positive and gram-negative bacteria was because they are easily transmissible through soil, food, water, animals and human [**17].** *Bacillus subtilis* is commonly found in soil and inhibits the guts, considered as a normal guts commensal[**18].** It is used in laboratory studies directed at discovering the fundamental properties and characteristics of gram-positive spore-forming bacteria [**19].**

S. aureus is a bacterium that is frequently found in the human respiratory tract and on the skin. It is the cause of common skin infections (e.g. boils), respiratory diseases (Sinusitis) and food poisoning [**20].** E. coli is a gram negative, rod like bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). It is also a normal flora of human body which causes a lot of vancomycin resistance, Enterococci and Methicllin resistant *Staphylococcus aureus* [**21].** It can cause serious food poisoning in humans and are occasionally responsible for product recalls [**22].** *P. aeruginosa* is a gram-negative aerobic coccobacillus with uni-polar motility that can cause disease such as urinary tract infection, burns, wounds and blood infections in humans, including animals [**23].** It is found in soil, water, skin flora and most made environments in most part of the world [**24].** The choice of *C. albicans* and *Asp. niger* as fungal organisms stems from the fact that they are diploid fungi that grow both as yeast and filamentous cell as well as causal agents of opportunistic oral and genital infections in humans. The choice of Ciprofloxacin and Ketoconazole as clinical standards is due to the fact that they possess broad spectrum of antibacterial and antifungal activities respectively. The results of the antifungal activities tests are shown below in **Table 1**.

Table 1: Results of Sensitivity Test of Compounds

Compd	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	B. Subtilis	*B. Cereus*	*S. Aureus*	*P. Aeruginosa*	*E. coli*	*K. Pneumoniae*	*C. Albican*	*A. Niger*
3	-	+	-	++	-	-	-	-
5	++	++	++	++	++	-	-	-
7	+	+++	++	+	+	+	+++	+++
9	+++	+++	+	++	-	-	+++	+
11	+	++	+	+	+	-	+	++
13	+	++	++	+	+	+	+	+
14	++	++	+++	++	+	++	-	-
16	++	-	++	-	++	-	+	+
17	-	-	+	-	-	+	-	+
18	+	+	+	+	++	+	+	+
20	++	+	++	-	+	+	++	+
21	+	++	+	+	++	+	-	+
22	++	+	++	++	+	+	++	+
CPFX	++	++	+++	++	+	++	-	-
KTCN	-	-	-	-	-	-	++	+++

Ciprofloxacin (CPFX) = reference drug for bacteria.
Ketoconazole (KTCN) = reference drug for fungi.

\+ = Slightly Sensitive
++ = Moderately Sensitive
+++ = Highly Sensitive and
\- = Resistant

From the result of the sensitivity testing, it was observed that compounds **7**, **13** and **18** showed sensitivity to both bacteria and fungi. Compounds **5** and **11** were sensitive to both bacteria and fungi except for *K. pneumoniae* which was resistant to compound **5**. Compound **16** was sensitive to bacteria and fungi except *B.cereus, P. aeruginosa* and *K. pneumoniae*. Compound **9** also showed sensitivity to all the bacteria and fungi except *E. coli* and *K. pneumoniae*. Compound **14** only showed sensitivity to bacteria. Compound **3** showed sensitivity to one gram-positive bacterium (*S. aureus)* and one gram-negative bacterium (*P. aeruginosa*). Compound **17** was slightly sensitive to the bacteria, *S. aureus, K. pneumoniae* and to the fungi, *A. niger*. CPFX and KTCN were sensitive to bacteria and fungi respectively.

4.2.2. Results of Minimum Inhibitory Concentration (MIC) (mg/mL) Testing of Compounds.

The compounds which were sensitive to the tested organisms were further diluted to get the MIC results as in **Table 2**.

Table 2: Results of the Minimum Inhibitory Test

Cpd	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	B.subtilis	*B. cereus*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*C. albicans*	*A. niger*
3	-	0.1905	-	0.0794	-	-	-	-
5	0.0832	0.100	0.0912	0.0661	0.1585	-	-	-
7	0.0758	0.0457	0.0794	0.1258	0.1047	0.1514	0.0912	0.1514
9	0.0457	0.0832	0.1585	0.1585	-	-	0.0501	0.1514
11	0.1659	0.1905	0.1514	0.1148	-	-	0.0794	0.1380
13	0.1905	0.0871	0.1995	0.1933	0.0871	0.1940	0.1906	0.1585
14	-	-	0.1905	0.1933	0.1913	-	-	-
16	0.0457	0.0955	0.1514	0.1258	-	-	0.100	0.1659
17	-	0.1514	0.1659	-	-	-	-	0.1738
18	0.1819	0.0661	0.1380	0.1738	0.1445	0.1538	0.1514	0.0794
20	0.1805	0.0874	0.1895	0.1933	0.0871	0.1945	0.1916	0.1685
21	0.1915	0.0771	0.1985	-	0.0891	0.1970	0.1909	0.1595
22	0.1605	0.0971	0.1885	0.1965	0.0971	-	0.1806	0.1585
CPFX	0.0212	0.0315	0.0213	0.0323	0.1677	0.0567	-	-
KTCN	-	-	-	-	-	-	0.0622	0.1356

From the results of MIC values obtained above, almost all the newly synthesized heterocyclic derivatives were active against the micro-organisms even at very low concentrations. This indicated that the lower the MIC values obtained, the higher the activity of the compound.

Compounds **9** and **16** were most the active against the bacteria *B.subtilis* with the MIC value of 0.0457 mg/mL while compound **7** was most active against *B. cereus* with the same MIC value. For the activity against *B. cereus*, Compounds **7** was the most active followed by compounds **21, 9**, **13** and **20** in that order (Table **2**). From *S. aureus*, compound **7** is the most active with MIC value of 0.0794 mg/mL followed by compound **5** the value of 0.0912 mg/mL. For the gram-negative bacteria, the activity against *P. aeruginosa, E. coli, K. pneumoniae* were highest for compounds **5, 20** and **7** with corresponding MIC values of 0.0661, 0.0871 and 0.1514 mg/mL respectively. Compounds **13** and **20** have comparable activity against E. coli with MIC value far below that of the standard drug **CPFX**. For the fungal test organisms, Compounds **3, 5** and **14** showed no activity. The activity against *C. albicans* was maximal for Compounds **9** with an MIC value of 0.0501mg/mL whereas Compound **18** showed the highest activity against A. *niger* with an MIC value of 0.0794 mg/mL.

Conclusion

The synthesized derivatives showed varying activities against the cultured bacteria and fungi used. But they were less active when compared with standard antibacterial (Ciprofloxacin) with the exception of compound **13** and **20** which were more active than the referent drug against *E. coli.* It is imperative to note that the standard antifungal drug (Ketoconazole) showed less activity against *C. albicans* and *A. niger* when compared to compounds **9** and **18**. We, therefore, conclude that the compounds which possess higher activities should be recommended for further preclinical screening which could be useful in combating the bacterial and fungal infections.

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