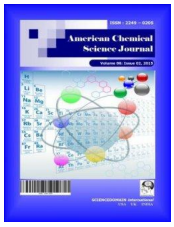

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Synthesis of Pent-aza Phenoxazines: A New Group of Anti-inflammatory Heterocycles. Their Effect and Other Analgesics on Pain

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The synthesis of novel 1-amino-7-methyl-2,4,6,8,9-pent-azaphenoxazine (**13**) and 2-aldehyde-7-methyl-4-phenyl-3,4,6,8,9-pent-aza phenoxazine (**17**) is reported. The pent-aza compound (**13**) was prepared by suspending acetamidine hydrochloride (**8**) in ethanol and treated with anhydrous hydrazine at 0°C to afford a pink solid compound acetamidrazone (**9**). Treatment of compound (**9**) with oxalyl chloride and heated at reflux, yielded 6-methyl-1,4,5-triazine-2,3-dione (**10**). This compound when reacted with 4,5-diamino-6-hydroxypyrimidine (**12**) in ethanol, provided a solid 1-amino-7-methyl-2,4,6,8,9- pent-azaphenoxazin (**13)** in excellent yield. The 2-aldehyde-7-methyl-4-phenyl-3,4,6,8,9- pent-aza phenoxazine (**17**) was obtained utilizing 3-aldehyde-5-amino-6-hydrxy-1-phenylpyridazine (**16**) which was produced by refluxing a mixture of furfural and phenylhydrazine in toluene. Treatment of compound (**16**) with 6-methyl-1,4,5-triazine-2,3.dione (**10)** in ethanol gave the pent-aza-phenoxazine (**17**) in good yield. These compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and MS spectrometry. The two novel products (**13)** and (**17**) were investigated for analgesic actions. The result compared favorably with three known analgesics: Paracetamol,

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Aspirin and Alabukun (a local analgesic). The doses of 0.125 to 0.150 g/kg injected into adult rabbits intraperetoneally lead to reduction of pain threshold. The result of the anti-inflammatory screening data revealed the dose and time dependant effect of these compounds in the carrageenan induced paw oedema. At time of 120mins, there was complete inhibition of oedema by 95.36% and 97.51% from the compounds **13** and **17** respectively, while the standard drug Zerodol (Aceclofenac) showed inhibition by 57.79%. The ability of these two compounds to antagonize carrageenan-induced oedema was correlated with anti-inflammatory potential.

Keywords: 1-amino-7-methyl-2,4,6,8,9-pent-azaphenoxazine; 2-aldehyde-7-methyl-4-phenyl-3,4,6,8,9-pent-azaphenoxazine; pain threshold; oedema; carrageenan-induced.

1. INTRODUCTION

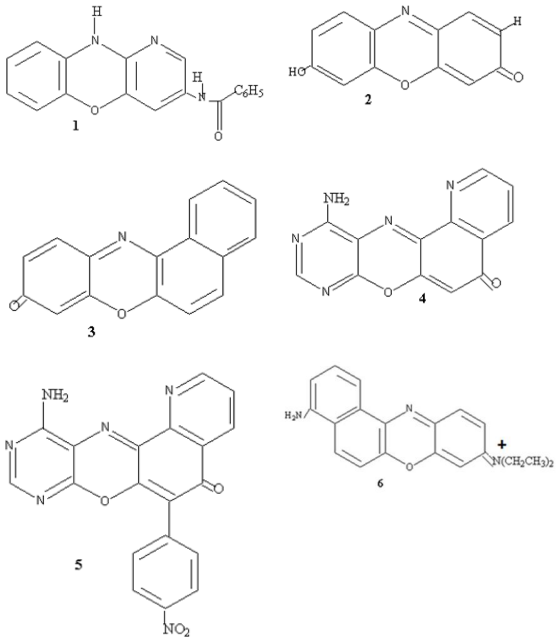
Phenoxazine and its derivatives have not received a great deal of attention either theoretically or commercially. The only derivatives of commercial importance are a few dyes, known as the oxazine dyes [1,2,3]. Some of the oxazine dyes, particularly the Nile Blue dyes, are known to stain brain cancer tissue, while decrease the rate of growth of tumors [1]. The use of phenoxazine derivatives in biological staining is closely related to their use in the treatment of cancer and tuberculosis. Nile Blue A and Nile Blue 2R have been reported to retard growth of cancer [1,4,5].

Phenoxazine derivatives have been reported for industrial application as acid-base indicator [6]. Phenoxazines containing pyrimidine nucleus have received significant pharmaceutical attention. Its ring is in clinical use such as anti-bacterial agents, anticancer, antiviral, antimalarial and antifungal agents [7].

Phenoxazine derivatives have been used also as antiperkinsonian, anticonvulsant, antihistaminic, antiviral and CNS depressants [7-13]. Phenoxazines have also been reported to be an effective multidrug resistant (MDR) modulator in cancer cells [7].

Phenoxazines containing pyridazine system, in recent times have received attention due to the biological activities. These pharmaceutical activities endowed in pyridazine have inspired chemists to synthsize phenoxazine systems containing pyridazine functionality in order to explore the usefulness of these heterocyclic template [14].

Phenoxazine systems of types (**1**): 3-benzamido azaphenoxazine [15]; (**2**): 7-hydroxy-phenoxazine-3one [16]; (**3**): Benzo[a]-phenoxazine-7-one[16]; (**4**): 11-amino-1,8,10-triazabenzo[a]-phenoxazine-5-one [17]; (**5**): 6-(4-nitrophenyl)-11-amino-1,8,10-triazabenzo[a] phenoxazine-5-one [17]; (**6**): Nile Blue A [18] and their known pharmacological and industrial applications have previously been documented.



In continuation of our research programme directed towards the preparation of heterocycles with pharmaceutical recognitions [19,20], Pent-azaphenoxazines of types (**13**) and (**17**) to the best of our knowledge have not been reported.

In this paper we report the synthesis of these novel heterocyclic systems as a new group of anti-inflammatory heterocycles and their effect and other analgesics on pain.

2. 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 melting points(m.p) were determined on a SMP3 melting point apparatus and were reported in °C uncorrected. Column chromatography was performed on Scharian Silica gel 60(70-230 mesh). Elemental analysis was performed using a Perking-Elmer 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 spectra were recorded on a Varian Germini 2000 spectophotometer operating 200 and 50 MH_z respectively. Chemical shifts were recorded as δ 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).

2.1 Animals

In this study we followed the principle of laboratory animal care (NIH publication No 85-23, revised 1985). We employed adult rabbits of either sex weighing between 0.7-1.2 kg for the toxicity and analgesic studies, while the anti-inflammatory studies employed adult Wister rats 200-350g. All the animals were obtained from the local market in Gwagwalada, Abuja, Nigeria and maintained at the Animal Facility Centre of Kogi State University at standard conditions and temperature (25°C) and fed with standard diet (Pfizer feed, PLC, Lagos) and *tridaxprocuben,* carbage and water for one week.

2.2 Synthesis

The synthetic routes for the two novel compounds are outlined in schemes **1** and **2**. The details are given below:

2.3 Acetamidine Hydrochloride (8)

This compound was prepared as in the literature [21]. Solid acetamido ethyl ether hydrochloride (98 g, 3 mol) was broken up and transferred to a dry mortar. 20 ml of super dry ethanol was added to it and grounded to paste before it was returned to the reaction vessel. Alcoholic ammonia solution (700 ml) was added to the contents in the vessel and Stirred mechanically for 4h, filtered and the filterate was 2389(CH_3). ^{1}H NMR: 5.71 (m, NH_2 protons), 5.76 (m, CH_3). ^{13} C NMR: 164 (C-NH), 139 (C=N), 89.5 (CH_3), 88.9 (C-H). Calculated for $C_2H_8N_2Cl$: C, 25.25; H, 8.48; N, 29.45:C.

2.4 Acetamidrazone (9)

9.5g, 1 mol of acetamidine hydrochloride **(8**) was suspended in super dry ethanol (50 ml) and treated with 3.2 g anhydrous hydrazine at 0°C for four minutes. The ethanol content was evaporated at 35°C to give a pink solid. M.p:261-265°c. Yield:30g, 91%. IR: 1446 (C=N), 817 (C-N), 3284-3180 (N-H), 2388 (CH_3 group), ^{1}H NMR: 5.70 (m. NH_2 protons), 5.75 (m. CH_3). ^{13}C NMR: 163 (C-NH), 138 (C=N), 89.4 (CH_3), 88.8 (C-H). Calculated for $C_2H_7N_2$: C, 40.64; H, 11.95; N, 47.41%. Found: C, 40.63; H, 11.93; N, 47.40%.

2.5 6-methyl-1,4,5-triazine-2.3-dione (10)

The synthetic route followed a modification of the methods already described by Naidu et al [22,23]. 0.03 mol, 2.18 g of acetamidrazone **(9**) was suspended in super dry ethanol (40 ml) and treated with oxalyl chloride (0.03 mol, 3.96 g) and heated while refluxing for 2 h. The reaction mixture was allowed to cool and filtered. The resulting solid was crystallized from ethanol/ water. M.p:203-206°C. Yield:22g, 79%. IR: 1556 (C=O), 1585 (C=N), 1170 (C-N), 3235 (N-H), 2389 (CH_3). ^{1}H NMR: 6.8 (s, 1H, NH), 7.6 (4H, s, NH), 5.73 (s, CH_3). ^{13}C NMR: 187.63 (C=O triazine); 182 (s, C6); 27.28 (CH_3); 171.30 (C-NH); 130.73 (C=N). Calculated for $C_4H_5N_3O_2$: C,37.78; H, 3.97; N, 33,06; O, 25.19% Found: C, 37.76; H, 3.46; N, 33.04; O, 25.17%.

2.6 1-amino-7-methyl-2,4,6,8,9-pent-azaphenoxazine (13)

0.1 mol, 12.7 g of 6-methyl- 1,4,5-triazine-2,3-dione (**10**) was mixed with 0.1 mol, 21.7 g 4,5-diamino-6-hydroxypyrimidine (**12**) in ethanol (100 ml) and heated at reflux for 3 h. The reaction mixture was cooled and the resulting solid filtered. The product was crystallized from ethanol/water. M.p:189°C. Yield: 32 g, 98%. IR: 3370 (N-H phenoxazine), 690 (C-O-C), 1439 (C=N0), 817 (C-N). ^{1}H NMR: 7.9 (1H, s,N-H phenoxazie), 5.79 (CH_3), 5.73 (s, NH_2 protons). ^{13} C NMR: 130.1 (C=N, C4), 131.20 (C=N, C8), 28.11(m,CH_3), 138.1(C-NH_2). Calculated for: $C_8H_7N_7O$: C, 14.19; H, 3.25; N,45.13; O,7.37%. Found: C,14.19; H, 3.24: N, 45.13; O, 7.36%.

2.7 3-aldehyde-5-amino-6-hydoxy-1-phenylpyridazine (16)

This was prepared using a modification of the methods already described by Dilek et al. [14] and Alberto et al. [24]. A mixture of 4-amino-5-hydroxyfurfural (1.7 ml 0.02 mol) **(14**) and phenylhydrazine 92.89 g, 0.02 mol) in toluene (20 ml) was heated for 4 h. The solvent (toluene) was removed by evaporation and the oily residue was treated with diethyl ether overnight. The crude product formed was crystallized from acetic acid. M.p: 356-358°C. Yield: 58%. IR: 3341 (N-H pyridazine); 816 (C-N); 1722, 1654 (C=O); 1382 (C=C arom.); 2408 (C-H arom.); 3430 (OH); 1631 (ArH); 3291 (NH_2); 658 (C-O). ^{1}H NMR: 5.39 (s, NH_2); 6.70 (OH); 7.54-7.97 (Ar-H); 5.29 (s, NH pyridazine); 6.7 (C-H). ^{13} C NMR: 138.3 (C-NH_2); 175.6 (s, C4); 137.28-130.20 (m, Ar.C); 129.6 (C-N, C6); 138.3 (C-NH_2); 192.28 (C-N, C6); 192.28 (C=O pyridazine); 78.9 (CH); 112.40 (C=C). Calculated for $C_{11}H_{11}N_3O_2$: C,60.80; H, 5.11; N, 19.35; O, 14.74%. Found: C,60.79; H,5.10; N,19.35; O,14.73%.

2.8 2-aldehyde-7-methyl-4-phenyl-3,4,6,8,9-pent-azaphenoxazine (17)

0.1 mol, 12.7 g of 6-methy-1,4,5-triazine-2,3-dione (**10**) was mixed with 0.1 mol, 32 g 3-aldehyde-5-amino-6-hydroxy-1-phenylhydrazine (**16**) in ethanol (100 ml) and heated at reflux for 3 h. The reaction mixture was cooled, the solid formed was filtered and crystallized from ethanol/water. M.p: 192°C, Yield: 41g.76%. IR: 3451 (N-H pyridazine); 3427 (N-H phenoxazine); 1631 (Ar-H); 1444 (C=N); 816 (C-N); 657 (C-O); 1651 (C=O); 2001 (C-H arom.); 1376 (C=C arom.); 658 (C-O phenoxazine); 2385 (CH_3 groups). ^{1}H NMR: 8.1 (s, NH phenoxazine); 6.9 (m, ArH); 5.36 (N-H pyridazine); 2.97 (s, CH_3). ^{13}C NMR: 186.63 (s, C1); 128.30-134.17 (m, Ar.C); 130.6 (C=N); 131.6 (C=C); 133.8 (C=O). Calculated for: $C_{15}H_{12}N_6O_2$: C, 58.42; H, 3.93; N, 27.26; O,10.39%. Found: C, 58.41; H, 3.92; N, 27.24; O, 10.37%.

2.9 Determination of LD_{50}

The acute toxicity (LD_{50}) of the novel phenoxazines (**13 &17**) was determined in adult rabbits using the methods of Lorke [25], Amos et al. [26], Azuine et al. [27], Gurad et al. [28], and Odin et al. [20]. The animals were divided randomly into eight groups of five rabbits each. Widely differing dozes of 10, 100, 1000, 1500, 2000, 3500 and 5000 mg/kg of the compounds were administered intraperitoneally and orally on groups 1-7. The eight group was administered normal saline (10 ml/kg) and served as control. The animals were monitored for 72 h for signs of toxicity such as spontaneous motor activity, exploratory activity, climbing behavior, paw-licking, convulsions, lacrimation and salivation [19,29-31]. At the end of the experiment, they were sacrificed and then autopsied and examined microscopically for any pathological changes. Pieces of organs, such as liver, heart and kidney were examined for histopathological changes.

2.10 Haemotological Parameters

The effect of the synthesized compounds (**13 &17**) on haemotology of adult rabbits after the administration of the compounds on day 3, (72^{nd} h) was determined. This include haemotological parameters such as Packed cell volume (PVC), haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC). The methods of McDonard et al. [32]; Subhasini et al. [33]; Olamide and Mathew, [29] were employed and modified. The RBC, WBC, Hb and PVC were measured by an auto Hemotology Analyzer. These parameters were quantified in each pent-azaphenoxazine compound (**13&17**) and control. The results are presented in Table 2.

2.11 Test for Analgesia

The hot plate method of Onabanjo et al. [34] was modified thus: The temperature of the flask placed in water-bath was maintained in the range of 55-60°C. The rabbits were shared into twelve groups of four each. Groups **1&2** were administered intraperitoneally 3 ml and 5 ml normal saline respectively, and served as controls. Groups **3&4** were administered 0.125 and 0.150 g/kg doses of aspirin respectively. Groups **5&6** had 0,125 and 0.150 g/kg doses of paracetamol respectively, while groups **7&8** were given 0.125 and 0.150 g/kg doses of alabukun respectively. The chosen concentrations were based on toxicity studies. Animals in groups **9&10** were injected with 0.125 and 0.150 g/kg compound **13**, while those in groups **11&12** were also injected with 0.125 and 0.150 g/kg compound **17**. All the treated animals were left for 30 min for drug digestion. They were then individually placed into the flask and the reaction time was monitored. The reaction time was recorded as the jump latency [35]. Time of maximum intolerance to the heat was regarded as the maximum analgesic period (Table 1).

2.12 Anti-inflammatory Studies

We employed the modified methods of Winter et al. [36], Azuine et al. [27], Ratheesh and Helen, [37], Adeyemi et al. [38] and Haruna and Cyril, [39]. Wister rat comprising male and female weighing between 180-300 g were divided into six groups of five rats each.

Inflamation of the right hind paw was induced by injecting 1% carrageenan suspension in 0.9% NaCl solution into the sub planter surface. Animals in group 1 received normal saline (20 ml/kg) and were labeled as negative control. Group **2** and **3** animals were administered intraperitonealy compound **13** doses of 50 mg/kg and 100 mg/kg respectively. The fourth and fifth group animals were administered compound **17** doses of 50 mg/kg and 100 mg/kg respectively while group six animals were given zerodol (aceclofenac-50 mg/kg) injected intraperitonealy and were designated as positive control. All the drugs were administered 60 minutes before the subplanter injection of the phlogistic agent. The paw volume was measured after every 20 minutes for a period of 120 minutes by a volume displacement methods [27] using a digital plethysmometer, Coulbouru instrument-AHarvard apparatus company (Table 3).

The average inflammation, percentage inflammation and percentage inhibition of Oedema were calculated on each dose and recorded (Table 4, 5, and 6). The average inflammation was calculated according to the methods of Azuine et al. [27]: $L_t - L_0$ where L_t is the linear circumference at the time t and L_o is the linear circumference at zero time (Table 4).

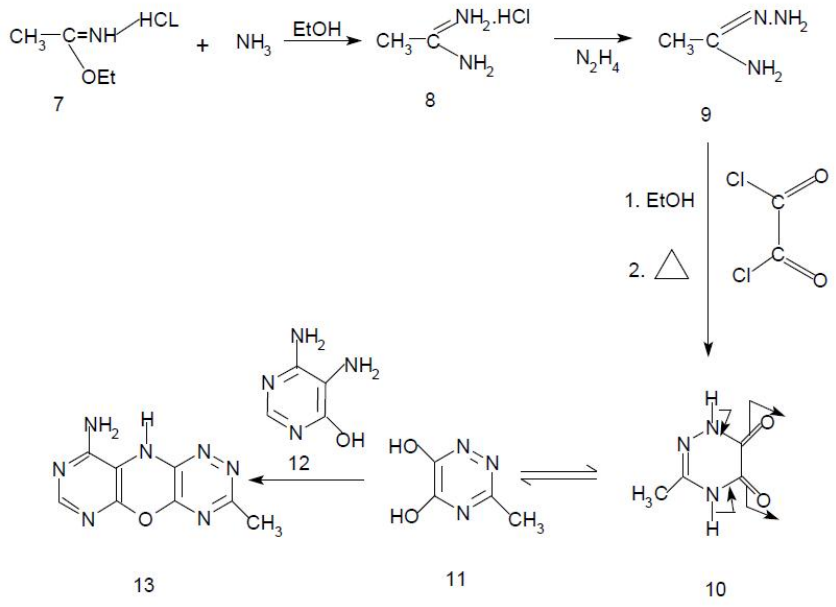
The percentage inflammation was also calculated according to the method of Azuine et al. [27]:

A/B x 100 where A is the average inflammation of treated group at time t while B is the average inflammation of the control at the same time (Table 5). The percentage inhibition of Oedema (Table 6) is calculated as follows:

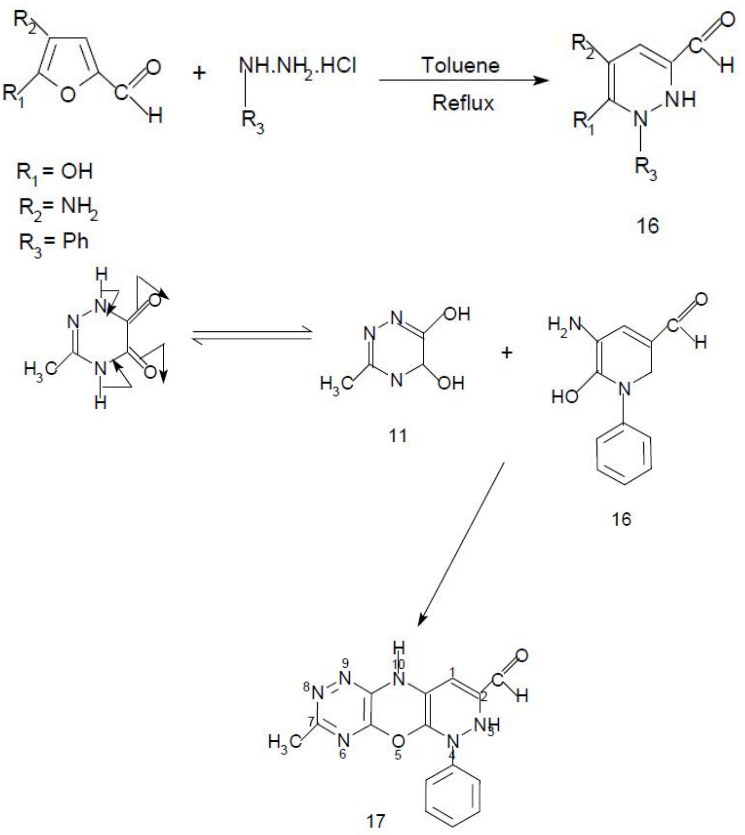
100 – percentage inflammation.

3. RESULTS

Acetamidine hydrochloride (**8**) was prepared from solid acetamido ethyl ether hydrochloride in super dry ethanol followed by the addition of alcoholic ammonia. Treatment of this compound (8) with anhydrous hydrazine afforded a pink solid acetamidrazone (**9**). 6-methyl-1,4,5-triazine-2,3-dione(**10**) was produced by suspending compound **(9**) in super dry ethanol treated with oxalyl chloride and refluxed for 2h.



Scheme 1



Scheme 2

Compound (**10**) when mixed with 4,5-diamino-6-hydroxylpyrimidine in ethanol yielded a solid 1-amino-7-methylpent-azaphenoxazine(**13**) (scheme 1). Compound (**16**), 3-aldehyde-5-amino-6-hydroxyl-1-phenylpyridazine was synthesized by heating a mixture of 4-amino-5-hydroxylfurfural and phenylhydrazine in toluene. The crude product was crystallized from acetic acid. When this compound (**16**) was mixed with 6-methyl-1,4,5-triazine-2,3-dione(**10**) in ethanol and crystallized in ethanol/water, the result was a solid compound 2-aldehyde-7-methyl-4-phenylpent-azaphenoxazine(**17**) (scheme 2).

The structural assignment of the synthesized compound is based on the spectral data. In the IR spectrum of compound (**8**), the hydrogen bonded N-H stretching appeared at 3283-3179 cm^{-1}. Absorption band at 2389 cm^{-1} represent CH_3 groups. The C=N absorption band appeared at 1445 cm^{-1}, while that at 816cm^{-1} is characteristic of C-N stretching. ^1H and ^{13}C NMR. Studies of this compound(8) confirmed the structure. In ^1H NMR spectra data, compound (8) showed multiplet at δ5.76. In ^{13}C NMR spectrum, a characteristic signal appeared for C-N in the range of δ164 while the signal for C=N, (CH_3) and (CH) appeared in the range of δ139, δ89.5 and δ88.9 respectively.

The IR spectrum of compound (**9**) showed broad band at 3284-3180 cm^{-1} for hydrogen bonded N-H stretching. The bands 1446cm^{-1} and 817cm^{-1} were noticed for (C=N) and (C-N) stretching respectively, while that for CH_3 stretching appeared at 2388 cm^{-1}. In the ^1H NMR spectral data, compound(9) showed multiplet at δ5.70 and δ5.75 due to NH_2 and CH_3 protons. A characteristics signals appeared for (C-NH), (C=N) (CH_3) and (C-H) in the range of δ163, δ138, δ89.4 and δ88.8 respectively in the ^{13}C NMR spectrum.

In the IR spectrum of compound (**10**), a broad band for hydrogen bonded NH stretching showed at 3235cm^{-1}. The band at 2556cm^{-1} indicated C=O stretching while the band at 1585cm^{-1} and 1170cm^{-1} were for (C=N) and (C-N) respectively. The absorption at 2389cm^{-1} were located for CH_3 groups.

In the ^{1}H NMR spectrum of this compound, the singlet for 2NH proton appeared in the region δ5.80 and δ7.6, while the CH_3 protons showed a singlet in the region δ5.73. The ^{13}C NMR studies of this compound confirmed the structure. A characteristic signal appeared for (C=O triazine) at δ183.67, while that of C=N were seen in the range δ130.73. The signal at δ27.78, δ171.30 and δ130.73 were located for (CH_3), (C-NH) and (C=N) respectively.

In compound (**13**) the band at 3370 cm^{-1} is assigned to N-H phenoxazine while the absorption band at 690 cm^{-1}, 1439 cm^{-1} and 817 cm^{-1} are characteristic of C-O-C, C=N and C-N stretching vibration. In the ^{1}H NMR spectrum, the singlet for NH, CH_3 and NH_2 protons appeared at δ7.9, δ5.79 and δ5.73 respectively. In the ^{13}C NMR spectrum, a characteristic signals appeared for (C-NH, C_4) and (C=N, C_8) in the range of δ130.1 and δ131.20 respectively, while that of (CH_3) and (C-NH_2) were detected in the range of δ28.11 and δ138.1 respectively.

In the IR spectrum of compound (**16**), the absorption band at 3341 cm^{-1} and 3291 cm^{-1} represent the hydrogen bonded N-H pyridazine and NH_2 stretching vibration. There were a number of peaks at 816 cm^{-1}, 1722 cm^{-1}, 1654 cm^{-1}, 1382 cm^{-1}, 2408cm^{-1}, 3430 cm^{-1} and 658 cm^{-1} representing C-N, C=O, C=C , OH and C-O respectively. The band at 1631 cm^{-1} is assigned to Ar-H stretching. In the ^{1}H NMR spectral data, compound(16) showed a singlet at δ5.39 and δ5.29 due to NH_2 and N-H protons. The Ar-H protons appeared in the region of δ7,54 while the OH protons in the compound were located in the range of δ6.70. In ^{13}C NMR spectrum, a characteristic signal appeared for (C-NH_2), (C_4), (C-N, 6), (C-NH_2), (C=O pyridazine), (CH) and (C=C) in the range of δ138.3, δ175.6, δ129.6, δ138.3, δ192.28, δ78.9, and δ112.40 respectively, while the (Ar-C) were in the range of δ137.28- δ3130.20.

The IR spectrum of compound **(17**) showed absorption bands at 4351 cm^{-1} and 3427 cm^{-1} representing the hydrogen bonded N-H pyridazine and N-H phenoxazine respectively. The absorption band at 1631 cm^{-1} was for Ar-H stretching, while 1444cm^{-1} and 816 cm^{-1} represent (C=N) and (C-N) stretching respectively. There were other peasks at 657 cm^{-1}, 1651 cm^{-1}, 2001cm^{-1}, 1376 cm^{-1}, 658 cm^{-1}, and 2385 cm^{-1} which were for C-O, C=O, stretching , aromatic C-H, aromatic C=C, C-O phenoxazine and CH_3 groups. In the ^{1}H NMR spectrum, compound (17) showed singlet for N-H phenoxazine, –CH_3 protons at δ8.1 and δ2.97 repectively and multiplet for Ar-H at δ6.9, while the N-H pyridazine protons were located at δ5.36. In the ^{13}C NMR spectrum, some signals were noticed at δ186.63, δ130.40, δ131.6 and δ133.8 for (s, C l), C=N, C=C, and C=O respectively. That of Ar.C were detected at δ128.30 -134.17.

The mass spectrophotometric studies performed on the synthesized compounds confirmed the molecular weight values. The result of the anti-inflammatory test on the novel heterocyclic systems and their effect and other analgesics on pain are as presented in Tables 1,2,3,4,5 and 6.

4. DISCUSSION

From the result of the acute toxicity (LD_{50}) of the novel phenoxazine compounds (**13 & 17**), differing doses of 10 mg/kg to 5000mg/kg of the compound were selected so that the entire range of toxicity from high acute toxicity to virtual non-toxicity could be tested. The weight of the animals after the text showed that they all gained weight. This was taken as a sign of having survived the acute intoxication. The mice treated intraperitoneally up to 2000 mg/kg did not show sign of toxicity compared with control animals. Similarly no significant effects was detected in animal treated orally with the compounds up to 5000 mg/kg. This high dose only produced intense quietness. This effect was reversed within 3 hrs. All the animals survived the text. That is no death was recovered. This clearly demonstrate that the two phenoxazine compounds (**13** and **17**) even at high dose of 5000 mg/kg (5 g/kg) were non-toxic.

The haemotological profile of the adult rabbits were affected by the two phenoxazine compounds. The WBC protect the body against foreign invaders such as bacteria. Therefore a low WBC count usually indicates that the neutrophile count is low and it becomes easier to get an infection and harder to recover from it. A low number of WBC is called Leukopenia. A WBC count below 4500 is below normal. The normal number of WBC in the blood is 4,500-10,000 per microlitre (mcl). From Table 2, it is observed that the number of WBC in the blood of the treated rabbits compared favourably with control treated animals. The observed significant increase in values of WBC at 1500-5000 mg/kg may be attributed to stimulation of the immune system. This significant in dose dependent manner can be an indication of the non-toxicity in

high doses. The compound therefore might be useful in the management of the inflammatory and tumor related disease.

The body needs oxygen to release energy and keep organs and tissues healthy. This is one of the functions of the RBC using a substance called haemoglobin (hb). A person with a low Hb level may have anemia [40]. A normal Hb level is between 12% and 16% while a normal RBC count is between 4.7-9.4 microlitre (mcl).

From Table 2, the significant decrease in the level of haemoglobin concentration and increase in the level of red blood cell count with increasing doses are within the normal level. This is an indication that the novel phenoxazine compounds cannot cause anemia in animals.

The PVC values is a measure of the oxygen carrying capacity of the blood. Table 2 revealed a decrease in the PVC values with increasing doses, indicating the degree of stress on the test animal health [41].

Results in Table 1 indicate that the pent-azaphenoxazines (**13** and **17**) administered to the rabbit were highly active compared to the human analgesic drugs employed in the study considering the mean reaction times. At a considerable concentration of 0.150 g/kg, the decreasing order of action of the human analgesics were alabukun, paracetamol and aspirin. At a lower concentration of 0.125 g/kg, compounds **13** and **17** compared favourably with the three human analgesics. This implies therefore that the active principle are equally present in the synthesized compounds. The doses of 0.125 to 0.150 g/kg injected into the adult rabbits intraperetoneally resulted in reduction of pain threshold. This level of activity is remarkable. It was previously observed that aspirin has a very poor analgesic action through the inhibition of prostaglandin synthesis [42]. This observation is supported by the result obtained in the study at aspirin concentration of 0.150 g/kg and the result of aspirin versus saline applications (Table 1).

The effect of the pent-azaphenoxazine compounds (**13** and **17**) on carrageenan-induced oedema in rats are shown in Tables 3,4,5 and 6.

From Table 4 it can be seen that in control animals, the sub-planter injection of carrageenan produced local oedema after 20 min. The two compounds at 50 mg/kg and 100 mg/kg demonstrated a significant anti-inflammatory effect. The dose dependent effect of these compounds in the carrageenan induced paw oedema showed that the effect was real and not due to counter irritant activity. Table 4 also revealed that apart from being dose and time dependent, the action of pent-azaphenoxazine compounds on carrageenan induced oedema also depends on the nature of the di-azine molecules and the type of substituent available on them (compound **13** and **17**). The average inflammation was 0.29 mm and 0.09mm when the rat was pretreated with 50 mg/kg and 100 mg/kg respectively with compound (**13**) in the first 20 min, while that of compound **17** was 0.28 mm and 0.08 mm.

From Table 6, at time 120 min there seems to be a complete inhibition of oedema by 95.36% and 97.51% for compounds **13** and **17** respectively. The standard drug Zerodol (Aceclofenac) at 50 mg/kg showed inhibition by 57.79% (Table 6).

This significant changes was probably due to the nature of the di-azine molecule in the pent-azaphenoxazine systems and the type of substituent available in them.

Table 1. Analgesic property of the pent-azaphenoxazines

Group	Test agent	Dose(g/kg)	Mean reaction time (sec)
1	Saline	-	12±6.32
2	Saline	-	13±8.23
3	Aspirin	0.125	14±6.6
4	Aspirin	0.150	16±2.32
5	Paracetamol	0.125	37±4.32
6	Paracetamol	0.150	40±3.12
7	Alabukun	0.125	42±4.52
8	Alabukun	0.150	48±4.52
9	Pent-azaphenoxazine(13)	0.125	69±14
10	Pent-azaphenoxazine(13)	0.150	73±20
11	Pent-azaphenoxazine(17)	0.125	72±11
12	Pent-azaphenoxazine(17)	0.150	78±64

Table 2. Effects of pent-azaphenoxazine (13 and 17) on haemotology of adult rabbits

	Compound 13 group							Compound 17 group						
Parameters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Control**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
RBC(mm³)	8.35±0.03	8.40± 0.36	8.46±0.94	8.59±0.49	8.68±0.93	8.89±0.36	9.12±0.37	9.28± 0.11	8.38±0.17	8.36±0.11	8.43±0.11	8.56±0.32	8.66±0.18	8.87±0.61	9.01±0.46
WBC(mm³)	6375±0.11	6492±0.30	7742±35	7901±72	8.058±0.21	8.079±0.86	8148±0.61	6580±41	6381±0.91	6499±0.12	7811±10	7981±33	8073±0.20	8106±	8192±
Hb(%)	14.3±0.37	13.9±0.14	13.3±0.58	12.7±0.34	12.4±0.71	12.09±0.80	11.7±0.43	15.9±0.19	14.01±0.42	13.6±0.18	13.09±0.64	12.5±0.16	12.2±0.93	12.0±0.11	11.5±0.36
PCV(%)	52.3±19	50.5±0.13	47.2±22	44.3±0.19	40.7±89	39.6±75	39.42±77	58.4±0.14	51.90±11	49.3±0.71	46.9±0.38	43.1±0.34	39.9±0.31	38.0±0.16	38.6±0.14

Table 3. Paw volume (mm³)

Treatment group	**0 min**	**20 min**	**40 min**	**60 min**	**80 min**	**100 min**	**120 min**
Normal saline (20 ml/kg)	0.50	1.08±0.07	1.13±0.10	1.14±0.03	1.05±0.02	1.03±0.04	1.01±0.12
Zerodol (aceclofenac) 50 mg/kg	0.53	0.85±0.03	0.86±0.13	0.84±0.17	0.78±0.72	0.77±0.10	0.75±0.19
Compound 13 (50 mg/kg)	0.55	0.84±0.41	0.85±0.11	0.84±0.01	0.78±0.14	0.77±0.00	0.78±0.04
Compound 13 (100 mg/kg)	0.63	0.72±0.06	0.71±0.31	0.70±0.47	0.68±0.13	0.67±0.11	0.65±0.10
Compound 17 (50 mg/kg)	0.56	0.84±0.20	0.84±0.17	0.82±0.16	0.78±0.48	0.76±0.13	0.74±0.91
Compound 17 (100 mg/kg	0.69	0.72±0.13	0.71±0.11	0.69±0.51	0.68±0.00	0.66±0.14	0.65±0.00

Table 4. Average inflammation (mm) of the right hind paw

Treatment group	20 min	40 min	60 min	80 min	100 min	120 min
Normal saline (20 ml/kg)	0.581	0.63	0.64	0.55	0.53	0.54
Zerodol (aceclofenac) 50 mg/kg	0.32	0.33	0.31	0.25	0.24	0.22
Compound 13 (50 mg/kg)	0.29	0.30	0.29	0.23	0.22	0.20
Compound 13 (100 mg/kg)	0.09	0.08	0.07	0.05	0.04	0.02
Compound 17 (50 mg/kg)	0.28	0.28	0.26	0.22	0.20	0.18
Compound 17 (100 mg/kg	0.08	0.07	0,05	0.04	0.02	0.01

Table 5. Percentage inflammation (%) of right hind paw

Treatment group	20 min	40 min	60 min	80 min	100 min	120 min
Zerodol (aceclofenac) 50mg/kg	54.89	51.88	48.50	46.18	46.00	42.21
Compound 13 (50mg/kg)	49.87	47.81	44.75	42.09	41.40	39.83
Compound 13 (100mg/kg)	15.40	12.58	11.43	9.30	7.19	4.64
Compound 17 (50mg/kg)	48.32	43.88	44.32	39.16	38.44	36.15
Compound 17 (100mg/kg)	14.25	11.84	8.47	6.82	4.71	2.40

Table 6. Percentage inhibition of oedema (%)

Treatment group	20 min	40 min	60 min	80 min	100 min	120 min
Zerodol (aceclofenac) 50 mg/kg	45.11	48.12	51.5	53.82	54.00	57.79
Compound 13 (50 mg/kg)	50.13	52.49	55.25	57.91	58.60	60.17
Compound 13 (100 mg/kg)	84.60	87.42	88.57	90.70	92.70	95.36
Compound 17 (50 mg/kg)	51.68	56.12	58.68	60.84	61.56	63.85
Compound 17 (100 mg/kg)	85.75	88.16	91.56	93.48	95.29	97.51

Compound **13** contains a pyrimidine structure with an amino substituent. Phenoxazines containing pyrimidine nucleus have received significant pharmaceutical attention [7]. This ring has aided the clinical use of phenoxazines as anti-cancer, anti-malarial, antibacterial agents, anti-viral and anti-fungal agents [7].

Compound **17** contains Pyridazine nucleus, one activating group and one carbonyl group that deactivates the pyridazine ring. Phenoxazines containing pyridazine systems, in recent times have been reported to posses biological activities [14]. These properties including the pyridazine functionality present in compound **17** probably have enhanced the percentage inhibition of this compound above that of compound **13**.

The result of this work as indicated in Tables 1,2,3,4,5 & 6 clearly demonstrate the significant anti-infammatory properties of these novel pent-azaphenoxazine compounds (**13 & 17**), their effect and other analgesics on pain.

The suppression of oedema by the compounds may be due to the presence of the diazine systems.

4.1 The Mode of Action

The carrageenan induced oedema in rats is due to the release of histamine, serotonin and prostaglandin [37,43]. The two pent-azaphenoxazines (**13 & 17**) including the control drug Zerodol (Aceclofenac) are among the NSAIDs (Non steroidal anti-inflammatory drugs). The mechanisms of action of these systems may be due to their ability to irreversibly inhibit prostagladin G/H synthesis by acting on the active site of the enzyme. They prevent the formation of prostagladin synthase including thromboxaze, prostacyclin and other prostaglandins [44]. When a tissue is injured or stimulated, protagladine synthesis in that tissue increases. The prostaglandins are mediators of inflammation and they also sensitize nerve endings, lowering their threshold of response to stimuli and the tenderness of inflammation [44].

The fact is that a drug that prevents the synthesis of prostaglandins is likely to be effective in relieving pains deu to inflammation of any kind. This is probably how Zerodol (aceclofenac) and other non steroidal anti-inflammatory drugs (NSAIDs) act [43]. Meaning that NSAIDs act by inhibiting cyclo-oxygenase (prostaglandin G/H synthase). This shows that the synthesized compounds (**13** &**17**) will relieve pain when there is some tissue injury with consequent inflammation.

5. CONCLUSION

Azaphenoxazines as anti-inflammatory drugs has long been recognized. Compounds 13 and 17 are the first pent-azaphenoxazines to possess

anti-inflammatory properties. The ant-inflammatory screening experiments of the two compounds on the carrgeenan induced paw oedema is a measure of the anti-inflammatory activity. Complete inhibition of oedema by 95.36% and 97.51% from these compounds revealed their anti-inflammatory effects. This procedure has been accepted as a parameter for evaluating inflammatory conditions. The ability of these compounds to antagonize carrgeenan-induced oedema in Wister rats has been correlated with anti-inflammatory potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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