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Metal Catalyzed Synthesis and Antimicrobial Studies of Some New Amido Derivatives of Non-Linear Polycyclic Diazapheno-thiazinone

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Metal Catalyzed Synthesis and Antimicrobial Studies of Some New Amido Derivatives of Non-Linear Polycyclic Diazaphenothiazinone

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Abstract- The synthesis of novel non-linear polycyclic diazaphenothiazine and its amido derivatives via Buchwald – Hartwig reaction protocol is reported. By reacting 2-amino-5-bromopyrazine-3[4*H*]-thione and 2, 3-dichloro-1, 4-naphthoquinone in the presence of anhydrous sodium carbonate, 9-bromo-6-chloro-8, 11-daiza-5*H*-benzo[a] phenothiazin-5-one was obtained. The arylation of 9-bromo-6-chloro-8, 11-daiza-5*H*-benzo[a]pheno-thiazin-5-one with some amides under palladium catalysis gave the amido derivatives in good yield. The stability of these cyclic quinoneimine systems, which has been attributed to ionic resonance effect, is noted. Structural assignments were based on UV, IR and NMR spectra as well as elemental analysis. These compounds showed significant antimicrobial activity against *E. coli*, *Staphylococcus spp*, *Bacillus spp*. and *Pseudomonas aeruginosa* at varying concentrations.

Keywords: synthesis, arylation, amido derivatives and palladium catalysis.

I. Background

It is a well-known fact [1] that transition metal-catalyzed reactions represent an elegant approach to complex molecular scaffolds. The use of reactions catalyzed by transition-metal complexes especially palladium has changed the face of modern organic synthesis and led to the development of radically new methods of building carbon-carbon and carbon-heteroatom bonds [2 – 5]. Besides their esthetics and increase in structural complexity, they have also become tools for combating the challenges posed by the difficulties in constructing C-C, C-N and C-O bonds. Palladium catalyzed substitution reactions forming carbon-carbon and carbon-heteroatom coupling reactions as well as nickel-catalyzed reactions [6-9] across multiple bonds are extensively used in the synthesis of complex organic moieties.

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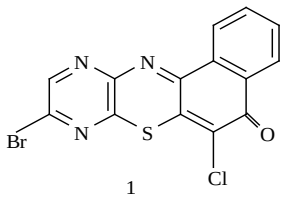
There exist two different aspects in the application of transition metal-catalyzed reactions to the chemistry of heterocyclic compounds. The first involves the building of the heterocyclic backbone and the second, using the heterocyclic fragment as one of the reactions components. Both groups of reactions are equally important though the second one usually has no individual features of its own, replicating the regularities observed in the chemistry of aromatic compounds.

Some of the transition-metal cross-coupling reactions that have revolutionized synthetic strategies include the (i) Buchwald-Hartwig coupling reactions (ii) Heck – Mizoroki Cross-coupling reactions (iii) Suzuki – Miyaura (SM) coupling reactions (iv) Sonogashira reaction of aryl halides using acetylene (alkylnylation), (v) Stille reaction of aryl halides using stannanes, (vi) Migita-Kosugi-Stille Cross-coupling reactions involving organotin compounds, (vii) Negishi pd-catalyzed allyl-alkenyl coupling reactions among others. The influence of metal catalyzed cross-coupling reactions was recognized with Richard Heck, Akira Suzuki and Ei-ichi Negishi being declared the 2010 Chemistry Nobel Laureates for their development of these reactions [10,11].

Although significant advances have occurred in the metal-catalyzed amination and amidation of aryl halides during the last decade, application of this coupling to various heterocyclic structures such as angular azaphenothiazines is still grossly understudied [6,12]. Palladium-catalyzed C-N bond forming reactions between 9-bromo-6-chloro-8, 11-diaza-5*H*-benzo[a]phenothiazin-5-one, 1 and nucleophiles such as amides and amines are still unknown despite continued interest in the chemistry of phenothiazines [13-16] with annular nitrogen atoms. More so, the diverse biological activities of these new compounds spurred our interest the more; hence, we report our studies on these useful reactions.



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II. Methods

a) General Information

Most of the chemicals were purchased from Sigma-Aldrich and were used as purchased because they were of analytical grade. Melting points of the novel compounds were determined using Fisher-Johns melting point apparatus and were uncorrected. ^{1}HNMR, ^{13}CNMR, IR, UV/Visible spectroscopic methods and elemental analysis were employed in their structural characterization. Nuclear Magnetic Resonance (^{1}HNMR and ^{13}CNMR) spectra were done using Varian NMR Mercury-200BB in Central Science Lab. Obafemi Awolowo University Ife. Chemical shifts are reported on the δ-scale (neat). Infrared spectra were recorded using SHIMADZU FTIR-8400S Fourier Transform Infrared Spectrophotometer (KBr discs), at NARICT, Zaria. UV-Visible spectra were done on a JENWAY 6405 UV/VIS Spectrophotometer using matched 1cm quartz cells at the Department of Pure and Industrial Chemistry, University of Nigeria Nsukka. The elemental analyses were carried out at the Central Science laboratory, University of Cairo, Egypt on a CE440 Elemental Analyzer.

b) Synthesis of 2-Amino-3,5-dibromopyrazine, 3

Preparation of compound **3** was achieved using literature procedure [13a,17,18]. 9.5 g, (100 mmol) 2-Aminopyrazine was placed in a three-necked flask into which glacial acetic acid (70 ml) has been added. The mixture was warmed on a steam bath until it dissolved. Sodium acetate trihydrate (33 g, 243 mmol) was then added with constant swirling. The reaction mixture was placed in an ice-salt bath and stirred, maintaining the temperature at -5°C while bromine (16 ml) was added drop wise over a 4 h period.

Stirring continued in the ice bath after the addition of bromine for 2 h and then at room temperature for 24 h. The entire crude was poured into ice (50 g) and neutralized with concentrated ammonia (pH 8). 2-amino-3,5-dibromopyrazine (16.8 g, 66%), m.p 113-114°C (Lit.[13a17, 18] 114-115°C) was recrystallized from methanol (Norit) as colourless needles

c) Synthesis of 2-Amino-5-bromopyrazine-3[4H]–thione, 4

This intermediate was prepared according to literature[13a, 18]. 2-amino-3,5-dibromopyrazine (7.69 g, 30 mmol) and sodium hydrosulphide (13.33 g, 238 mmol) were added to methanol (60 ml) in a 250 ml three-necked flask equipped with a reflux condenser and a magnetic stirring bar and the mixture was refluxed for 4.5 h.

The solvent (methanol) was then removed by distillation and the residual mixture worked up. Recrystallization using DMF (Norit) furnished pure 2-amino-5-bromopyrazine-3[4*H*]-thione (3.75g, 61%) as a yellow solid, m.p 209-211°C (dec); (lit.[18]208-210°C).

UV-Vis (MeOH): λ_{max} 222 (2.46), 249 (4.16), 340 (3.89), 425 (1.61) nm.

Ir (KBr): υmax 3446, 3173, 1563, 1524, 1318, 674cm^{-1}

d) Synthesis of 9-Bromo-6-chloro-8,11-diaza-5H-benzo[a]phenothiazin-5-one (also known as 9-bromo-6-chloro-8,11,12 triazabenzo[a]anthracen-5-one[18]), 1

Compound 4 (1.03 g, 5 mmol), anhydrous sodium carbonate (1.06 g, 10 mmol) and benzene (40ml) mixed with dimethylformamide (5 ml) were charged into 100 ml three-necked round-bottom flask fitted with a short magnetic stirring bar and a reflux condenser. The mixture was refluxed for 45 min on a water-bath at 70-75°c while stirring. 2,3-Dichloro-1,4-naphthoquinone (1.14 g, 5 mmol) was added and the mixture refluxed for 7h. At the end of the 7h., the solvent was removed by vacuum distillation. Crushed ice was added and filtration was carried out. The residue was recrystallized from DMF-acetone-water mixture (1:2:2) after treatment with activated charcoal to yield a purplish red crystalline solid which weighed 0.98 g (52%); melted at 238-240°c (lit.[18]>200°c) and identified as 9-bromo-6-chloro-8,11-diaza-5*H*-benzo[a]phenothiazin-5-one, 1.

UV-Vis (MeOH): λ_{max}245 (3.82), 285 (3.76), 335 (3.92) nm.

Ir (KBr): υmax 3172, 1674, 1562, 1220, 1121, 820, 702cm^{-1}

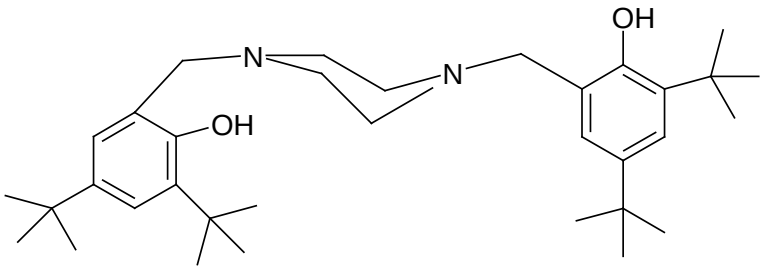
^{1}HNMR (DMSO): δ7.8 - 8.4 (4H, m), 7.2(1H, s),

^{13}CNMR (DMSO): δ160 (C=O), 143 (C-10), 134 (C-10), 127 (C-6), 76 -77(DMSO)

Anal. Calcld for $C_{14}H_5N_3BrSOCl$: C, 44.39; H, 1.32; N, 11.10; Br, 21.14; S, 8.45; Cl, 9.40

Found: $C_{14}H_5N_3BrSOCl$: C, 44.40; H, 1.29; N, 11.20, Br, 21.28; S, 8.50; Cl, 9.49

Preparation of 1,4-bis(2-hydroxy-3,5-di-tert-butylbenzyl)piperazine, $C_{34}H_{54}N_2O_2$,10.



10

The preparation of the ligand, 10 was achieved following Balakrishna *et al* method [22]. Piperazine (2.2 g, 25.54 mmol) was mixed with 40% aqueous formaldehyde solution (5.30 ml, 75.36 mmol) and dissolved in methanol (40 ml). The reaction mixture was refluxed until a clear solution was obtained. After cooling, 2,4-di-*tert*-butylphenol (10.3 g, 50.41 mmol) in methanol (60 ml) was added. The resulting solution was further refluxed for 12 h at 60°C. The mixture was allowed to cool to room temperature and colourless crystals of compound **10** were obtained. Yield: 64% (8.35 g, 15.98 mmol); m.p >250°C (dec) (lit[22]>250°C (dec).

e) General Procedure for the Palladium-Catalyzed C-N Cross-Coupling Reactions [20]

An oven-dried 50 ml three-necked flask equipped with a magnetic stir bar and a reflux condenser was charged with $Pd(OAc)_2$(1mol %) and ligand (3mol %). The reaction vessel was flashed with nitrogen and the solvent (2 ml) and degassed water (4mol %) were added via syringe. After the addition of the water, the solution was heated to 110°C for 1.5 min.

Another 50 ml three-necked flask equipped with a magnetic stir bar was charged with base (1.4 mmol), the phenothiazine (1.0 mmol) and amide/amine (1.4mmol) and flashed with nitrogen. The activated catalyst solution was transferred from the first reaction vessel into the second. The entire reaction mixture was heated to 110°C for 3h. At the end of the reflux period, the reaction mixture was cooled to room temperature. After removal of the solvent in vacuo, the compound of interest was recrystallized from ethyl acetate-water mixture.

f) 6-Chloro-9-ethanamido-8,11-diaza-5H-benzo[a]-phenothiazin-5-one, 9a.

A mixture of 9-bromo-6-chloro-8,11-diaza-5*H*-benzo[a]phenothiazin-5-one (0.378 g, 1.0mmol), acetamide (0.083 g, 1.4 mmol), K_3PO_4 (0.297 g, 1.4 mmol), $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), 1,4-bis(2-hydroxy-3,5-di-*tert*-butyl-benzyl)piperazine (0.016 g, 0.03 mmol), H_2O (1μl, 0.04 mmol) and t-BuOH (2 ml) was heated to 110°C for 3h. After removal of the solvent in vacuo, the compound of interest was recrystallized from ethyl acetate-water mixture as a dark brown solid (0.403 g, 80.4%), m.p 106-108°C.

UV-Vis (MeOH): λ_{max}221.8 (4.40), 251.2 (4.32), 345.6 (3.92), 514.4 (3.06) nm.

Ir (KBr): υmax 3300, 3158, 2861,1672, 1553, 705 cm^{-1}

^{1}HNMR (DMSO): δ8.5 (1H, s, N-H), 8.1 (4H, m), 7.0 (1H, s), 1.8 (3H, s, CH_3).

^{13}CNMR (DMSO): δ166 (C=O), 153 (C-9), 143 (C α to C-6), 135 (C-10), 127 (C-1 & C-4), 123 (C-6), 38.7 – 41.2 (DMSO).

*Anal.*Calcld for $C_{16}H_9N_4SO_2Cl$: C, 53.86; H, 2.52; N, 15.71; S, 8.97; Cl, 9.96

Found: $C_{16}H_9N_4SO_2Cl$: C, 53.80; H, 2.45; N, 15.60; S, 9.06; Cl, 10.09

g) 9-Benzamido-6-chloro-8,11-diaza-5H-benzo[a]phenothiazin-5-one, 9b

9-bromo-6-chloro-8,11-diaza-5*H*-benzo[a]phenothiazin-5-one (0.378 g, 1.0mmol), benzamide (0.169 g, 1.4 mmol), K_3PO_4 (0.297 g, 1.4 mmol), $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), ligand (0.016 g, 0.03 mmol), H_2O (1μl, 0.04 mmol) and t-BuOH (2 ml) as a mixture was heated to 110°C for 3h. After removal of the solvent in vacuo, the compound of interest was recrystallized from ethyl acetate-water mixture as a reddish-brown solid (0.421 g, 80%) m.p 115-117°C.

UV-Vis (MeOH): λ_{max}245.2 (4.38), 317 (4.13), 3661.6 (3.73), 502.4 (3.47) nm.

Ir (KBr): υmax 3300, 3173,1665, 1566, 1277, 697 cm^{-1}

^{1}HNMR (DMSO): δ8.3 (1H, s, N-H), 8.1(4H, m), 7.5 (4H, m), 6.9 (1H, s,).

^{13}CNMR (DMSO): δ168(C=O), 153.7 (C-9), 134 (C-2), 131(C-3), 129 (C-1, C-4 & C-10), 128(C at *m*-position in benzamido moiety), 115(C-6), 38.7 – 41.2 (DMSO).

*Anal.*Calcld for $C_{21}H_{11}N_4SO_2Cl$: C, 60.22; H, 2.63; N, 13.40; S, 7.65; Cl, 8.48

Found: $C_{21}H_{11}N_4SO_2Cl$: C, 60.35; H, 2.60; N, 13.42; S, 7.80; Cl, 8.36

h) 6-Chloro-9-(4-nitrobenzamido)-8,11-diaza-5H-benzo[a]phenothiazin-5-one, 9c.

A mixture of 9-bromo-6-chloro-8,11-diaza-5*H*-benzo[a]phenothiazin-5-one (0.378 g, 1.0mmol), 4-

nitrobenzamide (0.232 g, 1.4 mmol), K_3PO_4 (0.297 g, 1.4 mmol), $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), ligand (0.016 g, 0.03 mmol), H_2O (1 μl, 0.04 mmol) and t-BuOH (2 ml) was heated to 110°C for 3h. After removal of the solvent in vacuo, the compound of interest was recrystallized from ethyl acetate-water mixture as a reddish-brown solid (0.374 g, 57.7%), m.p 129-131°C.

UV-Vis (MeOH): λ_{max}222 (4.43), 249.8 (4.47), 338.6 (3.92), 494 (1.97) nm.

Ir (KBr): υmax 3452, 3167,1688, 1609, 1538, 1334, 711 cm^{-1}

^{1}HNMR (DMSO): δ8.5 (1H, s, N-H), 8.2 (4H, m), 7.8 (4H, m), 6.9 (1H, s).

^{13}CNMR (DMSO): δ166 (C=O), 153 (C-9), 150 (C-NO_2), 143.5 (C α to C-6), 136.9 (C α to C-4), 134 (C-2), 131 (C-3), 129 (C-1, C-4 & C-10),127.5 (C at *o*-position in the nitrobenzamido moiety), 124 (C-6 & C at *m*-position in the benzamido moiety), 38.7 – 41.2 (DMSO).

*Anal.*Calcld for $C_{21}H_{10}N_5SO_4Cl$: C, 54.37; H, 2.24; N, 15.10; S, 6.90; Cl, 7.66

Found: $C_{21}H_{10}N_5SO_4Cl$: C, 54.40; H, 2.19; N, 15.25; S, 6.80; Cl, 7.81

i) 6-Chloro-9-ureacyl-8,11-diaza-5H-benzo[a]phenol-thiazin-5-one, 9d.

A mixture of 9-bromo-6-chloro-8,11-diaza-5*H*-benzo[a]phenothiazin-5-one (0.378 g, 1.0mmol), urea (0.084 g, 1.4 mmol), K_3PO_4 (0.297 g, 1.4 mmol), $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), ligand (0.016 g, 0.03 mmol), H_2O (0.04 mmol) and t-BuOH (2 ml) was heated to 110°C for 3h. After removal of the solvent in vacuo, the title compound was recrystallized from ethyl acetate-water mixture as a brown solid (0.343 g, 47%), m.p 102-104°C (dec).

UV-Vis (MeOH): λ_{max}249.6 (4.50), 340.2 (4.08), 496.2 (3.16) nm.

Ir (KBr): υmax 3458, 3359, 3168, 1680, 1609, 1454, 1284, 712 cm^{-1}

^{1}HNMR (DMSO): δ8.5 (1H, s, N-H), 7.8 (4H, m), 6.9 (1H, s), 5.5 (2H, s, N-H).

^{13}CNMR (DMSO): δ180, 177 (C=O), 153 (C-9), 143 (C α to C-Cl), 135 (C-2), 133 (C-3), 127 (C-1, C-4 & C-10), 123 (C-6), 38.7 – 41.2 (DMSO).

*Anal.*Calcld for $C_{15}H_8N_5SO_2Cl$: C, 50.35; H, 2.24; N, 19.58; S, 8.98; Cl, 9.93

Found: $C_{15}H_8N_5SO_2Cl$: C, 50.50; H, 2.10; N, 19.60; S, 9.00; Cl, 9.95

j) 6-Chloro-9-(3-nitroanilino)-8,11-diaza-5H-benzo[a]-phenothiazin-5-one, 9e.

A mixture of 9-bromo-6-chloro-8,11-diaza-5*H*-benzo[a]phenothiazin-5-one (0.378 g, 1.0mmol), 3-nitroaniline (0.193 g, 1.4 mmol), K_3PO_4 (0.297 g, 1.4 mmol), $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), ligand (0.016 g, 0.03 mmol), H_2O (1 μl, 0.04 mmol) and t-BuOH (2 ml) was heated to 110°C for 3h. After removal of the solvent in vacuo, the compound of interest was recrystallized from ethyl acetate-water mixture as a dark brown solid (0.518 g, 85%), m.p, 96-98°C.

UV-Vis (MeOH): λ_{max}241.2 (4.80), 341.8 (4.19), 497 (3.12) nm.

Ir (KBr): υmax 3302, 3087,1674, 1523, 1342, 725 cm^{-1}

^{1}HNMR (DMSO): δ8.4 (1H, s), 8.2 (4H, m), 7.5 (1H, s), 7.3 (3H, m), 7.0 (1H, s), 5.8 (1H, s.N-H)

^{13}CNMR (DMSO): δ161(C=O), 150.5 (C-9), 143 (C α to C-Cl), 130 [(C -3 & to C-9-(5)], 120 [(C-6 & C-9-(6)], 107 [(C-9-(2)], 38.7 – 41.2 (DMSO).

*Anal.*Calcld for $C_{20}H_{10}N_5SO_3Cl$: C, 55.11; H, 2.30; N, 16.07; S, 7.35; Cl, 8.15

Found: $C_{20}H_{10}N_5SO_3Cl$: C, 55.21; H, 2.36; N, 16.00; S, 7.45; Cl, 8.20

k) 6-Chloro-9-phthalimido-8,11-diaza-5H-benzo[a]-phenothiazin-5-one, 9f.

A mixture of 9-bromo-6-chloro-8,11-diaza-5*H*-benzo[a]phenothiazin-5-one (0.378 g, 1.0mmol), phthalimide (0.206 g, 1.4 mmol), K_3PO_4 (0.297 g, 1.4 mmol), $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), 1,4-bis(2-hydroxy-3,5-di-*tert*-butyl-benzyl)piperazine (0.016 g, 0.03 mmol), H_2O (1μl, 0.04 mmol) and t-BuOH (2 ml) was heated to 110°C for 3h. After removal of the solvent in vacuo, the title compound was recrystallized from ethyl acetate-water mixture as a greyish-brown solid (0.420 g, 67.9%), m.p, 118-120°C.

UV-Vis (MeOH): λ_{max}222.2 (4.46), 245.8 (4.56), 273.8 (4.39), 338 (4.16), 360 (4.00), 498 (3.44) nm.

Ir (KBr): υmax 3156, 1674, 1674, 1494, 1367, 705 cm^{-1}

^{1}HNMR (DMSO): δ8.5 (1H, s), 8.1 (4H, m), 7.8 (4H, m), 6.9 (1H, s).

^{13}CNMR (DMSO): δ180, 176 (C=O), 153.7 (C-9), 143 (C α to C-6), 135 (C-2), 131(C-3), 127 (C at phthalimido moiety), 123 (C-6), 38.7 – 41.2 (DMSO).

*Anal.*Calcld for $C_{22}H_9N_4SO_3Cl$: C, 59.39; H, 2.02; N, 12.59; S, 7.20; Cl, 7.99

Found: $C_{22}H_9N_4SO_3Cl$: C, 59.25; H, 2.06; N, 12.58; S, 7.33; Cl, 8.00

l) Antibacterial Activity of the Synthesized Angular Diazaphenothiazinones

The antibacterial activity of the synthesized Angular Phenothiazinones was conducted using agar-well diffusion method [23]. Solution of each compound with a 20 mg/ml concentration was made by dissolving 0.04 g of each compound in 2 ml dimethyl sulfoxide (DMSO). A colony of each test bacteria isolate was picked and mixed with 2ml of sterile nutrient broth in a bijoux bottle. Each mixture of test bacteria isolate was standardized to correspond to 0.5 McFarland turbidity standards (approximately 10^8 cfu/ml). This suspension

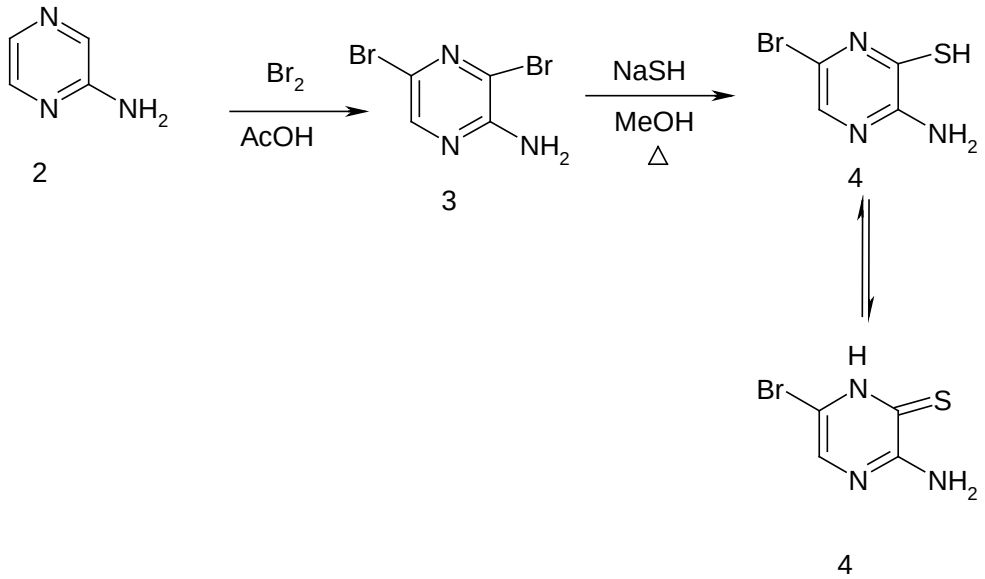
was used to inoculate onto the surface of iso-sensitest agar and the excess fluid drained into discard pot containing disinfectant. The inoculated agar was allowed to air dry and the petri dishes were appropriately labelled. A cork borer with a diameter of 6mm was used to bore wells onto the inoculated iso-sensitest agar. Then a micropipette was used to deliver 50μl of each test compound solution into each well. The petri dishes were left on the bench for 30 minutes so that the compound would diffuse into the solid agar. Thereafter, the petri dishes were put in the incubator at 37^{o}C for 24h. After 24h incubation, the petri dishes were checked for zone of inhibition around the wells. The inhibition zone diameters were measured with metre rule and recorded to the nearest whole millimetre.

m) Minimum Inhibitory Concentration (MIC) of the Synthesized Angular Diazapheno-thiazinones.

The minimum inhibitory concentration of these non-linear diazaphenothiazines was done using agar dilution method as stated by Clinical Laboratory Standards Institute (CLSI) [24]. One millilitre of DMSO was put into sterile test tubes that were lined up on a test tube rack. Two-fold serial dilution of each compound suspension was done by transferring 1 ml of each compound solution (20mg/ml) into the first test tube and from the first to the second test tube and so on till the seventh one. The concentration of the solutions in each test tubes were as follows: 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 (mg/ml)(i.e, graded concentrations of the compounds). After the dilutions, 1ml of each resultant solution was added into 19 ml each of sterilized molten agar, mixed very well and poured onto sterilized petri dishes. These petri dishes with compound amended agar were left to cool and gel on the bench. Then, a colony of each test bacteria isolate was picked and used to inoculate 2ml sterile nutrient broth making a suspension of each test bacterium. All the suspensions of the test bacteria were standardized. Ten microlitre of each bacterial suspension was spot-inoculated onto the surface of the agar using a micropipette. Agar without any of the test compound was also spot-inoculated as positive controls. The inoculated petri dishes were incubated at 37^{0}C for 24 h. After incubation they were checked for any visible bacterial growth. MIC was recorded as the least concentration of the compounds that showed no visible bacteria growth on the agar surface.

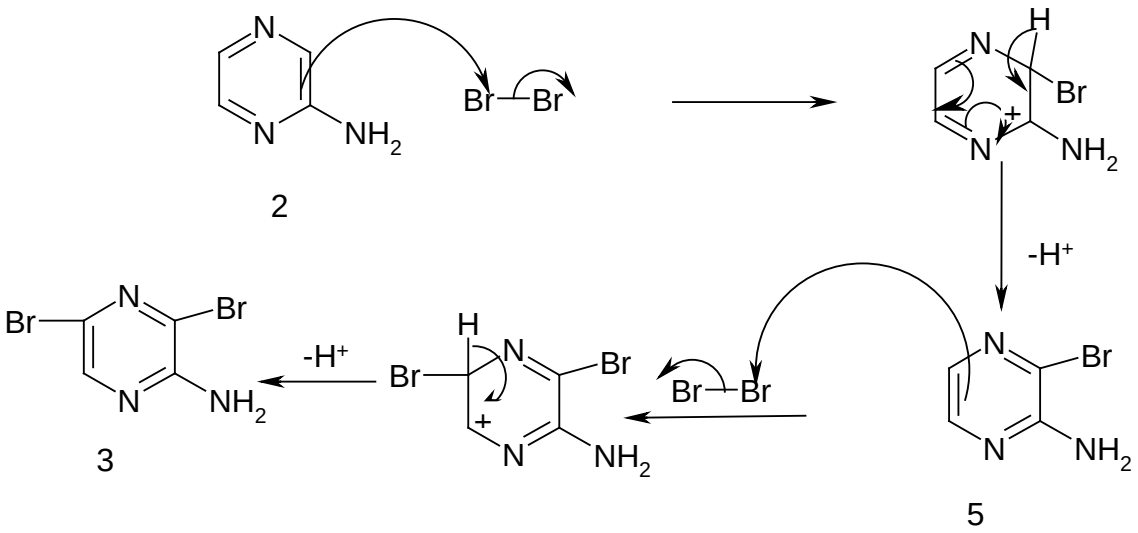
III. Results and Discussion

2-Aminopyrazine, 2, was converted to the 3,5-dibromo derivative, 3, by the action of bromine in glacial acetic acid as also reported by Okafor [17]. Thiation of product 3 using sodium hydrosulphide (NaSH) in methanol furnished 2-amino-5-bromopyrazine-3[4*H*]-thione, 4 (scheme 1).



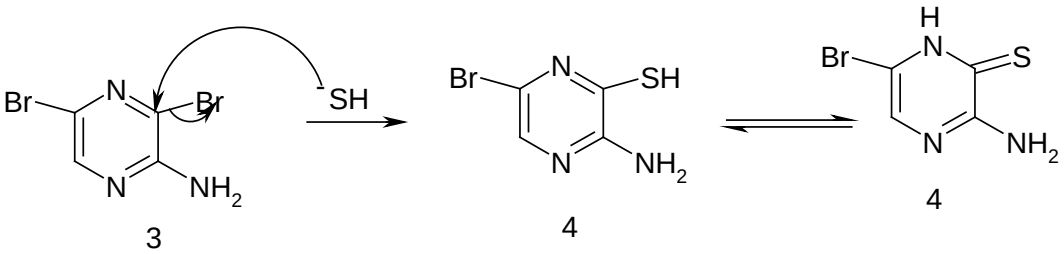
Scheme 1: Synthesis of compound **4**

The possible mechanisms of these reactions are shown in schemes 2 and 3. Under acidic condition, bromine mounts electrophilic attack on carbon in position 3 followed by proton elimination to give compound **5**. Another electrophilic attack is mounted on carbon in position 5 and with hydride elimination; compound 3 is produced as colourless needles on recrystallization from methanol (Norit). It melted at 113 – 115^{o}C (Lit[17], 114 – 115^{o}C).



Scheme 2: Possible mechanism of forming compound 3

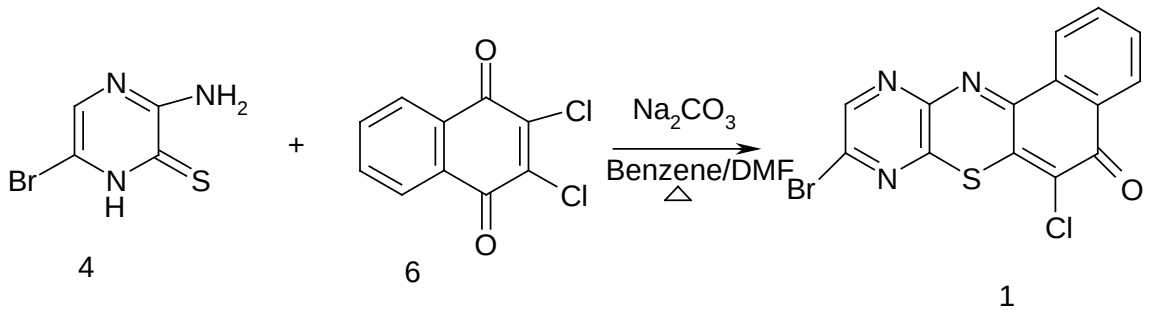
Because of the high reactivity of pyrazine derivatives with nucleophilic agents such as sodium hydrosulphide the displacement of bromine in bromopyrazines like compound 3 proceeds with relative ease. The mercapto group SH^- attacks the carbon at the 3-position displacing the bromine atom giving compound 4 and there could also be hydride shift.



Scheme 3: Possible mechanism for forming compound 4

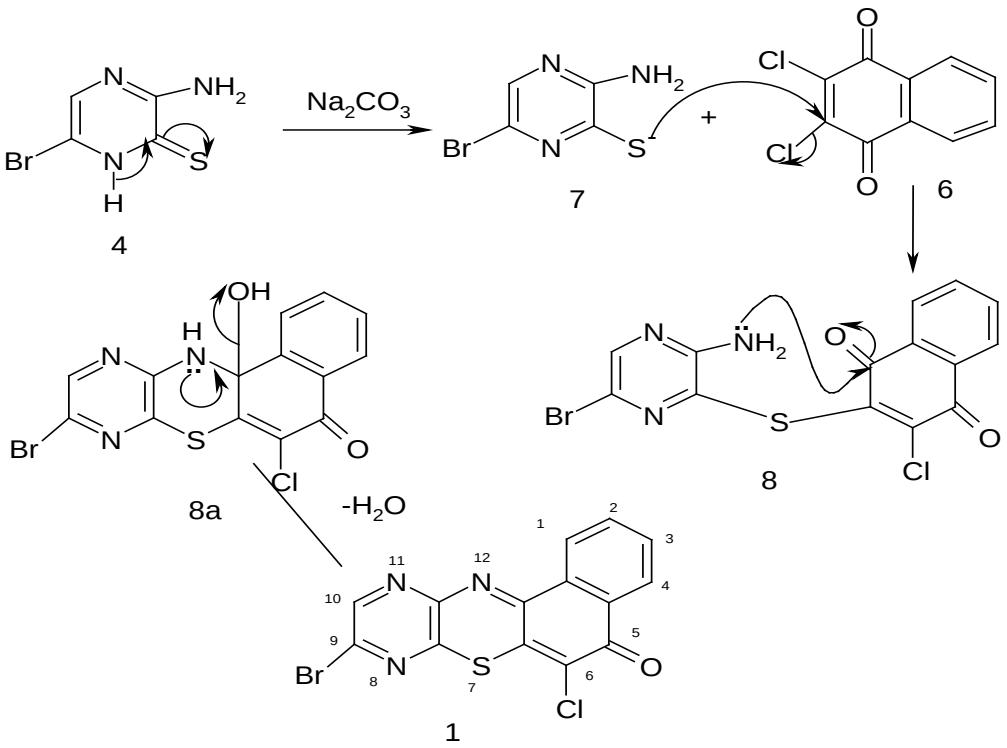
The UV-Vis spectrum gave absorptions at λmax 222 (ε=2.46), 249 (4.16), 340 (3.89) and 425 (1.61) nm. Infrared spectrum (cm^{-1}) showed absorption signals at 3446 (N-H stretch), 3173 (C-H aromatic rings), 1563, 1524 (C =C aromatic rings) and 674 (C – Br stretch). Other bands also agree with the assigned structure.

Condensation reaction (scheme 4) between 2-amino-5-bromopyrazin-3[4*H*]-thione, 4 and 2,3-dichloro-1, 4-naphthoquinone,6, in the presence of anhydrous sodium carbonate furnished compound 1 which was a purplish red crystalline solid melting at 238 – 240°C dec. (Lit[18], > 200°C dec.)



Scheme 4: Synthesis of compound 1

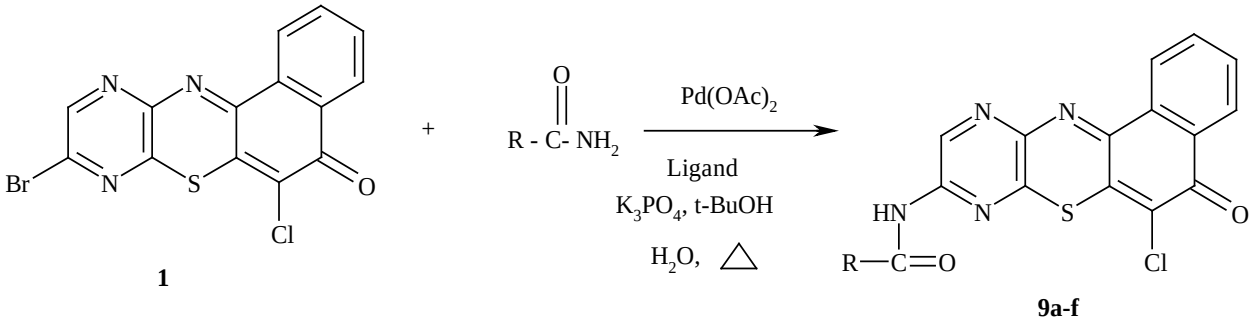
The proposed mechanism (scheme 5) for this reaction could be by an initial nucleophilic attack of the dichloronaphthoquinone, 6, by the mercaptide ion, 7, which results in the formation of the diaryl sulphide, 8. Cyclization takes place by the condensation of the amino group with the carbonyl group in the naphthoquinone moiety. Eliminationoccurs giving 9-bromo-6-chloro-8, 11-diaza-5*H*-benzo[a]phenothiazin-5-one, 1. Compound 1 is an intensely coloured solid due to its extended conjugated system and it is air stable.



Scheme 5

Microanalysis and spectroscopy agree with the assigned structure. The UV-Vis spectrum showed bands at λmax 245 (ε=3.82), 285 (3.76) and 335 (3.92) nm. The infrared spectrum gave signals (cm^{-1}) at 3172 (C-H aromatic rings), 1674 (C=O), 1562 (C=C aromatic rings), 1220, 1121 (C=-N stretch), 820 (C-Cl and 720 (C – Br). The ^{1}HNMR gave peaks at 8.4 (m, 4H) and 7.2 (s,1H). The absorption at δ2.3 is due to dimethyl sulfoxide (DMSO). The ^{13}CNMR showed signals at δ160 (C = O), 143 (C – 10), 134 (C – 2), 127 (C – 6). Peaks at δ76 - 77 were attributed to the solvent used (DMSO).

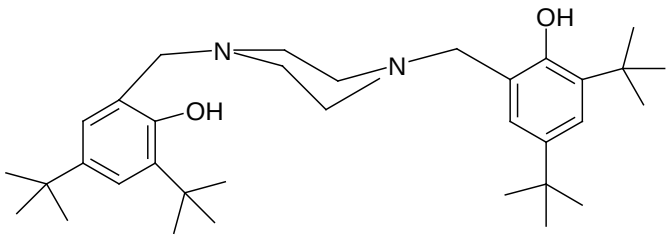
Using $Pd(OAc)_2$, 1,4-bis(2-hydroxy-3,5-di-*tert*-butylbenzyl)piperazine, t-BuOH, K_3PO_4, water and at 110°C, cross-coupling reactions were carried out between compound 1 and some amides/amines (Buchwald-Hartwig reaction) to furnish compounds 9a-f (scheme 6).



R = Me, Phenyl or substituted phenyl

Scheme 6: Synthesis of compounds 9a-f

The protocol utilized water and piperazine ligand, 10, to reduce $Pd(OAc)_2$ and generate the active LnPd(0) complex. The activation was monitored visually by colour change.



10

a) 1,4-bis(2-hydroxy-3,5-di-tert-butylbenzyl)piperazine, $C_{34}H_{54}N_2O_2$, 10

This type of activation was first published in 1992 by Ozawa and Hayashi [19] in which they were able to reduce $Pd(OAc)_2$ in the presence of 3 equivalents of BINAP. According to their report, in the absence of water, the reduction did not proceed; however by adding extra equivalents of water, the rate of activation could be accelerated. Similarly, Buchwald et al [20] successfully coupled even electron-deficient 4-nitroaniline and 2-nitroaniline with 4-n-butylchloro-benzene in excellent yield by performing a water-mediated catalyst pre-activation of $Pd(OAc)_2$. The reaction sequence of this type of activation can be deduced as follows:

$$2L + Pd(II)(OAc)_2 \rightleftharpoons L_2Pd(II)(OAc)_2$$

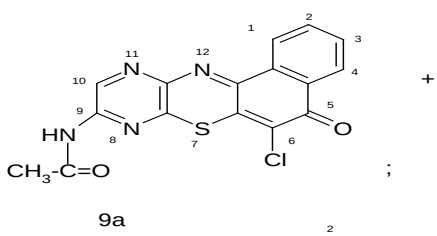
$$LPd(0) + L(oxide) \xleftarrow[-2HOAc]{H_2O} LPd(0)(OAc)^- + AcOL^+$$

This can be simplified as:

$$dX_2 + Ph_3P + H_2O \longrightarrow Pd(0) + Ph_3PO + 2HX$$

The achievement here is that we have been able to utilize piperazine compound (a bidentate ligand) instead of Ph_3P to achieve the purpose of reduction of Pd(II) to Pd(0). This is in agreement with an earlier report by Buchwald *et al*[20]who used XPhos as their ligand, although XPhos is a monodentate ligand.

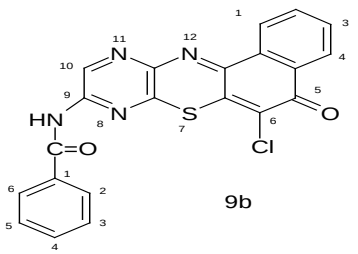
Compounds 9a-f were identified based on their microanalysis and the spectroscopic data available as 6-chloro-9-ethanamido-8,11-diaza-5*H*benzo[a]pheno-thiazin-5-one, 9a; 9-benzamido-6-chloro-8, 11-diaza-5*H*-benzo{a}phenothiazin-5-one, 9b; 6-chloro-9- (4-nitro-benzamido)-8, 11-diaza-5*H*-benzo[a]phenothiazin-5-one, 9c; 6-chloro-9-ureacyl-8, 11-diaza-5*H*-benzo[a]phenothiazin-5-one, 9d; 6-chloro-9-(3-nitroanilino)-8, 11-diaza-5*H*-benzo[a]phenothiazin-5-one, 9e and 6-chloro-9-phthalimido-8, 11-diaza-5*H*-benzo[a]phenothiazin-5-one,9f.



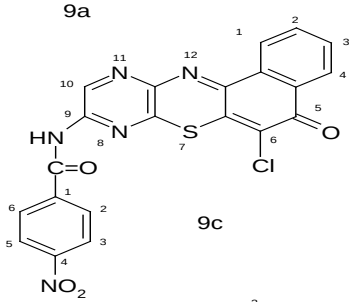
9a

;

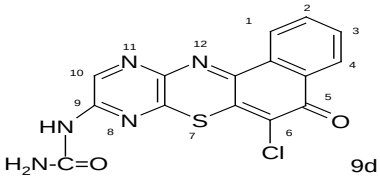
+



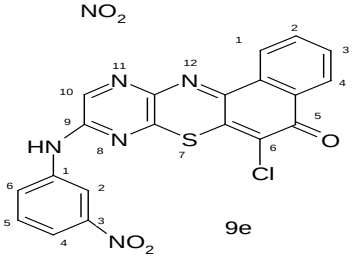
9b



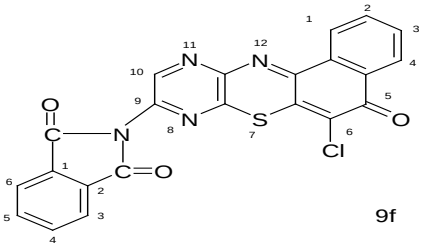
9c



9d



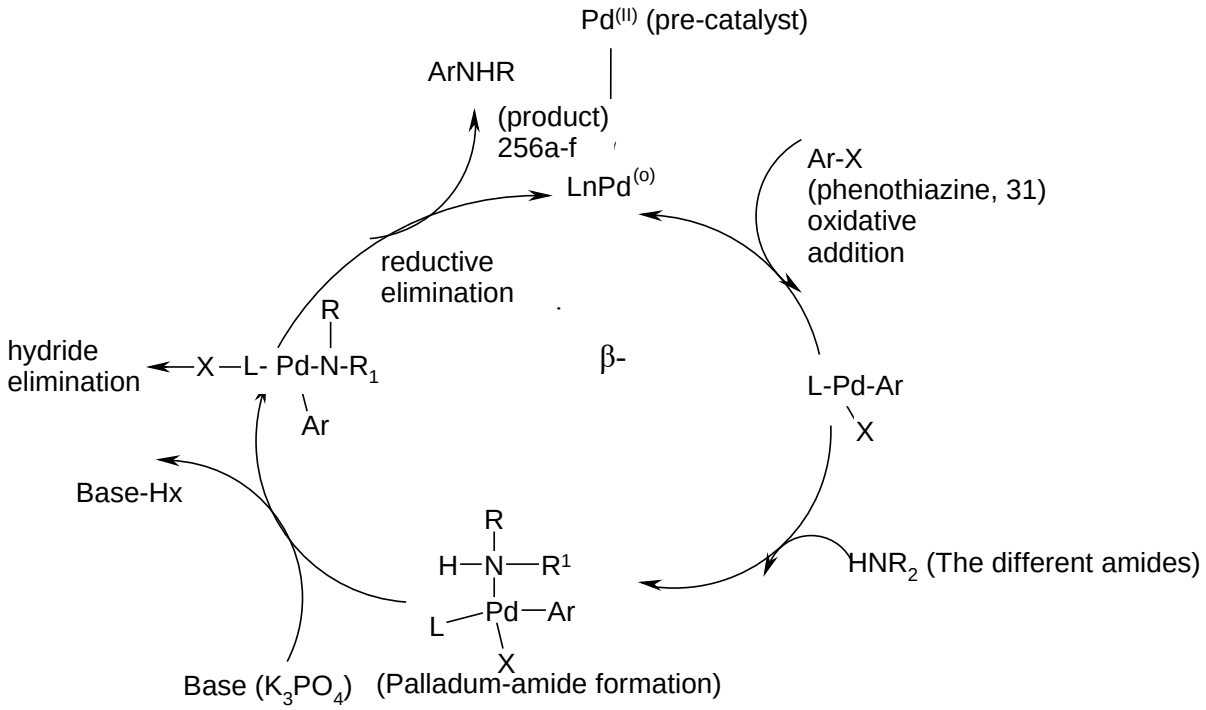
9e



9f

The physical, analytical and spectroscopic data for compounds 9a-f are summarized in the experimental section.

The mechanism of these reactions (scheme 7) follows the general mechanism[21] for Buchwald-Hartwig coupling reactions. The reaction mechanism has been demonstrated to proceed through steps similar to those known for palladium-catalyzed C-C coupling reactions. The steps involve oxidative addition; palladium-amide formation and finally reductive elimination.



Scheme 7: Possible mechanism for the synthesis of compounds 9a-f

The antimicrobial activity of these newly synthesized compounds was evaluated and the data obtained showed they had significant inhibitory effects. An effective approach of antimicrobial therapy of an infection is based on the isolation and identification of the infected organism and determining its sensitivity to antimicrobial drugs. The bacteria tested were three*E. coli* strains (Eco 3, Eco 4 and Eco 12), 2 *Staphylococcus species (Staphylococcus pseudointermedius* and *Staphylococcus scuiri)*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Table 1 below shows the result of the antibacterial susceptibility test. Seven compounds tested against seven bacteria isolates comprising of Gram Positive and Gram negative bacteria. The result of the preliminary screening of the seven compounds tested against the above mentioned Gram negative and Gram positive bacteria are shown in Table 1.

Table 1: Antibacterial Activity of Synthesized Angular Phenothiazinones showing Inhibition Zone Diameter (mm) produced by the Compounds tested (20mg/ml)

Cpd	*S. pseudointermedius*	*S. scuiri*	Eco 3	Eco 4	Eco 12	*Bacillus subtilis*	*Pseudomonas aeruginosa*
1	16	19	14	12	11	16	0
9a	17	16	12	12	15	18	0
9b	15	15	11	11	11	18	0
9c	17	17	17	18	16	17	0
9d	19	20	17	18	18	30	0
9e	15	16	11	12	12	16	0
9f	16	14	11	12	12	16	0

Cpd = compound

The figures in Table 1 show the inhibition zone diameter (IZD) produced by each compound against the test bacteria at 20 mg/ml concentration. It ranged between 11 and 30 mm in diametre; the higher the IZD, the higher the sensitivity. The test compounds produced good activity against all the test bacteria except *Pseudomonas aeruginosa*. This implies that the

compounds that produced high IZD have high antibacterial activity against the test bacteria.

The results of the MIC of the test compounds ranged between 0.625 and 5mg/ml. Hence, Table 2 below shows the result of MIC of the test compounds, Ampicillin and Gentamicin against Eco 3, *S. scuiri* and *Bacillus subtilis*.

Table 2: Minimum Inhibitory Concentration (mg/ml) of the Synthesized Compounds, Ampicillin and Gentamicin against test Bacteria

Compound/Drug	Eco3 (mm)	*S. scuiri*(mm)	*Bacillus subtilis*(mm)
1	5	1.25	1.25
9a	5	1.25	1.25
9b	5	1.25	1.25
9c	2.5	1.25	1.25
9d	1.25	1.25	0.625
9e	5	1.25	1.25
9f	5	1.25	1.25
Ampicillin	100	2.5	1.25
Gentamicin	6.25	2.5	0.15625

The MIC against the test bacteria ranged from 0.625 mg/ml to 5 mg/ml. It became necessary to compare the MICs of the synthesized compounds with some antibiotics against the bacteria tested. Ampicillin and Gentamicin were used as positive control because they are among the drugs used in the treatment of infections caused by similar strains of bacteria while DMSO served as the negative control.

The MIC of the drugs used ranged between 0.15625 mg/ml and100 mg/ml. From Table 2, most of the synthesized phenothiazine derivatives were active against E. coli strain (Eco 3) at very low concentrations whereas Ampicillin has its MIC against Eco 3 as 100 mg/ml which shows that the E. coli strain is highly resistant to Ampicillin. The MIC of Gentamicin showed MIC of 6.25 mg/ml against Eco 3 which is still greater than that of the test compounds especially compounds **9c** and **9d**. This same explanation goes for G84. It was only in the case of *Bacillussubtilis* that the MIC values of novel compounds compared favourably with those of the standard drugs with Gentamicin having the lowest MIC value, 0.15625 mg/ml. All these show that the synthesized phenothiazine derivatives are highly biologically active; hence, they are of pharmaceutical interest.

IV. Conclusion

The syntheses of these biologically active novel angular diazaphenothiazinones have in no small measure extended the list of phenothiazine derivatives of medicinal importance. Again, the carbon-nitrogen coupling reaction catalyzed by transition metal (palladium $^{(0)}$) leading to the synthesis of these derivatives has opened a new route of synthesis.

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Authors' Contributions

EUGN and UCO designed the work, carried out the syntheses and characterization of all the compounds. ICU and EUGN did the antimicrobial screening. EUGN, UCO and ICU contributed in the manuscript write-up. MAE and FNI edited and scrutinized the article. All authors read and approved the submission of this manuscript.

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