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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, computational and biological study of novel azabenzo[a]phenothiazine and azabenzo[b]phenoxazine heterocycles as potential antibiotic agent

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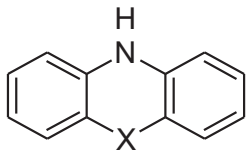
Abstract

Two angular phenothiazines and one angular phenoxazine were successfully synthesized via anhydrous base condensation reaction of 2,6-diamino-4-chloropyrimidine-5-thiol, with 7-chloro-5,8-quinolinequinone,2-aminothiophenol and 2-aminophenol, respectively. Condensation reaction between 2-6-diamino-4-chloropyrimidine-5-thiol and 7-chloro-5,8-quinolinequinone in the presence of anhydrous sodium carbonate yielded 10-amino-8-chloro-1,9,11-triaza-5*H*-benze[a]phenothiazine-5-one, 1-aza-5*H*-benzo[*a*]phenothiazine-5-one and 1-aza-5*H*-benzo[a]phenoxazine-5-one were produced with anhydrous basic condensation between 7-chloro-5,8-quinolinequinone and 2-aminothiophenol and 2-aminophenol respectively. These angular azaphenothiazin-5-ones and angular azaphenoxazine-5-one were converted to their derivatives via palladium(o)/piperazine ligand utilizing Mizoroki–Heck cross coupling tandem reaction to obtain six derivatized compounds. The synthesized compounds are intensely coloured and their structural elucidation were established by combined spectroscopic and elemental analytical data. In silico and in vitro screening methods were used to investigate the antibacterial potencies of the compounds. All the compounds, except one, interacted with Type I SPase, an unconventional validated antibiotic enzyme targeted in combating antibacterial resistant, at low micromolar range. They also showed activity against the tested bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. In fact, *B. cereus* exhibited more susceptibility towards four of the compounds than the standard drug—ciprofloxacin. The predicted binding modes of four compounds with outstanding activities were finally studied to identify vital ligand–protein interactions, which can serve as template during activity optimization process.

Keywords Phenoxazine · Phenothiazine · Benzophenothiazine · Benzophenoxazine · Antimicrobial · Antibiotic.

Introduction

Previous reports have highlighted the numerous usefulness of phenothiazine **1** and phenoxazine **2** and their derivatives most especially as drugs (Pluta et al. 2011; Okafor 1986; Onoabedje et al. 2017) (Scheme 1).



X = 1 (S) ; 2 (O)

The phenothiazine and phenoxazine nuclei are the pharmacophores in various antimicrobial (Onoabedje et al. 2016; Wainwright et al. 2012) antimalarial (Ge et al. 2010; Wesolowska et al. 2006), sedative, tranquilizer (Okafor

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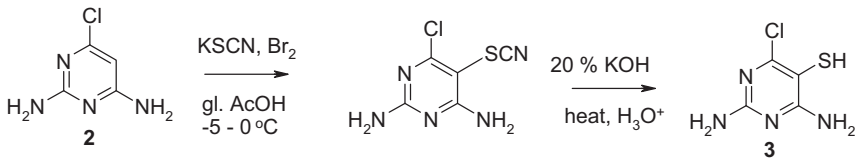
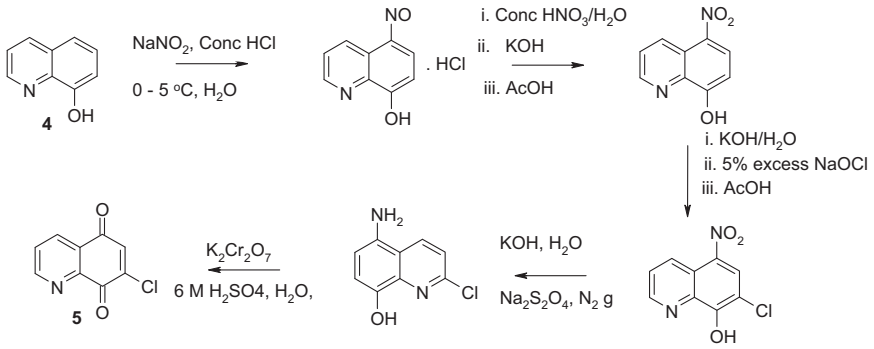
Scheme 1 Synthesis of 2,4-diamino-6-chloropyrimidine- 5-thiol



Scheme 2 Synthesis of 7-chloroquinoline-5,8-dione



1977), antituberculotic (Okafor et al. 1981; Sridhar et al. 2015), antiproliferative, immunosupressive, antiviral (Wesolowska et al. 2006) and anticancer (Pluta et al. 2010; Thimmaiah et al. 1992) agents. While phenothiazine does not have their core in the world of natural compounds, phenoxazine is well known components of actinomycin D, an antibiotic produced by *Streptomyces antibioticus* (Pluta et al. 2010; Jaszczyn et al. 2012). Natural products containing phenoxazine moiety exhibit anticancer, antibacterial, antiviral and antifugal properties (Barness et al. 2015). Besides the numerous biological importance, many phenothiazine and phenoxazine derivatives are fluoresecent, they form stable radical cations which are used as chromophores for photo-induced electron transfer experiments and organic light emitting diodes (Turdean et al. 2009; Sailer et al. 2006; Zhu et al. 2005; Okamoto et al. 2005). The versatile utility of phenothiazine and phenoxazine have motivated their intensive and extensive structural modifications in order to obtain better drugs and scientific materials. We have recently reported the synthesis of new aryl, styryl, alkynyl, thiophenyl and furanyl derivatives of phenoxazine and phenothiazines which possesses broad and potent antimicrobial activity (Onoabedje et al. 2016) (Scheme 2). Therefore, as an extension of the previous work, we are reporting a convenient protocol for the transformation of angular chlorophenothiazines and angular phenoxazines and their antimicrobial properties.

Experimental section

General information

Starting materials and reagents were purchased from Sigma-Aldrich chemical company and were used without further purification. All compounds were synthesized in the senior research chemistry laboratory of Department of Pure and Industrial chemistry, University of Nigeria, Nsukka. Melting points were determined with a Fisher-John apparatus and were uncorrected. Most of the reactions were carried out in inert atmosphere (Nitogen atmosphere). UV-visible and IR spectra were recorded on UV2500PC series using matched 1 cm quartz cells and on a Shimadzu FTIR-8400s Fourier Transform Infrared (KBr pellets) respectively in National Research Institute Chemical Technology, Zaria. Nuclear Magnetic resonance (^{1}HNMR and ^{13}C NMR) spectra were determined using a Bruker AV-400 and a JEOL-JNM LA-400 spectrometer from Strathclyde Institute of Pharmacy and Biomedical Science, University of Strathclyde, Glasglow, Scotland. Chemical shifts are recorded on the δ-scale(neat) and coupling constant (J) reported in hertz. Multiplicity indicated using the following abbreviations; d for doublet, dd for doublet of doublet, t for triplet and m for multiplet. Elemental analysis was carried out at the Central Science Laboratory, University of Cairo, Egypt on a CE440 Elemental Analyzer.

Molecular simulation

X-ray crystal structure of bacteria Type I signal peptidase (Type I SPase) in complex with its substrate (PDB code 4WVI) was retrieved from protein databank (Berman et al. 2000). Molecular operating environment (MOE) software was used to treat the protein as described in our earlier work (Ntie-Kang et al. 2014). The graphical user interface and QuSAR module implemented in MOE package were respectively used to generate the three dimensional structures and calculate the following molecular descriptors (after energy minimizing the molecules to a 0.001 kcal/mol

energy gradient using Merck Molecular (MMFF94) force-field (Halgren 1996): molecular weight (MW), lipophilicity (log P), hydrogen bond acceptor/donor (HBA/HBD) and number of rotatable bond (NRB) of the newly synthesized phenothiazines and phenoxazines.

AutoDock 4.2.0 was used to carry out docking computation (Morris et al. 1998). A grid box size of 40 40 40 A^3 points (spacing between the grid points of 0.375 Å) was employed which centered on the mass center (10.631, 29.727, −3.746) of the crystallographic macromolecule encompassing all the active site atoms. The dock protocol used was validated by ensuring that it gives a docked pose of the substrate which differs from that of the X-ray crystallography by less than 2.0 Å.

10-Amino-8-chloro-1,9,11-triaza-5*H*-benzo[a] phenothiazine-5-one 6

To a suspension of 2,6-diamino-4-chloropyrimidine-5-thiol (0.88 g, 5 mmol) in benzene (40 ml) mixed with dimethylformamide (DMF), (5 ml) was added Na_2CO_3 (1.06 g,10 mmol).The mixture was heated to boiling temperature before adding 7-chloro-5,8-quinolinequinone (0.97 g, 5 mM) and entire mixture refluxed for 6 h while stirring in a round bottom flask. At the end of the reflux period the mixture was filtered and the filtrate exposed in a dish for the solvent to evaporate. The yellow-brown solid product was recrystallized from acetone, after treatment with activated charcoal, filtered and air-dried. Yield = 0.90 g (57%), mp 198–200 °C. UV-visible (DMSO) λ_{max} 366.5 (4.00), 440 (3.76), 753(2.05). IR (ν_{max}, cm^{-1}): 3380 (–NH_2), 1657 (C=O), 1589,1566 (C=C), 1494, 1274,1172, 925, 881, 806, 746, 677, 630. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 9.42 9.19 (1 H, m, Ar-H); 8.63–8.44 (2 H, m, Ar-H), 7.94–7.80 (1 H, m, Ar-H) 4.87(2 H, s, br, NH_2). ^{13}C NMR (DMSO-d_6, 100): 181.5 (C=O), 167.7 (C), 162.9 (C), 148.5(C), 146.7 (C), 136.1 (CH), 135.4 (CH), 132.1 (CH), 130.7 (CH), 128.4 (CH), 126.4 (CH), 124.5 (CH), 115.97 (CH). (Anal. Calcd. for $C_{13}H_6ClN_5OS$: C, 64.56; H, 2.81. Found: C, 64.60; H, 2.77).

1-Aza-*5H*-benzo[a]phenothiazin-5-one 10

Compound **10** was prepared by coupling of 2-aminothiophenol (0.63 g 5 mmol) with 7-chloro-5,8-quinolinequinone (0.97 g, 5 Mm) following similar procedure for synthesis of compound **6**. The crude product was recrystallized from acetone after treatment with activated charcoal to give brown coloured solid. Yield = 1.20 g (91%), mp 130–132 °C. UV-visible (DMSO) λ_{max} 366.5 (4.00). 410 (2.44). IR(ν_{max}, cm^{-1}): 1648 (C=O), 1609 (C=C), 1568, 1479, 1392, 1276, 1178, 1147, 1018, 926, 876, 750, 678. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 9.48 - 9.40 (1 H, m, Ar-H), 9.32–9.19 (1 H, m, Ar-H), 8.66–8.56 (2 H, m, Ar-H), 7.94–7.34 (4 H, m, Ar-H). ^{13}C NMR (DMSO$_6$, 100 MHz): 180.7 (C=O), 149.4 (C), 148.8 (C), 147.9 (C), 144.6 (C), 142.4 (C), 141.1 (C), 140.3 (C), 132.1 (CH), 129.3 (CH), 128.5 (CH), 126.4 (CH), 125.8 (CH), 120.0 (CH), 117.0 (CH). (Anal.calcd. for $C_{15}H_8N_2OS$: C, 68.18; H, 3.03. Found: C, 68.30; H, 2.98).

1-Aza-5*H*-benzo[a]phenoxazin-5-one 13

A reaction mixture containing 2–aminophenol (0. 47 g, 5 mmol), anhydrous sodium carbonate (1.06 g, 10 mmol) and benzene (40 ml) mixed with DMF (5 ml) in a round bottom flask was refluxed at 70–75 °C for 45 min while stirring. 7–Chloro–5,8–quinolinequinone (0.97 g, 5 mmol) was added and stirring continued at the same temperature for 6 h. At the end of the reaction, solvent evaporated in vacuum, and the crude product recrystallized from acetone. Yield = 1.15 g (87%), mp 210–212 °C (dec.). UV-visible (DMSO) λ_{max} 366 nm (4.00), 450 (3.22). IR(ν_{max},cm^{-1}): 3109 (Ar-H); 1656 (C=O), 1575, 1276, 1200, 910, 876, 780 710. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 9.42–9.21 (1 H, m, Ar-H), 8.64–8.20 (2H, m, Ar-H), 7.94–7.76 (1 H, m, Ar-H), 7.10–7.00 (1 H, m, Ar-H), 6.76–6.42 (3H, m, Ar-H). ^{13}C NMR (DMSO$_6$, 100 MHz): 175.1 (C=O), 152.4 (C), 150.3 (C), 149.5 (C), 145.8 (C), 143.6 (C), 142.0 (C), 132.1 (CH), 131.7 (CH), 129.0 (CH), 126.3 (CH), 124.5 (CH), 117.1 (CH), 116.7 (CH), 115.4 (CH). (Anal.calcd. for $C_{15}H_8N_2O_2$: C, 72.58; H, 3.32. Found C,73.15; H, 3.32).

The general procedure for the Mizoroki–Heck cross-coupling reactions

In a round bottom flask equipped with a stirrer, piperazine ligand (0.005 g, 0.01 mmol), dppb($PdCl_2$) catalyst (0.006 g, 0.01 mmol) and methanol (10 ml) were stirred at room temperature under nitrogen atmosphere. After stirring for 5 min an intimate mixture of 10-amino-8-chloro-1,9,11-triaza-5-benzo[a]phenothiazin-5-one (0.947 g, 3 mmol), aryl halide (1 mmol) and K_2CO_3 (0.193 g, 1.4 mmol) were added. The reaction mixture was heated to 60 °C for 4 h. After the reflux period, the solvent was removed by vacuum distillation. Crushed ice (5 g) was added to the resultant residual mixture which precipitated out the solid product. The product was further recrystallized from aqueous ethanol. The general procedure was employed in synthesing compounds **7, 8, 11–15**.

10-Amino-8-chloro-6(4-nitrophenyl)1,9,11-triaza-5*H*-benzo[a]phenothiazin-5-one 7

The coupling of 10-amino-8-chloro-1,9,11-triaza-5*H*-benzo [a]phenothiazin-5-one (0.947 g, 3 mmol), and

4-iodonitrobenzene (0.248 g, 1 mmol) afforded compound **7** as brown solid, % yield (1.10 g, 84%). Mp > 250 °C (dec). UV-visible (DMSO) λ_{max} 367(4.00), 500 (3.42), 508 (3.42), 750(1.07), 779(1.072). IR (ν_{max},cm^{-1}): 3350 (–NH_2), 1645 (C = O), 1591, 1404, 1334, 1255, 1168, 1004, 848, 744, 682. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 9.45–9.42 (1 H, dd, J = 8.93, 18.84 Hz), 8.19–8.04 (1 H, d, J = 8.50 Hz), 7.97–7.55 (3 H, m), 6.73–6.58 (2 H, m), 3.50 (2 H, s, –NH_2). ^{13}C NMR ($DMSO_6$, 100 MHz): 178.22 (C=O), 156.3 (C), 155.10 (C), 153.0 (C), 150.9 (C), 142.9 (C), 143.91 (C), 142.45 (C), 139.3 (C), 135.9 (C), 134.1 (C), 132.2 (C), 130.9 (C), 126.4 (CH), 125.0 (CH), 122.7 (CH), 119.3 (CH), 119.7 (CH), 112.6 (CH). (Anal. calcd. for $C_{19}H_9N_5SO_3Cl$: C, 52.12; H, 2.30; N, 19.19; S, 7.32. Found: C, 51.72; H, 2.01; N,19.10; S, 7.02).

10-Amino-8-chloro-6(4-carboxyphenyl)-1,9,11-triaza-5*H*-benzo[a] phenothiazin-5-one 8

The reaction of 10-amino-8-chloro-1,9,11-triaza-5-benzo[a] phenothiazin-5-one, **6** (0.947 g, 3 mmol) with 4–iodobenzoic acid (0.248 g, 1 mmol) afforded compound **8** as yellowish brown powdered solid after recrystallized from aqueous ethanol. Yield = 1.1 g (85%), mp > 250 °C (dec). UV-visible (DMSO) λ_{max} 343 (4.92); 378 (4.00); 556 (1.09), 661(1.08); 744 (1.18); 794 (1.22). IR (ν_{max}, cm^{-1}): 3430 (–NH_2), 1740 (COOH), 1660 (C=O), 1570, 1404, 1334, 1260, 1178, 1053, 923, 881, 767, 678. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 12.1 (1 H, s, br, -COOH), 9.45–9.43 (1 H, m, Ar-H), 9.33–9.20 (1 H, m, Ar-H), 9.07–8.57 (5 H, m, Ar-H), 3.50 (2 H, br, -NH_2). ^{13}C NMR ($DMSO_6$, 100 MHz): 190.4 (COOH), 177.5 (C=O), 154.5 (C), 152.1 (C), 150.6 (C), 147.7 (C), 145.5 (C), 141.3 (C), 140.9 (C), 136.6 (C), 132.0 (C), 130.8 (C), 128.32 (CH), 125.4 (CH), 123.7 (CH), 120.9 (CH), 119.2 (CH), 115.8 (CH), 112.1 (CH). (Anal.calcd. for $C_{20}H_{10}N_4SO_3Cl$: C, 54.99; H, 2.54. Found: C, 55.39; H, 2.94).

6-(4-Nitrophenyl)-1-aza-5*H*-benzo[a]phenothiazin-5-one, 11

Compound **11** was obtained by reaction of 1-aza-5*H*-benzo [a]phenothiazin-5-one **10**, (0.792 g, 3 mmol) with 4-iodonitrobenzene (0.248 g, 1 mmol) as a orange solid. Yield = 1.06 g (86%), mp > 250–252 °C. (dec). UV-visible (DMSO) λ_{max} 290 (4.00); 366 (3.00); 511(1.38). IR (ν_{max}, cm^{-1}): 3430 (–NH_2), 1626 (C=O), 1589, 1479, 1313, 1267, 1192, 1097, 977, 844, 750, 669, 621. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 8.13–7.93 (4 H, m, Ar-H), 7.43–7.40 (4 H, m, Ar-H), 7.35–7.12 (3 H, m, Ar-H). ^{13}C NMR ($DMSO_6$, 100 MHz): 181.0 (C=O), 156.3 (C), 151.4 (C), 148.0 (C), 146.9 (C), 145.2 (C), 143.9 (C), 140.5 (C), 137.6 (C), 136.1 (C), 132.7 (C), 123.8 (CH), 127.0 (CH), 126.1 (CH), 129.4 (CH), 127.8 (CH), 124.6 (CH), 120.2 (CH), 119.2 (CH), 118.7 (CH), 115.9 (CH). Anal. calcd. for $C_{21}H_{11}N_3SO_3$: C,65.45; H,2.85. Found: C, 65.05; H, 2.45.

6-(4-Carboxyphenyl)-1-aza-5*H*-benzo[a] phenothiazin-5-one 12

Reaction of 1-aza-5*H*-benzo[a] phenothiazin-5-one **10** (0.792 g, 3 mmol), 4-iodobenzoic acid (0.248 g, 1 mmol) afforded as greenish yellow solid after recrystallized from aqueous ethanol. Yield = 0.94 g (78%), mp > 250 °C (dec). UV-visible (DMSO) λ_{max} 367 (5.00), 433 (2.70). IR (ν_{max}, cm^{-1}): 1710 (COOH), 1634 (C=O), 1560, 1473, 1394, 1271, 1147, 1010, 922, 879, 746, 680, 623. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 11.35 (1 H, s, –COOH), 9.46–9.18 (2 H, m, Ar-H), 8.70–8.57 (2 H, m, Ar-H), 7.12–6.99 (3 H, m, Ar-H), 6.74–6.41 (4 H, m, Ar-H). ^{13}C NMR ($DMSO_6$, 100 MHz): 188.5 (COOH), 171.9 (C = O), 150.8(C), 148.0 (C), 146.1 (C), 145.4 (C), 136.4 (C), 136.0 (2 C), 132.0 (2 C), 131.9 (CH), 131.7 (CH), 128.3 (CH), 125.8 (CH), 124.1 (CH), 123.6 (CH), 120.3 (CH), 118.4 (CH), 117.0 (CH), 115.9 (CH), 112.7 (CH). (Anal. Calcd. for $C_{22}H_{12}N_2SO_3$: C, 68.74; H, 3.15. Found: C, 68.78; H, 3.10).

6–(4–Nitrophenyl)–1-aza-5*H*–benzo[a]–phenoxazin-5-one 14

Compound 14 was obtained by reaction of 1-aza-5H-benzo [a]phenoxazin-5-one **13** (0.744 g, 3 mmol) and 4–iodonitrobenzene (0.248 g, 1 mmol) to afford greenish solid after recrystallization from aqueous ethanol. Yield = 0.92 g (70%), mp 216–218 °C (dec). UV-visible (DMSO) λ_{max} 366(4.00), 540 (3.15). IR (ν_{max}, cm^{-1}): 1644 (C=O), 1593, 1504, 1400, 1330, 1259, 1165, 1010, 846, 746, 682, & 624. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 8.12 8.06 (4 H, m, Ar-H), 7.99–7.93 (4 H, m, Ar-H), 7.48–7.47 (1 H, m, Ar-H), 6.71–6.59 (2 H, m, Ar-H). ^{13}C NMR ($DMSO_6$, 100 MHz): 177.0 (C=O), 150.5(C), 145.3 (C), 144.1 (C), 142.3 (C), 140.5 (C), 139.9 (C), 136.7 (C), 135.4 (C), 132.9 (C), 133.7 (CH), 130.1 (CH), 129.6 (CH), 127.0 (CH), 126.9 (CH), 124.3 (CH), 122.5 (CH), 120.8 (CH), 119.7(CH), 113.1 (CH). (Anal. calcd. for $C_{21}H_{11}N_3O_4$: C, 68.29; H,3.00. Found: C,67.89; H, 2.60).

6-(4-Carboxyphenyl)-1-aza-5*H*-benzo[a]phenoxazin-5-one 15

The reaction of 1-aza-5*H*-benzo[a]phenoxazin-5-one **13** (0.744 g, 3 mmol) with 4–iodo–benzoic acid (0.248 g, 1 mmol) gave compound **15** as dark brown solid. Yield = 0.95 g (82%), mp 240–242 °C (dec). UV-visible (λ_{max}, DMSO-d_6): 332(1.48), 339(1.49), 442(2.50). IR (ν_{max}, cm

$^{-1}$): 1755 (COOH), 1667 (C = O), 1589, 1554, 1494, 1392, 1271, 1174, 1143, 1006, 923, 877, 829, 752, 682, 621. ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 12.41 (1 H, s, COOH), 9.50–9.21 (3 H, m, Ar-H), 8.70–8.32 (4 H, m Ar-H), 7.77–7.49 (4 H, m, Ar-H). ^{13}C NMR ($DMSO_6$, 100 MHz): 191(COOH), 168.7 (C=O), 154.6 (C), 152.2 (C), 150.4 (C), 149.5 (C), 148.3 (C), 145.5 (C), 144.5 (C), 140.6 (C), 138.2 (CH), 137.7(CH), 136.3(CH), 134.5 (CH), 132.9(CH), 131.9(CH), 130.4(CH), 128.8(CH), 126.6(CH), 125.1(CH), 120.7(CH), 115.3(CH). Anal. calcd. for $C_{22}H_{12}N_2O_4$: C,71.74;H,3.28; N,7.60. Found: C, 71.34; H, 3.20; N, 7.21.

Antimicrobial screening test of the synthesized angular phenothiazines and phenoxazines

Antimicrobial analyses were carried out using the agar-well diffusion method Barry and Theornsberry 1985; Bauer et al. 1966). Fresh and pure clinical isolates of *Bacillus subtillis, staphylococcus aureus, Enterococcus faecalis, Pseudomones aeruginosa, Escherichia coli, Candida albicans* and *Aspergilus niger* from Faculty of Pharmaceustical Sciences, Univeristy of Nigeria, Nsukka were used for the tests. Stock solutions of the respectives synthesized compounds were prepared by initially dissolving 0.04 g in 2 ml of dimethyl sulphoxide, DMSO, to obtain stock solutions of concentration 20 mg/ml each. From the stock solution, concentration of 10, 5, 2.5, 1.25, 0.125 and 0.3125 mg/ml were prepared by serial dilution. Inoculation of the prepared agar plates with the organism was done using a wire loop to transfer a strand of the organism into the plate followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. The bores (8 mm in diameter) were aseptically made into the plates using sterilized cork borer. A synthesized compound of known concentration was introduced into the well using a sterilized syringe. The plates were incubated at 37 °C for bacteria and 25 °C for fungi for 24 h. At the expiration of the time the plates were examined for inhibition zones and the observed zones were measured and recorded in millimeters.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined using the agar dilution method. A set of six capped small test tubes were used for each synthesized sample against each organism. Nutrient agar solution was prepared according to the level of turbidity of the solution in the test tubes. The test tube containing the solution of lowest concentration of the sample that produced a clear solution was taken and recorded as the MIC of the synthesized sample. The screening effect of the synthesized compounds was compared with the standard drugs, ciprofloxacin and ketoconazole.

Results and discussion

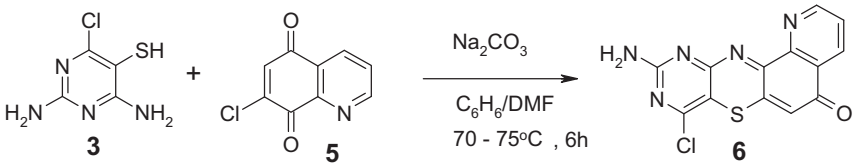
Synthesis and characterization of the compounds

The syntheses of the novel derivatives of angular azaphenothiazines and angular azaphenoxazines started with synthesis of key intermediates. 4-Choro-2,6-diaminopyrimidine-5-thiol **3** with melting point 201–203 °C (Lit mp 202–203 °C) (Okafor 1975), was synthesized by three-step thionation of 4-chloro-2,6-diaminopyrimidine **2** (Scheme 1) (Okafor 1973).

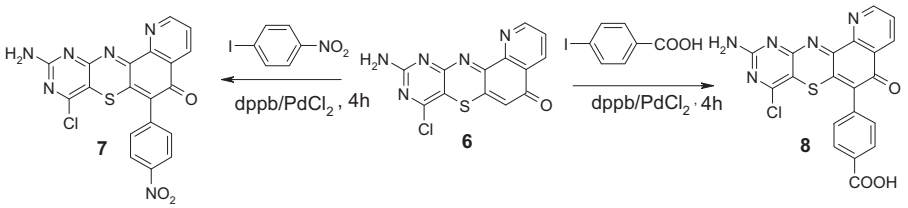
The second intermediate, 7-chloroquinoline-5,8-dione **5**, with melting point 172–174 °C (Lit. mp 173.5–174.5 °C), was prepared by multistep conversion of 8-hydroxylnapthoquinoline **4** (Petrow and Sturgeon 1954) (Scheme 2).

The cross-coupling of equimolar amounts of 4-chloro-2, 6-diaminopyrimidine-5-thiol **3** with 7-chloroquinoline-5,

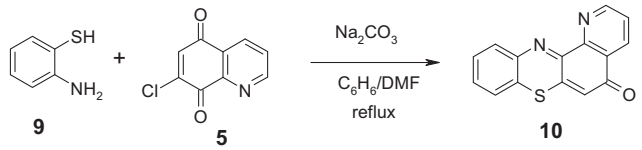
Scheme 3 Synthesis of 10-amino-8-chloro-1,9,11-triaza-5*H* benzo[a]phenothiazine-5-one **6**



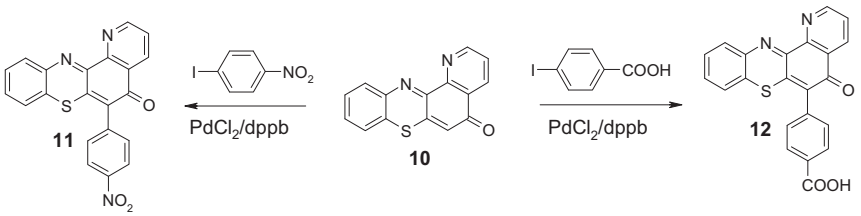
Scheme 4 The synthesis of 4-nitro-phenyl and 4-carboxylphenyl derivatives of compound **6**



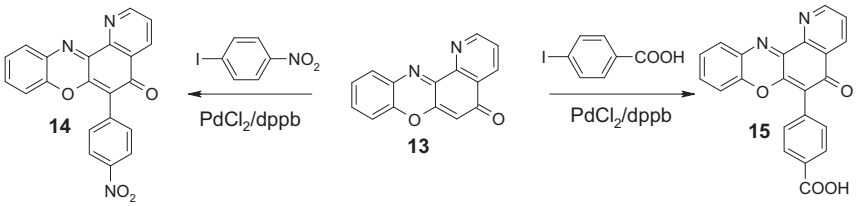
Scheme 5 Synthesis of 1-aza-5*H*-benzo[a]phenothiazin-5-one **10**



Scheme 6 The synthesis of 4-nitro-phenyl and 4-carboxylphenyl derivatives of compound **10**



Scheme 7 The synthesis of 4-nitro-phenyl and 4-carboxylphenyl derivatives of compound **13**



8-dione **5** under anhydrous base catalyzed reaction afforded a brownish solid compound, identified as 10-amino-8-chloro-1,9,11-triaza-5*H*-benzo[a]phenothiazine-5-one **6** melted at 130–132 °C (Scheme 3). The IR showed characteristic absorptions at 3380, 1653 cm−1 which were assigned to –NH_2 and C=O, respectively. The multiplet signals at δ 9.48–7.34 were assigned to aromatic protons.

The palladium(0)/piperazine catalyzed cross-coupling of 10-amino-8-chloro-7-thia-1,9,11,12-tetrabenzo[a]anthracen-5-one **6** with 1-iodo-4-nitrobenzoic acid and 4-iodobenzoic acid afforded new 10-amino-8-chloro-6-(4-nitrophenyl)-1,9,11-triaza-5*H*-benzo[a]phenothiazin-5-one **7** and 10-amino-8-chloro-6-(4-carboxyphenyl)-1,9,11-triaza-5*H*-benzo[a]phenothiazin-5-one **8**, respectively (Scheme 4) in high yields. In this reaction, a 1:1 mixture of 1,4-bis(diphenylphosphino)butane-palladium(II)chloride precatalyst and a synthesized 1,4-bis(2-hydroxy-3,5-di-tertbutylbenzyl)piperazine ligand (Mohanty et al. 2008) were used in combination with K_2CO_3 under refluxing methanol provided the optimal yields of the products. The UV-visible spectra of compounds **7** and **8** exhibited bathochromic shifts due to extended conjugation. The characteristic absortions for –NH_2 and C=O functional groups were found at 3350 and 1645, respectively for compound **7** and at 3430 and 1660 for compound **8**. The ^{1}H NMR signals for the carboxyl proton in compound **8** appeared offset as a weak peak at 2.10 ppm. Other spectral and analytical data were in agreement with the synthesized compounds.

In a similar procedure to the above, the reaction of equimolar mixture of 2-aminothiophenol and 7-chloroquinoline-5,8-dione supplied a yellow powder solid identified as 7-oxa-1,12-diazabenzo[a]anthracene-5-one **10**, melting point at 210–212 °C (Scheme 5).

The structure of compound **10** is in agreement with spectral and elemental analytical data. Besides the aromatic protons that integrated for eight protons as multiplet at δ 9.48–7.34 the IR displayed absorption for C=O at 1648 cm^{-1}.

The Mizoroki-Heck cross coupling of compound **10** with 1-iodo-4-nitrobenzoic acid and 4-iodobenzoic acid afforded brownish coloured solids identified as compounds 6-(4-nitrophenyl)-1-aza-5*H*-benzo[a]phenothiazin-5-one **11** and 6-(4-carboxyphenyl)-1-aza-5*H*-benzo[a]phenothiazin-5-one **12**, respectively in excellent yields after recrystallization from ethanol (Scheme 6).

The spectral data are in agreement with the assign structures and molecular formula of the synthesized compounds. Compounds **11** and **12** showed red shifts in the UV-visible spectra due to extended conjugations. The ^{1}H NMR –COOH absorption appeared offset in compound **12**. The IR bands at 1710, 1634 for compound **12** were assigned to –COOH and C=O, respectively.

Similar procedure used in synthesis of compounds **6** and **10** was employed to prepare compound **13**. The coupling of equimolar amount of aminothiophenol with 7-chloroquinoline-5,8-dione supplied 7-oxa-1,12-diazabenzo[a]athracen-5-one **13**, which was converted into 6-(4-nitropenyl)-1-aza-5*H*-benzo[a]phenoxazin-5-one **14** and 6-(4-carboxyl)-1-aza-5*H*-benzo[a]phenoxazin-5-one **15** via Heck reactions. Like compounds **6** and **10**, compound **13** IR band for carbonyl (Scheme 7) 1656 cm^{-1} (C = O) is lower than

that expected for 7-chloroquinoline-5,8-dione (1690 cm^{-1}) probably due to resonant effects in which the ionic resonance form contribute appreciably to the ground state (Agrawal and Mital 1975). The same lowering of carbonyl IR frequencies were observes in all the synthesized derivatives. The molecular structures of the synthesized compounds were established by NMR, IR, UV and elemental analysis data.

How drug-like are the compounds

Several cases are widely reported in literature where compounds with interesting pharmacological activities failed to make it to the shelves of market due to poor/bad pharmacokinetic properties (Ibezim et al. 2015). This has resulted to investigation of these properties early in drug development process. One of the most investigated of these properties is oral bioavailability profile. This is of particular importance because most drugs are formulated in oral administrable forms (Ibezim et al. 2017). Oral bioavailability, also known as drug-likeness, of molecules is determined computationally through calculation of certain molecular descriptors and comparing the results to a set of criteria outlined by Lipinski. Lipinski proposed rules, now generally referred to as 'Lipinski's rule of five' (ro5) which qualifies a compound to be drug-like if it possesses the following properties; MW less than five, lipophilicity (log P) less than five, number of HBA/HBD groups less than ten/five and NRB less than five (Lipinski et al. 1997).

All the compounds have physical-chemical parameters which fall within the acceptable ranges for a drug-like molecule according Lipinski ro5 (Table 1). However, the MW of compound **7** and **8** seems quite high (436.839 and 435.851 Da respectively) and could be of concern if more functional groups or moieties will be added during chemical structural modification process for activity optimization. Also, it was observed that the low HBD property of the compounds was greatly compensated by their relatively high number of HBA and this is reflected in the lipophilicity values. This account for the reason compounds (**10**, **11**, **13** and **14**) with zero HBD value had relatively good lipophilicity (at the range of 3.029–4.735). Overall, drug-likeness check revealed all the compounds will be well exposed in systemic circulation when administered orally.

Table 1 Physicochemical properties for evaluating drug-likeness according to ro5

Compounds	NRB	MW	HBA	HBD	logP (o/w)
6	0	315.744	6	1	1.158
7	2	436.839	9	1	2.864
8	2	435.851	8	2	2.606
10	0	298.753	3	0	3.029
11	2	419.848	6	0	4.735
12	2	418.860	5	1	4.477
13	0	282.686	4	0	2.393
14	2	403.781	7	0	4.099
15	2	402.793	6	1	3.841

Antibacteria testing

Docking calculations

Rising cases of drug resistant bacteria (DRB)is now an obvious global public health threat which has got the attention of World Health Organization and Infectious Disease Society of America (Worthington and Melander 2013). In the United States alone, a review performed by Klein and Coworkers revealed that, more people died from methicillin-resistant *Staphylococcus aureus* infections than those who died of HIV/AIDS, emphysema, Parkinson's disease and homicide combined (Klein et al. 2007). Scientists in both academia and industry seem to agree that one of the effective ways of designing new molecules to combat DRB is through tackling unconventional targets (Smitha Rao et al. 2014 one of which is Type I signal peptidase (Type I SPase).Type I SPase is an essential validated antibacterial drug target which plays key roles in bacterial secretory pathway through catalyzing the cleavage of amino-terminal signal peptide from the translocated preprotein and to release exported proteins from the membrane to reach their correct cellular or extra-cellular destination (Tuteja 2005).

The best docked conformations of the test compounds representing the lowest theoretical binding free energy of the ligand–target complexes showed that all the compounds interacted favourably with Type I SPase (Table 2). All inhibited the activity of the enzyme at micromolar concentration except **6** which did at millimolar range. Throughout, it appears attachment of benzoic acid and nitrobenzene to either parent scaffolds afforded increased interaction and therefore stronger binding with Type I SPase. Although none of the new compounds bound to Type 1 SPase stronger than its cocrystallized substrate (maltose; −9.30 kcal/mol), their physicochemical properties, low inhibitory concentration and good ligand efficiency present them as promising/potential antibacterial agents. Therefore, it was necessary to confirm their predicted potencies experimentally through whole bacteria assay.

Experimental screening of the compounds against selected bacteria

In vitro antibacterial screening results of the compounds are shown in Table 3. It was observed that the compounds

demonstrated varying activity against the studied organisms. *B. cereus* was most susceptible to the compounds being inhibited at a concentration as low as 0.25 µg/mL by compound **6**. Only compound **10** showed activity against E. coli at a measurable MIC level (1.38 µg/mL). In comparison, the compounds were more active against the tested Gram positive bacteria (*B. cereus* and *S. aureus*) than the tested Gram negative bacteria (*P.aeruginosa*and *E. coli*). Many research findings attribute the relatively poor antibiotic effect of compound candidates against Gram negative bacteria to the presence of lipopolysaccharide (LPS) layer enveloping their cell wall. However, except for compound **6** which might get stocked in the LPS layer due to its very low lipophilicity (log P = 1.158), the lipophilicity of the rest of the compounds apparently guarantees easy passage of this layer to reach intercellular targets. The discrepancies between docking predictions and experimental assay is more obvious in the performance of compound **12** where docking scores it high whereas it performed very poorly experimentally. The reason for differing results between these two techniques is due to the fact that the in vitro screening was not carried out directly on the enzyme used in docking. Notwithstanding, the in silico result suggests that in a situation where antibiotic resistant occurs, these compounds would possibly thrive because they have different target enzyme (Type I SPase) other than the ones known to contribute/participate in bacteria resistant.

In the overall, compounds **7**, **10**, **11** and **15** emerged as the best potent against the studied bacteria because of inhibition of both Gram positive and negative bacteria at low MIC some even lower than the used approved antibiotic drug (ciprofloxacin).

Table 2 Dock scores of the newly synthesized compoundstoward Type I SPase binding site

Compounds	Inhibition constant	Binding free energy	Ligand efficiency
6	3.23	−3.40	0.16
7	492.55	−4.51	0.15
8	378.48	−4.67	0.16
10	60.99	−5.75	0.29
11	7.08	−7.03	0.24
12	1.97	−7.78	0.27
13	35.13	−6.08	0.3
14	16.15	−6.54	0.23
15	15.24	−6.57	0.23

Inhibition constant (Ki) values are in µM except for compound 6 which is in mM concentration

Binding mode prediction of 7, 10, 11 and 15

Understanding the intermolecular interaction existing between a putative drug molecule and a protein target goes a long way to facilitate activity optimization of that molecule. In that light, analysis of the binding conformation of selected better performing compounds were studied as shown in Fig. 1a–d.

Interactions of the compounds with Type I SPase were grossly characterized with π-π contacts with aromatic rings of Tyr161, Tyr216, Trp68 and Trp236 residues and hydrogen bond relationships with Arg72 and Arg350 NH groups. Compound **7** gained stronger binding with the protein by forming extra lipophilic and hydrogen bonding with the Trp346 aromatic ring and Asp20 carboxylate moiety through its nitrophenyl aromatic ring and amino group respectively. It appears the pose of compound **10** enabled it to make more hydrogen contact with Lys21 and Ala69 NH through its chlorine atom. Both compounds **7**

Table 3 Results of inhibition zone diameter (IZD) in millimetre (mm) of synthesized compounds (20 mg/mL)

Compounds	bacteria			
	Gram positive		Gram negative	
	B.c.	S.a.	P.a	E.c.
6	22 ± 0.55 (0.25)	19 ± 1.20 (0.80)	4	6
7	14 ± 0.11 (0.63)	12 ± 1.50 (1.00)	18 ± 0.44 (0.40)	3
8	10 ± 0.44 (1.00)	–	16 ± 0.07 (0.89)	-
10	27 ± 0.55 (0.44)	20 ± 0.07 (0.42)	–	14 ± 0.35 (1.38)
11	16 ± 0.55 (0.50)	–	23 ± 1.44 (0.40)	–
12	7	–	7	–
13	–	17 ± 0.22 (0.63)	4	5
14	15 ± 0.25 (0.96)	12 ± 0.55 (0.91)	5	4
15	28 ± 0.10 (0.40)	22 ± 0.40 (0.45)	5	4
Ciprofloxacin	31 ± 0.08 (0.55)	25 ± 0.22 (0.50)	25 ± 0.10 (0.44)	22 ± 0.22 (0.63)

Minimum inhibitory concentration (MIC) in µg/mL in bracket. B. c (*Bacillus cereus*); S. a (*Staphylococcus aureus*); E. c (*Escherichia coli*); P. s (*Pseudomonas aeruginosa*); Conc. = µg/mL of DMSO

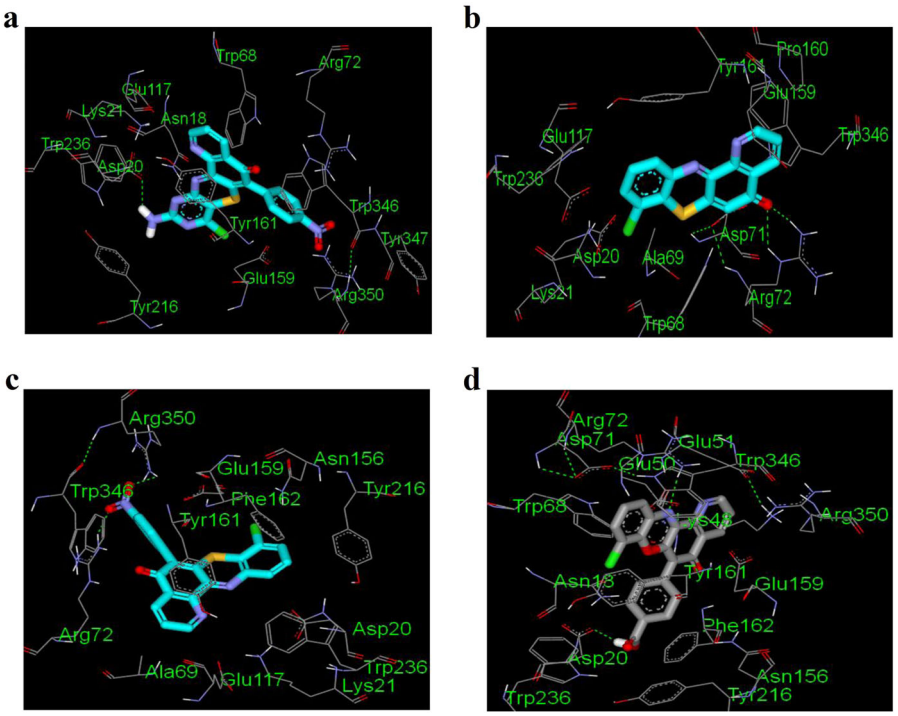


Fig. 1 Dock pose of compounds **7** (**a**), **10** (**b**), **11** (**c**) and **15** (**d**) toward Type I SPase binding site. The atoms are in their standard colours for both protein residues and ligands, i.e., oxygen atom in red, chlorine atom in green, hydrogen atom in white, sulphur atom in yellow etc. Polar contacts are shown in dash lines

and **11** made intercourse with same residues with the exception of that initiated by the amino group in **7**. The best dock pose of compound **15** gave a pose which made contact, although with the same protein residues as the rest, but using different part of the compound. Unlike the hetero nitrogen atom of either phenothiazine or phenoxazine that participated in hydrogen bonding, there was no observable contribution made by sulphur and oxygen atoms in the interaction of the compounds with the test protein. Moreover, it appears that the four candidates hydrogen bond with lysine (Lys21 for **7**, **10** and **11** and Lys48 for **15**), an essential residue for signal peptidase 1 activity (Dalbey 2013). Also, the compounds (**7**, **10**, **11** and **15**) made contact with Type I SPase through the same residues as did the maltose which suggests they might be acting as activators rather than inhibitors. However, their activity in vitro indicated that they are inhibitors (Ting et al. 2016).

Conclusion

We have reported convenient protocol for functionalisation of heterocyclic phenothiazine as well as phenoxazine in moderate to high yields to afford highly coloured derivatives at low temperatures and short time via Mizoroki–Heck reaction system. The reaction conditions tolerated both carbonyl functional groups and unprotected $-NH_2$ moieties in substrates. The 10-amino-8-chloro-1,9,11-triaza-5*H*-benzo[a]phenothiazine, 1-aza-5*H*-benzo[a]phenothiazin-5-one and 1-aza-5*H*-benzo[a]phenonoxazin-5-one and their derivatives were particularly susceptible to *Bacillus subtilis* and *Staphyllocccus aureus*. Further research work is in progress to optimize the reaction conditions and develop a better catalytic system to explore related cross-coupling protocols so as to diversify target molecular motifs. In addition, results of computational and biological screening respectively showed that the new compounds interacted favourably with an unconventional validated antibiotic enzyme targeted to treat drug resistant bacteria—Type I SPase and inhibited the activities of both Gram positive and Gram negative bacteria with some having lower MICs than the used approved drug—ciprofloxacin.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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