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Synthesis, characterization and neuropharmacological activity of novel angular pentacyclic phenothiazine

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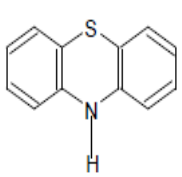
13H-5,14-dihydroquinoxalino[2,3-a]phenothiazine (a new pentacyclic ring system) was synthesized by condensation of diphenylamine and sulphur, which on nitration yielded 1-nitrophenothiazine. Reduction of this compound afforded 1-aminophenothiazine dihydrochloride and subsequent protection of this product with acetic anhydride gave 1-acetylaminophenothiazine. The nitration of this acetylated compound yielded two isomeric compounds: 1-amino-2-nitrophenothiazine and 1-amino-4-nitrophenothiazine. The reduction of the ortho isomer furnished 1,2-diaminophenothiazine which when added to catecol and refluxed with ethanol gave the pentacyclic product 13H-5,14-dihydroquinoxalino[2,3-a]phenothiazine. Structures were established by analytical and spectral data. The results of the neuropharmacological screening data revealed that the novel system possessed neurosedative properties. The ability of this compound to antagonise fortwin – induced climbing behaviour in mice was correlated with neuroleptic potential.

Key words: 13H-5,14-dihydroquinoxalino[2,3-a]phenothiazine, pentacyclic, phenothiazine, neurosedative, fortwin-induced.

INTRODUCTION

The chemistry of phenothiazines has generated intensive scientific interest due to their biological properties (Ujuwala et al., 2012). Great work has been done on the bioactivity of phenothiazine and its derivatives. Phenothiazine itself is found to be a worming agent for livestock. The pesticidal action of phenothiazine results from the fact that they affect the nervous system of insects by inhibiting the breakdown of acetylcholine. The derivatives of phenothiazine have been studied for their antipsychotic properties (Luiza et al., 2007; Whitaker, 2004). They constitute the largest of the five classes of antipsychotic drugs. The antipsychotic activities of phenothiazine have been attributed to the basic nitrogen of the thiazine ring which donates electrons to the biological receptors by a charge transfer mechanism and also the ability of substituting the hydrogen atom to the nitrogen atom by substituent groups which further enhances the pharmacological property (Abdel-Monem and Portoghese, 1972; Martina et al., 2007).

The chemistry of the linear phenothiazine (**1**) is well developed (Abdel-Rahman et al., 2013; Okafor 1971; 1978). The non-linear aza phenothiazine is relatively understudied in spite of their pharmacological importance in medicine, agriculture and industry (Ezema et al., 2012; Okoro et al., 2009).



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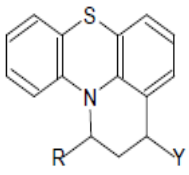

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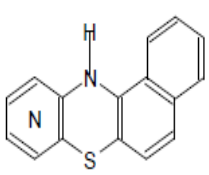

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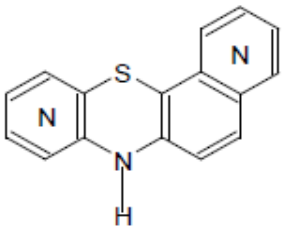
While tetracyclic, N-(2,3-dihydro-1H-pyrido[3,2,1-kl]phenothiazines) of type (**2**) have previously been reported (Alan et al., 1999; Tim et al., 2012), very few of the non linear tetracyclic phenothiazine of type **3** benzo[a]azaphenothiazine and its diaza-analogue type **4** have been reported (Chuan et al., 2012; Okoro et al., 2009).



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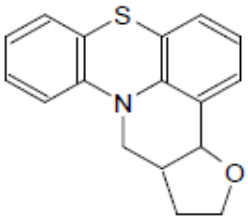

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Still grossly understudied are the non-linear (angular) pentacyclic phenothiazine systems in spite of their known pharmaceutical and industrial applications. Pentacyclic phenothiazines of type **5** (Tetrahydro-3aH-furo[2,3:4,5]pyrido[3,2,1-kl]phenothiazine) have previously been prepared by the treatment of 10-[benzotriazol-1-yl] methyl phenothiazine with cyclic hydrofuran and 3,4-2H-dihydropyran (Abdel-Rahman et al., 2013; Alan et al., 1999). The non-linear pentacyclic aza phenothiazine of type **13** to the best of our knowledge has not been reported. In this paper we report the synthesis of the pentacyclic phenothiazine **13** (a new pentacyclic ring system) and its pharmacological properties.

MATERIALS AND METHODS

All chemicals were obtained from different sources (Lavans, Aldrich, Merck) and were used without further purification. The melting points were determined on a SMP3 melting point apparatus and are reported in degree Celsius uncorrected. Column chromatography was performed in Scharlan silica gel 60 (70 – 230 mesh).^{1}H and ^{13}C –NMR spectra were recorded on a Varian Gemini 2000 spectrophotometer operating 200 and 50 MHz respectively. Chemical shifts were recorded as ∂ values in ppm referenced to the solvent. HPLC separations were performed in a Bulk Scientific 500 apparatus using a reverse phase Lichrospher 100RP-18(5 µm) column at room temperature (eluent: methanol/water-8:2, v/v). The Infrared (IR) spectra were recorded in cm^{-1} on a Bulk Scientific 500 Spectrophotometer. The mass spectra were recorded on a Schimadzu GCMS-QP-1000E, mass spectrometer at 70 eV and elementary analysis for C, H, S and N on a Perkin-Elmer analyzer 2400.

Drugs

Diazepam and Nitrazepam were obtained from Roche Nigeria ltd, while pentobarbital and apomorphine were obtained from Sigma Chemical Company, USA. All drugs were freshly prepared. Parallel control experiments were done in each case to correct possible effects caused by the vehicle alone.

Animals

All experiments performed on laboratory animals in this study followed the “Principle of laboratory animal care” (NIH publication No 85-23, revised 1985). Swiss albino mice (20 to 30 g) and wistar rats (180 to 200 g) of either sex were used. All the animals were maintained at the Animal Facility Centre of Kogi State University at standard conditions and temperature (25°C) and fed with standard diet (Ladokum feeds, Ibadan and water *ad libitum.*

Synthesis

The synthetic routes for all compounds are outlined in the scheme 1 and the details are given below:

Phenothiazine (1)

A mixture of the corresponding diphenylamine (13 g, 3.0 mol) and sulphur (1.2 g, 0.834 mol) was heated in a glycerol oil bath to 195°C. After cooling to 100°C, elemental iodine was added while heating continued. The separation of hydrogen sulphide was observed at 170°C and was decomposed by leading in 5% aqueous sodium hydroxide solution. The mixture was heated to 185°C and held at this temperature for 45 min. The bath was cooled to 50°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 evaporator. The product was dried and purified by column chromatography. Yield 11.06 g (80.5%), m.p. 184-186°C. IR (Vmax/cm^{-1}): 2999 (NH), 2859-2861 (C-H arom.), 717(C-H bending), 1197-1211 (C-H in-plane) and 1300-1411 (C-N arom.). UV: 311 (log Ɛ 3.3281)nm. ^{1}H NMR (200 MHz, DMSO): 7.32 (d, 2H), 7.34 (d, 4H), 7.29 (s, 6H), 7.28 (s, 8H), 7.04 (s, 3H), 7.06 (s, 7H), 6.99-6.92 (m, 1H), 6.98-6.91(m, 9H), 11.48 (s, -NH proton). ^{13}C NMR (50MHz, DMSO): 140.7 (C. Arom. 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 $C_{12}H_9NS$: C, 72.40; H, 6.30; N, 9.72; S, 22.22%. Found: C.72.29; H, 6.34; N, 9.69; S, 22.14%.

1-Nitrophenothiazine (7)

Concentrated nitric acid (10 ml, 0.5 mol) was placed in a 200 ml round bottom flask, while concentrated sulphuric acid (10 ml, 0.5 mol) was added to it portion wise over 30 min. With efficient stirring at room temperature, compound (**1**)(15 g, mol) was added. The mixture was refluxed in a water bath while the temperature was held at 50°C for 40 min. The product was washed with 500 ml cold water and filtered with suction on a Buchner funnel, dried and purified by column chromatography. Yield 20.40 g (95%), m.p. 160-161°C, IR: 2910 (C-H stretch), 972 (C-H bend), 1611-1462 (Arom. Skeleton), 1580-1550 and 134s4-1332 (aromatic nitro gr. Vibrations),UV: 320 nm. ^{1}H NMR: 7.21 (d,1H), 6.95 (d,2H), 6.91 (s,6H), 6.80 (m, 7H), 6.92 (d,8H), 8.82 (d, 9H), 10.30-9.82 (s, NH protons).^{13}C NMR: 141.8 (C arom. Ring), 121.9 (CNH), 130.8 (NO_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 $C_{12}H_8N_2S$: C, 67.89; H, 3.80; N, 13.20; S, 15.11%. Found: C, 67.83; H, 3.78; N, 13.18; S, 15.09%.

1-Aminophenothiazine dihydrochloride (8)

Iron powder (20 g, 0.36 mol) was added portionwise to 1-nitrophenothiazine (17 g, 0.07 mol) suspended in 100 ml warm water containing 5 ml concentrated hydrochloric acid. The mixture was heated to 60°C and held at this temperature for 1½ 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: 13.50 g (79.4%); m.p. 151-158°C. IR: 3541 (N-H stretch), 2819-2821 (C-H stretch), 1093 (C-H inplane), 1320 (C-N stretch), 1684-1698 (Arom. skeletal system). UV 312 nm. ^{1}H NMR: 1.15-1.31 (m, 9H), 3.02-3.21 (m, 3H), 4.14-4.50 (m, 2H), 7.00-7.32(m, 8H), 10.30(br. s, 7H), 1.17-1.33 (m, 5H), 3.16-3.39(m, 1H), 3.57(m, 3H), 4.17-4.44(m,10H) 9.76 (s, NH protons), 5.70 (m,NH_2 protons). ^{13}C NMR: 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 $C_{12}H_{10}N_2S$: C, 67.25; H, 4.70; N, 13.08; S, 14.97%. Found: C, 67.18; H, 4.50; N, 13.02; S, 14.09%.

1-Acetylaminophenothiazine (9)

In a 100 ml beaker, 3.20 g (0.13 mol) of 1-aminophenothiazine dihydrochloride was added to 30 ml water. The solution was warmed to 50°C and 1.5 ml acetic anhydride added. Aqueous lead acetate prepared from 5 g (0.015 mol) lead acetate in 10 ml water was quickly added to the mixture. The beaker was swirled intermittently and placed in an ice bath for 20 min, filtered and the crystals were washed with cold water, dried and purified by column chromatography (silica gel, DMSO). Yield: 14.80 g (82.31%); m.p. 162-163°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), 2385 (-CH_2 groups). UV: 262.0 nm. ^{1}H NMR: 7.29 (d, 1H), 7.09 (d, 2H), 6.97 (d, 6H), 6.84 (d, 7H), 6.83 (d, 8H), 7.20 (d, 9H), 6.53-8.36 (m, NH protons), 3.98-3.94 (s, OCH_3), 2.19-2.26(s, -CH_3). ^{13}C NMR: 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 $C_{14}H_{12}N_2OS$: C,65.76; H,4.70; N,10.88; O,6.21; S,12.45%. Found: C,65.70; H,4.67; N,10.78; O,6.18; S,12.42%.

1-Amino-2-Nitrophenothiazine (10)

Powdered 1-acetylaminophenothiazine (0.41 g, 0.002 mol) was added to glacial acetic acid (0.4 ml) in a 100 ml beaker. While stirring, concentrated sulphuric acid (0.8 ml) was added to the mixture surrounded by a freezing mixture of ice and salt. At 0°C, a cold mixture of concentrated nitric acid 90.2 ml) and was added dropwise. The mixture was held at room temperature for one hour. After cooling to room temperature, the reaction mixture was poured into 500 ml cold water and allowed to cool for 15 min, then filtered with suction in a Buchner funnel and washed with cold water. The filtrate was heated for 2 h to obtain oily product of two layers which were separated to give two isomeric compounds. Purification was by column chromatographic method (Scheme 1). Yield: 210 ml (96.8%). UV: 540 nm. IR: 3698-3100 (hydrogen bonded N-H), 2912 (Ar,C-H); 1370 (Ar, C-N), 1644, 1473 (Aromatic skeleton). ^{1}H NMR: 7.23 (d, 1H), 6.98 (d, 2H), 6.95 (s,6H), 6.82 (m, 7H), 6.92 (d, 8H), 8.80 (d,9H), 8.30 (m, NH protons), 6.71 (m, NH_2 protons). ^{13}C NMR: 142.8 (C aromatic ring), 121.7 (CNH), 167.3 (CNH_2), 130.6 (CNO_2), 111.6, 112.5, 121.4, 123.2, 126.7, 140.1, 115.2, 117.3, 122.8, 124.1 (CH and C). Anal. Cal. For $C_{12}H_9N_2O_2S$: C, 58.76; H, 3.70; N, 11.42; S,13.07; O, 13.05%. Found: C, 58.72; H, 3.56; N, 11.38; S, 13.02; O, 13.01%.

1-amino-4-nitrophenothiazine (11)

Compound 11 was synthesized by using similar method as in 10 above. Yield: 68 ml (31.3%). UV: 490 nm. IR: 3692-3100 (hydrogen bonded N-H), 2899 (Ar. C-H), 1376 (Ar. C-N), 1642, 1472 (Ar. Skeleton). ^{1}H NMR: 6.80 (d, 1H), 6.20 (d,2H), 7.92 (s,6H), 5,89 (m,7H), 7.32 (d,8H), 7.50 (d,9H), 8.11 (m, NH protons), 6.67 (m ,NH_2 protons), ^{13}C NMR: 138.6 (C aromatic ring), 119,2 (CNH), 165.5 (CNH_2), 148.4 (CNO_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 $C_{12}H_9N_2O_2S$: C, 58.76; H, 3.70; N, 11.42; S, 13.07; O, 13.05%. Found: C, 58.70; H, 3.54; N, 11.30; S, 13.00; O, 13.02%.

1,2-diaminophenothiazine trihydrochloride(12)

4 g (0.07 mol) of iron powder was added to a warm suspension of 2-nitro-1-aminophenothiazine (10 ml) in water (40 ml) containing 3 ml concentrated hydrochloric acid. 2 g (0.036 mol) of iron powder was added to the reaction mixture and heated for 50 min in a water bath. The resulting suspension was filtered hot and the filtrate treated with excess concentrated hydrochloric acid. Yield: 290 ml. (94.7%). UV: 312 nm. IR: 3671-3200 (N-H stretch), 809-781 (C-H out of plane), 1051 (C-H in-plane), 1477 (C-N stretch), 1641 and 1477 (Arom. skeletal system). ^{1}H NMR: 7.29 (d, 1H), 7.09 (d, 2H), 6.97 (d, 6H), 6.84 (d, 7H), 6.83 (d, 8H), 7.20 (d, 9H), 7.60 (m, NH protons), 5.70 (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: $C_{12}H_{11}N_3S$: C, 63.02; H, 4.81; N, 18.24; S, 13.92%. Found: C, 63.01; H, 4.79; N, 18.22; S, 13.90%.

13H-5,14-dihydroquinoxalino[2,3-a]phenothiazine (13)

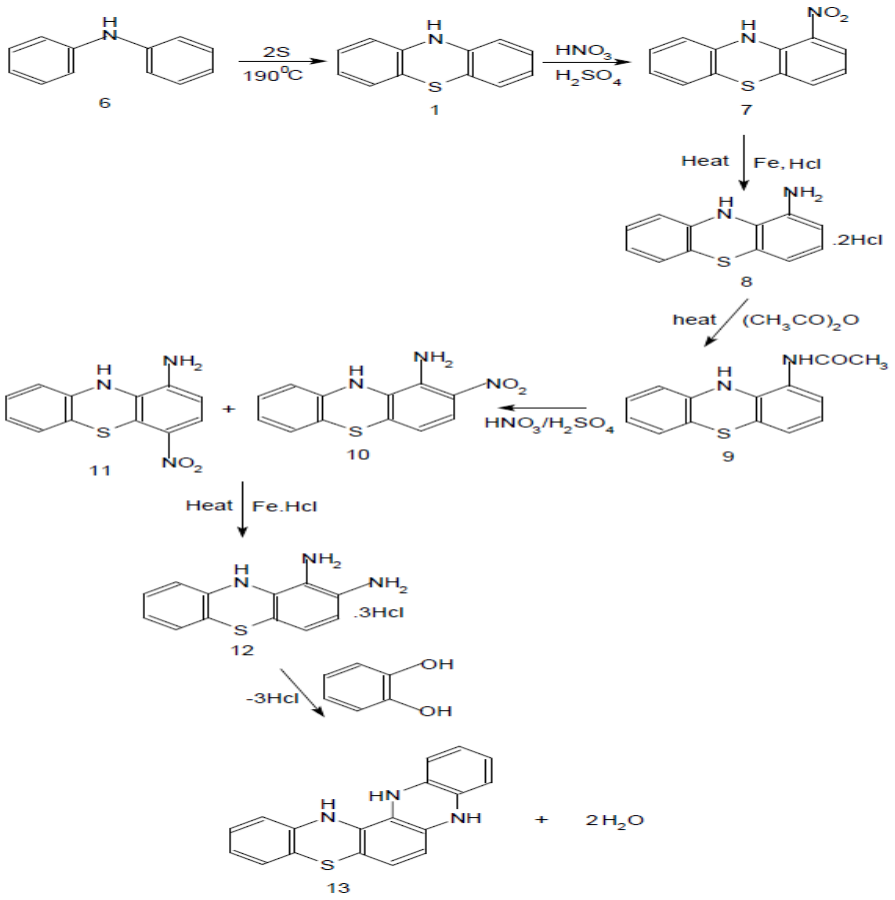
A mixture of 1,2-diaminophenothiazine hydrochloride (10 ml) and catechol (7.5 g, mol) was refluxed with ethanol (30 ml, 3 times for 1 h) and filtered off. The product was dried and purified by column chromatography. Yield; 15.32 g (98.4%). m.p. 174-175°C. UV: 320 nm. IR: 3773 (N-H stretch), 2910-2819 (Ar, C-H), 713 (C-H out of plane bending), 1098 (C-H in plane bend). ^{1}H NMR: 6.83 (d, 1H), 7.89 (d, 2H), 7.62 (s, 4H), 5.88 (m, NH protons), 7.29 (dd, 8H), 7.08 (d, 9H), 6.98 (dd, 13H), 6.84 (d, 15H), 7.19(d, 16H), 7.93 (s, 3H), 6.85 (d, 14H).^{13}C NMR: 145.7 (C-aromatic ring); 119.7 (CNH), 116.11 (C=C), 126.9, 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 $C_{20}H_{15}N_3S$: C, 72.92; H, 4.59; N, 12.76; S, 9.74%. Found: C, 72.89; H, 4.55; N, 12.57; S, 9.71%.

Acute toxicity studies

The acute toxicity (LD_{50}) was determined following the method described by (Amos et al., 2002; Gurad et al., 2011; Lorke, 1983). Animals were divided randomly into six groups of six mice each. The sample was administered intraperitoneally in the range of doses 10, 100, 1000, 1500, 2000, 3500 and 5000 mg/kg. The animals were observed for 72 h. At the end of the experiment, the animals were sacrificed and then autopsied and examined microscopically for any pathological changes.

Studies on exploratory activity in mice

Mice were divided into four groups of six mice each. Groups 1 and 2 were treated with sample at doses 50 and 100 mg/kg i.p respectively, while group 3 received normal saline (10 ml/kg) which served as control. Animals in group 4 were treated with diazepam (a known neurosedative) 2 mg/kg i.p (File and Pellow, 1985; Kota et al., 2010). 30 min after the drugs were administered; the animals were placed individually in an automatic Letica board with 16 evenly spaced holes with a counter (Letica LE3333). The number of head dips by the mice into the holes over a period of 5 min was automatically counted (Koofreh et al., 2012; Perez et al., 1998;



Scheme 1. Synthetic routes for all the compounds.

Wolfman et al., 1994).

Studies on spontaneous motor activity

Adult mice were randomly divided into three groups of 6 mice each. Groups 1 and 2 received the sample at doses 50 and 100 mg/kg i.p, while group 3 received normal saline (10 ml/kg p.o). Motor activity of the mice was recorded using a Letica activity cage floor. The animals were singly placed in the cage and their activity was recorded for 6 min at 30 min intervals for a period of 120 min (Odin et al., 2003). In another experiment, the effect of aminophiline 2 mg/kg i.p was recorded. The effects of the sample on aminophiline – induced hyperactivity were compared to that of chlorpromazine 2 mg/kg i.p.

Studies on pentobarbital induced sleep

Adult rats were divided into 4 groups of 6 rats each. Groups 1 and 2 received 50 and 100 mg/kg i.p of the sample and group 3 was administered normal saline (10 ml/kg p.o) and served as control (Jae-wook et al., 2012; Kaul and Kulerni, 1978; Ngouemo et al., 1994). Diazepam 1 mg/kg i.p was administered to animals in group 4. All the animals were injected with Phenobarbital sodium (phenobarbitone 35 mg/kg), 30 min after the drug treatment. The onset and duration of sleeping time were recorded (Hong et al., 2009; Wambebe, 1985).

Studies on apomorphine (fortwin) – induced climbing in mice

Adult mice were randomly divided into 3 groups of 10 mice each. The first group received normal saline (10 ml/kg p.o) and served as control. Groups 2 and 3 received the sample at doses of 50 and 100 mg/kg i.p. 30 min after treatment, all mice were treated with apomorphine (fortwin) 3 mg/kg (Hong et al., 2009; Protais et al., 1976). Readings were taken at 10, 20, and 30 min after apomorphine administration. The mice were observed for climbing and scored as follows:

0= fore paws on the floor
1= fore feet holding the vertical bars
2= fore feet holding the bars.

RESULTS

Phenothiazine (**1**) was synthesized from the fusion of diphenylamine (**6**) with excess elemental sulphur. The sulphur residue was removed by heating the mixture after benzene was added and was filtered hot. The filtrate is phenothiazine in benzene which was recovered on heating to dryness.The phenothiazine (**1**) was nitrated with a mixed acid at 50°C while avoiding polynitration to give 1- nitrophenothiazine (**7**).

Reduction of (**7**) with iron in dilute hydrochloric acid furnished 1-aminophenothiazine dihydrochloride (**8**), which was subsequently reacted with acetic anhydride to achieve (**9**). This was done to protect the amino group in (**8**) from further nitration. Nitrating compound (**9**) in mixed acid yielded ortho and para nitro phenothiazines, (**10**) and (**11**) respectively. Reduction of (**10**) with iron in dilute hydrochloric acid furnished 1,2-diaminophenothiazine trihydrochloride (**12**), which when added to catecol and refluxed with ethanol gave the novel pentacyclic product:13H-5,14-dihydroquinoxalino[2,3-a]phenothiazine (**13**) (Scheme 1).

The structural assignment of the synthesized compounds is based on the spectral data. In the IR spectrum of Compound **1,** absorption band at 2999 cm^{-1} represents the hydrogen bonded N-H stretching. There were number of peaks at 2859-2861 cm^{-1}, 717 cm^{-1}, 1197-1211 cm^{-1}, 1300-1411 cm^{-1} for aromatic C-H stretching, out of plane C-H bending, in-plane C-H bending and the aromatic C-N stretching respectively. Only two of the aromatic skeletal stretching bands were readily visible at 1300-1473 cm^{-1}. The ^{1}H and ^{13}C NMR studies of Compound 1 confirmed the structure. The Compound **1** reacting with mixed acids (nitration) yielded 1-nitrophenothiazine (**7**). The IR spectrum of compound (**7**) showed absorption for aromatic C-H stretching and in-plane C-H bending at 2910 and 972 cm^{-1} respectively. The aromatic skeletal system is responsible for the bands at 1611 and 1462 cm^{-1}.

Compound (**7**) was reduced with iron to give **(8**)-1-aminophenothiazine dihydrochloride. The N-H stretching and the aromatic C-H stretching appeared at 3541, 2819 and 2821 cm^{-1} respectively. The in-plane C-H band appeared weakly at 1093 cm^{-1}, while the C-N stretching showed at 1320 cm^{-1} which is characteristic of aromatic amines. The bands at 1684 to 1698 cm^{-1} are that of aromatic skeletal system. In ^{1}H NMR spectra data, Compound (7) shows a singlet at δ 10.30 to 9.81 due to N-H proton. This was shifted to δ 9.76 in 1-aminophenothiazine hydrochloride (8). This shifting towards upfield in compound 8 is ascribed to intramolecular hydrogen bonding as –NH...O=N in Compound 7.

Compound (**8**) was subsequently reacted with acetic anhydride to protect the amino group from further nitration. This yielded 1-acetylaminophenothiazine (**9**). The hydrogen bonded N-H stretching appeared at 3670 cm^{-1}, while the bands at 2929 and 2861 cm^{-1} were for C-H stretching for aromatic systems. The bands at 1354 cm^{-1} is characteristic of aromatic C-N stretching, while bands at 2385 and 2671 cm^{-1} indicated C=O stretching and methylene CH_2 groups. In the ^{1}H NMR spectrum of Compound 9, the multiplet for –NH protons appeared in the region δ 6.53 to 8.36. The $–OCH_3$ protons and $–CH_3$ protons in the compound showed a singlet in the region δ 3.98-3.94 and δ 2.19 to 2.26 respectively, indicating a complete acylation of Compound 8.

The nitration of Compound (**9**) yielded two isomers: 2-nitro-1-aminophenothiazine and 4-nitro-1-aminophenoyhiazine (Compounds **10** and **11** respectively). The IR spectra of compounds **10** and **11** showed broad bands at 3698 to 3100 cm^{-1} indicating hydrogen bonded N-H stretching. The absorption band at 2912 cm^{-1} was for aromatic C-H stretching. The band at 1370 cm^{-1} is characteristic of aromatic C-N stretching, while the aromatic skeletal was found at 1644 cm^{-1} and 1473 cm^{-1}. Similarly, in compound 10, $N-H_2$ proton appeared as multiplet at δ 6.71, while in ^{13}C NMR spectrum, a characteristic signal appeared for (CNH_2) and (CNO_2) in the range of δ 167.3 and δ 130.6 respectively. These were found absent in compound 9 indicating a successful nitration of this compound. The nitro group is responsible for the broad shoulder at 1195 cm^{-1}.

Reduction of (**10**) furnished 1,2-diaminophenothiazine hydrochloride (**12**). The IR spectrum of (**12**) showed a broad band at 3671 to 3200 cm^{-1} for hydrogen bonded N-H stretching. The band at 1477 cm^{-1} indicated C-N stretching, while the aromatic skeletal system was located at 1641 and 14777 cm^{-1}. In the ^{1}H NMR spectrum, Compound 12 showed two signals for NH and NH_2 at δ 7.60 to 5.70 respectively. A characteristic signal appeared for CH_2 in the range of δ 119.5 in the ^{13}C NMR spectrum, while that of CNH_2 was located at δ 169.5.

Refluxing a mixture of Compound (12) and catechol with ethanol yielded the pentacyclic product-13H-5,14-dihydroquinoxalino[2,3-a]phenothiazine (**13**). The IR spectrum of Compound (**13**) showed N-H stretching at 3773 cm^{-1}. The bands at 2910 to 2819 cm^{-1} appeared for aromatic C-H stretching, while bands at 713 and 1098 cm^{-1} were for C-H out-of-plane bending and in-plane bending respectively. These clearly support the fact that Compound (**11**) was not used in the synthesis of the novel product (**13**). The absorption at 205 nm in the UV-visible spectrum of Compound (1) resembles that of benzene while the shift in wavelength to 311 nm indicated the presence of auxochrome type –NHR in phenothiazine.

The UV spectrum of (**8**) showed maximum absorption at 312 nm. No appreciable bathochromic shift because the compound is in the form of hydrochloride. Compound **(10**) showed a UV maximum at 540 nm. This powerful bathochromic shift is probably due to the presence of free amino group, while the pentacyclic product **(13**) exhibited a UV maximum at 320 nm characteristic of phenothiazine systems. In the ^{1}H NMR spectrum, compound 13 displayed a signal at δ 5.88 for NH protons, while the multiplet for aromatic protons appeared in the region between δ 6.54-8.36. In ^{13}C NMR spectrum of compound 13, a characteristic signal appeared for (CNH) in the range of δ 119.7. The mass spectrophotometric studies performed on the phenothiazines confirmed the molecular weight values. The results of the pharmacological tests are as presented in Tables 1, 2, 3, 4 and 5.

Table 1. Effect of sample on exploratory activity in mice.

Treatment	Dose mg/kg	Mean score
Normal saline	10 ml/kg	40.8±5.8
Sample	50	21.3±2.4
Sample	100	12.2±2.9
Diazepam	2	15.0±3.5

Table 2. Effect of sample on spontaneous motor activity in mice.

Treatment	Dose mg/kg	Time (minutes)				
		0	30	60	90	120
Normal saline	10 ml/kg	91.2± 3.6	86.2± 2.5	82.8± 1.8	81.8± 2.0	75.5± 2.8
Sample	50	91.8±4.1	35.8±3.1	31.8± 2.3	22.5± 2.8	13.7±2.0
Sample	100	90.7± 1.2	33.0± 2.4	14.7± 1.7	8.2± 1.3	5.8±1.1

Table 3. Effect of sample on aminophilin induced hypermotility in mice.

Treatment	Dose mg/kg	Time (minutes)				
		0	30	60	90	120
Normal saline	10 ml/kg	90.3±2.9	85.2± 2.5	82.5±2.4	81.0±1.5	76.0± 2.9
Aminophillin	2	90.0±1.6	102.1±4.4	126±5.2	118±3.1	104 ±2.9
Aminiphillin + sample	50	91.8±1.6	73.5±2.9	53.5±2.6	33.7±3.1	17.3±2.7
Aminophillin +sample	100	90.5±2.2	62.5±2.2	34.2±2.4	17.3±2.6	14.7±2.5

DISCUSSION

The structural assignment of the synthesized compounds was based on the spectral data. The IR spectrum of the pentacyclic Compound (13) clearly showed that the isomeric Compound (11) was not used in the synthesis of the final product. This was further buttressed by the disappearance of C-O absorption (1200 cm^{-1}) in the spectrum of (13). The pentacyclic product exhibited a UV maximum at 320 nm characteristic of phenothiazine systems. The angular pentacyclic ring system was further identified by the information from the ^{1}H and ^{13}CNMR spectral with the resonances assigned to hydrogen and carbon. Compound **13** (sample) when administered, inhibited the exploratory behaviour in mice dose dependently. The effect was similar to that of diazepam (2 mg/kg), a known neurosedative and significantly different from those of control (Table 1). From Table 2, at 50 and 100 mg/kg i.p, the sample caused a significant time and dose dependent decrease in the spontaneous motor activity in mice.

Similarly, aminophilin induced hypermotility was reduced dose and time dependently (Table 3). When aminophilin alone is administered, at 60 min the mice was very active, 12±5.2, while the combination of 50 mg/kg sample with aminophilin reduced the activities of the mice, 53.5±2.6. The activities of the mice were further reduced, 34.2±2.4 when the dose was increased to 100 mg/kg. Similarly at 120 min, it was noticed that at 50 mg/kg the activities of the mice reduced to 17.3±27, while 100 mg/kg sample plus aminophilin further reduced the activities of mice to 14.7±2.4. Table 4 recorded that the administration of the sample at 50 and 100 mg/kg i.p did not affect the onset of sleep, but significantly prolonged the duration of pentobarbital sleep dose dependently. Similarly, Table 5 showed that 50 and 100 mg/kg administered i.p inhibited fortwin induced climbing dose dependently.

Conclusion

The importance of linear phenothiazine compounds as antipsychotic drugs has long been recognised. Compound **13** is the first angular pentacyclic phenothiazine to possess neurosedative properties. The hole board experiment is a measure of exploratory activity and a decrease in this parameter revealed sedative effects. The procedure has been accepted as a parameter for evaluating anxiety condition. The decrease

Table 4. Effect of sample on pentobarbital induced sleep in mice.

Treatment	Dose{(mg/kg}	Duration of sleep (minutes)
Normal saline	10 ml/kg	52.6±4.2
Sample	50	92.8± 5.6s
Sample	100	126±2.6
Diazepam	1	82.8±3.4

Table 5. Effect of sample on fortwin induced climbing in mice.

Treatment	Time (minutes)		
	1 0	20	30
normal saline 10ml/kg	0	1	2
Sample 50 mg/kg	1	0	0
Sample 100 ml/kg	0	0	0

The values are expressed as follows: 0= four paws on the floor, 1= fore feet holding the vertical bars, 2= fore feet holding the bars.

in spontaneous motor activity and potentiation of pentobarbital induced sleep strongly suggest central depressant activity. The ability of Compound **13** to antagonise fortwin - induced climbing behaviour in mice has been correlated with neuroleptic potential.

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