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Palladium Catalyzed Cross-Coupling of Pentacyclic Phenothiazines: Novel Therapeutic Target for Positive and Negative Symptoms of Schizophrenia in Experimental Animal Models

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ABSTRACT

8-Ethynylbenzene 12H-5,14-dihydroquinoxalino[2,3-a]pentacyclic phenothiazine (22) was prepared from the reaction of ethenylbenzene with stoichiometric amount of 8-chloro-12H-5,14-dihydroquinoxalino[2,3-a] pentacyclic phenothiazine according to water mediated catalyst preactivation. Structures were established by analytical and spectra data. In this study we used pharmacological methods to evaluate the effects of the novel phenothiazine compound (22) on PCP-induced behaviours. The compound was found to decrease exploratory activity in animals, which is a parameter for evaluating anxiety conditions. The novel compound also attenuated hyper-locomotion caused by PCP challenge dose and time-dependently. Treatment of animals with a combination of PCP and compound (22) significantly enhanced their social behaviour dose-dependently. The study provided evidence that the novel compound (22) mediated procognitive effects, attenuating PCP-induced deficits in Novel Object Recognition (NOR) in albino mice. The ability to reverse PCP-induced Novel Object Recognition deficits evidenced neuro-protective effects rested in the novel phenothiazine compound.

Keywords: 8-Ethynylbenzene 12H-5,14-dihydroquinoxalino[2,3-a] penta-cyclic phenothiazine; Compound 22; PCP-induced; Novel object recognition; Exploratory activity

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INTRODUCTION

Nitrogen containing structures are recognized in numerous bio-active natural and synthetic products. The principle of palladium catalyzed cross-couplings is that two molecules are assembled on the metal via the formation of the metal-carbon bonds, which is followed by the coupling of molecules to one another leading to the formation of a new carbon-carbon bond [1].

Phenothiazine compounds are one of the most recognized from the view of biological activity in many areas of medicine. Particularly in neuro degenerated diseases [2-5], and their anti-dopaminergic effect. They non-selectively inhibit all the five types of dopamine receptors particularly their D2 receptor blocking activity. In this regard, phenothiazine possessing antipsychotic properties can inhibit symptom of schizophrenia [6,7]. Schizophrenia is a severe mental illness characterized by disintegration of the process of thinking and emotional responsiveness [8]. There are two types: The positive symptoms which are delusions and hallucination, while the negative symptoms are social withdrawal, impairment of ego boundaries and loss or energy and initiative.

It was established that excess dopamine in the brain was the main cause for the condition of schizophrenia [9-12]. The claim was further supported by recent research that psychotic patents release more dopamine when stimulated with amphetamine [13-18].

Dopamine is a neurotransmitter responsible for the regulation of man's body activities such as sleep, motion control, cognition memory and host of other body functions [19]. It is synthesized by the anterior part of the brain.

As a neurotransmitter, dopamine is released by neurons to pass on signals to other neurons in the brain, acting as a chemical messenger in the central nervous system. The dysfunction of the dopamine system is responsible for various nervous disorder such as schizophrenia [19].

The chemistry of the linear aza phenothiazines of types (Figure 1):

1. 2,3,6,9-tetra-aza phenothiazine [20];
2. 3-Cl-1,2,6,7-tetra-aza phenothiazine [21];
3. 3-Cl-1,2,7,8-tetra-aza phenothiazine [22];
4. 3-R1-[benzoa], 11-R2,-1,6,8,10-tetra-cyclic phenothaizine, is well developed [23-33];

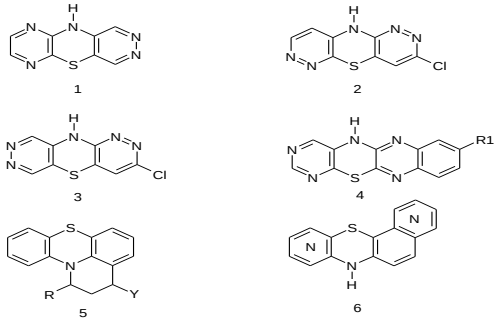


Figure 1. Tetracyclic phenothiazine structures of types 1-5

While tetracyclic phenothiazine of type 5: (N-(2,3-dihydro-1H-pyrido[3,2,1K] phenothiazine have previously been reported [34]. The nonlinear phenothiazine of type 6 has been documented also. [35].

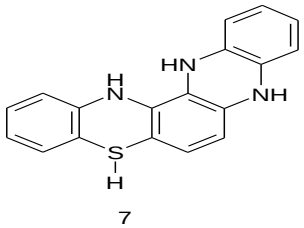


Figure 2. Penta-cyclic phenothiazines structure of type 7

Still grossly understudied are the nonlinear penta-cyclic phenothiazines of type 7: 13 H-5,14-dihydroquinoxalino[2,3-a] phenothiazine [36], in spite of their known neuro pharmacological activity (Figure 2).

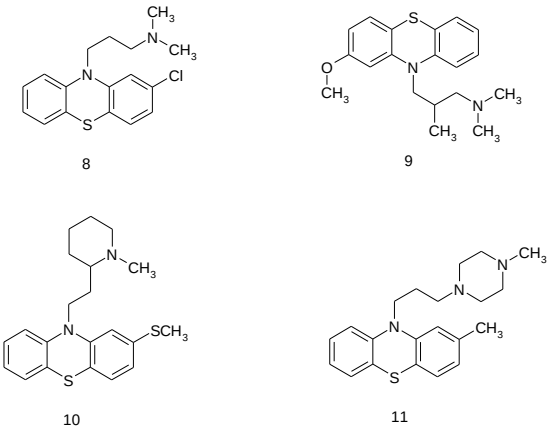


Figure 3. Linear phenothiazines structure of types 8-11

Linear phenothiazines of types 8 (Chlorpromazine); 9 (Levomepromazine); 10 (Thioridazine) and 11 (Tyfluoperazine) have been documented as phenothiazine-based antipsychotics in various forms and effective inhibitors of dopamine receptors (Figure 3) [36-39].

The dihydroquinoxalino pentacyclic phenothiazine of type (22) to the best of our knowledge has not been documented.

In this paper we have synthesized a new penatcyclic phenothiazine (22) with excellent yield, under mild conditions and reported its robust schizophrenic inhibitory behavior.

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. All the chemicals were obtained from different sources (Lavans, Aldrich, Merck) and were used without further purification.

The melting points (M.P) were determined on a SMP3 melting point apparatus and were reported in °C on Scharian silica gel 60(70-230 mesh). Elemental analysis was performed using a Perking-Elmer 2400 CHN analyzer. The Infra Red (IR) spectra were recorded in cm^{-1} on a Bulk-Scientific 500 spectrophotometer. The ^{1}H NMR and 13CNMR was 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 (5 m) column at room temperature (eluent: methanol/water 8:2 v/v).

Drugs

Diazepam and Nitrazepam were obtained from Roche Nigeria Ltd. While pentobarbital, phencyclidine hydrochloride, apomorphine were obtained from Sigma Chemical Company, USA. 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 (180-200 g) of either sex were used. All the animals were maintained at the Animal Facility Centre of Kogi State University at standards conditions and temperature (25°C) and fed with standard diet and water ad Libitum.

Acute Toxicity Studies

Acute toxicity (LD50) was determined following the method described earlier [40-45]. Animals were divided randomly into six groups of six mice each. The samples were administered intraperitoneally (i.p.) in the range of doses 2.5, 5, 10, 25, 50, 75,100, 250, 500, and 1000 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.

Synthesis

The synthetic routes for the novel anti-schizophrenia compounds are outline in Schemes 1 and 2.

8-Chlorophenothiazine (13): A mixture of the corresponding 3-chlorodiphenylamine 12 (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 evaporative. The product was dried and purified by column chromatography. Yield 13:11 g (83:20%). M.p.`187-189°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 MHz, DMSO): 7.0 6 (s.7H), 6.99-6.92 (m,1H), 6.98-6.91 (m,9H), 11.48(s,NH proton). 13C NMR (50 MHz ,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 C12H8NSCl: 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 (14): Concentrated nitric acid (10 ml, 0.5 mol) was placed in 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 (13) (15 g, 0.07 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 23.42 g (96%). M.P. 160-161°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: 320 nm. ^{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). 13C NMR: 141.8 (C. arom. ring), 121.9 (CNH), 130.8 (CN02), 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. C12H7N2O2SCl: 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 (15): Iron powder (20 g. 036 mol) was added portion wise to 8-chloro-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 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: 15.61 g (81.3%). 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. `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,NH2 protons). 13CNMR: 141.5 (Arom. Ring C), 118.6 (CNH), 163.9 (CNH2), 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. C12H11N2SCl2: 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 (16): In a 100 ml beaker, 3.20 g (0.13 mol) of 8-Chloro-1-amino phenothiazine 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 mol 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: 18.43 g (85.34%); m.p 163-165°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(CH3 groups) 1400-1000 (C-Cl stretch) UV: 262.0 nm. ^{1}H NMR: 7.29(d.2H), 7.09 (d.3H), 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,-CH3).

13CNMR: 144.6 (C aromatic ring), 54.6 (CNH), 55.3 (OCH3), 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 C14H11N2OSCl: 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-cloro-1-amino-2-nitrophenothiazine (17): Powdered 8-chloro-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 00C, 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 500 ml cold water and allowed to cool for 15 mins, 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 compound. Purification was by column chromatographic method. Yield 219 ml (97.7%), UV: 540 nm, 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}H NMR: 7.23 (d.1H), 6.98 (d.3H), 6.95 (s.6H), 6.82 (m, 7H) 8.80 (d.9H), 8.30 (m, NH protons), 6.71 (m, NH_2 protons).

13CNMR: 142.8 (C aromatic ring), 121.7 (CNH), 167 (CNH2), 130.6 (CNO2), 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 C12H8N3O2Scl: 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 (18): Compound 18 which is an isomer of compound 17 was synthesized by using similar method as in 17 above. Yield: 69 ml (33.4%). UV: 490 nm; IR: 3693-3100 (hydrogen bounded N-H). 2899 (Ar. C-H), 1376 (Ar. C-N), 1642 (Ar Skeleton). ^{1}H NMR: 6.80 (d.1H), 6.20 (d.2H), 7.92 (s.6H), 5.89 (m.7H), 7.50 (d.9H), 8.11 (m, NH protons), 6.67 (m.NH2 protons).

13C NMR: 38.6 (C. arom. ring), 119.2 (C-NH), 165.5 (C-NH2), 148.4 (C-NO2), 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 C12H8N3O2SCl:

8-Chloro-1,2-diaminophenothiazine trihydrochloride (19): 4 g (0.07 mol) of iron powder was added to a warm suspension of 8-chloro-1-amino-2-nitrophenothiazine (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 mins in a water bath. The resulting suspension was filtered hot and the filtrate treated with excess concentrated hydrochloric acid.

Yield: 300 ml (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}H NMR: 7.29 (d.1H), 7.60 (m .NH protons), 570 (m, NH2 protons).

13C NMR: 144.6 (C-aromatic ring), 119.5 (CNH), 169.5 (CH2) 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: C12H13N3SCl4: 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] penta-cychlic phenothiazine (20): A mixture of 8-chloro-1,2-diaminophenothiaine trihydrochloride, 19 (10 ml) and catecol (7.5 g) was refluxed with ethanol (30 ml, 3 times for 1 h) and filtered off. The product was dried and purified by column chromatography. Yield: 18.41 g (98.6%). M. P. 174-175°C. UV: 320 nm. 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}H NMR: 6.83 (d.1H), 7.89 (d.2H), 7.62 (s.44), 5.88 (m NH protons), 7.29 (dd.7H), 7.08 (d.9H), 6.98 (dd.13H), 6.54-8.36 (Aromatic protons), 7.93 (S. 3H), 6.85 (d.14H).

13C 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 C18H16N3SClO2: 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-ethenylbenzene-12H-5,14-dihydroquinozalino [2,3-a] pentacyclic phenothiazine (22): The carbon-carbon bond was constructed according to previously documented reports [5,17,26,28,33].

The arylated styrene (compound 22) was prepared from the reaction of ethenyl benzene (21) with stoichiometric amount of 8-chloro-12H-5,14-dihydroquinozalino [2,3-a] pentacyclic phenothiazine (20) according to water mediated catalyst preactivation method as previously reported [5,33]. The reaction mechanism involved a syn migratory insertion of the ethenyl benzene (21) into the oxidative addition product. This was followed by a syn β-hydride elimination of the hydridopalladium.

The reaction detail is given in Scheme 2, while the reaction mechanism is represented by the catalytic cycle shown in Figure 4.

Formation of Pd(0) from $Pd(OAc)_2$: The active catalyst was formed by heating a mixture of Pd(OAc)2 (0.80 g, 5.30 mmol), 9 mmol H_2O and triphenylphosphine ligand (4.01 g, 0.071 mmol) in benzene (5 ml) for 1.5 min.

Oxidative addition of 8-chloro-12H-5,14-dihydroquinoxalino [2,3-a] pentacyclic phenothiazine (20) and insertion of ethenylbenzene (21): The reaction mixture containing $Pd^0L_2(OAc)$ was transferred into a vessel containing a mixture of (5.10 g, 2.2 mmol) 8-chloro-12H-5,14-dihydroquinoxalino [2,3-a] pentacyclic phenothiazine (20), NEt_3 (2.2 mmol) and ethenylbenzene (21) (0.62 g, 2.61 mmol). The entire mixture was heated to 100°C for 2 min and refluxed for 2 h. The product was recrystalized from DMF/H_2O to afford the corresponding 8-ethenylbenzene-12H-5,14-dihydroquinoxalino [2,3-a] pentacyclic phenothiazine (22)**:**

Yield: 21.26 g (97.3%). M.P.181-183°C. UV: 460 nm

IR: 3773 (N-H stretch), 2100-2260 (C=C stretch),
2910-2519 (Ar.CH), 1660-2000 (Ar. ring stretch),
1030-1230 (C-N).

^{1}H NMR: 590 (m NH protons), 7.10-8.36 (Ar. protons)
1.90 (d.5H), 6.85 (d.14H)
^{13}CNMR: 119.7 (C-NH), 116 (C=C), 145.7 (C-Ar. ring),
120, 9, 128.2, 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,
147.3 (CH and C).

Anal. Cal. For $C_{26}H_{19}N_3S$: C, 53.06; H, 38.78;
N, 6.12; S, 2.04%. Found: C, 53.04; H, 38.76;
N, 6.10; S, 2.03 %.

POSSITIVE SYMPTOMS OF SCHIZOPHRENIA TEST

Apomorphine (Fortwin)-induced Hyperactivity

Adult mice were randomly divided into 3 groups of 10 mice each. The first group received normal saline (100 ml/p.o) and served as control. Groups 2 and 3 received the phenothiazine compound (22) at doses of 5 and 10 mg/kg i.p.

30 min after treatment all the mice were treated with Apo morphine 3 mg/kg [39-43]. Readings were taking at 10, 20 and 30 min after apo morphine administration.

The mice were observed for climbing and scored as follows:

0=four paws on the floor
1=forefeet holding the vertical bars
2=forefeet holding the bars

Test on Exploratory Activity in Mice

We adopted the method as previously reported [23,35,40]. Mice were divided into four groups of six mice each. Groups 1 and 2 animals were treated with compound **22** at doses 5 and 10 mg/kg i.p respectively, while group 3 received normal saline (10 ml/kg, i.p) and served as control. Animals in group 4 were treated with diazepam (a known neuro sedative) 2 mg/kg i.p [12,22].

30 min after the drugs were administered, the animals were placed individually in an automatic Letca 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.

NEGATIVE AND COGNITIVE SYMPTOMS OF SCHIZOPHRENIA TEST

Phencyclidine (PCP)-induced Social Behavior Deficits

Locomoto experiment: The method of previous studies [10,29,31] were adopted but with slight modification. Wista rats (180 to 200 g at the start of the experiment) were selected ad used for the study.

The animals were randomly divided into three groups. They were accommodated 5 rats per cage. All the cages were kept in a room maintained at 25 ± 1°C, 12 h light and 12 h dark cycle. They were allowed free access to food and water ad libitum in their respective cages. The rats were left for one week environmental habituation.

Groups 1 animal were injected (10 ml/kg i.p) saline. Motor activity of the rat 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.

In the same experiment, animals in group 2 were administered phencyclidine hydrochloride 10 mg/kg dissolved in saline, intraperitonealy (i.p). The effect of phencyclidine-induced motor activity was recorded as above [32] for a period of 120 min at 30 min interval. Group 3 animals were administered (5 and 10 mg/kg i.p) phenothiazine compound (22). 10 days after the administration, the rats received (10 mg/kg i.p) phencyclidine hydrochloride and immediately returned to the Letica activity cage floor. The activity was also recorded for 6 min at 30 min intervals for a period of 120 min.

Social interaction test: This assay is designed to measure the ability of a mouse to make contact with a new one [30,31].

Wister rats (male) weighing between 200-210 g at the start of this study were selected. They were randomly divided into three groups and accommodated 5 rats per cage. The room housing the cages was maintained at 25 ± 1°C, 12 h light-dark cycle and fed with food and water *ad Libitum* [46-49]. All the rats underwent one week of habituation to the environment.

Animals in group 1 received (10 ml/kg, i.p) saline and served as control, while those in group 2 were administered 10 mg/kg intraperitoneally (i.p) Phencyclidine hydrochloride twice daily (morning/evening) for a period of 7 days. The animals were allowed to undergo two weeks washout period [10,48-50].

After the washout period the animals were subjected to social interaction test as reported by Maple *et al* 2017 but with modifications.

Group 3 Wister rats received for 7 days 10 mg/kg i.p compound 22. 10 days later, the rats received phencyclidine hydrochloride (10 mg/kgi.p).

Social interaction testing was carried out during the light time of the light-dark cycle [46,47]. All the animals were weighed again on the day of social testing. The rats (one white and one black) that had received identical treatment and that were not familiar to each other were placed into an unfamiliar room 90-100 cm apart [44].

Interaction between rats was recorded under red lights using video camera to detect its locomotion tested after over 10 mins [30,50-55].

The camera detected the contacts between the animals when they were 3.5 cm apart from each other. Based on previous report earlier [31], the social contacts between the experimental rats were rated by the velocity of the animals' approach. The velocity of approach was classified to be either active or passive contact.

PCP-induced cognitive dysfunction (learning and memory impairments)

This experiment allows the evaluation of cognitive searching behavior as a negative index for learning and memory impairment of schizophrenia.

1. **Test for novel object recognition (NOR)**

Animals: Swiss Albino mice (20-30 g) at the beginning of this experiment were employed for this study. The animals were randomly housed in three groups of five in standard cages and kept in a room maintained under controlled laboratory conditions at 25 ± 1 °C, 12 h light and 12 h dark cycle. Standard rodent food and water were provided ad libitum.

Groups 1 and 2 animals were injected subcutaneously (S.C) 3 ml/kg saline and PCP (10 mg/kg) once daily for 10 days [24,56-59].

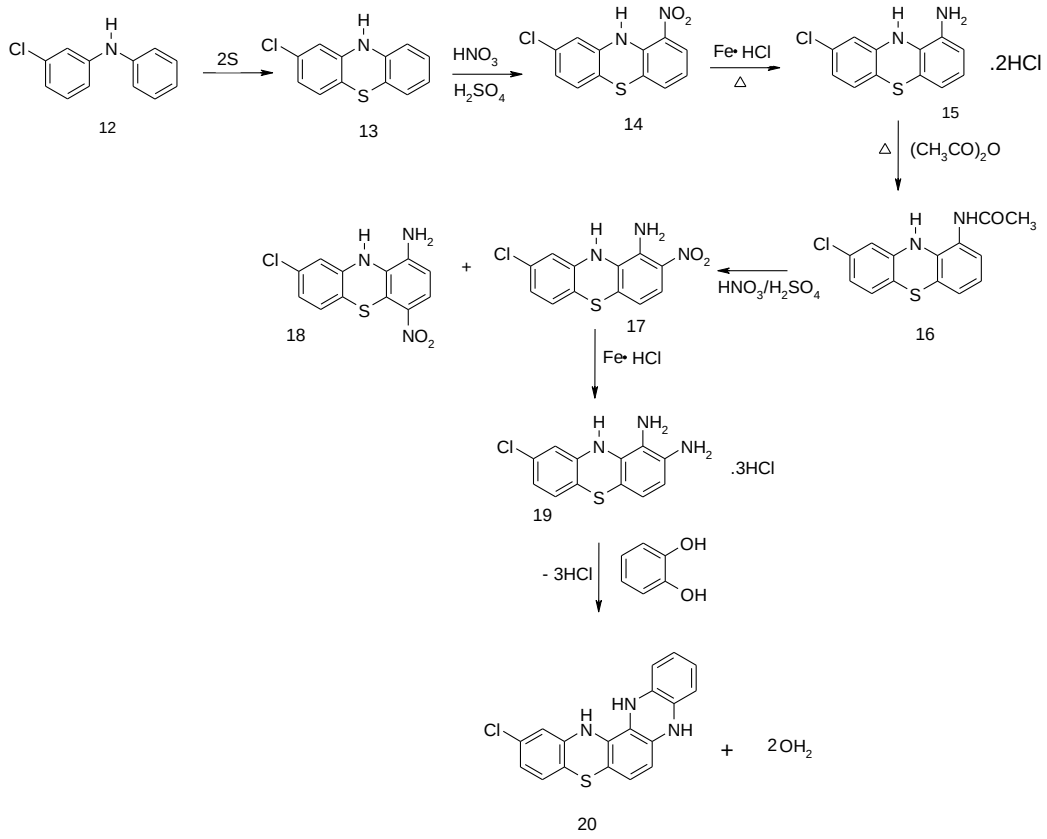
The mice in group 3 were administered i.p 10 mg/kg compound 22 for 10 days also. This was followed by i.p administration of 10 mg/kg PCP for another 10 days.

At the end of drug treatment, the animals were subjected to 10 min habituation to a wooden box. The NOR testing followed the principle as reported by [11,15,16,24,31].

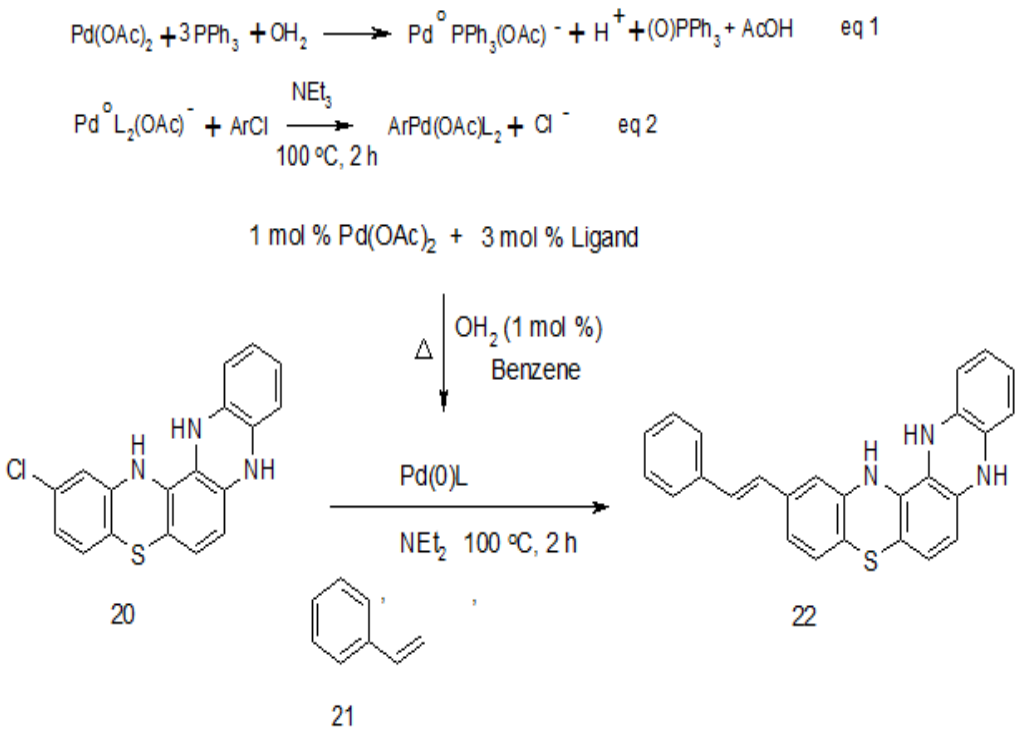
The aim of the habituation stage was to familiarize the mice with the new chamber, and were subsequently allowed to explore the chamber for 10 min for 4 consecutive days (Training test session). At the end of the 4 days training test period, each animal was exposed to two identical objects (one coloured Red, while the other was coloured Black, placed in the chamber) for a period of 120 min.

While in the chamber, exploration by the animals was scored after every 20 min. The animal touching the object with its fore paws while sniffing was measured [24].

24 h later, the object coloured Red was replaced with another one coloured Yellow and the mice were reintroduced to the chamber. The percentage exploration preference during this retention test session was also measured after every 20 min for a period of 120 min. The results are as shown in Figures 5 and 6.



Scheme 1. Synthetic routes for all the compounds



Scheme 2. Palaldium catalyzed cross-coupling of 8-chloro-12 H-5, 14-dihydroquinoxaline[2,3-a]pentacyclic phenothiazine

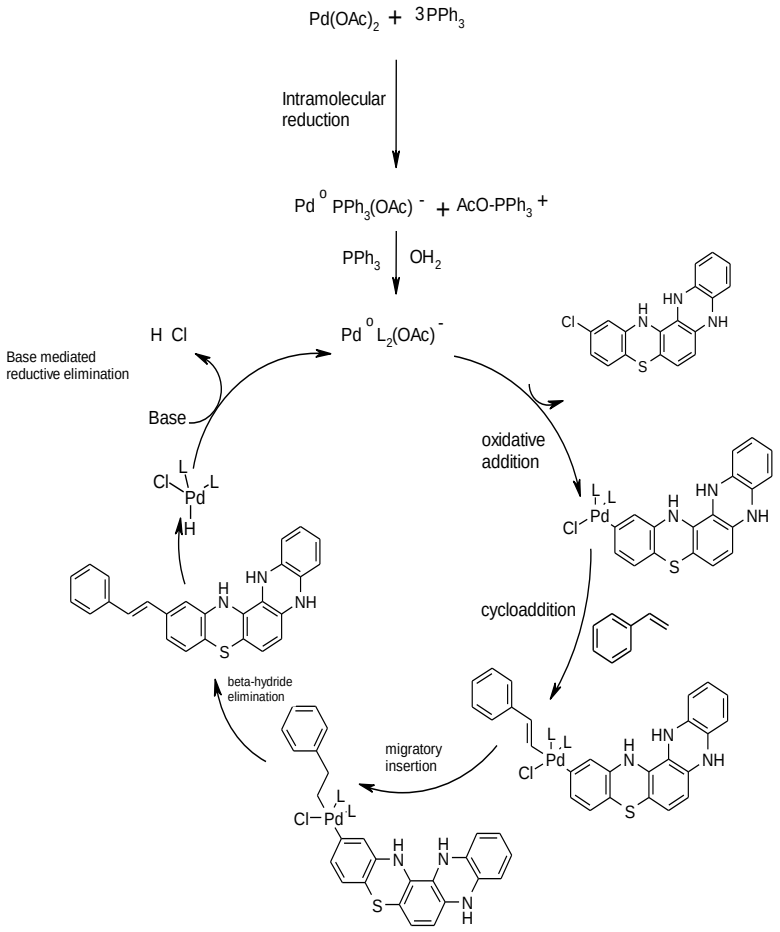


Figure 4. Catalytic cycle for palladium catalyzed cross-coupling of 8-ethenylbenzene-12H-5,14-dihydroquinoxalino [2,3-a] pentacyclic phenothiazine

RESULTS

8-chlorophenothiazine (13) was synthesized by fusing 3-chlorodiphenyl amine (12) 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 (13) was nitrated with mixed acid at 50°C while avoiding polynitration to afford 8-chloro-1-nitro phenothiazine (14).

Reduction of (14) with iron in dilute hydrochloric acid while heating gave 8-chloro-1-aminophenothiazine dihydrochloride (15).

The amino group of this compound was subsequently protected using acetic anhydride to afford (16). Nitrating compound (16) in mixed acid yielded two isomeric compounds: (17) and (18). Reduction of (17) using iron in dilute hydrochloric acid furnished 8-chloro-1,2-diaminophenothiazine trihydrochloride (19), 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 (20): (Scheme 1).

The structural assignment of the synthesized compounds is based on the spectral data. In the IR spectrum of compound (13), the hydrogen bonded N-H stretching appeared at 2999 cm^{-1}. The C-N aromatic was located at 1300-

1411 cm^{-1}, while that at 1400-1000 cm^{-1} is characteristic of C-C stretching. There were number of peaks at 2859-2861 cm^{-1}, 717 cm^{-1}, 1197-1211 cm^{-1} representing aromatic C-H, C-H bending and C-H in plane respectively.

The ^{1}H and ^{13}C NMR studies of compound (13) confirmed the structure.

Compound (13) when nitrated using mixed acids yielded 8-chloro-1-nitrophenothiazine (14). The IR spectrum of this compound showed broad band at 3284-3180 cm^{-1} for hydrogen bonded N-H stretching. The bands 2910 cm^{-1}, 972 cm^{-1} and 1611-1462 cm^{-1} were noticed for C-H stretching, C-H bonding and aromatic skeleton respectively. The aromatic nitro group vibration was shown at 1580-1550 cm^{-1} and 1344-1322 cm^{-1}, while that for C-C stretching appeared at 1400-1000 cm^{-1}.

Compound (14) was reduced with iron to give 8-chloro-1-aminophenothiazine dihydrochloride (15).

The N-H stretching and aromatic C-H stretching of this compound 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 characteristics of aromatic amines. The bands at 1684 to 1698 cm^{-1} are that of aromatic skeletal system.

In ^{1}H NMR spectra data, compound (14) showed a singlet at ∂10.30-9.82 due to N-H protons. This was shifted to ∂ 89.76 in 8-chloro-1-amino phenothiazine dihydrochloride (15). This shifting towards up field in compound (15) is ascribed to intramolecular hydrogen bonding as –NH--O=N in compound (14). The ^{13}C NMR studies of this compound confirmed the structure. A characteristics signal appeared for aromatic ring carbon (Arom. ring C) at ∂ 8141.5, while the signal at ∂ 8118.6 and ∂ 8163.9 were located for (C-NH) and (C-NH_2) respectively. Subsequently, compound (15) was reacted with acetic anhydride to protect the amino group from further nitration. This reaction afforded 8-chloro-1-acetylaminophenothiazine (16).

In compound (16), the hydrogen bonded N-H stretching appeared at 3670 cm^{-1}, while the bonds 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 2671 cm^{-1} indicated C=O stretching, while CH_3 stretching appeared at 2388 cm^{-1}.

In the ^{1}H NMR spectrum of compound (16), the multiplet for –NH protons appeared in the region ∂ 6.53 to 8.36. The –OCH_3 protons and –CH_3 protons in the compound showed a singlet in the region ∂3.98-3.94 and ∂2.19 to 2.26 respectively, indicating a complete acylation of compound (15).

A characteristic signal appeared for (C-aromatic ring), (C-NH) and (O-CH_3) in the range of ∂144.6, ∂54.6 and ∂55.3 respectively in the ^{13}C NMR spectrum.

The nitration of compound (16) yielded two isomeric phenothiazines: 8-chloro-1-amino-2-nitrophenothiazine (17) and 8-chloro-1-amino-4-nitrophenothiazine (18). The IR spectrum of compounds (17) and (18) showed broad bands at 3698 to 3100 cm^{-1} indicating hydrogen bonded N-H stretching. The absorption band at 2912 cm^{-1} is characteristic of aromatic C-H stretching. The band at 1370 cm^{-1} was for aromatic C-N stretching, while the aromatic skeletal was located at 1644 cm^{-1} and 1473 cm^{-1}. Similarly, in compound (17), N-H_2 proton appeared as multiplet at ∂6.71, while in ^{13}C NMR spectrum, a characteristic signal appeared for (C NH_2) and (C NO_2) in the range of ∂167.3 and ∂130.6 respectively. These signals were found absent in compound (16) indicating a successful nitration of compound (17). The nitro group is responsible for the broad shoulder at 1195 cm^{-1}.

Subsequent reduction of compound (17) furnished 8-chloro-1,2-diamino phenothiazine trihydrochloride (19). The IR spectrum of (19) showed a broad band at 3671-3200 cm^{-1} for hydrogen bonded N-H stretching. The band at 1477

cm^{-1} is assigned to C-N stretching, while the aromatic skeletal system was located at 1641 and 1477 cm^{-1}. In the ^{1}H NMR spectrum, compound (19) showed two signals for NH and NH_2 protons at ∂7.60 and ∂5.70 respectively. A characteristic signal appeared for CH_2 in the range of ∂119.5 in the ^{13}C NMR spectrum, while that of C-NH_2 was established at ∂169.5.

Refluxing a mixture of compounds (19) and catechol with ethanol yielded the pentacyclic products-8-chloro-12H-5, 14-dihydroquinoxalino [2,3-a]pentacyclic phenothiazine (20). The IR spectrum of compound (20) 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 located for C-H out-of-plane bending and in-plane bending respectively. The absorption bands at 1660-2000 cm^{-1} was for Aromatic ring stretching. There were other peaks at 1030-1230 cm^{-1} and 600-800 cm^{-1} which were for C-N and C-Cl respectively. These clearly support the fact that compound (18) was not used in the synthesis of the novel product (20).

In the ^{1}H NMR spectrum, compound (20) showed singlet for N-H phenothiazine at ∂5.88.Tthe ^{13}C NMR spectrum detected some signals at ∂145.7, ∂119.7 and ∂116.11 for C-aromatic ring, C-NH and C=C respectively.

The absorption at 211 nm in the UV-visible spectrum of compound (13) resembles that of benzene, while the shift in wavelength to 320 nm in compound (14) indicated the presence of auxochrome type-NMR in phenothiazine.

The UV spectrum of compound (15) showed maximum absorption at 312 nm. No appreciable bathochromic shift because the compound is in the form of hydrochloride. Compound (17) 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 (20) exhibited a UV maximum at 320 nm characteristics of phenothiazine systems.

In the ^{1}H NMR spectrum, compound (20) displayed a signal at ∂5.88 for NH protons, while the multiplet for aromatic protons appeared in the region between ∂6.54-8.36.

In the ^{13}C NMR spectrum of compound (20), a characteristic signal appeared for (C NH) in the range of ∂119.7.

8-ethenylbenzene-12H-5,14-dihydroquinozalino(2,3-a)Pentacyclic phenothiazine (22) was prepared according to water mediated catalyst pre-activation method as previously reported and shown in Scheme 2 and depicted by the catalytic cycle in Figure 4.

The active catalyst was formed by heating a mixture of $Pd(OAc)_2$, H_2O and triphenylphosphine ligand.

The reaction mixture was transferred into vessel containing a mixture of compound (20), NEt_3 and ethenylbenzene (21). The entire mixture was heated and refluxed. The refluxed product was recrystallized to afford compound (22).

The structural assignment of this compound is based on the spectra data. In the IR spectrum of compound (22), the hydrogen bonded N-H strectching appeared at 3773 cm^{-1}, while that at 2100-2260 cm^{-1} is characteristic C=C stretching. The bands at 2910-2519 cm^{-1} appeared for aromatic C-H out-of-plane bending and in-plane bending respectively. The absorption band at 1660-2000 cm^{-1} was for aromatic ring stretching.

^{1}H and ^{13}C NMR studies for this compound (22) confirmed the structure. In the ^{1}H NMR Spectral data, this compound showed multiplet at ∂5.90 and ∂7.10 due to NH and Ar-H protons. A characteristic signal appeared for (C-NH), (C=C) and (C-aromatic ring) in the range of ∂119.7, ∂116 and ∂145.7 respectively in the ^{13}C NMR spectrum.

The results for Fortwin-induced hyperacivity and test on exploratory activity in mice (positive symptoms of schizophrenia) are as presented in Tables 1 and 2, while those for negative symptoms are given in Tables 3 and 4.

The acute toxicity studies of the phenothiazine compound (22) was found to be relatively safe as no lethality was observed at even 1000 mg/kg i.p. in mice.

On the exploratory activity, the synthesized compound was found to be dose-dependently inhibiting exploratory activity in mice. The effect was similar to that of diazepam (2 mg/kg). The observed effect was statistically significant from normal saline that served as control (Table 2). From Table 1, apart from the compound (22) to be dose-dependent in inhibiting Apo morphine (fortwin)-induced hyperactivity, the inhibition was also found to be time-dependent.

Table 3 results showed that the phenothiazine compound (22) also exhibited a dose and time-dependent decrease in locomotor activity in rats.

During the social interaction test, the normal saline treated rats as recorded by the camera made inspection of the entire arena together. The two unfamiliar animals explored and interacted. The amount of time the rats engaged in the interaction was recorded.

On the other hand the group two animals that received phencyclidine hydrochloride (PCP) moved along the corners of the chamber avoiding each other, while displaying a high level of unfriendliness.

The administration of the phenothiazine compound (22) was captured by the video camera to have significantly increased the level of social interaction. Statistical comparison between normal saline and PCP treated rats, as well as those that received phenothiazine (22) treatment revealed that the amount of time the group 1 rats engaged in social interaction was noted to have reduced when compared to the amount of time the group 2 animals that received phencyclidine hydrochloride.

However, the reduction in the amount of time was reversed in the group 3 rats treated with compound (22). This camera captured reduction showed that the novel phenothiazine reversed the effect of phencyclidine-induced social deficits. The results are tabulated in Table 4.

The results of the novel object recognition test are presented in Figures 5 and 6.

The percentage exploratory preference during the training test session and retention test session were calculated as a measure of cognitive performance [16,24].

All the experimental animals in the various treatment groups displayed remarkable preference while exploring the RED or BLACK object (Training test session). However during the retention test session, when one of the objects was replaced by a novel one coloured YELLOW, the animals in the various treatment groups also exhibited remarked differences in exploration [15,16,24]. From Figures 5 and 6, the group 2 mice that received phencyclidine hydrochloride (PCP) displayed significant reduction in novel object recognition compared to group 1 treated animals that received normal saline and served as control [15,16,24].

The animals in group 3 that were administered 10 mg/kg phenothiazine compound (**22**) for 10 days followed by intraperitoneal (i.p.) administration of 10 mg/kg phencyclidine hydrochloride (PCP) for another 10 days, displayed greater novel object recognition compared to group 2 mice that receive only 10 mg/kg phencyclidine hydrochloride (PCP) for 10 days also.

Table 1. Apomorphine (Fortwin)-induced hyperactivity

Treatment	Time (Minutes)		
	10	20	30
Normal saline 10 ml/kg	0	1	2
Compound (22) 5 mg/kg	1	0	0
Compound (22) 10 mg/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

Table 2. Effect of compound (22) on exploratory activity in mice

Treatment	Dose mg/kg	Mean score
Normal saline	10.0 ml/kg	48.3 ± 4.7
Compound (22)	5.0 mg/kg	23.6 ± 1.9
Compound (22)	10.0 mg/kg	14.4 ± 5.3
Diazepam	2.0 mg/kg	18.0 ± 6.1

Table 3. Effect of compound (22) on Locomotor activity in rats

Time (minutes)	Dose	Time (minutes)				
Treatment	mg/kg	0	30	60	90	120
Normal saline	10 ml/kg	90.4 ± 1.9	86.3 ± 1.9	83.5 ± 2.5	81.4 ± .1.8	75 ± 2.8
Phencychidine	10 mg/kg	92.0 ± 3.1	104. ± 3.0	128 ± 3.1	120.3 ± 40	107 ± 5.3
Phencyclidine +Compound (22)	5 mg/kg	90.4 ± 1.3	75.5 ± 2.3	54.3 ± 1.4	34.3 ± 1.8	17.3 ± 4.5
Phencyclidine + Compound (22)	10 mg/kg	88.4 ± 2.8	63.5 ± 1.7	36.8 ± 2.4	18.3 ± 2.6	13.7 ± 2.8

Table 4. Effect of Phenothiazine compound (22) on PCP-Induced Active Social Behaviour in rats

Treatment	Dose mg/kg	Time (Mean sec ± SEM)
Normal saline	10 ml/kg	83.43 ± 6.5.2
Phencyclidine	10 mg/kg	81.61 ± 3.41
Phencyclidine +Compound (22)	5 mg/kg	90.53 ± 7.20
Phencyclidine +Compound (22)	10 mg/kg	96.81 ± 9.52

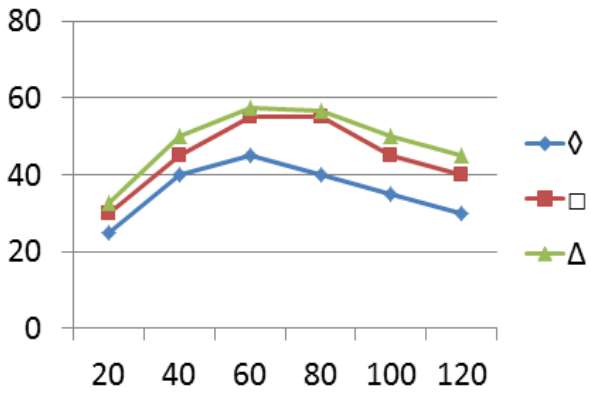


Figure 5: Novel Object Recognition (NOR) Comparative Test (Training Session) Key: ◊=PCP only; □=PCP + Phenothiazine (22); Δ=Normal Saline

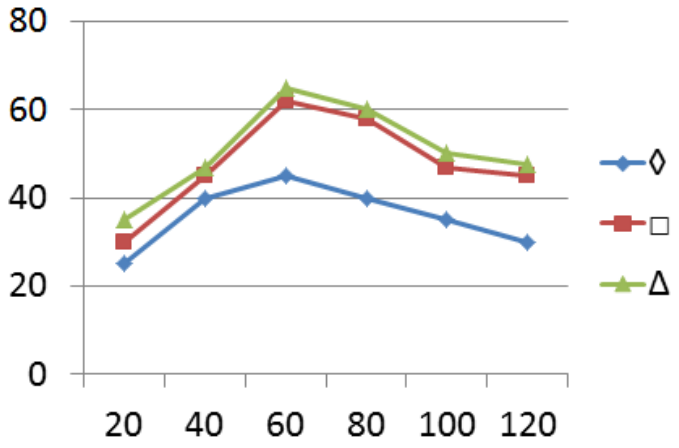


Figure 6: Novel Object Recognition (NOR) Comparative Test (Retention Session) Key: ◊=PCP only; □=PCP + Phenothiazine (22); Δ=Normal Saline

DISCUSSION

The structural assignment of the synthesized compounds was based on the spectra data. The IR spectrum of compound (19) indicated clearly that the isomeric compound (18) was not used in the synthesis of the final product (20). This was further established by the disappearance of C-O absorption (1200 cm^{-1}) in the spectrum of (20).

The pentacyclic product (20) 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 cross-coupling of this compound (20) afforded position-8 substituted compound (22).

The structural assignment of this novel compound (22) was also based on the spectra data. This pentacyclic compound was evaluated for positive and negative symptoms of schizophrenia. The results of this study revealed that the compound has sedative activities on the central nervous system of rodents. The compound was found to decrease exploratory activity (Table 2). File and Wardill (1975) have shown that the hole-board experiment is a measure of exploratory behaviour in animals.

A decrease in this parameter reveals sedative [4], which has also been accepted as a parameter for evaluating anxiety conditions in animals [8].

In this study, we observed that Phencyclidine increased locomotion time-dependently (Table 3). However, treatment with Phenothiazine compound (22) attenuated hyper-locomotion caused by the PCP challenge and decreased locomotion dose and time-dependently. Meaning that the compound produced recovery of agonist-induced by PCP. Locomotor activity is an indication of the level of excitability of the central nervous system. A decrease in this parameter exhibited by phenothiazine compound (22) may be closely related to sedation resulting from depression of the central nervous system [4,38].

The ability of the novel compound to antagonize Fortwin-induced climbing behaviour (Table 1) in mice is correlated with neuroleptic potential.

From Table 4, PCP treated animals engaged in significantly less social interactions compared to those treated with normal saline. Treatment with combination of PCP and compound (22) significantly enhanced their social behaviour dose-dependently.

Our study on phencyclidine-induced cognitive dysfunction provided evidence that the novel phenothiazine compound (22) mediated pro-cognitive effects, attenuating PCP-induced deficits in novel object recognition in albino mice (Figures 5 and 6). The ability of this compound to reverse PCP-induced novel object recognition deficits evidenced a neuro-protective effects rested in the novel compound [24]. However, exploratory preference during the training and retention sessions was time-dependent. This parameter decreased at the end of the 60^{th} minute of exploration which may be related to tiredness of the animals.

CONCLUSION

Nitrogen containing structures has long been recognized in numerous bio-active natural and synthetic products. Compound 22 is the first angular pentacyclic phenothiazine to attenuate hyper-locomotion caused by PCP challenge. Locomotion activity is an indication of the level of excitability of the central nervous system. A decrease in this parameter is closely related to sedation resulting from depression of the central nervous system.

The ability of the novel compound to reverse PCP-induced novel object recognition deficits evidenced neuroprotective effects rested in it.

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