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Palladium Catalyzed Anilino Analogues of Angular Penta-Cyclic Phenothiazines: Novel Human Cholinesterases Inhibitors

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Abstract The synthesis of novel 8-chloro12H- 5,14- di hydro- quinoxalino [2,3, a] – penta cyclic phenothiazine (16) and its anilino derivatives (18 a-h) are reported. The preparation followed the water-mediated catalyst pre-activation method as reported by Buchwald and co-workers. Structures were established by analytical and spectral data. The compounds (18 a-h) were evaluated for inhibitory potency against Acetylcholineesterase (AChE) and Butyrylchlolinesterase (BChE). The tested pentacyclic phenothiazines showed IC_{50} values for both AChE and BChE in the range of 1.26 nM to 84.69 nM. Compound 18c exhibited the most persuasive inhibitor of AChE, having an IC_{50} value of 1.26 nM, while (18h) gave the least potent inhibitor at IC_{50} = 52.11 nM. The inhibitory values of the tested compounds for repression of BChE showed concentration range of 4.38-84.69 nM. The presence of substituent groups in the aromatic ring was noted to have influenced the inhibitory potency of the eight phenothiazine systems and as such demonstrating Structure Activity Relationship (SAR among the compounds. The results were compared with Rivastigmine and Galantamine, which are well known human cholinesterase inhibitors. All the tested phenothiazine systems showed excellent inhibition of AChE than Rivastigmine. Galanthamine was more potent on AChE than the tested compounds, except (18 a, 18c, 18f and 18g). Only five compounds exhibited inhibition of BChE than Galantamine.

Keywords 8-anilino-12H-5,14-dihydroquinoxalino-[2,3-a] penta cyclic phenothiazine, acetylcholinesterase, butyryl cholinesterase, inhibition, substituent groups

Introduction

Many transition metals are effective promoters and catalysts for cross-coupling reactions. These include copper, palladium, nickel and iron [1-2], Palladium catalyst has been chosen from the aforementioned in this study because of its high selectivity. It is a good organic partner, particularly with other reaction species such as ligands, solvents, bases etc [3]. The principle of palladium catalyzed cross-couplings is that two molecules are assembled on the metal via the formation of metal-carbon bonds, which is followed by the coupling of molecules to one another, leading to the formation of a new carbon-carbon bond [3].

Palladium- catalysed amination of hetero-aryl halides has rapidly emerged as a valuable tool in the synthesis of pharmaceuticals and natural products.

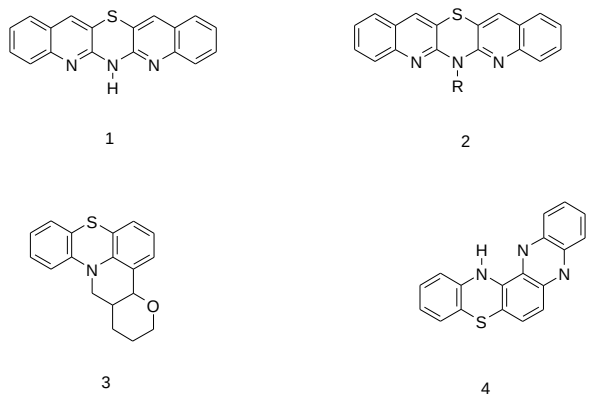


Figure 1: Pentacyclic phenothiazine systems of types 1, 2, 3 & 4 [4-7]

They are still grossly under studied in spite of their known neuro pharmacologigal activity.

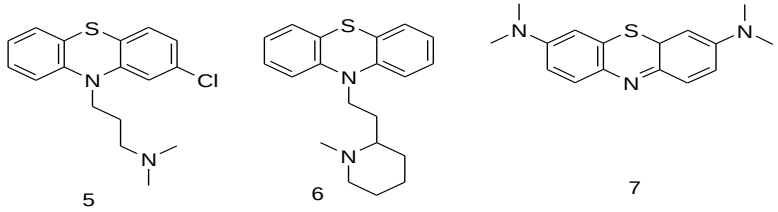


Figure 2: Other phenothiazine systems

Various derivatives of phenothiazine which are inhibitors of human cholinesterase of types (5): Chlorpromazine; (6): Thioridazine and (7): MTC(3) have been reported for their anti-Alzheimer properties [8-10].

Alzheimer is a brain disease that causes problems with memory, thinking and behavior. It gets worse over time. Its first symptom is forgetfulness. It is the most common form of dementia which is associated with loss of memory [11]. It is a progression brain disease that destroys cognitive function memory, thinking and reasoning. Victims of this disease are unable to carryout simple daily responsibilities. The signs and symptoms of alzheimer are in varying degrees and stages. In the first stage, the individual does not experience any symptoms. In stage two, very mild impairment will be noticed. While in stage three, some deficiencies will begin to show up. These include difficultly in planning, remembering names of close friends and retention of matters is gradually lost [12].

Acetyl cholinesterase (AChE) is the main enzyme responsible for the hydrolysis of Acetylcholine (ACh), while Butyryl cholinesterase (BChE) plays a supportive role in the process [10, 13].

The inhibition of AChE causes an increase in the concentration of Ach, leading to the alleviation of Alzheimer disease [10, 13].

Phenothiazine compounds have been reported to possess anti-Alzheimer's properties due to their ability to inhibit acetyl and butyrychohinesterases [10, 14-15] and neuro protective activity [16-17].

The dihydroquinoxalino pentacyclic phenothiazines of types (**18a-h)** to the best of our knowledge have not been documented.

Previous reports showed clinical benefits of cholinesterases inhibition with rivastigmine in Alzheimer disease. Unlike galantamine, that selectively inhibits AChE, rivastigmine has proven to inhibit both AChE and BChE [18]. Hence it was chosen as BACB.

In this paper we have synthesized new pentacyclic phenothiazines (**18a-h)** with excellent yield, under mild conditions. Herein, we have also reported their robust cholinesterases inhibitory behavior and the Structure Activity Relationship (SAR) of the compounds.

Results and Discussion

8-chlorophenothiazine **(9)** was synthesized by fusing 3-chlorodiphenyl amine (**8**) with excess elemental sulphur, heated in glycerol oil bath. This was followed by adding elemental iodine while heating. The excess sulphur residue

was removed by heating the mixture after the addition of benzene and filtered hot. The filtrate was recovered on heating to dryness. The chloro phenothiazine **(9)** was nitrated with mixed acid at 50 ^{o}C while avoiding polynitration to afford 8-chloro-1-nitro phenothiazine (**10)**.

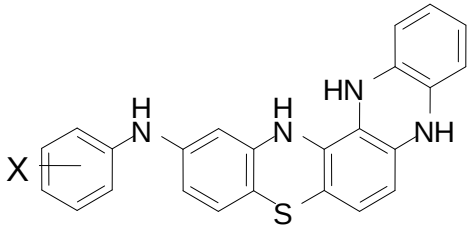
Reduction of (**10)** with iron in dilute hydrochloric acid while heating gave 8-chloro-1-aminophenothiazine dihydrochloride **(11).**

The amino group of this compound was subsequently protected using acetic anhydride to afford **(12).** Nitrating compound **(12**) in mixed acid yielded two isomeric compounds: (**13**) and (**14)**. Reduction of (**13)** using iron in dilute hydrochloric acid furnished 8-chloro-1,2-diaminophenothiazine trihydrochloride **(15),** which when added to catecol and refluxed with ethanol gave the novel pentacyclic product: 8-Chloro-12H-5,14-dihydroquinoxalino [2,3-a] pentacyclic phenothiazine (**16):** (scheme 1).

The structural assignment of the synthesized compounds was based on the spectral data. The IR spectrum of compound **(15)** indicated clearly that the Isomeric compound (**14)** was not used in the synthesis of the final product **(16)**. This was further established by the disappearance of C-O absorption (1200 Cm^{-1}) in the spectrum of (**16**).

The pentacyclic product (**16)** exhibited a UV maximum at 320 nm characteristic of phenothiazine systems. The angular pentacyclic system was further identified by the information from the ^{1}H and ^{13}CNMR spectra with the resonance assigned to hydrogen and carbon.

Palladium catalyzed amination of compound **(16)** afforded position -8 substituted derivatives (**18a-h).**



18(a-h)

X= 2-CH_3 (18a); X= 2-NO_2 (18b); X= 4-COOEt (18c); X= 3-NO_2 (18d); X= 4-CN (18e); X= 4-COMe (18f); X= 4-CHO (18g); X= 4-NO_2 (18h)

Figure 3: Structure of Angular Penta-Cyclic Phenothiazines

The structural assignment of these anilino derivatives was also based on the spectral data.

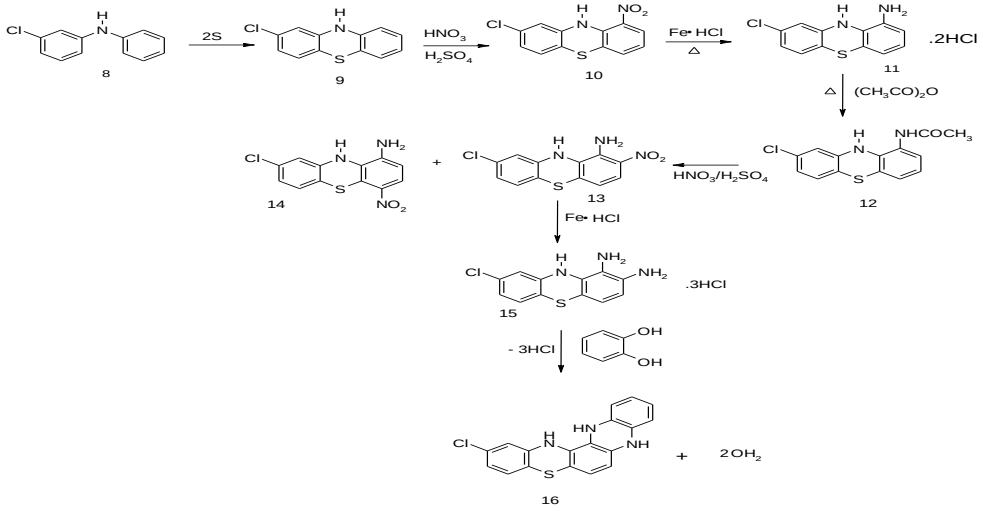
These compounds (**18a-h**) were evaluated for inhibitory potency against Acetylcholinesterase (AChE) and Butylrylcholinesterase (BChE). The tested pentacyclic phenothiazines showed IC_{50} values for both AChE and BChE in the range of 1.26 nm to 84.69 nm. The inhibitory values (IC_{50}) of all the tested compounds for repression of BChE showed concentration range of 4.38-84.69 nM. Compound (**18c)** with inhibitory concentration (IC_{50} = 4.38nM) was the most effective inhibitor of BChE, while Compound **(18h**) IC_{50} value of 84.69 nM was recorded as the least inhibitor in BChE.

On the other hand, IC_{50} values for AChE are in contrast with those of BChE. The repression values for AChE are in the range of 1.26-52.11 nM. Compound **(18c)** exhibited the most persuasive inhibition of AChE, having an IC_{50} value of 1.26 nM, while compound (**18h)** exhibited the least potent inhibition at IC_{50} = 52.11nM.

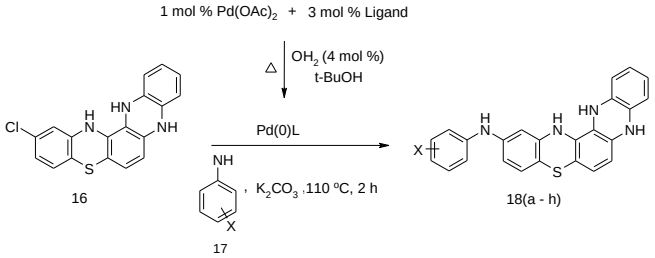
It was observed that the presence of substituent groups at position-8 on the aromatic ring of phenothiazine compounds **(18a-h)** influenced the inhibitory potency of the eight phenothiazine systems.. These structural effects on inhibition of the two human cholinesterases were also evaluated. Compounds **18b, 18d, 18h, 18e** and **18g** displayed structural effect on inhibition of AChE at IC_{50} values in the range of 21.54 to 52.11nM.

In contrast, IC_{50} values for BCHE are in the range 22.72 – 84.69 nM. These high pronouncements of IC_{50} inhibitory concentration of the two human cholinesterases were probably due to the moderate electron-withdrawing effect of the substituent groups, consequently deactivating the ring and as such the inhibitory reactions proceeded much slower in the compounds bearing these groups.

Similar pronouncements were also noted for compounds **(18a)** and **(18c**). The inhibitory concentrations (IC_{50}) of AChE and BChE by compound **18a** was recorded at 14.77 nM and 17.89 nM respectively. Compound **(18c**) IC_{50} values for AChE was noted at 1.26 nM, while its IC_{50} inhibition concentration for BChE was at 4.38 nM. The two IC_{50} values from compound **18c** for both enzymes (AChE, BChE) remain the least inhibitory concentration in the study. This confirmes the fact that variations in one part of a molecule can affect the chemistry and properties of another part of the same molecule (Robert, C. Nenman). The results are as shown in **Table 1.**



Scheme 1: Synthetic routes for all the compounds



Scheme 2: Palladium catalyzed amination of 8-chloro-12H-5,14-dihydroquinoxalino[2,3-a]pentacyclicphenothiazine

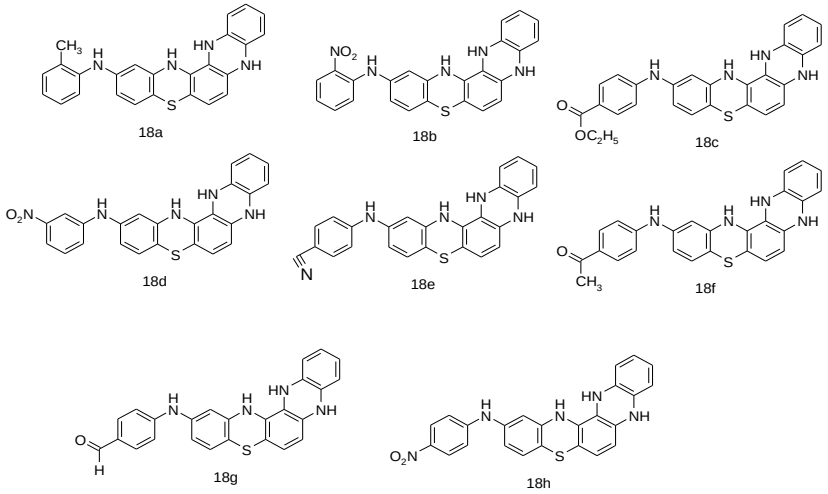


Figure 4: Structures of synthesized compounds

Table1: The Half Maximal inhibitory Concentration (IC_{50})-values for Acetylcolinesterse and Butyrylcholinesterase

Phenothiazine	IC_{50} (ACHE) Nm	IC_{50} (BCHE) nM
18a	14.77±1.21	17.89±0.16
18b	41.23±1.39	74.44±1.37
18c	1.26±1.84	4.38±1.41
18d	35.49± 2.36	38.13±1.83
18e	29.14±3.11	30.60±2.61
18f	24.83±0.34	28.47±1.36
18g	21.54±1.84	22.72±2.29
18h	52.11± 2.41	84.69±1.88
Rivastigmine	89.18±1.36	84.90±15
Galantamine	25.13±4.12	36.74±18

The inhibition results were compared with Rivastigmine and Galantamine, two human Cholinesterase inhibitors. All the tested phenothiazine systems showed excellent inhibition of AChE than Rivastigmine while Galantamine was more potent on AChE than compounds **18b, 18e** and **18h**. The remaining five phenothiazines (**18a, 18c, 18e, 18f,** and **18g**) showed inhibition of BChE than Galanthamine, while all the compounds, except **18h** exhibited inhibition of BChE than Galanthamine.

Determination of Cholinesterase Inhibition

The method of Aykul and Erik, [19]; Davidson *et al* [20]; Copeland [21-23]; Darvesh *et.al,* [24-26]; Debord *et.al,* [8]; Kratky *et.al,* [27] and Stojak *et.al,* [28] were adopted and modified for the evaluation of Cholinesterase activity. The IC_{50} values were determined using a high and constant concentration of the Phenothiazine compounds **(18a-h),** which enabled the cholinesterase enzymes react at an appreciable rate.

The inhibitory potency of the eight pentacyclic phenothiazines was evaluated for Acetycholinesterase (AChE) and Butyrylcholinesterase (BChE). The concentration range used for each phenothiazine compound was from 10-1000 nM. The experiment was carried out in three replicates. The various experiments were plotted as viability (V) versus concentration (C) which gave a sigmoidal Curve. The midpoint of this sigmoidal shape gave the viability value of 0.5; corresponding to 50 % inhibition (IC_{50}) as indicated in Table 1. The inhibitory potency results were compared with Rivarstigmine and Galantamine.

Materials and Methods

All chemicals used complied with International Standards on Health and Safety as approved for commercial use by OECD (organization of Economic Cooperation and Development), UNEP (United Nation Environmental programmes). This provides the environmental credentials of the chemicals.

The chemicals were obtained from different sources (Lavans, Aldrich, Merck) and used without further purification. Acetyl cholinesterase (AChE) and Butyrylcholesterase (BChE) from *Electropherus electricus* and horse serum respectively were specifically obtained from Adrich, USA.

The melting points (M.P) were determined on a SMP3 melting point apparatus and were reported in ^{O}C on Scharian Silica gel 60 (70-230 mesh). Elemental analysis was performed using a Perking –Elemer 2400 CHN analyzer. The infrared (IR) spectra were recorded in cm^{-1} on a Bulk Scientific 500 spectrophotometer. The ^{1}H NMR and ^{13}C NMR were recorded on a Varian Germini 2000 spectrophotometer operating 200 and 50 MHz respectively.

Chemical shifts were recorded as ∂ values in PPM referenced to the solvent. HPLC separations were performed on a Bulk Scientific 500 apparatus using a reverse phase Lichrospher 100 RP- 18(5m) column at room temperature (eluent: methanol/water 8:2, V/V.

8-Chlorophenothiazine (9)

A mixture of the corresponding 3-chlorodiphenylamine **8,** (13g, 3.0 mol) and sulphur (1.2g, 0.834 mol) was heated in a glycerol oil bath to 195^{o}C. After cooling to 100^{o}C, elemental iodine was added while heating continued. The

separation of hydrogen sulphide was observed at 170^{o}C and was decomposed by leading in 5% aqueous sodium hydroxide solution. The mixture was heated to 185^{o}C and held at this temperature for 45 min: The bath was cooled to 50^{o}C and was diluted with benzene (100 ml). This was filtered hot in a vacuum pump to remove the excess sulphur. The yellowish filtrate was concentrated using a rotary evaporative. The product was dried and purified by column chromatography. Yield 13:11g (83:20%). M.p.`187-189^{o}C. IR (Vmax/Cm-^{1}): 2999 (NH), 2859-2861 (C-H aromatic), 717(C-H bending), 1197-1211 (C-H in-plane) and 1300-1411 (C-N arom). 1400-1000 (C-H strength) UV: 211 nm. ^{1}H NMR (200 MH$_{z}$, DMSO): 7.0 6 (s .7H), 6.99-6.92 (m 1H, *J* = 7.6, 1.4), 6.98 – 6.91 (m, 9H), 11.48 (s, NH proton). ^{13}C NMR (50 MHz, DMSo): 140.7 (C.a rom-ring) 120.8 (CNH), 110.2, 113.3, 121.4, 122.6, 125.2, 139.6, 103.2, 118.9, 124.9, 130.6 (CH and C). Anal. Cal. for $C_{12}H_8NSCl$: C, 61.62; H, 3.42; N, 5.99;S, 13.74; Cl, 15.20%. Found: C, 6.62; H, 3.41; N, 5.97; S, 13.73; Cl, 15.21%.

8- Chloro-1-nitro phenothiazine (10)

Concentrated nitric acid (10ml, 0.5mol) was placed in 200ml round bottom flask, while concentrated sulphuric acid (10ml, 0.5mol) was added to it portion wise over 30min with efficient stirring at room temperature, compound **(9)** (15g, 0.07mol) was added. The mixture was refluxed in a water bath while the temperature was held at 50^{o}C for 40 min. The product was washed with 500ml cold water and filtered with suction on a Buchner funnel, dried and purified by column chromatography. Yield 23.42g (96%). M.P. 160-161^{o}C, IR: 2910 (C-H stretch), 972 (C-H bend), 1611-1462 (Arom skeleton). 1580-1550 and 1344-1332 (Arom nitro group vibrations) 1400-1000 (C-Cl stretch), 3284-3180 (N-H Stretch) UV: 320nm. ^{1}H NMR: 7.21 (d.3H), 6.95 (d.6H) 6.91 (s.6H), 6.80 (m.7H), 6.92 (d.9H), 10.30-9.82(s, NH protons). ^{13}C NMR: 141.8 (C. arom. ring), 121.9 (CNH), 130.8 (CNO_2), 111.5, 112.4, 121.5, 123.2, 126.7, 140.1, 115.1, 117.3, 122.8, 123.4 (CH and C). Anal. Cal. For. $C_{12}H_7N_2O_2SCl$: C, 51.76; H, 2.52; N, 10.06; S, 11.54; Cl, 12.62; O, 11.50%. Found: C, 51.74; H, 2.52; N, 10.04; S, 11.52; Cl, 12.61; O, 11.48%.

8-Chlro-1-amino phenothiazine (11)

Iron powder (20g. 036 mol) was added portion wise to 8-chloro-1-nitrophenothiazine (17g. 0.07mol) suspended in 100ml warm water containing 5ml concentrated hydrochloric acid. The mixture was heated to 60^{o}C and held at this temperature for 1 ½ h. The reaction mixture was filtered hot and the filtrate treated with excess concentrated hydrochloric acid, dried and purified by column chromatography (silica gel, DMSO. Yield: 15.61g (81.3%). M.P 151-158^{o}C.

IR: 3541 (N-H stretch), 2819-2821 (C-H stretch), 1093 (C-H implane), 1320 (C-N stretch), 1684-1698 (Arom. skeletal system). UV 312nm. `HNMR:1.15-1.31 (M, 9H), 3.02-3.21 (m,3H), 4.14-4.50 (m.2H), 10.30 (br.S.7H), 1.17-1.33 (m.4H), 3.16-3.9 (m.1H), 3.57 (m,3H), 4.17-4.44 (m,10H), 9.76 (S,NH protons), 5.70 (m,NH_2 protons). ^{13}CNMR: 141.5 (Arom.Ring C), 118.6 (CNH), 163.9 (CNH_2), 114.3, 112.2, 119.5, 122.5, 123.6, 141.5, 115.2, 118.2, 121.5, 1248 (CH and C). Anal.Cal. For. $C_{12}H_{11}N_2SCl_2$: C, 50.33; H, 3.84; N, 9.79; S, 11.22; Cl, 24.82%. Found: C, 50.31; H, 3.82; N, 9.77; S, 11.21; Cl, 24.81%.

8-Chloro-1-acetyl amino Phenothiazine (12)

In a 100ml beaker, 3.20g (0.13 mol) of 8-Chloro-1-amino phenothiazine dihydrochloride was added to 30ml water. The solution was warmed to 50^{o}C and 1.5ml acetic anhydride added. Aqueous lead acetate prepared from 5g (0.015 mol) lead acetate in 10mol water was quickly added to the mixture. The beaker was swirled intermittently and placed in an ice bath for 20min, filtered and the crystals were washed with cold water, dried and purified by column chromatography (Silica gel, DMSO). Yield: 18.43g (85.34%); m.p 163 – 165^{o}C. IR: 3670 (N-H stretch), 2929-2861 (C-H stretch), 979-713 (C-H out of plane), 1462 (C-H in plane), 1354 (C-N stretch), 1611-1462 (Arom skeletal system), 2671 (C=O stretch). 2388(CH_3 groups) 1400-1000 (C-Cll stretch) UV: 262.0 nm. ^{1}HNMR: 7.29(d.2H), 7.09 (d.3H *J* = 12.2 Hz), 6.97 (d.6H), 6.84 (d.7H), 7.20 (d.9H), 6.53-8.36 (m. NH protons), 3.98-3.94 (s.OCH3), 2.19-2.26 (s,-CH_3 *J* = 8.8 Hz). ^{13}CNMR: 144.6 (C aromatic ring), 54.6 (CNH), 55.3 (OCH_3), 115.6, 113.4, 120.1, 122.6, 124.6, 141.8, 115.10, 118.4, 121.6, 124.9 (CH and C). Anal. Cal. For $C_{14}H_{11}N_2OSCl$: C, 57.83; H, 3.79, N, 9.64; S, 11.02; Cl, 12.22; O, 5.51%. Found: C, 57.81; H, 3.77; N, 9.63; S, 11.01; Cl, 12.20: O, 5.49%.

8-Chloro-1-amino-2-nitrophenothiazine (13)

Powdered 8-chloro-1-acetylaminophenothiazine (0.41g.002 mol) was added to glacial acetic acid (0.4ml) in a 100 ml beaker. While stirring, concentrated sulphuric acid (0.8ml) was added to the mixture surrounded by a freezing mixture of ice and salt. At 0^oC, a cold mixture of concentrated nitric acid 90.2 ml and sulphuric acid was added drop wise. The mixture was held at room temperature for one hour. After which, the reaction mixtures was poured into 500ml cold water and allowed to cool for 15mins, then filtered with suction in a Buchner funnel and washed with cold water. The filtrate was heated for 2h to obtain oily product of two layers which were separated to give two isomeric compound. Purification was by column chromatographic method. Yield 219 ml (97.7%), UV: 540nm, IR: 3698-3100 (hydrogen bounded N-H), 2912 (Ar C-H), 1370 (Ar. C-N), 1644, 1473 (Arom. Skeleton), 1400-1000 (C-Cl stretch), 1195 (NO_2 group). ^{1}HNMR: 7.23 (d.1H *J* = 7.7, 1.5 Hz), 6.98 (d.3H), 6.95 (s.6H), 6.82 (m, 7H *J* = 12.1 Hz) 8.80 (d.9H), 8.30 (m, NH protons), 6.71 (m, NH_2 protons). ^{13}CNMR: 142.8 (C aromatic ring), 121.7 (CNH), 167 (CNH_2), 130.6 (CNO_2), 111.6, 112.5, 121.4, 123.4, 126.7, 140.1, 115.2, 117.3, 122.8, 124.1 (CH and C). Anal. Cal. For $C_{12}H_8N_3O_2SCl$: C, 49.06; H, 2.73; N, 14.31; S, 10.90; Cl, 12.10; O, 10.90%. Found: C, 49.03; H, 2.72; N, 14.30; S, 10.89; Cl, 12.08, O, 10.89%.

8-Chloro-1-amino-4-Nitrophenothiazine (14)

Compound 14 which is an isomer of compound 13 was synthesized by using similar method as in 13 above. Yield: 69ml (33.4%). UV: 490 nm; IR: 3693-3100 (hydrogen bounded N-H). 2899 (Ar. C-H), 1376 (Ar. C-N), 1642 (Ar Skeleton). ^{1}HNMR: 6.80 (d.1H *J* = 1.45, 7.7), 6.20 (d.2H *J* = 7.8 Hz), 7.92 (s.6H), 5.89 (m.7H), 7.50 (d.9H *J* = 8.8 Hz), 8.11 (m, NH protons), 6.67 (m.NH_2 protons). ^{13}C NMR: 38.6 (C. arom ring), 119.2 (C-NH), 165.5 (C-NH_2), 148.4 (C-NO_2), 112.5, 106.5, 123.4. 132.1, 116.7, 138.6, 116.3, 117.6, 123.9, 120.4 (CH and C). Anal. Cal. For $C_{12}H_8N_3O_2SCl$: C, 49.06; H, 2.73; N, 14.31; S, 10.90; Cl, 12.10; O, 10.90%. Found: C, 49.00; H, 2.61; N, 14.10; S, 10.44; Cl, 12.18, O, 10.67 %.

8-Chloro-1,2-diaminophenothiazine trihydrochlride (15)

4g (0.07 mol) of iron powder was added to a warm suspension of 8-chloro-1-amino-2-nitrophenothiazine (10ml) in water (40 ml) containing 3ml concentrated hydrochloric acid. 2g (0.036mol) of iron powder was added to the reaction mixture and heated for 50mins in a water bath. The resulting suspension was filtered hot and the filtrate treated with excess concentrated hydrochloric acid.

Yield: 300ml (98.1%). UV: 312 nm. IR: 3671-3200 (N-H stretch), 809-781 (C-H out of place). 1477 (C-N stretch), 1641 and 1477 (Arom. Skeletal system). ^{1}HNMR: 7.29 (d.1H *J* = 8.1, 1.5 Hz), 7.60 (m .NH protons), 570 (m, NH_2 protons). ^{13}C NMR: 144.6 (C- aromatic ring), 119.5 (CNH), 169.5 (CH_2) 115.6, 113.4, 120.1, 122.6, 124.6, 141.8, 115.1, 118.4, 121.6, 124.9 (CH and C), Anal. Cal. For: $C_{12}H_{13}N_3SCl_4$: C, 38.61; H, 3.50; N, 11.26; S, 8.58; Cl, 38.07%. Found: C, 38.60; H, 3.48; N, 11.24; S, 8.57; Cl, 38.06%.

8-Chloro-12H-5, 14-dihydroquinoxalino [2,3,-a] pentacychlic phonothiazine (16)

A mixture of 8-chloro-1,2-diaminophenothiaine trihydrochloride (10ml) and catecol (7.5g) was refluxed with ethanol (30ml, 3 times for 1 h) and filtered off. The product was dried and purified by column chromatography. Yield: 18.41g (98.6%). M. P. 174-175 ^oC. UV: 32nm. IR: 3773 (N-H stretch), 2910-2819 (Ar, C. H.), 713 (CH out of plane bending) 1098 (C-H in plane bend), 600-800 (C-Cl), 1660-2000 (Aromatic ring), 1030-1230 (C-N). ^{1}HNMR: 6.83 (d.1H *J* = 7.5, 1.48 Hz), 7.89 (d.2H *J* = 12.5 Hz), 7.62 (s.4H *J* = 13.3 Hz), 5.88 (m NH protons), 7.29 (dd.7H *J* = 12.3 Hz), 7.08 (d.9H *J* =8.8 Hz), 6.98 (dd.13H), 6.54-8.36 (Aromatic protons), 7.93 (S. 3H), 6.85 (d.14H). ^{13}C NMR: 145.7 (C-aromatic ring), 119.7 (C-NH), 116.11 (C=C) 120.9, 128.2, 127.3, 127.8, 122.7, 115.8, 116.3, 112.3, 121.1, 123.4, 123.9, 142.4, 116.11, 149.3, 122.4, 125.2 (CH and C). Anal. Cal. For $C_{18}H_{16}N_3SClO_2$: C, 57.83; H, 4.28; N, 11.24; S, 8.57; Cl, 9.50; O, 8.57%. Found: C, 57.81; H, 4.27; N, 11.23; S, 8.56; Cl, 9.49; O, 8.55%.

8-Anilino -12H-5, 14-dihydroquinoxalino [2,3-a] pentacyclic phenothiazine derivatives (18a-h)

General procedure

Preparation of the 8-anilino derivatives was according to water mediated catalyst pre activation method as described by Brisco *et. al* [29]; Zhu *et. al*, [30], Fors *et.al*, [31-34]; Fors and Buchwald, [35], Furstner *et.al*, [2]; Ian *et.al*, [36]; Melford, [37]; Surry and Buchwald, [38]; Surry and Buchwald, [39].

The active catalyst was formed by heating a mixture of 0.058g, 4.04 mmol of Pd $(OAc)_2$, 8mmol H_20 and biaryldialkylphisphine ligand (3.20g, 0.06 mmol) in t-BuOH (4ml) for 1 min. The reaction mixture containing the activated catalyst solution was transferred into a vessel containing a mixture of 8-chloro-12H-5, 14-dihydroquinoxalino[2,3,a]pentacyclic phenothiazine (4.68g, 2.0mmol), KCO_3 (0.38g, 3.2mmol) and aniline derivative (0.26g, 2.1 mmol). The entire mixture was heated to 110^{o}C for 2 min and refluxed for 2h. The product was recrystalised from dimethylformamide/water to afford the corresponding 8-amilino-12H-5,14-dihydroquinoxalino [2,3-a] pent acyclic phenothiazine derivatives (**18a-h**) in good yield.

The reaction detail is as shown in scheme 2, while the reaction mechanism is depicted by the catalytic circle given in fig. 1.

(o-tolyl)-13,14-dihydro-8H-quinoxalino[2,3-a]phenothiazin-2-amine (18a)

Yield: 21:32g (89.7%). M.P. 179-182^{o}C, UV: 328 nm. IR: 3773 (N-H stretch), 2910-2819 (Ar. C-H); 713 (CH out of plane bending), 1098 (C-H in plane bending), 1660-2000 (Ar. Ring); 1030 – 1230 (C-N), 2100-2260 (C=C), 1370 -1390 (CH_3 bending), 3200-3400 (Ar – N H Stretch). ^{1}H NMR: 6.83 (d.1H *J* = 7.5 Hz), 7.89 (d.2H *J* = 9.1 Hz), 7.62 (S.4H), 5.88 (m.NH protons), 7.29 (dd.7H), 7.08(d.9H), 6.98 (dd.13H), 7.93 (S.3H), 6.85 (d.14H *J* = 13.1 Hz), 1.50 (s. Ar- CH_3), 7.1 (m. Ar-H). ^{13}C NMR: 145.7 (C-aromatic ring), 119.7 (C-NH), 116.11 (C=C), 120.9, 128.2, 127.3, 127.8, 122.7, 115.8, 116.3, 112.3, 121.1, 123.4, 123.9, 142.4, 116.11, 149.3, 122.4, 125.2 (CH and C); 59.5 (CH_3). Anal. Cal. For $C_{25}H_{20}N_4S$: C, 73.53; H, 13.73; N, 13.73; S, 7.84%. Found: C, 73.51; H, 4.88; N, 13.72; S, 7.83%.

8(2-nitro-anilino)–12H-5,14-dihydroquinoxalino-(2,3-a)pentacyclic phenothiazine (18b)

Yield: 23.68g (91.3%), M.P. 181-181.3 ^{o}C. UV: 332 nm. IR: 1335 (Ar-NO_2), 1031-1230 (C-N), 3773 (N-H stretch) 2910-2819 (Ar. C-H), 713 (CH out of plane bending) 1098 (C-H in plane bending), 1660-2000 (Ar-ring), 1030-1230 (C-N), 2100-2260 (C=C), 3200-3400 (Ar-NH stretch). ^{1}HNMR: 6.83 (d.1H *J* = 1.6, 7.5 Hz), 7.90 (d.2H), 7.62 (S. 4H *J* = 12.2 Hz) 5.90 (m. NH protons), 7.29 (dd.7H), 7.10(d.9H *J* = 13.2 Hz), 6.98 (dd.13H), 7.93 (S.3H), 6.85 (d.14H), 7.1(m.Ar-H). ^{13} CNMR: 145.7(C aromatic ring), 119.7(C-NH), 116.11 (C=C), 120.9, 129.2, 127.3, 127.8, 122.7, 115.8, 116.11, 149.3, 122.4, 125.2 (CH and C). Anal. Cal. For $C_{24}H_{17}N_5O_2S$.: C, 65.60; H, 3.87; N, 15.95; S, 7.29; O, 7.29%. Found: C, 65.58; H, 3.86; N, 15.94; S, 7.28; O, 7.28%.

Ethyl-4-((13,14-dihydro-8H-quinoxalino[2,3-a]phenothiazin-2-yl)amino)benzoate (18c)

Yield: 22.72g (89.6%). M. P. 193-195.5 ^{o}C. UV: 322 nm. IR: 1715-1730 (C=O stretch), 3773 (N-H stretch), 2910-2819 (Ar-C-H), 713 (CH out of plane bending), 1098 (C-H inplane bending), 1660-2000 (Ar-ring), 1070-1230 (C-N), 2100 – 2260 (C=C), 3200-3400 (Ar-NH stretch), 1760-1790 (Ar-C (O)-O-R). ^{1}H NMR: 3.7 (-C-O-CH_5 protons), 6.83 (d. 1H *J* = 7.5, 1.5 Hz), 7.50 (d. 2H), 7.62 (s.4H *J* = 12.1 Hz), 5.50 (m. NH protons), 7.29 (dd.7H *J* = 13.3 Hz), 7.10 (d.9H), 6.58 (dd.13H), 7.93 (s. 3H), 6.85 (d. 14H), 7.1 (m. Ar-H). ^{13}C NMR: 145.7 (C-aromatic ring), 119.7 (C-NH), 183.67 (C=O Stretch), 116.11 (C=C), 120.9, 129.2, 127.3, 127.8, 122.7, 115.8, 116.3, 112.3, 121.1, 123.4, 123.9, 142.4, 116.11, 149.3, 122.4, 125.2 (CH and C). Anal. Cal. For $C_{27}H_{22}N_4SO_2$:C, 69.53; H, 4.72; N, 12.02; S, 6.87; O, 6.87%. Found: C, 69.51; H, 4.71; N, 12.01; S, 6.86; O, 6.85%.

8(3-NO_2-anilino)–12H-5,14-dihydroquinoxalino (2,3-a) pentacyclic phenothiazine (18d)

Yield: 23.68g (86.9%), M. P.189-191.2^{o}C. UV: 350 nm. IR: 1335 (Ar-$N0_2$), 1031-1230 (C-N), 3773 (N-H stretch), 2910-2819 (Ar-CH), 713 (CH out of plane bending), 1098 (C-H inplane bending), 1660-2000 (Ar-ring), 1030-1230 (C-N), 2100-2260 (C=C), 3200 – 3400 (Ar-NH stretch). ^{1}HNMR: 6.83 (d.1H), 7.90 (d. 2H), 7.62 (s. 4H), 5.90 (m. NH protons), 7.29 (dd. 7H *J = 4.7*), 710 (d.9H), 6.98 (dd. 13H), 7.93 (s. 3H), 6.85 (d.14H), 7.1 (m. Ar. H). ^{13}CNMR: 145.7 (C-aromatic ring), 119.7 (C-NH) 116.11 (C=C), 120.9, 129.2, 127.3, 127.8, 122.7, 115.8, 116.3,

112.3, 121.1, 123.4, 123.9, 142.4, 116.11, 149.3, 122.4, 125.2 (CH and C). Anal. Cal. For $C_{24}H_{17}N_50_2S$: C, 65.60; H, 3.87; N, 15.94; S, 7.29; O, 7.29%. Found: C, 65.59; H, 3.86; N, 15.92; S. 7.28; O, 7.28%.

8(4-ntrile-anilino)–12H–5,14-dihydroquinoxalino-[2,3-a]Pentacyclic phenothiazine (18e)

Yield: 27.8g (91. 6%). M,P: 191-193.4^{o}C. UV: 316 nm. IR: 2202-2240 (Ar-C≡N), 2210-2260 (C≡N), 3773 (N-H stretch), 2910-2819 (Ar. C-H), 713(CH out of plane bending), 1098 (C-H in plane bending). 1660-2000 (Ar-ring), 1030-1230 (C-N), 2100-2260 (C=C), 3200-3400 (Ar. NH Stretch). ^{1}HNMR: 6.83 (d.1H), 7.90 (d. *J = 2.7*, 2H), 7.62 (s.4H), 5.90 (m.NH protons), 7.29 (dd. *J = 4.0, J = 6.3, J = 5.9*, 7H), 710 (d.9H), 6.98 (dd.13H), 7.93 (s.3H), 6.85 (d.14H), 7.1. (m. Ar-H). ^{13}CNMR: 145.7 (C-aromatic ring), 119.7 (C-NH), 131.0 (C≡C), 116.11 (C=C), 120.9, 129.2, 127.3, 127.8, 123.9, 142.4, 116.11, 149.3, 122.4. 125.2 (CH and C). Anal. Cal. For $C_{25}H_{18}N_5S$: C, 71.43; H,4.29; N, 16.67; S, 7.62%. Found: C, 71.41; H, 4.28; N, 16.65; S, 7.61%.

8(4-methylketone-anilino)–12H-5,14-dihydroquinoxalino-(2,3-a)pentacyclic phenothiazine (18f)

Yield: 30.25 (92.4%). M.P 189-192.6^{o}C. UV: 315 nm. IR: 1370-1390 (CH_3 bending), 1715-1730 (C=) stretch), 1675-1695 (Ar-C (O) – CH_3), 3773 (N-H stretch). 2910-2819 (Ar. C-H), 713 (Ch out of plane bending, 1098 (C-H in plane bending), 1660-2000 (Ar. ring) 1030-1230 (C-N), 2100-2260 (C=C). 3200-3400 (Ar. NH stretch). ^{1}HNMR: 6.83 (d. 1H), 7.90 (d.2H), 7. 62 (s. 4H), 5.90 (m.NH protons), 7.29 (dd.7H *J* = 13.5 Hz), 710 (d. 9H *J* = 12.3 Hz), 6.98 (dd. 13H *J* = 13.5 Hz), 7.93 (S. 3 H *J* = 4.8 Hz), 6.85 (d. 14H), 7.1 (m-Ar-H), 2.3 (R-C≡CH_3 protons). ^{13}CNMR: 145.7 (C-Aromatic ring), 119.7 (C-NH). 116.11 (C=C), 120.9, 129.2, 127.3, 127.8, 122.7, 115.8, 116.3, 112.3, 121.1, 123.4, 123.9, 142.4, 116.11, 149.3, 122.4, 125.2 (CH and C). Anal, Cal. For $C_{26}H_{22}N_4SO$: C, 71.33; H, 5.02; N, 12.79; S; 7.31; O, 3.65%. Found: C, 71.31; H, 5.01; N, 12.78; S, 7.30; O, 3.64%.

8(4-aldehyde-anilino)–12H-5,14-dihydroquinoxalino-[2,3-a]pentacyclic phenothiazine (18g)

Yield 28.3g. (88.9%). M. P.: 189-192.6^{o}C. UV: 319 nm. IR: 1685-1710 (Ar-CH=O), 1715-1730 (C=O stretch), 3773 (N-H stretch), 2910-2819 (Ar. C-H), 713 (CH out of plane bending), 1098 (C-H in plane bending), 1660-2000 (Ar. ring), 1030-1230 (C-N), 2100-2260 (C=C), 3200-3400 (Ar. NH stretch). ^{1}HNMR: 9.2 (Ar – CHO), 6.83 (d.1H), 7.90 (d.2H *J* = 8.7 Hz), 7.62 (S.4H), 5.90 (m. NH protons), 7.29 (dd.7H *J* = 12.1 Hz), 7.10 (d.9H), 6.98 (dd.13H, *J* = 13.1 Hz), 7.93 (S.3H), 6.85 (d.14H), 7.1 (m.Ar-H). ^{13}C NMR: 145.7 (C- aromatic ring), 119.7 (C-NH), 116.11 (C=C), 120.9 129.2, 127.3, 127.8, 122.7, 115.8, 116.3, 112.3, 121.1, 123.4, 123.9, 142.4, 116.11, 149.3, 122.4, 125.2 (CH and C). Anal. Cal. For $C_{25}H_{18}N_4OS$: C, 71.09; H, 4.27; N, 13.29;S, 7.58; O, 3.78%. Found: C, 71.07; H, 4.26; N, 13.26; S, 7.56; O, 3.78%.

8(4-NO_2-anilino)–12H-5,14-dihydroquinoxalino-(2,3-a)pentacyclic phenothiazine (18h)

Yield: 26.8g (88.4%), m.p 188-190^{o}C. UV: 354 nm. IR: 1335 (Ar. N0. 2), 1031-1230 (C-N), 3773 (N-H stretch), 2910-2819 (Ar-C-H), 713 (CH out of plane bending), 1098 (C-H in plane bending), 1660-2000 (Ar. ring), 1030-1230 (C-N), 2100-2260 (C=C), 3200 – 3400 (Ar.- NH stretch). ^{1}H NMR: 6.83 (d. 1H), 7.90 (d. 2H), 7.62 (S. 4H), 5.90 (m. NH protons), 7.29 (dd.7H *J* = 14.1 Hz), 7.10 (d. 9H *J* = 14.3 Hz), 6.98 (dd.13H), 7.93 (S.3H), 6.85 (d.14H), 7.1 (m. Ar-H). ^{13}C NMR: 145.7 (C-aromatic ring), 119.7 (C-NH), 116.11 (C=C), 120.9, 129.2 127.3, 127.8, 122.7, 115.8, 116.3, 112.3, 121.1, 123.4, 123.9, 142.4, 116.11, 149.3, 122.4, 125.2 (CH and C). Anal. Cal. For $C_{24}H_{17}N_5O_2S$: C, 65.60; H, 3.87; N, 15.95; S, 7.29; O, 7.29%. Found: C, 65.59, H, 3.86; N, 15.94; S, 7.28; O, 7.27%.

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