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Utility of Suzuki-Miyaura Cross-Coupling Reaction in Synthesis of Benzo[a]phenothiazine and Benzo[a]phenoxazine Derivatives and their Antimicrobial Screening

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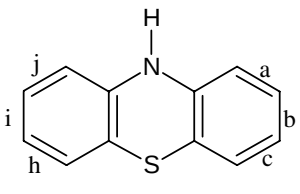
Palladium-catalyzed Suzuki-Miyaura cross-coupling reactions of 6-chloro-5*H*-benzo[a]phenothiazin-5-one (**1**), 11-amino-6-chloro-9-thio-5*H*-naphtho[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (**2**) and 6-chloro-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (**3**) with phenylboronic acid and 3-nitrophenylboronic acid were thoroughly investigated. The above intermediates were prepared by the reactions of 2-aminothiophenol, 4,5-diamino-6-hydroxylpyrimidine-2-thiol and 2-aminopyridin-3-ol each with 2,3-dichloronaphthalene-1,4-dione in a basic medium using benzene/DMF as the solvent. Thereafter, each was subjected to the Suzuki-Miyaura coupling reaction with phenylboronic acid and 3-nitrophenyl boronic acid, refluxing for 7-8 h at 110 °C using *tris*(dibenzylideneacetone)palladium(0), dicyclohexyl-phosphino-2',6'-dimethoxybiphenyl (SPhos), potassium phosphate and toluene as the catalyst, ligand, base and solvent correspondingly to yield the derivatives (**1a-b**), (**2a-b**) and (**3a-b**), respectively. Structures of the compounds were characterized using UV/visible spectrophotometry, FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy and elemental analysis. The compounds were screened against six micro-organisms, *viz*: *Bacillus subtitis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Aspergillus niger* and were shown to have significant activity against some Gram-positive micro-organisms.

Keywords: Suzuki-Miyaura cross-coupling reaction, Benzo[a]phenothiazine, Benzo[a]phenoxazine, Antimicrobial screening.

INTRODUCTION

The emergence of new catalytic systems, utilizing palladacyclic complexes, electron-rich trialkylphosphine ligands and the bulky biphenyl-based phosphines developed by Buchwald and co-workers[1], has virtually transformed the trend in organic synthesis. This has led to the development of some novel compounds from unreactive aryl and heteroaryl chlorides that exhibit strong actions against drug-resistant microbes and other potentials. However, the applications of phenothiazine (**1**) and phenoxazine (**2**) compounds and their derivatives in drug, textile, agriculture and other related industries have long been recognized.



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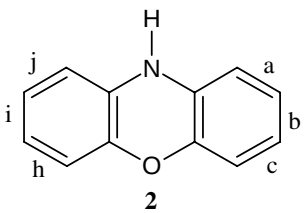

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Phenothiazine, one of the most frequently encountered bioactive heterocycles in compounds of biological interest[2] and its derivatives have been found to show tremendous biological activities such as antiparkinsonian[3], anticonversant[4], antidepressant[5], neuroleptic[6], anti-inflamatory[7-9], antimalarial[10-12], antipsychotic[13-15], antimicrobial[16,17], anti-tubercular[18-21], anti-tumor[22,23], antihistaminic[24,25], analgesic[26], prion disease drug[27]. In textile, paint and plastic industries, they are used as dyes and pigments[28] and in agricultural industries as insecticides[29]. In petroleum industries, they are used as antioxidants in lubricants and fuels[30]. It has been observed that some phenothiazines inhibit intracellular replication of viruses including human immunodeficiency viruses (HIV)[31]. On the other hand, some have been reported to exhibit significant anticancer activity[32,33].

Similarly, phenoxazine and related compound have been reported to possess various biological activities such as anti-parkinsonian[34,35], anticonvulsant[36], antihistamic[37], antihelmatic[38], antiviral[36], antitumor[39], anticancer[40], antiparasitic[41], antibacterial[41,42] and CNS depressant[31]. Other applications include their use as antioxidants and biological stains, laser

dyes, indicators and especially as chromophoric compound[43-49] in host guest artificial protonic antenna system.

Although several synthetic routes to linear and angular phenoxazines and phenothiazines have been reported, methods are often not applicable for the preparation of a wide variety of derivatives with excellent yields and good biological activity. Therefore, it becomes imperative to investigate elegant and facile reaction procedures to synthesize possible derivatives with variety of functionalities.

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-2500PC series spectrophotometer using matched 1 cm quartz cells. The IR spectra (cm^{-1}) were recorded on 8400S FT-IR spectrometer using KBr discs (at NARICT, ZARIA). The ^1H NMR and ^{13}C NMR were 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 CHN rapid analyzer and the antimicrobial screening was done at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.

6-Chloro-5*H*-benzo[a]phenothiazin-5-one (1): To a mixture of 2-aminothiophenol (4 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) λ_{max} (nm) log(ε): 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); ^{13}C NMR (DMSO, 700 MHz) δ: 178.0 (carbonyl carbon), 164.6, 149.0, 138.0, 136.3, 134.6, 131.1, 131.5, 130.2, 129.5, 127.5, 126.4, 124.7, 121.1, 116.5; IR (KBr, ν_{max}, cm^{-1}): 582, 651(C-S str), 752 (C-Cl str), 880 (C-H out-of-plane bend), 1141, 1084 (arom. in-plane C-H bend), 1297 (C-O str), 1472 (C=C str, aromatic), 1628 (C=N str), 1732 (C=O str), 3390, 3623 cm^{-1} (>NH str); Anal. calcd. for $C_{16}H_8NOSCl$: C 64.45, H 2.69, Cl 11.92, N 4.70, S 10.75; Found: C 65.59, H 3.77, Cl 11.71, N 5.40, S 10.99.

11-Amino-6-chloro-9-mercapto-5*H*-naphtho[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (2): To a mixture of 4,5-diamino-5-hydroxyl-2-mercaptopyrimidine (2.00 g, 13 mmol) and Na_2CO_3 (1.34 g, 13 mmol) in benzene (120 mL) and DMF (10 mL) stirred at room temperature for 0.5 h in a 250 mL two-necked flask equipped with magnetic stirrer, thermometer and reflux condenser, was added 2,3-dichloro-1,4-naphthoquinone (2.87 g, 13 mmol). The entire reaction mixture was refluxed in a water bath at 75-80 °C for about 6 h. Thereafter, solvent was distilled off in vacuum and water (50 mL) added to the crude product, stirred and filtered. The pure product was obtained as dark orange solid upon recrystallization with benzene-toluene after treatment with activated charcoal. yield = 7.18 g (86 %); m.p: dec. 290 °C; UV-visible (MeOH) λ_{max}: 220, 317, 414, 451,731; ^{13}C NMR (DMSO, 700 MHz) δ: 178.0 (carbonyl carbon), 170.1, 168.0, 150.4, 147.1, 141.3, 134.6, 131.7, 131.5, 131.1, 130.8, 126.4, 125.9, 124.7 (sp^2 hybridized carbon atoms); IR (KBr, ν_{max}, cm^{-1}): 627, 569 (C-S-C str), 749 (C-Cl str), 1159 (C-N str, oxazine ring), 1266 (C-N str, aromatic primary amine), 1454 (ArC=C str), 1553 (C=N str, pyrimidine ring), 1647 (C=O str), 2354 (S-H str, thiol), 3395 cm^{-1} (N-H str, amine). Anal. cald. for $C_{14}H_7N_4O_2SCl$: C 50.79, H 2.12, Cl 10.73, N 16.93, O 9.68, S 9.68; Found: C 51.02, H 2.01, Cl 10.79, N 16.70, S 9.68.

6-Chloro-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (3): By similar method, 6-chloro-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one was afforded as yellowish brown solid using 2-aminopyridin-3-ol (2 g, 18 mmol) and 2,3-dichloro-1,4-naphthoquinone(3.45 g, 15 mmol) and after recrystallization from acetone and treatment with activated carbon. Yield = 4.47 g (81 %); m.p. 208-209 °C; UV-visible (MeOH) λ_{max}: 207, 219, 346, 443, 732; ^{13}C NMR (DMSO, 700 MHz) δ: 177.2(carbonyl carbon), 150.2, 147.2, 146.0, 144.3, 140.6, 133.1, 132.9, 131.3, 129.8, 126.8, 126.2, 125.9, 124.5, 115.9; IR (KBr, ν_{max}, cm^{-1}): 780 (C-Cl str), 940 (C-H out-of-plane bend) 1125, 1055 (arom. in-plane C-H bend), 1220, 1261 (C-O str), 1467 (C=C str, aromatic), 1555 (C=N str, pyridine), 1645(C=N str, oxazine ring), 1703 (C=O str), 3101 cm^{-1} (C-H str, arom.); Anal. cald for $C_{15}H_7N_2O_2Cl$: C 63.68, H 2.48, Cl 12.56, N 9.91; Found: C 63.23, H 3.16, Cl 12.47, N 9.84.

General procedure for derivatives synthesis using Suzuki Cross-coupling reaction: Dried and degassed toluene (2 mL) was charged to a mixture of RX (1 mmol), $RB(OH)_2$ (1.5 mmol), K_3PO_4 (0.58 g, 4 mmol), $Pd_2(dba)_3$ (1 mol % Pd) and SPhos (Pd/L = 1:2) in round bottom flask (100 mL). The mixture was flushed with nitrogen three times and stirred at 110 °C under nitrogen for 6-8 h. It was then cooled to room temperature and partitioned with water (10 mL) and chloroform (10 mL). The organic layer was separated, dried over sodium tetraoxosulphate (VI), concentrated and purified by column chromatography (10 % EtOAc/90 % petroleum ether eluent) to provide the analytical pure title product.

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

6-Phenyl-5*H*-benzo[a]phenothiazin-5-one (1a): The above method was employed using 6-chlorobenzo-5*H*-phenoxazin-5-one (0.30 g, 1 mmol), boronic acid (1.5 mmol), $Pd_2(dba)_3$ (0.03 g, 1 mol %), S-Phos (0.06 g, 2 mol %), K_3PO_4 (0.64 g, 3 mmol) and product was afforded as crystalline reddish brown solid. Yield = 146 mg (43 %); m.p. 220 °C. UV-visible (MeOH) λ_{max}: 206, 219, 318, 358, 371, 484, 737; ^1H NMR (DMSO, 700 MHz) δ: 8.90-8.89 (1H, d), 8.21 -8.20 (2H, d), 7.94-7.80 (2H, t), 8.99-8.98 (2H,d), 7.67-7.65(2H, dd), 7.58- 7.56 (2H, t), 7.52-7.49 (2H, m, aromatic protons), 7.36-7.35 (2H,d); ^{13}C NMR (DMSO, 700 MHz) δ (ppm): 178.1 (carbonyl carbon), 144.6, 137.9, 135.8, 134.9, 134.4, 133.0, 132.5, 132.2, 132.1, 131.9, 130.9, 129.7, 129.6, 129.1, 128.6, 126.0, 125.9, 125.7,

123.6; IR (KBr, ν_{max}, cm^{-1}): 582 (C-S str.), 741 (C-H def.), 1078 (aromatic C-H in-plane bend), 1338 (C-N str.), 1511, 1621 (C=N str., thiazine ring), 1728 cm^{-1} (C=O str.); Anal. cald for $C_{22}H_{13}NOS$: C 77.78, H 3.83, N 4.12, S 9.43; Found: C 78.02, H 4.25, N 4.24, S 9.56.

6-(3-Nitrophenyl)-5*H*-benzo[a]phenothiazin-5-one (1b): The product was obtained as yellowish red solid. Yield = 173 mg (45 %); m.p 230-231 °C; UV-visible (MeOH) λ_{max}: 314, 370, 495; ^{1}H NMR (DMSO, 700 MHz) δ: 8.99-8.97 (1H, d); 8.90-8.89 (1H, d); 8.21-8.20 (1H, d); 7.99-7.98 (2H, d); 7.94-7.93 (2H, m); 7.90-7.89 (2H, d); 7.67-7.65 (2H, dd); 7.36-7.35 (1H, d). ^{13}C NMR (DMSO, 700 MHz) δ: 178.1 (carbonyl carbon), 147.8, 146.8, 137.9, 135.8, 134.9, 134.4, 133.0, 132.5, 132.2, 132.1, 131.9, 130.9, 129.7, 129.6, 129.1, 126.0, 125.9, 123.1, 121.0; IR (KBr, ν_{max}, cm^{-1}): 755 (C-H bend), 1080 (aromatic ring str.), 1319 (C-N str.), 1504 (-NO_2 str., aromatic), 1610 (C=N str., thiazine ring), 1722 (C=O str.), 3071 cm^{-1} (aromatics C-H str.); Anal. cald. for $C_{22}H_{12}N_2O_3S$: C 68.68, H 3.12, N 7.28, S 8.32; Found: C 68.92, H 3.55, N 7.40, S 8.45.

Synthesis of derivatives of 11-amino-6-chloro-9-mercapto-5*H*-naphtho[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (2)

11-Amino-9-mercapto-6-phenyl-5*H*-naphtho[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (2a): 11-Amino-6-chloro-9-mercapto-5H-naphtho[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (0.33 g, 1 mmol), boronic acid (1.5 mmol), $Pd_2(dba)_3$ (0.03 g, 1 mol %), SPhos (0.06 g, 2 mol %), K_3PO_4 (0.64 g, 3 mmol) was uses and the product obtained as dark purple solid; Yields = 190 mg (51 %); m.p: dec. 290 °C; UV-visible (MeOH) λ_{max}: 207, 223, 737, 764, 780; ^{1}H NMR (DMSO, 700 MHz) δ: 12.15 (IH, s); 8.53 (2H, s); 8.03-8.00 (2H, d); 7.78- 7.69 (2H, m); 7.35-7.17 (5H, m); ^{13}C NMR (DMSO, 700 MHz) δ: 170.1 (carbonyl carbon), 165.7, 150.4, 147.3, 136.4, 134.5, 132.5, 131.5, 131.1, 130.6, 128.9, 128.6, 126.4, 125.9, 124.7, 123.2; IR (KBr, ν_{max}, cm^{-1}): 580 (C-S str), 618, 883 (C-H out-of-plane bending), 919, 1076 (in-plane C-H bending vibr. of arom. compd), 1275 (C-N str, aromatic amine), 1239 (C-O-C str), 1644 (C=N str), 1714 (C=O str), 2346 cm^{-1} (S-H str, thiol); Anal. cald. for $C_{20}H_{12}N_4O_2S$. C 64.45, H 3.22, N 15.04, S 8.59; Found: C 65.00, H 3.31, N 15.10, S 8.55.

11-Amino-9-mercapto-6-(3-nitrophenyl)-5*H*-naphtho-[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (2b): As yellowish purple solid. Yields = 232 mg (60 %); m.p.: dec. above 300 °C; UV-visible (MeOH) λ_{max}: 219, 285, 734; ^{1}H NMR (DMSO, 700 MHz) δ: 12.15 (IH, s); 8.53 (2H, s); 8.14 (1H, d); 8.03-8.00 (2H, d); 7.91 (1H,s); 7.78- 7.69 (2H, m); 7.66-7.56 (2H, m) 7.35-7.17 (5H, m); ^{13}C NMR (DMSO, 700 MHz) δ: 168.7, 158.0, 141.2 few peaks because of insolubility; IR (KBr, ν_{max}, cm^{-1}): 617 (C-S str), 747, 880 (aromatic out-of-plane bend), 1192 (aromatic C-H in-plane bend), 1267 (C-O-C str), 1375 (-NO_2 str), 1460 (C=C str, aromatic) 1558 (C=C + C=N str), 3398 cm^{-1} (NH str, arom. prim. amine); Anal. cald for $C_{20}H_{11}N_5O_4S$: C 57.50, H 2.64, N 16.77, S 7.67; Found: C 58.00, H 2.52, N 16.87, S 7.72.

Synthesis of derivatives of 6-chloro-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (3)

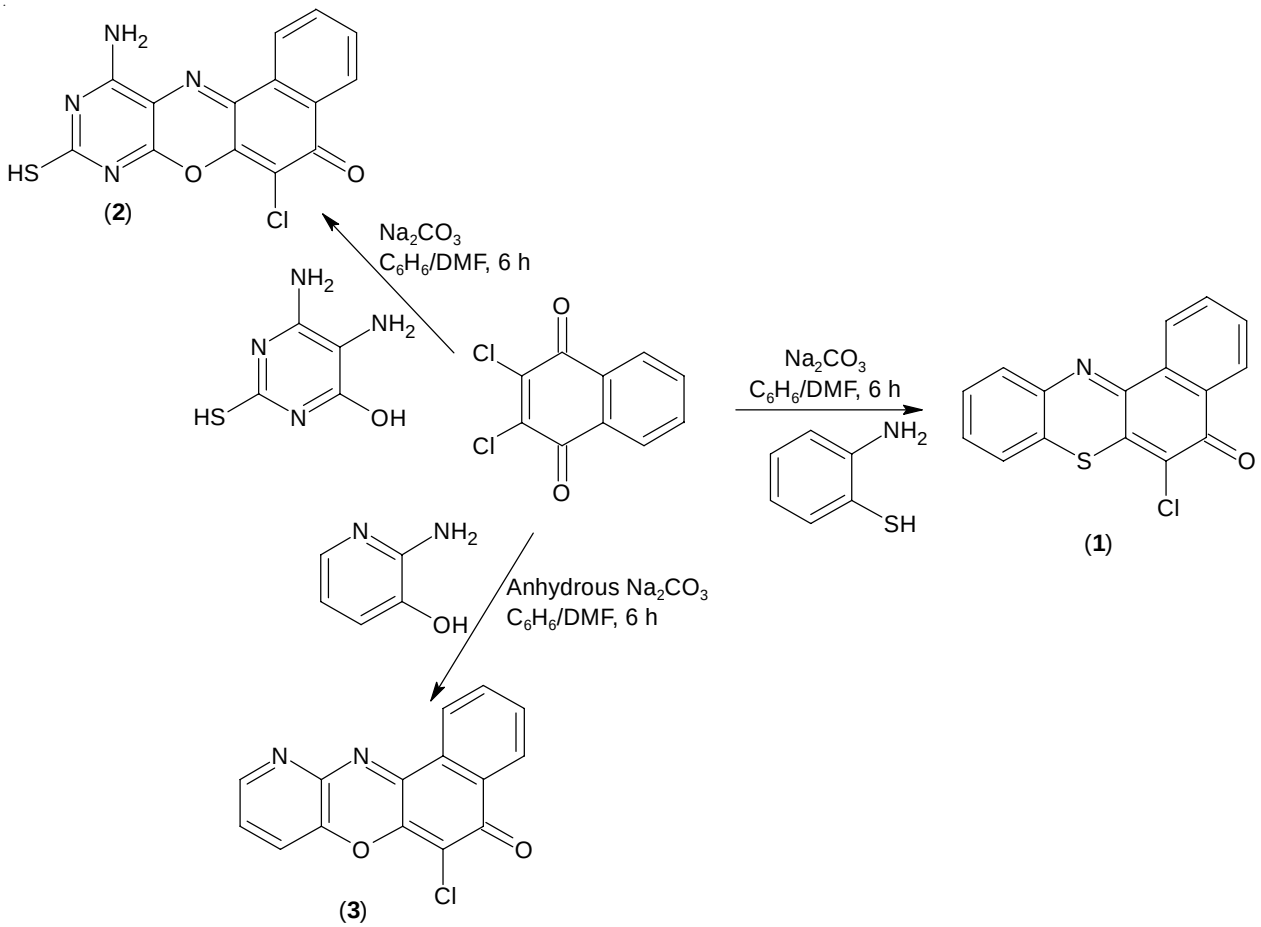
6-Phenyl-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (3a): The above general protocol was employed using 6-chloro-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (0.28 g, 1 mmol), boronic acid (1.5 mmol), $Pd_2(dba)_3$ (0.03 g, 1 mol %), SPhos (0.06 g, 2 mol %), K_3PO_4 (0.64 g, 3 mmol) and the product was afforded as black solid. Yield = 143 mg (44 %); m.p: dec. 237 °C. ^{1}H NMR (DMSO, 700 MHz) δ: 8.88-8.85 (1H, m); 8.53-8.51 (1H, dd,); 8.31-8.29 (1H, m); 7.78-7.75 (2H, m); 7.47-7.44 (5H, m); 7.40-7.37 (1H, m), 7.32-7.29(1H, dd); ^{13}C NMR (DMSO, 700 MHz) δ: 182.4 (Carbonyl carbon), 151.7, 146.4, 144.6, 140.9, 135.6, 132.9, 132.5, 131.9, 130.6, 130.5, 130.3, 129.5, 128.4, 128.3, 128.0, 127.5, 127.2, 126.6, 125.5, 124.4, 120.6, 115.4,; IR (KBr, ν_{max}, cm^{-1}): 748 (C-H def. benzene ring with 4 adjacent H atoms), 877, 1076 (aromatic C-H out-of-plane bend), 1237 (C-O-C str.), 1386 (C-N str.), 1457 (C=C str., aromatic), 1714 cm^{-1} (C=O str.); Anal. cald for $C_{21}H_{12}N_2O_2$: C 77.70, H 3.70, N 8.63; Found: C 78.20, H 3.62, N 8.58.

6-(3-Nitrophenyl)-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (3b): As yellowish red solid; Yield = 143 mg (44 %); m.p: dec. above 270 °C; UV-visible (MeOH) λ_{max}: 342, 448, 738, 758; ^{1}H NMR (DMSO, 700 MHz) δ: 8.88-8.85 (1H, m); 8.53-8.51 (1H, dd,); 8.31-8.29 (1H, m); 7.78-7.75 (2H, m); 7.47-7.44 (4H, m); 7.40-7.37(1H, m), 7.32-7.29(1H, dd); ^{13}C NMR (DMSO, 700 MHz) δ: 182.4 (carbonyl carbon), 151.7, 148.4, 145.9, 144.6, 140.9, 135.6, 132.9, 132.5, 131.9, 130.6, 130.5, 130.3, 129.5, 128.4, 128.3, 128.0, 127.5, 127.2, 126.6, 125.7, 125.5, 124.4, 120.6, 115.4; IR (KBr, ν_{max}, cm^{-1}): 751, 886 (aromatic C-H out-of-plane bend), 1123 (C-O str), 1264 (C-N str), 1443 (C=C str, aromatic), 1545 (-NO_2 str, aromatic), 1646 cm^{-1} (C=C str); Anal. cald for $C_{21}H_{11}N_3O_4$: C 68.23, H 2.98, N 11.37; Found: C 68.56, H 3.00, N 11.31.

RESULTS AND DISCUSSION

The intermediates, **1-3** were prepared by the condensation of 2-aminothiophenol, 4,5-diamino-6-hydroxylpyrimidine-2-thiol and 2-aminopyridin-3-ol each with 2,3-dichloronaphthalene-1,4-dione in a basic medium using benzene/DMF as the solvent (**Scheme-I**). They were obtained as shiny red, dark orange and yellowish brown solids respectively after recrystallization from toluene-benzene. The structures assigned to the intermediates were consistent with their spectra and these were confirmed by the microanalysis.

Synthesis of coupled products *via* SMC: The bulky biphenyl-based phosphine developed by Buchwald and co-workers was applied in successful synthesis of phenothiazine and phenoxazine derivatives (**1a-a**, **2a-b** and **3a-b**). Like Buchwald, we applied $Pd_2(dba)_3$ and SPhos (Pd/L = 1:2) to form our catalytic system in combination with either of the bases, Na_2CO_3 and K_3PO_4 and non-polar solvents (toluene). We started our preliminary experiment by coupling 6-chloro-benzo[a]phenothiazine 600 mg (1 mmol) with phenyl- and 3-nitrophenyl boronic acid 180 mg (1.5 mmol), 2 mol % of $Pd_2(dba)_3$ (3 mg), 4 mol % of SPhos (6 mg) and 637 mg (3 mmol) K_3PO_4 and run the reaction in toluene at temperature of 110 °C while the progress of reaction monitored on 1 h bases by TLC. We observed product formation after 1 h with trace conversion which increases with time. However, after 7 h there was no change in TLC spot of product even after the reaction was left to run overnight. We obtained an isolated



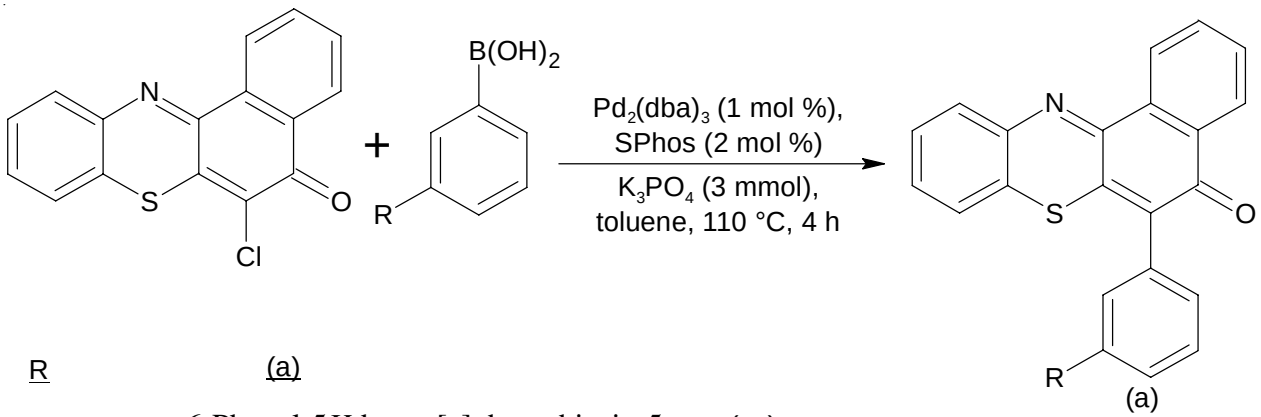
Scheme-I

product yield of 53 % with a conversion of over 70 % after work-up and purification by column chromatography on silica gel using 5 % EtOAc/95 % petroleum ether solvent mixture (**Scheme-II**).

The spectra and elemental analysis confirmed the identity of the isolated product of each of the coupling reaction of 6-chloro-5*H*-phenothiazin-5-one (**1**) with phenyl- and 3-nitrophenyl boronic acids as 6-phenyl-5*H*-phenothiazin-5-one (**1a**) and 6-(3-nitrophenyl)-5*H*-phenothiazin-5-one (**1b**) with molecular formula $C_{22}H_{13}NOS$ and $C_{22}H_{12}N_2O_3S$, respectively.

The structure assigned to the 6-phenyl-5*H*-benzo[a]phenothiazine was consistent with the UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectra. The structural assignment was confirmed by the micro-analytical result of compound. Likewise, the structural assignment of the 6-(3-ntrophenyl)-5*H*-benzo[a]phenothiazine was consistent with spectroscopic data.

The reaction ran without addition of the ligand practically gave no conversion even when it was left over night (24 h). Equally, the use of triphenylphosphine (PPh_3) instead of SPhos gave no conversion within the reaction time of 8 h and conver-



R	(a)
H	6-Phenyl-5*H*-benzo[a]phenothiazin-5-one (**1a**)
NO_2	6-(3-Nitrophenyl)-5*H*-benzo[a]phenothiazin-5-one (**1b**)

Scheme-II

sion of > 10 % when reaction was left over night (24 h). Consquently, the ligand plays a crucial role in Suzuki-Miyaura cross-coupling reactions. Besides the bulkiness which has been corroborated to assist in generation of monoligated complex ion (L_1Pd) that was believed to undergo oxidative reaction more readily than [PdL_2] complex, the strong σ-electron donating character of SPhos equally contributes to the ease with which chloro-phenothiazine and phenoxazines were coupled to boronic acids. We believed that the electron richness of SPhos minimized the rate of potential binding of the heteroatoms in the reactants and/or product to metal center thereby increasing the rate of the reaction as well as yield of expected product. We used slight excess of boronic acids to compensate for the fractions that may be converted to side products such as homocoupled products. While the use of Na_2CO_3 in place K_3PO_4 gave lower product yields (24 %), K_2CO_3 gave comparable yield to K_3PO_4 under the same conditions. It is also noticed that the reactions performed without prior solvent degassing gave comparable yield of products under these conditions. Although we used only $Pd_2(dba)_3$ as palladium source. It is also believed that other palladium species such as $PdCl_2$, $Pd(NO)_2$ and $Pd(OAc)_2$ together with SPhos will effect similar transformations.

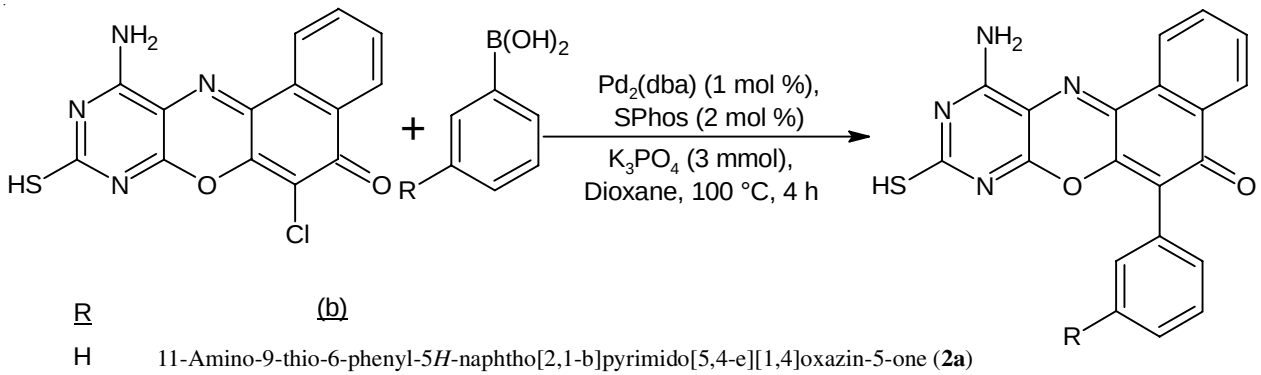
Consequently, the procedure was applied in coupling other intermediates (**2** and **3**) to phenyl and 3-nitrophenyl boronic acids, running the reaction for a maximum of 8 h under refux (**Schemes III** and **IV**).

Each completed reaction was work-up by first extracting the crude product from water with chloroform followed by purification by column chromatography on silica gel.

The numbers of aromatic protons were accounted for in the ^{1}H NMR of the compounds prepared by Suzuki-Miyaura cross-coupling and were found at 8.919-6.003 ppm. The carbon nuclei were nicely represented in the carbon nuclear magnetic spectra for **1a** with the carbonyl carbon signals found in a far distance low field from the others sp^2 hybridized carbon peaks except in **2a, 3a** and **3b**. The IR absorption maxima for carbonyl functional groups in synthesized compounds were in the range of 1725-1610 cm^{-1}.

Furthermore the UV-visible spectra data of compounds revealed bathochromic shifts as a result of increase in conjugation with compound **2a** exhibiting the highest shifts. However, results from elemental analysis of compounds undoubtedly confirmed their molecular and structural formulae assignment.

Antimicrobial activity: All the synthesized compounds were screened for their antimicrobial activity at concentration 10 mg/disc in agar media following the method of Bauer *et al.*[50]. Using ciprofloxacin, an antibacterial and ketoconazole, an antifungal activity as reference drugs, the compounds were screened against six micro-organisms, *viz*: *Bacillus subtitis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Aspergillus niger.* This was carried under sensitivity test and minimum inhibitory concentration (MIC).

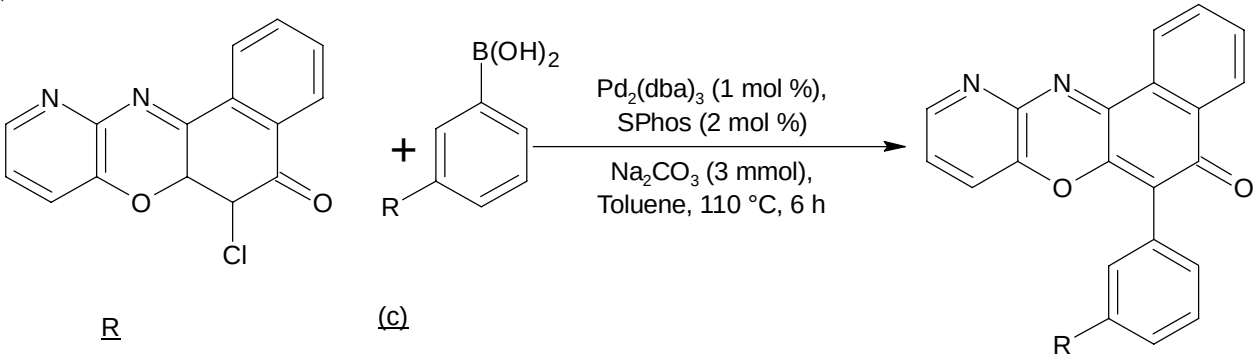


R (b)

H 11-Amino-9-thio-6-phenyl-5*H*-naphtho[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (**2a**)

NO_2 11-Amino-9-thio-6-(3-nitrophenyl)-5*H*-naphtho[2,1-b]pyrimido[5,4-e][1,4]oxazin-5-one (**2b**)

Scheme-III



R (c)

H 6-Phenyl-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (**3a**)

NO_2 6-(3-Ntrophenyl)-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one (**3b**)

Scheme-IV

TABLE-1
ANTIMICROBIAL SENSITIVITY RESULTS OF SYNTHESIZED COMPOUNDS AND THE VALUES ARE GIVEN IN INHIBITION ZONES DIAMETER (mm)

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi Organism	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
1	10	9	11	4	–	10
2	–	–	6	9	15	12
3	–	–	9	5	11	8
1a	25	–	–	–	–	–
1b	23	–	–	–	–	–
2a	–	–	–	–	–	–
2b	17	–	–	–	–	–
3a	21	–	–	16	–	–
3b	25	–	–	13	–	–
Ciprofloxacin	17	20	13	8	–	–
Ketoconazole	–	–	–	–	16	21

(–) Resistance

TABLE-2
MINIMUM INHIBITORY CONCENTRATION OF THE COMPOUNDS (mg/mL)

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi Organism	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
1	0.17380	0.12020	0.15140	0.41635	–	0.09120
2	–	–	0.69960	0.13200	0.08500	0.13000
3	–	–	0.46320	0.20200	0.06500	0.15000
1a	0.01442	–	–	–	–	–
1b	0.01567	–	–	–	–	–
2a	–	–	–	–	–	–
2b	0.02121	–	–	–	–	–
3a	0.01716	–	–	0.08385	–	–
3b	0.01442	–	–	0.10320	–	–
Ciprofloxacin	0.02120	0.02130	0.0323	0.16770	–	–
Ketoconazole	–	–	–	–	0.06220	0.13560

Sensitivity test: The assay was carried out by applying agar-well diffusion method[51] using a concentration of 10 mg/mL of each of the compounds as described in the experimental. From the results (Table-1), the synthesized derivatives showed substantial activity against *Bacillus subtilis* and *E. coli* only, except compound **2a** which is inactive against *B. subtilis* as well as *E. coli* and compound **1a**, **1b, 2a** and **2b** which are inactive against *E. coli*. All the derivatives were found to be insensitive to *S. aureus, P. aeruginosa, C. albicans* and *A. niger.* In the same vein, the intermediates (**1-3**) are all sensitive to the test organisms except **1** which is inactive to *C. albicans;* and compound **2** and **3** that are insensitive to the Gram-positive bacteria (Table-1).

Minimum inhibitory concentration (MIC) determination result: Here, serial dilutions of 5, 2.5, 1.25 and 0.625 mg/mL of each of the sensitive compounds obtained from the sensitivity test were used following the procedure outlined by Chemical Laboratory Standards Institute (CLSI)[52]. Almost all the sensitive synthesized phenothiazine and phenoxazine 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 **1a** and **3b** have the lowest MIC value (0.01442 mg/mL) and hence, most activity against *B. subtilis* (Table-2) when compared to the referent drug. Similarly, activity against *E. coli* was much pronounced in compound **3a** followed by compound **3b** and then the intermediates. Intermediate **1** has no MIC values for *C. albicans* since it is insensitive to the test organism. All the synthesized compounds were insensitive to fungal organisms except the intermediates. The intermediate, **1** has the stongest activity against *A. niger* consequent on its lowest MIC value. However, it has no activity against *C. albicans*. From the forgoing, it could be inferred that the synthesized compounds can be used as antibacteria against *E. coli* and *B. subtilis* since they have better activity compared to the standard reference drugs (Table-2).

Conclusion

This study introduced new reactions for derivatization of benzophenothiazine and benzophenoxazine rings. Palladium-SPhos mediated Suzuki-Miyaura cross-coupling reaction was developed as a facile and mild reaction procedure for functionalization of the phenothiazines and phenoxazines in good to excellent yields. The prepared compounds exhibited high activities against Gram-positive bacteria (*B. subtilis* and *E. coli*) and the activity was much pronounced for 6-phenyl-5*H*-benzo[a]phenothiazin-5-one and 6-(3-nitrophenyl)-5*H*-naphtho[2,1-b]pyrido[2,3-e][1,4]oxazin-5-one than the others.

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