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Palladium Catalyzed Transformation and Antimicrobial Screening of Novel Angular Azaphenothiazines

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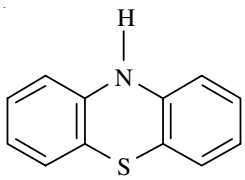
Base mediated condensation reaction between 2-amino-5-bromopyrazine-3[4*H*]-thione and 7-chloro-5,8-quinolinequinone under anhydrous condition gave 9-bromo-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one. Palladium catalyzed cross-coupling reaction between 9-bromo-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one and four arylated halogeno compounds utilizing Heck-Mizoroki protocol furnished 6-substituted derivatives of the angular tetracyclic heterocycle. Structures were assigned based on spectroscopic and elemental analytical data. Antimicrobial screening of these compounds showed they were biologically active.

Keywords: Phenothiazine, Palladium catalysis, Arylated halogeno compounds, Angular azaphenothiazines.

INTRODUCTION

The chemistry of phenothiazine (**1**), dates more than a century ago [1], yet interest in the synthesis of its derivatives especially the aza analogues remains unabated because they have been found to have better biological and chemical properties than their carboxylic counterparts [2-4] which have been attributed to the basic nitrogen of the ring donating electrons to the biological receptors by a charge-transfer mechanism and the stabilities of the charge transfer complexes have been determined [3].



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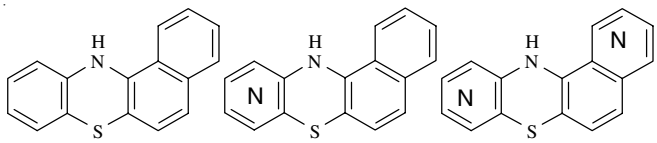
Although benzo[a]phenothiazine (**2**), the prototype of angular phenothiazines has been known for over ten decades. Its chemistry remains poorly developed [5] especially the aza analogues of this ring system. In spite of early reports on phenothiazines [1,5b], little is relatively known about the chemistry of the angular systems. Only recently did the synthesis of a few aza analogues possessing annular nitrogen atom(s) appear in the literature [3,6-8]. Only few of aza-analogues of type 3 are known [6,9-11]. Also grossly under studied are angular phenothiazines possessing annular nitrogen atoms simultaneously in rings A and D, 4; only a few cases have been reported [12-14] albeit their reported uses in medicine [15,16], agriculture [17] and industry [5,18-20]. At present, only two isomeric forms of angular triazaphenothiazine of type 4 out of forty-five possible isomeric forms of this ring system have been reported [14].



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In furtherance of studies of triaza analogues of type 4, we now report the synthesis of new angular triazaphenothiazine ring systems where the annular nitrogen atoms are located in rings A and D and their biological activities. Besides, the route to the synthesis of the 6-substituted derivatives utilized Heck-Mizoroki catalysis which is an entirely new and a relatively unexplored process [21,22].

EXPERIMENTAL

Solvents and chemicals used were of analytical grade from Sigma-Aldrich. The reactions were monitored by TLC using Merck pre-coated TLC silica gel plates. Pure products were

obtained by recrystallization. Melting points were taken using Fisher-Johns melting point apparatus and they remain uncorrected. Compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, UV/visible spectroscopy and elemental analysis. UV-visible spectra were recorded on a JENWAY 6405 UV/VIS spectrophotometer using matched 1cm quartz cells, IR spectra on a Shimadzu FTIR-8400S Fourier transform infrared spectrophotometer (KBr Pellets), nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were determined using Varian NMR Mercury-200BB. Chemical shifts were recorded on the δ scale (neat). The elemental analyses were done on a CE440 elemental analyzer. Antimicrobial screening was done using agar-well diffusion method.

Preparation of 8-hydroxy-5-nitrosoquinoline hydrochloride (10): This compound was prepared following the procedure of Pratt and Drake [23].

To a solution of 8-hydroxyquinoline (58 g, 0.4 mol) in 200 mL of water, 75 mL of concentrated HCl and 200 g of ice was added a solution of 30 g of sodium nitrite in 100 mL of water in portions with vigorous shaking over 1 h at 0 to 4 °C. The mixture was allowed to stand overnight at 0 °C before the product was filtered off and washed with cold water. The product was air-dried giving a yield of 76 g (87 %) of the hydrochloride of 8-hydroxy-5-nitrosoquinoline (m.p. 181-183 °C, dec.) as fine yellow compound. The compound loses its brightness in time and darkens due to auto-oxidation.

Preparation of 8-hydroxy-5-nitroquinoline (11): Compound **11** was prepared as described by Petrow and Sturgeon [24].

Finely powdered 8-hydroxy-5-nitrosoquinoline (3 g, 0.017 mol) was added with vigorous stirring to a mixture of concentrated HNO_3 (9 mL) and water (6 mL) at about 17 °C. Oxides of nitrogen were evolved and the nitrosoquinoline was rapidly converted into insoluble 8-hydroxy-5-nitroquinoline nitrate. After 75 min with occasional stirring, the mixture was diluted with water (20 mL) and cooled to 0 °C. The cooled mixture was made alkaline by the addition of cold KOH solution. The red potassium salt was neutralized with acetic acid (pH = 7). Filtration was done and the product washed with water and recrystallized from aqueous ethanol to give fine yellow needles of 8-hydroxy-5-nitroquinoline; m.p. 180-182 °C (lit. 180 °C [24]; 179.5-181.5 °C [23]).

Preparation of 7-chloro-8-hydroxy-5-nitroquinoline (12): 8-Hydroxy-5-nitroquinoline (20 g, 115 mmol) suspended in water (2 L) and one equivalent of 1 M potassium hydroxide solution (107 mL) were vigorously stirred while 5 % excess sodium hypochlorite solution (143 mL) was added over 90 min. During the course of the addition, all the starting material dissolved and soon the salt of 7-chloro-8-hydroxy-5-nitroquinoline began to precipitate [18]. Stirring continued for another 2 h, before the mixture was neutralized with acetic acid (pH = 7) and stirred to permit complete conversion of the precipitate to the free quinolinol. The product was filtered, washed with water and air-dried. The crude product was recrystallized from ethyl acetate (500 mL) by continuous extraction to yield 16.9 g (86 %) of bright orange 7-chloro-8-hydroxy-5-nitroquinoline; m.p. 239-241 °C, dec. (lit. 239-240.5 °C).

Preparation of 5-amino-7-chloro-8-hydroxyquinoline (13): 7-Chloro-8-hydroxy-5-nitroquinoline (22.4 g, 0.1 mol) was ground in a mortar with 1 M KOH (110 mL) to ensure complete reduction of the insoluble potassium salt. The suspension was transferred to a 1 L three-necked flask equipped with a long magnetic stirrer with the aid of water (280 mL). The mixture was heated in a water-bath with vigorous stirring. 8 M KOH (70 mL) was added at 50 °C, the reaction mixture was treated with sodium dithionite (70 g). The mixture was then reheated and maintained at 80 °C for 10 min with rapid stirring while a stream of nitrogen was passed into the flask. After 10 min, more sodium dithionite (10 g) was added and the reaction allowed to continue 10 min longer. At the end of this period, the resulting suspension was cooled in ice under nitrogen and the precipitate was filtered off, washed with cold water containing a trace of sodium dithionite and dried rapidly in a vacuum oven to furnish 5-amino-7-chloro-8-hydroxyquinoline (17.4 g, 77 %) as a gold-yellow solid [(m.p.: 171-173 °C, dec) (lit. 172-173 °C [24]; 173-174 °C [23] dec.)].

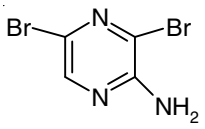
Preparation of 7-chloro-5,8-quinolinequinone (6): 5-Amino-7-chloro-8-hydroxyquinoline (17.9 g, 0.09 mol) was suspended in water (600 mL) in a 1 L flask equipped with a long magnetic stirring bar. One equivalent of 6 M sulphuric acid (18 mL) was added to dissolve the amine and vigorous stirring continued while the solution was cooled to 2 °C and the salt precipitated in a finely divided form. An ice-cold solution made up of 10 % potassium dichromate solution (103 mL) and 6 M sulphuric acid (71 mL) was then added. The mixture was stirred and cooled in an ice-bath for 15 min. The precipitated crude product was filtered on a Büchner funnel containing ice and washed with cold water and dried. The light tan coloured product was recrystallized from DMF treated with activated charcoal and it furnished fine yellow crystals of 7-chloro-5,8-quinolinequinone (12.6 g, 74.5 %); m.p.: 173-174 °C, dec. (lit. 173.5-174.5 °C [25]). UV-visible (MeOH) λ_{max} (nm): 240 (3.79), 290 (3.95) and 395 (4.17). IR (KBr, ν_{max}, cm^{-1}): 3104, 1654, 1584, 1501, 1296, 752.

Preparation of 5-amino-8-hydroxyquinoline sulphate (14): This compound was prepared from 8-hydroxy-5-nitrosoquinoline hydrochloride, whose synthesis has been described previously. Freshly prepared hydrochloride of 8-hydroxy-5-nitrosoquinoline (20 g, 0.11 mol) was dissolved in a mixture of water (80 mL) and 5 M NaOH (130 mL) placed in a three-necked flask (500 mL) equipped with a long magnetic bar. The entire mixture was warmed to 40 °C with stirring and sodium dithionite (48 g) was added portion wise. The temperature of the solution rose spontaneously to 75-80 °C and a rapid stream of nitrogen was introduced into the solution. The solution was allowed to cool slowly to about 50 °C and 6 M sulphuric acid (125 mL) was then added which led to evolution of sulphur dioxide. When the evolution of sulphur dioxide had subsided the solution was maintained under diminished pressure (pressure of the nitrogen gas reduced) with magnetic stirring until most of the dissolved gases had been removed. The mixture was cooled in an ice-bath and the resulting precipitate filtered without washing. Reddish-brown crystals of 5-amino-8-hydroxyquinoline sulphate (16.5 g, 82.5 %) was obtained, m.p.: 218-219 °C (lit. [25] 220 °C).

Equally, satisfactory results were obtained using 12 M HCl in place of 6 M H_2SO_4 which gave the dihydrochloride.

Preparation of 6,7-dibromo-5,8-quinolinequinone (7): Compound **7** was prepared by reported methods [27-29]. 5-Amino-8-hydroxyquinoline sulphate (54 g, 0.34 mol) was dissolved in 24 % hydrobromic acid (400 mL) in a 1 L three-necked flask equipped with reflux condenser, quick fit thermometer, magnetic bar and a dropping funnel. The reaction mixture was heated to 50 °C. A solution of sodium bromate (38.5 g, 0.225 mol) in water (150 mL) was added at such a rate that the temperature did not exceed 50-60 °C. After the addition, the reaction mixture was heated at that temperature with gentle stirring for 0.5 h and then cooled. The cooled mixture was poured onto ice (500 g) and further chilled in an ice-salt mixture. The resulting precipitate was filtered and recrystallized from ethanol to give yellow precipitate of 6,7-dibromo-5,8-quinolinequinone (71.3 g, 66 %); m.p.: 242-243 °C (lit. [27-29] 243-245 °C). UV-visible (MeOH) λ_{max} (nm): 265 (3.69), 290 (3.92), 345 (3.45) and 505 (2.20). IR (KBr, ν_{max}, cm^{-1}): 3093, 1686, 1541, 1289, 664, 662.

Preparation of 2-amino-3,5-dibromopyrazine: This compound was prepared following Okafor and Okoro's procedure [12,26].



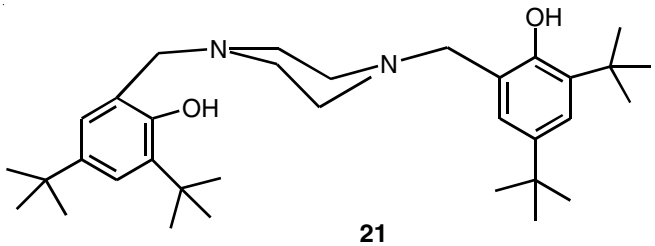
2-Aminopyrazine (9.5 g, 100 mmol) was placed in a reaction flask containing glacial acetic acid (70 mL) and warmed on a steam bath until it dissolved. Sodium acetate trihydrate (33 g, 243 mmol) was added with constant swirling. The slurry was stirred in an ice-salt bath maintained at -5 °C and bromine (16 mL) was added dropwise over a 4 h period (if the bromine addition was speeded up the reaction became turbulent and potentially hazardous).

The mixture was stirred in the ice bath for 2 h and then at room temperature for 24 h. It was then poured into ice (50 g) and neutralized with concentrated ammonia (pH 8). The crude product was collected and recrystallized from methanol (Norit) to give colourless needles of 2-amino-3,5-dibromopyrazine (16.8 g, 66 %), m.p.: 113-114 °C (lit. [13,26] 114-115 °C).

Preparation of 2-amino-5-bromopyrazine-3[4*H*]–thione (5): A mixture of 2-amino-3,5-dibromopyrazine (7.69 g, 30 mmol) and sodium hydrosulphide (13.33 g, 238 mmol) was added to methanol (60 mL) and the mixture was refluxed for 4.5 h. Methanol was then removed by distillation and the residual dark moist solid was dissolved in water (150 mL), treated with activated charcoal, boiled and filtered. The filtrate was cooled and the product filtered. Recrystallization from DMF (Norit) yielded pure 2-amino-5-bromopyrazine-3[4*H*]-thione (3.75 g, 61 %) as a yellow solid, m.p.: 209-211 °C (dec); (lit. [13] 208-210 °C). UV-visible (MeOH) λ_{max} (nm): 222 (2.46), 249 (4.16), 340 (3.89), 425 (1.61). IR (KBr, ν_{max}, cm^{-1}): 3446, 3173, 1563, 1524, 1318, 674.

Synthesis of 9-bromo-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one (19): 2-Amino-5-bromopyrazine-3[4*H*]-thione (1.03 g, 5 mmol) and anhydrous sodium carbonate (1.06 g, 10 mmol) were placed in a 100 mL three-necked flask equipped with a short magnetic stirring bar, a reflux condenser and a thermometer. A mixture of benzene (40 mL) and DMF (5 mL) was added into the flask. The mixture was refluxed on a water-bath at 70-75 °C for 45 min with stirring. 7-Chloro-5,8-quinolonequinone (0.97 g, 5 mmol) was added and the stirring continued for 7 h still maintaining the temperature of the reaction mixture at 70-75 °C. At the end of the reflux period, the resultant mixture was filtered and the solvent evaporated. Analytical sample was obtained by column chromatography using methanol and acetone (1:3) as the eluting solvent. The reddish-yellow solid compound, 9-bromo-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one weighed 1.28 g (74 %) and melted at 218-220 °C (dec.). UV-visible (MeOH) λ_{max} (nm): 285 (3.92), 345 (4.34), 435 (5.06). IR (KBr, ν_{max}, cm^{-1}): 3167, 1618, 1504, 1290, 691. ^{1}H NMR (DMSO) δ: 9.2 (3H, m), 8.2 (1H, s), 6.8 (1H, s). ^{13}C NMR (DMSO) δ: 168 (C=O), 153 (C-2), 143 (C-10), 142 (C-9) 123 (C-3), 120 (C-6), 38.7-41.2 (DMSO). Anal. calcld. (%) for $C_{13}H_5N_4BrSO$: C, 45.22; H, 1.45; N, 16.23; Br, 23.19; S, 9.28. Found (%): $C_{13}H_5N_4SOBr$: C, 45.30; H, 1.48; N, 16.30; Br, 23.20; S, 9.40.

Preparation of 1,4-*bis*(2-hydroxy-3,5-di-*tert*-butylbenzyl)piperazine ($C_{34}H_{54}N_2O_2$) (21): The bulky ligand, **21** was prepared following Balakrishna *et al*. method [30]. A mixture of piperazine (2.2 g, 25.54 mmol) and 40 % aqueous formaldehyde solution (5.30 mL, 75.36 mmol) was dissolved in methanol (40 mL) and heated to reflux for 2 h to get a clear solution. To the cooled solution was added 2,4-di-*tert*-butylphenol (10.3 g, 50.41 mmol) in methanol (60 mL). The resulting solution was refluxed for further 12 h at 60 °C. The reaction mixture was cooled to room temperature and filtered off to get compound **21** as colourless crystalline compound. Yield: 64 % (8.35 g, 15.98 mmol); m.p.: > 250 °C (dec.) (lit. [30] >250 °C (dec.).



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General procedure for Heck-Mizoroki reaction [30]: Under nitrogen atmosphere, piperazine ligand (0.005 g, 0.01 mmol), dppb/$PdCl_2$ (0.006 g, 0.01 mmol) and methanol (10 mL) were placed in a two-necked 100 mL round bottom flask equipped with a magnetic stirrer. After stirring for 5 min, an intimate mixture of 9-bromo-1,8,11-triaza-5*H*-benzo[a]-phenothiazin-5-one (1.04 g, 3 mmol), aryl halide (1 mmol) and K_2CO_3 (0.193 g, 1.4 mmol) was added to the reaction flask. 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. It was further recrystallized from aqueous ethanol.

9-Bromo-6-(4-nitrophenyl)-1,8,11-triaza-5*H*-benzo[a]-phenothiazin-5-one (20a): Following the general procedure, a

mixture of 1,4-*bis*(2-hydroxy-3,5-di-*tert*-butylbenzyl)piperazine (0.005 g, 0.01 mmol) dppb/$PdCl_2$ (0.006 g, 0.01 mmol), 9-bromo-1,8,11-triaza-5*H*-benza[a]phenothiazin-5-one (1.04 g, 3 mmol), 4-iodonitrobenzene (0.249 g, 1 mmol), K_2CO_3 (0.193 g, 1.4 mmol) and methanol (10 mL) was refluxed for 4 h. After removal of the solvent under reduced pressure, crushed ice (5 g) was added which precipitated out the solid product. It was further recrystallized from aqueous ethanol to yield a yellow solid (1.20 g, 85.7 %), m.p.: (114-116 °C, dec.). UV-visible (MeOH) λ_{max} (nm): 245.2 (4.52), 334.8 (4.25), 419 (4.13). IR (KBr, ν_{max}, cm^{-1}) 3148, 1618, 1504, 1447, 1290, 692. ^{1}H NMR (DMSO) δ: 9.4 (1H, d), 8.58 (1H, d), 8.32 (4H, m), 7.72 (1H, d), 7.0 (1H, s), 6.75 (2H, s). ^{13}C NMR (DMSO) δ: 188 (C=O), 153 (C-2), 146 (C-10), 143 [(C-6-(1)], 136.6 (C-9), 134 [(C-4), 127 [(C-6-(2)], 123 [(C-9-(2)], 120 [(C-6-(3)], 38.7-71.4 (DMSO). Anal. calcld. (%) for $C_{19}H_8N_5SO_3Br$: C, 48.93; H, 1.72; N, 15.02; S, 6.87; Br, 17.17. Found (%): C, 49.00; H, 1.80; N, 15.00; S, 6.68; Br; 17.20.

9-Bromo-6-(4-hydroxyphenyl)-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one (20b): Following the general procedure, a mixture of 1,4-*bis*(2-hydroxy-3,5-di-*tert*-butylbenzyl)piperazine (0.005 g, 0.01 mmol) dppb/$PdCl_2$ (0.006 g, 0.01 mmol), 9-bromo-1,8,11-triaza-5*H*-benza[a]phenothiazin-5-one (1.04 g, 3 mmol), 4-iodophenol (0.220 g, 1 mmol), K_2CO_3 (0.193 g, 1.4 mmol) and methanol (10 mL) was refluxed for 4 h. After removal of the solvent under reduced pressure, crushed ice (5 g) was added which precipitated out the solid product. It was further recrystallized from aqueous ethanol to yield a dark brown solid (1.11 g, 84.7 %), m.p.: (> 300 °C, dec). UV-visible (MeOH) λ_{max} (nm): 221.4 (4.21), 249.4 (4.31), 341 (4.01), 360 (3.78), 444.8 (3.87). IR (KBr, ν_{max}, cm^{-1}): 3452, 3153, 1623, 1505, 1298, 695. ^{1}H NMR (DMSO) δ: 9.3 (1H, d), 8.6 (1H, s), 8.1 (1H, d), 7.5 (2H, d), 6.8 (2H, d), 5.5 (O-H). ^{13}C NMR (DMSO) δ: 190 (C=O), 153 (C-2), 143 (C-10), 131 (Cβ to C-4), 124 (C-3), 116 [(C-6-(3 &5)], 38.3-41.2 (DMSO). Anal. calcld. (%) for $C_{19}H_9N_4SO_2Br$: C, 52.17; H, 2.06; N, 12.81; S, 7.32; Br, 18.31. Found (%): C, 52.00; H, 2.04; N, 12.90; S, 7.36; Br, 18.20.

9-Bromo-6-(4-anilino)-1,8,11-triaza-5*H*-benzo[a]-phenothiazin-5-one (20c): Following the general procedure, a mixture of 1,4-*bis*(2-hydroxy-3,5-di-*tert*-butylbenzyl)-piperazine (0.005 g, 0.01 mmol) dppb/$PdCl_2$ (0.006 g, 0.01 mmol), 9-bromo-1,8,11-triaza-5*H*-benza[a]phenothiazin-5-one (1.04 g, 3 mmol), 4-bromoaniline (0.172 g, 1 mmol), K_2CO_3 (0.193 g, 1.4 mmol) and methanol (10 mL) was refluxed for 4 h. After removal of the solvent under reduced pressure, crushed ice (5 g) was added which precipitated out the solid product. It was further recrystallized from aqueous ethanol to yield a yellow solid (0.89 g, 68 %), m.p.: (138-140 °C, dec.). UV-visible (MeOH) λ_{max} (nm): 221.4 (4.26), 247.6 (4.17), 327.8 (3.90), 360 (3.64), 441.4 (3.27), 498.4 (2.54), 656.2 (2.42). IR (KBr, ν_{max}, cm^{-1}): 3475, 3145, 1628, 1500, 1283, 692. ^{1}H NMR (DMSO) δ: 9.3 (1H, d), 8.6 (1H, s), 8.2 (1H, s), 7.52 (1H, s), 7.0 (2H, s), 6.5 (2H, d), 3.96 (2H, s,-NH_2). ^{13}C NMR (DMSO) δ: 172 (C=O), 153 (C-2), 147 (C-10), 145 (C-NH_2), 134 (C-4 & C-9), 132 (C β to C-4), 131 (C-6), 124 (C-3), 118 [(C-6-(3 &5)], 24.1-63.6 (DMSO). Anal. calcld. (%) for $C_{19}H_{10}N_5SOBr$: C, 52.29; H, 2.29; N, 16.05; S, 7.3; Br, 18.35. Found (%): $C_{19}H_{10}N_5SOBr$: C, 52.30; H, 2.10; N, 16.13; S, 7,34; Br, 18.40.

9-Bromo-6-(2-carboxyphenyl)-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one (20d): Following the general procedure, a mixture of 1,4-*bis*(2-hydroxy-3,5-di-*tert*-butyl-benzyl)piperazine (0.005 g, 0.01 mmol) dppb/$PdCl_2$ (0.006 g, 0.01 mmol), 9-bromo-1,8,11-triaza-5*H*-benza[a]phenothiazin-5-one (1.04 g, 3 mmol), 2-iodobenzoic acid (0.248 g, 1 mmol), K_2CO_3 (0.193 g, 1.4 mmol) and methanol (10 mL) was refluxed for 4 h. After removal of the solvent under reduced pressure, crushed ice (5 g) was added which precipitated out the solid product. It was further recrystallized from aqueous ethanol to give a reddish brown solid (1.17 g, 84 %), m.p.: (118-120 °C, dec.). UV-visible (MeOH) λ_{max} (nm): 228.4 (4.19), 246.6 (4.46), 339.4 (4.12), 443.8 (3.97). IR (KBr, ν_{max}, cm^{-1}): 3455, 3168, 1621, 1512, 1282, 687. ^{1}H NMR (DMSO) δ: 9.6 (1H, O-H), 8.6 (1H, s), 8.2 (1H, d), 7.1 (2H, d), 6.6 (2H, s). ^{13}C NMR (DMSO) δ: 168 (C=O), 153 (C-2), 146 (C-10), 134 [(C-4 & C-6-(5)], 131 [(C-6-(3)], 123 [(C-3 & C-6-(2)], 38.5-54.5 (DMSO). Anal. calcld. (%) for $C_{20}H_9N_4SO_3Br$: C, 51.61; H, 1.94; N, 12.04; S, 6.88; Br, 17.20. Found (%): $C_{20}H_9N_4SO_3Br$: C, 51.58; H, 1.95; N, 12.10; S, 6.79; Br, 17.25.

Evaluation of synthesized angular phenothiazinones for antibacterial activity: The assay was conducted using agar-well diffusion method [31]. A 20 mg/mL concentration of each compound was constituted by dissolving 0.04 g of each in 2 mL dimethyl sulfoxide. A single colony of each test isolate was suspended in 2 mL of sterile nutrient broth. The suspension of each isolate was standardized by adjusting to correspond to 0.5 McFarland turbidity standards corresponding to approximately 10^8 cfu/mL and used to inocu-late the surface of the iso-sensitest nutrient agar and the excess fluid drained into discard pot containing disinfectant. The inoculated agar surface was allowed to dry and the plates appropriately labelled. Using a cork borer of 6 mm in diameter, wells were bored in the inoculated iso-sensitest nutrient agar. With a micropipette, 50 µL of each test compound solution was deli-vered into each well. The plates were left on the bench for 30 min to allow the compound to diffuse into the agar. Thereafter, the plates were incubated at 37 °C for 24 h. After incubation, the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with metre rule to the nearest whole millimetre.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of synthesized angular triazaphenothiazines: This was carried out using agar dilution following the procedure outlined by Chemical Laboratory Standards Institute (CLSI) [32]. Sterile test tubes were arranged on a test tube rack and 1 mL of DMSO was dispensed into each of them. From the stock compound solutions, 1 mL was transferred into the first test tube and two-fold serial dilution of each compound solution was carried out and the resultant concentration in the test tubes were 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 (mg/mL) (*i.e.*, graded concentrations of the compounds). A single colony of each test isolate was suspended in 2 mL of sterile nutrient broth. The suspension of each isolate was standardized and used to inoculate the

surface of the nutrient agar and the excess fluid drained into discard pot containing disinfectant. The inoculated agar surface was allowed to dry and the plates appropriately labelled. Using a cork borer of 6 mm in diameter, wells were bored in the inoculated nutrient agar. With a micropipette, 50 µL of each test compound solution was delivered into each well. The plates were left on the bench for 30 min to allow the compound to diffuse into the agar. Thereafter, the plates were incubated at 37 °C for 24 h. After incubation, the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with metre rule to the nearest whole millimetre.

RESULTS AND DISCUSSION

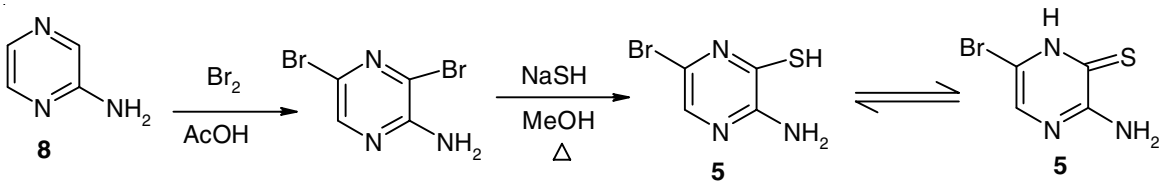
Intermediate **5** was obtained in good yield according to literature [13,26] as colourless needles by treating 2-amino-pyrazine (**8**), with bromine in glacial acetic acid at -5 to 0 °C followed by thiation using sodium hydrosulphide in methanol and recrystallization using DMF (Norit) (**Scheme-I**).

Compound **6** was prepared by multi-step conversion [23-25] of 8-hydroxyquinoline (**9**) (**Scheme-II**). In reaction sequence, nitrosation of compound **9** yielded 8-hydroxy-5-nitroso-quinoline hydrochloride (**10**) which was oxidized with nitric acid to give 8-hydroxy-5-nitroquinoline (**11**). Haloge-nation of compound **11** with sodium hypochlorite produced, 7-chloro-8-hydroxy-5-nitroquinoline (**12**) which on reduction with sodium dithionite under inert atmosphere gave 5-amino-7-chloro-8-hydroxyquinoline (**13**). Oxidation of compound **13** using acidified potassium dichromate and recystallization from DMF (Norit) furnished fine yellow crystals of 7-chloro-5, 8-quinolinequinone, **6**, in good yield.

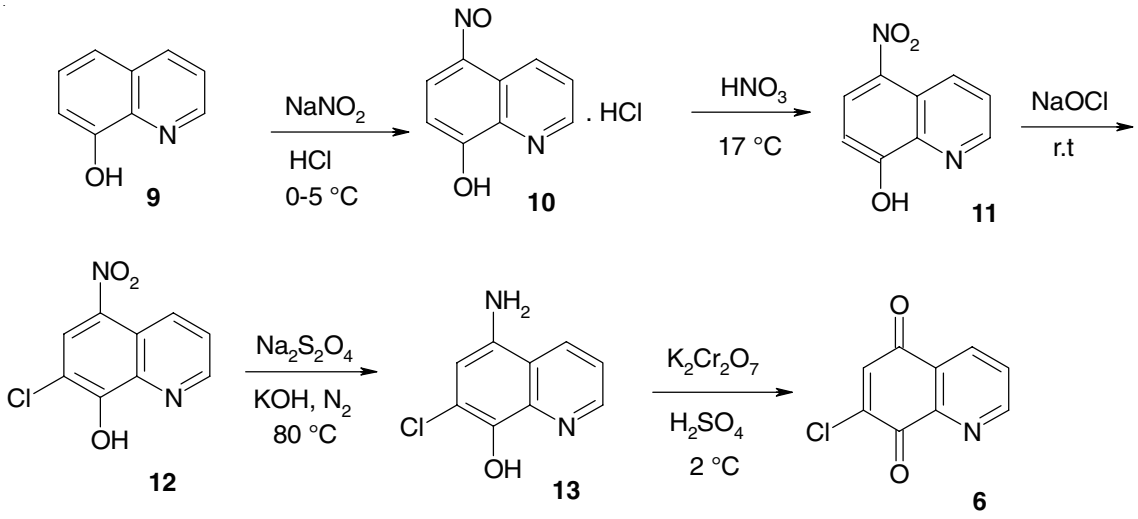
Under similar conditions, the coupling of compounds **5** and 7-chloro-5,8-quinolinequinone (**6**), in the presence of anhydrous sodium carbonate gave 9-bromo, 1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one (**19**) (**Scheme-III**).

The molecular formula, $C_{13}H_5N_4OSBr$ and assigned structure **19** were established based on microanalysis, UV, IR and NMR spectroscopy of compound **19**. The UV-visible (MeOH) showed signals at λ_{max} 285 (ε = 3.92), 345 (4.34) and 435 (5.06) nm. The IR showed signals at 3167 (C–H, aromatic), 1618 (C=O), 1504 (C=C, aromatic rings), 1290 (C–N stretch) and 691 (C–Br stretch) cm^{-1}. The ^1H NMR spectra showed signals at δ 9.2 (3H, arom), 8.2(s, 10-H), 6.8 (s, 6-H). The ^{13}C NMR spectra showed signals at δ 168 (C=O), 153 (C-2), 143 (C-10), 142 (C-9), 123 (C-3) and 120 (C-6).

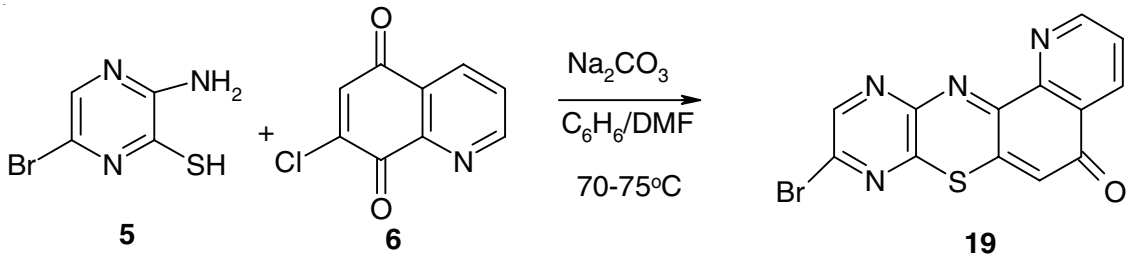
In a similar development, base mediated reaction of the non-linear triazaphenothiazinone with aryl iodo-/bromo-



Scheme-I: Synthesis of intermediate **5**

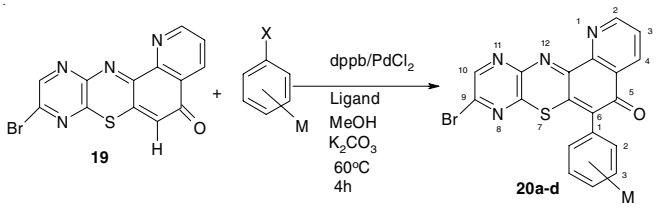


Scheme-II: Synthesis of compound **6**



Scheme-III: Synthesis of compound **19**

derivatives under the catalytic influence of palladium(II) chloride (Heck-Mizoroki protocol) furnished the 6-substituted derivatives (**20a-d**), leading to C–C bond elongation (**Scheme-IV**). These reactions were successful because compound **19** has olefinic hydrogen at position 6 which is a basic condition for Heck-Mizoroki coupling reactions to take place.



Scheme-IV: Synthesis of compounds **20a-d**

From microanalysis and spectroscopic data, the compounds were identified as 9-bromo-6-(4-nitrophenyl)-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one (**20a**), 9-bromo-6-(4-hydroxyphenyl)-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one (**20b**), 9-bromo-6-(4-anilino)-1,8,11-triaza-5*H*-benzo[a]phenothiazin-5-one (**20c**) and 9-bromo-6-(2-carboxyphenyl)-1,8,11-traiza-5*H*-benzo[a]phenothiazin-5-one (**20d**).

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 microorganisms tested were *E. coli* (strains used were coded Eco 3, Eco 4 and Eco 12), *Staphylococcus pseudointermedius* and *Staphylococcus scuiri* (G101 and G84 respectively), *Bacillus spp*. and *Pseudomonas aeruginosa*. The assay was conducted using agar-well diffusion method [31] in which a 20 mg/mL concentration of each compound was constituted by dissolving 0.04 g of each compound in 2 mL of dimethyl sulfoxide. A single colony of each test isolate was suspended in 2 mL of sterile nutrients broth. Inoculation was done followed by incu-bation at 37 °C for 24 h after which the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with metre rule to the nearest whole millimetre. The result of the preliminary screening of the novel compounds tested against some Gram-negative and Gram-positive bacteria are shown in Table-1.

Table-1 shows the inhibition zone diameter (IZD) produced by each compound against each bacterial organism at 20 mg/mL concentration. The IZD ranged between 10 and 37 mm in diameter. Higher the IZD, then sensitivity would be higher. The compounds showed significant activity against the test organisms except *Pseudomonas aeruginosa* which was only sensitive to compounds **10**, **20c** and **20d** with IZD of 15 mm. G101 (*Staphylococcus*) was highly sensitive to all the compounds with IZD of 35 and 37 mm. Eco 4 and Eco 12 were sensitive to all the compounds with IZD ranging between 15 and 27 mm.

Minimum inhibitory concentration (MIC) of the compounds was also determined by the agar-well diffusion method (already described above) with various concentrations ranging between 20 mg/mL and 0.15625 mg/mL. Minimum inhibitory concentration (MIC) is the minimal concentration of drug that inhibits visible growth after overnight incubation [33]. The lowest concentration of each compound that produced no zone was regarded as MIC. Hence, the essence of MIC is to determine the least concentration of drug that can inhibit the growth of the micro-organism. The graded concentrations (mg/mL) of the compounds numbered **1-7** are 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 respectively. Table-2 represents the inhibition zone diameter in millimetre.

Table-3 shows the result of minimum inhibitory concentration (MIC) of the synthesized compounds compared with those of ampicillin and gentamycin (standard drugs) against Eco 3, G84 and *Baccilus* spp.

The MIC for the novel compounds against Eco 3 ranged between 0.625 and 5 mg/mL. The MIC for the test compounds against G84 were 1.25 mg/mL and 2.5 mg/mL; for *Bacillus spp*, the MIC ranges between 1.25 mg/mL and 0.15625 mg/mL. Ampicillin and gentamycin are standard antibiotics were used as positive control because they are standard drugs used for treating such strains of microorganisms but DMSO which was the solvent used served as the negative control because it had no effect against the isolates as it showed no zone of inhibition.

TABLE-1
ANTIBACTERIAL SUSCEPTIBILITY TEST (TESTED WITH 20 mg/mL OF EACH COMPOUND)

Compound	G101	G84	Eco 3	Eco 4	Eco 12	*Bacillus* spp.	*Pseudomonas aeruginosa*
10	37	27	29	24	27	34	15
20a	37	24	26	18	26	33	0
20b	35	21	30	20	15	35	0
20c	37	28	27	23	27	36	15
20d	35	28	30	22	26	35	15

TABLE-2
INHIBITION ZONE DIAMETER (IZD) OF THE COMPOUNDS AT DIFFERENT CONCENTRATIONS

Compd.	Eco 3						G84							*Bacillus spp*						
	1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	6	7
10	22	19	15	0	–	–	15	12	11	0	–	–	–	32	30	26	15	0	–	–
20a	26	24	20	15	11	0	20	15	13	0	–	–	–	33	30	27	25	21	17	13
20b	22	10	0	–	–	–	14	12	12	0	–	–	–	30	30	28	20	14	0	–
20c	25	15	0	–	–	–	20	15	14	12	0	–	–	32	32	30	30	25	20	16
20d	22	20	18	10	0	–	17	14	11	0	–	–	–	35	32	30	24	18	13	0

TABLE-3
MINIMUM INHIBITORY CONCENTRATION (mg/mL) OF THE SYNTHESIZED COMPOUNDS AND SOME STANDARD DRUGS

Compound/Drug	Eco3	G84	*Bacillus* spp.
10	2.500	2.50	1.25000
20a	0.625	2.50	0.15625
20b	5.000	2.50	0.62500
20c	5.000	1.25	0.15625
20d	1.250	2.50	0.31250
Ampicillin	100.000	2.50	1.25000
Gentamycin	6.250	2.50	0.15625

The MIC of the standard drugs ranges between 100 and 0.15625 mg/mL. Table-3 suggested that synthesized phenothiazine derivatives were active against Eco 3 even at very low concentrations; the MIC for compound **20a**, being as low as 0.625 mg/mL whereas ampicillin has its MIC against Eco 3 as 100 mg/mL indicating that Eco 3 is highly resistant to ampicillin. The MIC of gentamycin against Eco 3 is 6.25 mg/mL which is still higher than the MIC values for most of the synthesized phenothiazine compounds especially compounds **10**, **20a** and **20d** whose MIC values ranged from 0.625 to 2.5 mg/mL.

The MIC value of gentamycin against *Bacillus* spp. is 0.1562 mg/mL and this is the same for compounds **20a** and **20c**. These results show that the synthesized phenothiazine compounds are highly biologically active.

Conclusion

It is shown that base mediated coupling reactions under anhydrous conditions and using benzene/DMF as solvent offer excellent routes to the synthesis of angular (non-linear) phenothiazines. Also, utility of Heck-Mizoroki palladium catalyzed protocol in the synthesis of derivatives of these heterocycles proved a reliable and facile route to the formation of C–C bond as it requires shorter time than the mineral acid catalyzed reactions. The study has opened up a new route for possible synthesis of other isomeric non-linear polycyclic triazaphenothiazine compounds. These novel compounds have also shown high level of biological activity which should not be ignored.

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