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*Keywords:* *bridgehead-fused1,2,3-trizolo[1,5-c]1,2,4-triazines, novel therapeutic systems, anti-inflammatory, egg-albumin, oedema.*

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# Synthesis of Bridgehead-Fused 1,2,3-Triazolo [1,5-c]-1,2,4-Triazines: Novel Anti-Inflammatory and Analgesic Therapeutic Systems

Odin, E. M. [α] & Onoja, P. K. [σ]

*Abstract-* An efficient method has been developed for the preparation of three novel heterocyclic compounds: Bridgehead-fused-5-methyl-6-methylketone-1, 2, 3 triazolo [1,5- c]-1, 2, 4-triazine(12),Bridgehead-fused-5-methoxyl -6-methylester-1, 2, 3-triazolo-[1,5-c]-1,2,4-triazine (14) and Bridgehead - fused - 5-methyl- 6-methylester-1,2,3-triazolo-[1,5-c] -1,2,4-triazine (16). The new heterocyclic systems were obtained utilizing 1H -1,2,3 – triazolo -5- diazonium salt (10) which was produced via thiourea sulphanilic acid(7). A mixture of this compound(7) with hydrazine in anhydrous acetonitrile, followed by continuous stirring afforded a solid compound: hydrazine carboximidamide (8). Addition of this hydrazine derivative to trimethyl orthoformate in a sealed vessel gave 5-amino – 1H- 1, 2,3 – triazole (9). Diazotization of this aminotriazole compound while maintaining the pH at 2, yielded the 1H – 1,2,3 – triazole -5- diazonium salt (10) in excellent yield.

The three fused heterocyclic systems were produced by coupling compound (10) with active methylene compounds: β-diketone, β-diester (dimethyl malonate) and β-keto ester respectively and heated under reflux in acetic anhydride. Recrystallization of the products in DMF – water, afforded pure colourless compound 12, light yellow system 14 and colourless fused compound 16 respectively. Structures were established by analytical and spectral data. The results of the anti-inflammatory and analgesic screening data revealed the potential analgesic values residing in the novel compounds which placed them as very strong drug candidate. The dose and time dependant effects of these compounds in the egg-albumin induced paw oedema showed the effect was real. The average inflammation was below 0.20 mm in the first 20min. At time 120min, there was a complete inhibition of oedema by 91.38%, 84.48% and 63.79% from the compounds(16,14 & 12) respectively, while the standard drug acetyl salicylic acid showed inhibition by 41.38%.The effect of substituent on inhibition was also recorded. The suppression of oedema by the compounds was correlated with anti-inflammatory potential.

*Keywords: bridgehead-fused1,2,3-trizolo[1,5-c]1,2,4-triazines, novel therapeutic systems, anti-inflammatory, egg-albumin, oedema.*

## I. Introduction

Triazolo-Triazines are well known class of aza-bridgehead fused heterocyclic compounds which have miscellaneous pharmaceutical applications (Akpanisi, 2004, Katrizky *et al*, 2004; Mohammed, 2009). Their structural pattern is well established in pharmaceutical agents, particularly psychotropic agents such as risperidone and paliperidone (Khan. 1997; Jeste *et al* 2000).

Triazolo heterocycles occupy a central position in modern heterocyclic chemistry, because they form an important recognition element in biologically active molecules (Romain *et al*.,2010).

Bridgehead-fused triazolo triazines contain three nitrogen atoms in both a five membered ring and a six membered ring fused together to form a bridgehead-fused ring system (Akpanisi, 2004).The triazolotriazine is capable of exhibiting diazoakyllideneamine-triazole ring chain tautomerism. This isomerism is also known as the Dimroth rearrangement (Akpanisi, 2004). These heterocyclic fused systems have attracted much interest since the last decades.

The global effort to eradicate cancer has motivated us to search for new products with analgesic and anti-inflammatory activities that could join the list of non steroidal anti-inflammatory drugs (NSAIDs) that could provide better therapeutic activity. Research into inflammation has shown that it is a complex process involving many biochemical pathways and a variety of agents and mediators (Davies et al, 1989). Inflammation is a tissue reaction by the body to injury which is classically characterized by swelling (tumor), pain (dolor), redness (rubor) as well as loss of function ( Macpheson, 1992). The anti-inflammatory activities of bridgehead-fused heterocycles have been attributed to their ability to irreversibly inhibit prostaglandin G/H synthase by acting on the active site of the enzyme (Laurence, *et al*,1997).

The chemistry of bridgehead fused 1,2,3-triazolo [1,5-a] pyrimidine **(1)** is well documented (Akpanisi, 2004 and Odin & Akpanisi, 2007).

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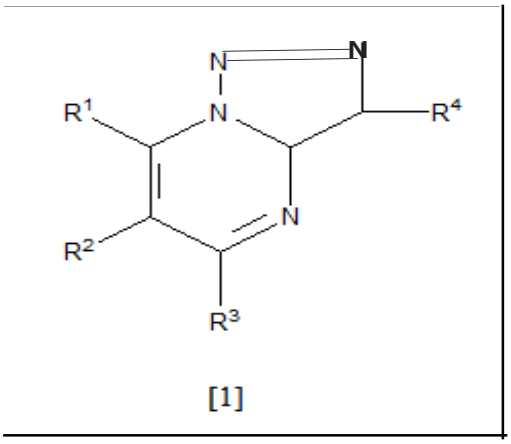
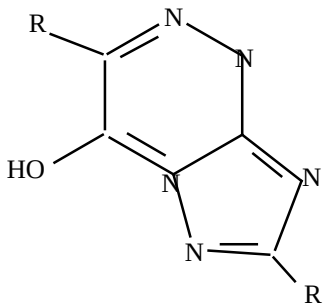


[1]

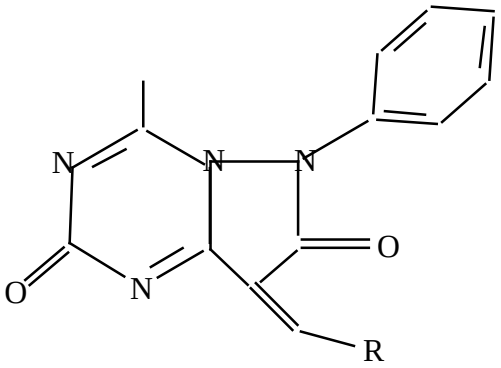
The bridgehead-fused triazolo triazines systems of types (2) : 1,2,4-triazolo[3,4-c]-1,2,4-triazine (Reda *et al*, 2010 and Duanis *et al*,1975; (3):2,4-diarylpyrazolo[1,5-a]-1,3,5-triazine (Sun, 2013); (4): 2-amino-4-thioxo-3-aryl-pyrazolo[1,5-a]-triazine (Sun, 2013); (5): 1,2,4-triazolo[1,5-a]-1,3,5-trizine (Federica *et al*, 2011) and their known pharmacological applications have previously been prepared.

In continuation of our research programme directed towards the preparation of heterocycles with pharmaceutical importance (Odin, *et al*, 2013), Bridgehead-fused-1,2,3,triazolo[1,5-c]-1,2,4-triazines of types 12,14 and 16 to the best of our knowledge have not been reported . In this paper we report the total synthesis of these novel fused heterocyclic systems (12, 14 and 16) and their analgesic/anti-inflammatory properties.



R = Me

[2]



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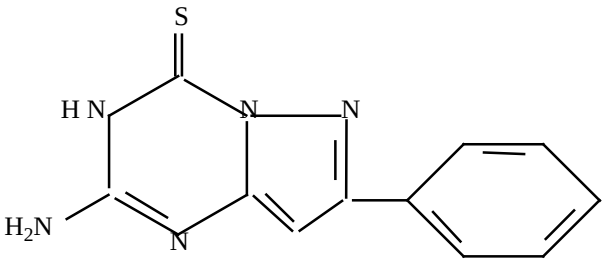

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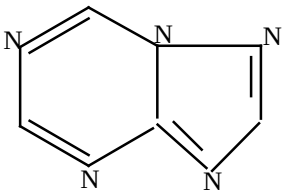


[5]

## II. Materials and Methods

All chemicals were obtained from different sources and were used without further purification. The melting points were determined on a SMP3 melting apparatus and are reported in °C uncorrected. Column chromatography was performed in Scharian silica gel 60 (70-230 mesh). HPLC separations were performed in a Bulk scientific 500 apparatus using a reverce phase lichrosper 100RP-18 (5$\mu$m) column at room temperature (eluent: methanol :water 8:2 v/v).

*a) Spectra analysis*

The methods of Predrag et al, 2000; Hujo et al, 1957 and Sigites et al, 2005 were adopted and modified.

The identity of the compounds was confirmed by IR and MS methods as reported by Predrag et al,

2000, Mohammed et al, 2009 and were recorded in $Cm^{-1}$ on a Bulk Scientific 500 Spectrophotometer and Schimadzu GCMS-QP-1000E mass spectrophotometer at 700eV respectively. Elemental analysis was on a Perkin-Elmer analyzer 2400. Proton Spectra ($^1H$ NMR) and $^{13}C$ NMR were recorded on a Varian Gemini 2000 spectrophotometer operating at 200 and 50 MHz respectively.

*b) Animals*

In this study we followed the" principle of laboratory animal care" (NIH publication No 85-23, revised 1985). We employed Swiss Albino mice (16-40g) for the toxicity and analgesic studies, while the anti-inflammatory studies employed adult Wister rats (16-300g). 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 (Pfizer feed, PLC, Lagos) and water *adlibitum.*

*c) Synthesis*

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

*d) Thioureasulphanilic acid(7)*

This compound was produced according to the methods of Maryanoff *et al*, 1986 and Romain *et al*, 2010. Thiourea (4 g, 0.08 mol) and sodium molybdate (5.2 g, 0.057 mol) were dissolved in a 1:1 mixture of chloroform/methanol (16 ml). This solution was added to $H_2O_2$ (0.5 ml, 12 mmol ). The reaction mixture was stirred at 25°C for 1h and was separated by preparative silica gel thin layer chromatography (eluent: 40-60 petroleum ether/ethyl acetate (2:3 v/v) to give thioureasulphanilic acid. Yield :15.72 g (82.4%). m.p. 163-164°C. IR: 3290 ($NH_2$), 2990 (C-N), 3600 ($O\text{-}H_{aliphatny}$). $^1H$ NMR: 2.4 (s, $NH_2$), 5.26 (s, C-H). $^{13}C$ NMR: 90 (C). Anal. Cal. For $C_1H_6N_2O_3S$ : C, 8,20, H, 4.14, N,32.86, O, 48.0, S, 21.94%. Found: C, 8.18, H,4.12, N, 32.84, O, 47,1, S, 21.93%.

*e) Hydrazine carboximidamide (8)*

The The method of Romain *et al*, 2010 was adopted and modified. 300 mg, 1.5 mmol of thioureasulphanilic acid (7) was mixed with hydrazine (110 mg, 1.1 mmol) in anhydrous acetonitrile (1.2 mL). The mixture was stirred at 35°C for 2 h. The reaction mixture was concentrated to a faint blue crystals. Yield: 7.8 g (76.3%). m. p 159-161°C. 1R: 3292 ($NH_2$), 3351 (NH), 1650 (C=N)

$^1H$ NMR : 4.62(s.$NH_2$), 4.65(s.NH).

$^{13}C$ NMR :163 (C-NH) .

Calculated for $C_1H_6N_4$: C, 16.21,H, 8.07, N,75. 63%. Found : C, 16.19, H, 8.05, N,75.60%

*f) 5-amino-1H-1,2,3-triazole (9)*

The method of Romain *et al*, 2010 was employed. Trimethylortho formate (1.5 ml) was added to 420 mg, 2.8 mol compound **(8)** and heated for 24 h at 145°C in a sealed vessel. The resulting mixture was cooled to room temperature and filtered. The filterate was washed with 30% ethanol in $CH_2Cl_2$, concentrated and purified using column chromatography (eluent : n-hexene/ethyl acetate 5:2 v/v). The result was yellow solid. Yield 180 mg, 70%. m.p 143-147°C. IR: 3293 ($NH_2$), 3311 (NH), 3021 (C-N). $^1H$ NMR: 3.9 (d 4H), 4.0 ( m, NH protons), 4.20 (m, $NH_2$ protons), 5.8 (m. C-H). $^{13}C$ NMR: 79.9 (CH), 80.2 (CNH).

Calculated for $C_2H_6N_4$: C,27.90, H,7.03, N,65.08%. Found: C,27.70, H,7.02, N,65.06%.

*g) 1H-1,2,3-triazolo-5-diazonium salt (10)*

This was prepared according to Draganov and Naicheva, 1981 and Odin *et al*, 2004. 200 ml of 8% aqueous HCl was added to 0.10 mol 5-amino-1H-1,2,3-triazole **(9)** in a reaction vessel while stirring. The mixture was cooled to 5-10°C for a period of 1 h. At this temperature, a solution of sodium nitrite (3.5 g dissolved in 20 ml $H_2O$) was added while the pH was held at 2.

*h) Reaction of 1H-1,2,3-triazolo-5-diazonium salt(10) with active methylene compounds*

i. *General Procedure*

The methods adopted were that of Akpanisi, 2004, Ahmad et al, 2004 Nataliya et al, 2010, Kin and Yoon, 2004, Brehme et al, 2007, Parmeter, 1959 and Patentent US 7122548, 2006. A solution of compound **10** (0.88 g, 0.006 mol) in aqueous ethanolic solution (20.0 ml) was added to cooled solutions of β-diketone (0.2 mol), β-diester (0.2 mol) and β-keto ester (0.2 mol) respectively. The diazonium salt was added proportion-wise while stirring over a period of 35 min at temperature 0-5°C. The pH of the reaction medium was lowered by adding sodium acetate. At the end, the reaction mixtures were intermittently stirred for another 2.5 h. The crude products were filtered, washed with cooled water and recrystallized from dimethylformamide-water to afford the corresponding hydrazones **(11)**, **(13)** and **(15)** respectively.

*i) 1H-1,2,3-triazolo-2-methyl-2-hydrazonoketone (11)*

Yield: 2.1 g (63%), m.p. :283-285°C. IR: 3020 (C-N arom), 1670 (C=O), 3252 (CH arom), 2929-2861 (C-H stretch), 2385($CH_3$ groups), 3140-3138 (2NH), 3022 (C-N), 1645 (C=N). $^1H$ NMR: 9.11 (s, NH), 7.21-7.19 (d, Ar-H), 10.12 (s, CHOO), 2.16 (s, $CH_3$). $^{13}C$ NMR: 169.4 (-$COCH_3$), 138 (C=N), 88.6 ($CH_3$). Anal. Calculated for $C_6H_{10}N_5O$: C, 42.84, H, 6.0, N, 41.65, O, 9.51%. Found: C,42.82, H, 5.9, N,41.63, O,9.49%.

*j) 1H-1,2,3-triazolo-2-methoxy-2-hydrazonoester (13)*

Yield: 3.2 g (71%), m.p. 289-290°C. IR: 3159, 3143 (2NH), 1623 (C=O), 3024 (C-N), 1644 (C=N), 2928-2918 (C-H stretch). $^1H$ NMR: 9.14 (s, NH), 2.14 (s, CH3), 720-756 (Ar-H). $^{13}C$ NMR: 69.5 (C=O), 149 ($COCH_3$), 139 (C=N). Anal. Calculated for $C_6H_{10}N_5O_3$: C,

36.00, H, 5.04, N, 35.03, O,23.98%. Found: C,35.98, H,5.02, N, 35.01, O, 23.96%.

*k) 1H-1,2,3-triazolo-2-methyl-2 hydrazonomethylester (15)*

Yield: 2.9 g (77%), m.p. 294-298°C. IR: 3154, 3148 (2NH), 1620 (C=O), 3023 (C-N), 1644 (C=N), 2927-2867 (C-H stretch). $^{1}$H NMR: 9.16 (s, NH), 2.16 (s, $CH_3$), 721-725 (Ar-H). $^{13}$C NMR: 69.8 (C=O), 148 ($COCH_3$), 138 (C=N). Anal. Calculated for $C_6H_{10}N_5O_2$: C, 39.12, H, 5.47, N, 38.03, O, 17.37%. Found: C, 39.10, H, 5.45, N, 38.01, O, 17.35%.

*l) Synthesis of Bridgehead-fused heterocyclic compounds 12, 14 and 16*

i. *General Procedure*

We employed the general procedures of Akpanisi, 2004; Ahmad, *et al*, 2004 and Nataliya, *et al*, 2010. A solution of the hydrazones **11**, **13** and **15** ( 2 mmol) respectively in acetic anhydride was refluxed for 4 h. The mixture was allowed to cool to room temperature, the solvent evaporated and the residue left was re-crystalized from dimethyformamide-water to afford the corresponding fused heterocyclic systems **12** (pure colourless), **14** (pure light yellow) and 16 (pure colourless).

*m) Bridgehead-fused-5-methyl -6- methylketone 1,2,3-triazolo[1,5-c]-1,2,4-triazine (12):*

Yield: 1.8 g ( 70%).
m.p. 298-300°C. IR: 1500 (C=Carom), 3020 (C-Narom), 1690 (C= 0), 3250 (CH arom),
2929-2861(C-H stretch), 2 671 (C=0 stretch),
2385 (CH3 groups).
$^{1}$H NMR :7.21-7.59 (d, Ar-H),
4.6 (d,2H), 3.7 (m, 4H),
$^{13}$C NMR :
111.20 (C=C) , 67.0 (C=0), 88.6 (CH).
Anal. calculated for :$C_7H_7N_5O$
C, 47.45, H, 3.98, N 39.54, O, 9.03%
Found: C, 45.43, H, 3.96, N, 39.52, O.9.01%.

*n) Bridgehead-fused -5- methoxy -6- methylester,1,2,3 triazolo[1,5-c]-1,2,4-triazine (14)*

Yield: 2.2 g (73%). m.p. 299-301°C. IR: 1500 (C=C arom)
1501 (C=C), 3021 (C-N arom), 1692 (C=0), 3250 (CH arom)
2928-2860 (C-H stretch), 2691, (C=0 stretch)
2385 ($CH_3$ groups)
$^{1}$H NMR :
4. 62 (S, 1H), 3.7 (m, 2H), 6.1 (m, CH2 ), 5. 76 (m, CH3)
$^{13}$ C NMR : 159.0 (C- arom), 111.2 (C=C),
169.4 (-O-C=O), 67.0 (C=O), 64.4 (-CH2), 16.2 , 55. 0 ($CH_3$), 88. 6 (CH).
Anal calculated for $C_7H_7N_5O_3$
C, 40.19, H, 3.37, N,33.49, O,22.95%
Found: C, 40.17, H,3.35, N,33.45, O, 22.93%

*o) Bridgehead-fused-5-methyl-6-methylester-1,2,3-triazolo[5,1-c]-1,2,4-triazine (16)*

Yield: 2.45 g (77.3%).
m.p. 297-299°C. 1R : 2911-2818 (Arom, C-H).
714 (C-H out of plane bending). 1096 (C-H in a plane bend).
1504 (C=C arom), 3022 (C-N arom), 1093 (C=0), 2928-2860 (C-H stretch).
$^{1}$H NMR : 4. 62 (s, 1H), 3.7 (m, 2H)
6. 19 (m, CH2 ), 5.76 (m. CH3, 9.07, (d.3H), 9.23 (d, 4H),
9.07 (s.5H), 9.23, (s.6H), 9.07, (3.H)
$^{13}$ C NMR : 159.0. (C triazine), 113.4 ( C=C), 169.4 ( -COO), 67.0 (C=O), 64.4 (-CH2), 16.2 ( CH3), 131.2, 127.6, 123.6, 151.4 ( CH and C).
Anal. calculated for: $C_7H_7N_5O_2$:C,43.12,
H, 3.65, N, 36.26, O, 16.56%
Found: C,43.10, H, 3.35, N, 33.45, 0.22.93%.

*p) Acute toxicity test ($LD_{50}$)*

This was determined in Swiss Albino mice by intraperitoneal (i.p) and oral routes according to the methods of Amos et al ,2002, Azuine et al, 1996,Gurad et al, 2011and Lork, 1983 .The animals were divided randomly into seven groups of five mice each. Widely differing doses of 10, 100, 1000, 1500, 2000, 3500 and 5000 mg/kg were administered intraperitoneally and orally. The animals were monitored for 72 h. At the end of the experiment, the animals were sacrificed and then autopsied and examined microscopically for any pathological changes. This was established for the three compounds **12, 14** and **16**.

*q) Test for Analgesia*

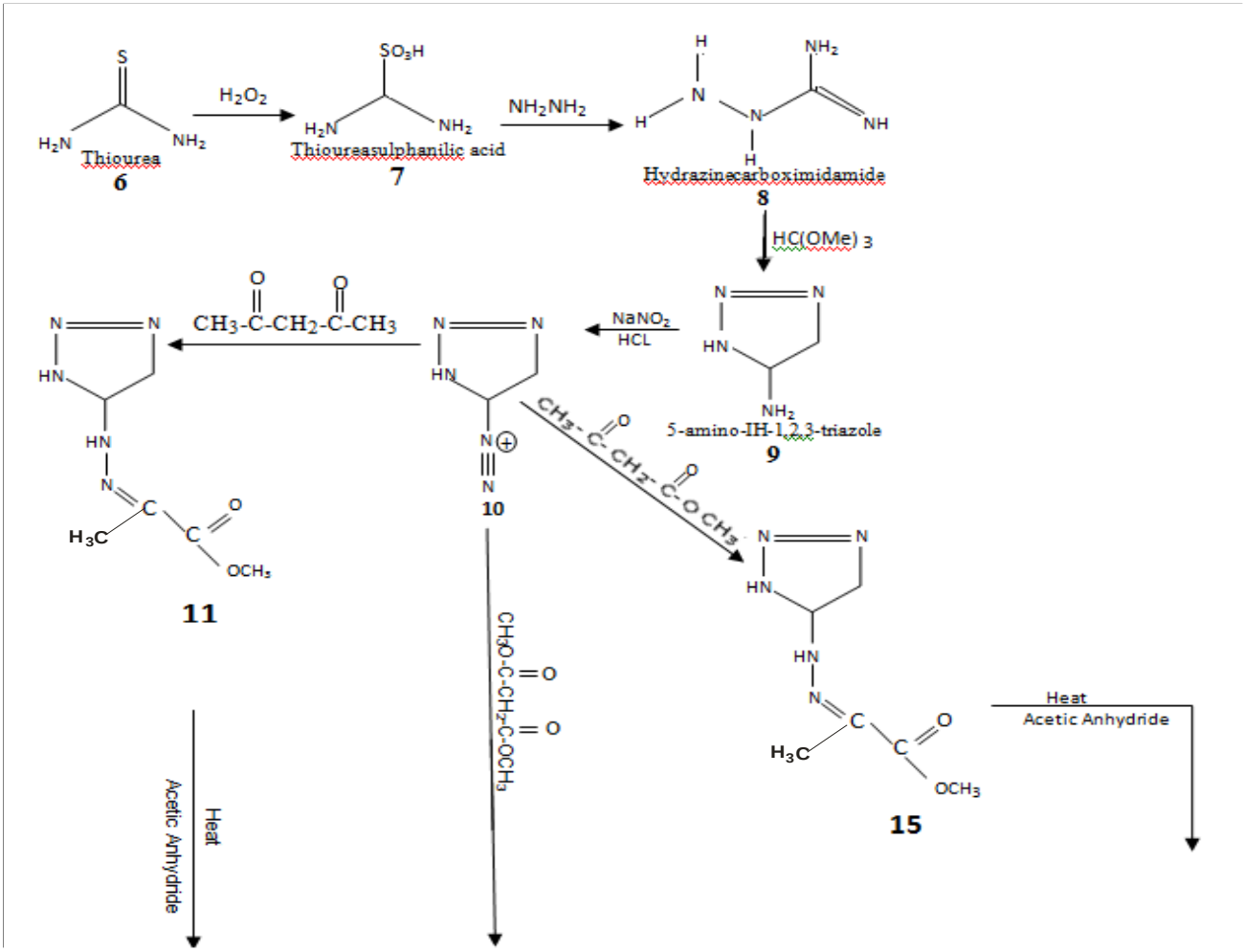
The analgesic property of the compounds was determined on Swiss Albino mice intraperitoneally as described by Koster *et al*, 1959 and Azuine *et al*, 1996. Twenty four (24) mice were treated with compound 12 (100 and 200 mg/kg) intraperitoneally 30 min, 60 min and 90 minutes prior to injection of 0.75% acetic acid (10 ml/kg i.p) (Tables 1and 2). This was to determine the dose and time dependence of the compound. The degree of suppression of writhen episodes were measured and compared to the negative control (10 ml/kg) acetic acid. Indomethacin (10 mg/kg) treated animals were used as the positive control. The test was also repeated for compounds **14** and **16** respectively. This was to establish the effect of substituent on analgesia (Tables 3 and 4).

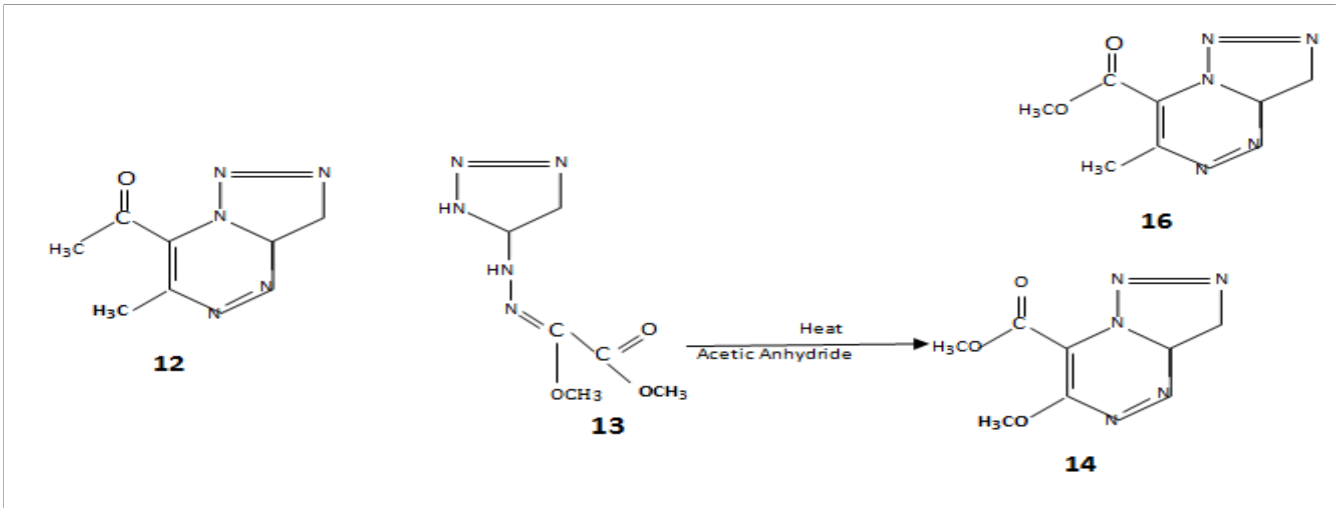
*r) Anti-inflammatory Studies*

The methods of Winter *et al*,1962 and Azuine *et al*, 1996 and Ratheesh and Helen, 2007 were employed and modified. Wister rats of either sex weighing between 160-275 g were divided into six groups of five rats each. Inflammation of the right hind paw was induced by injecting 0.05 ml of 50% solution of fresh egg albumin into the sub planter surface. Group 1 animals received

normal saline (20 ml/kg) and were designated as negative control. The second and third groups were administered compound **12** doses of 100 mg/kg and 200 mg/kg respectively by intraperitoneal route. Groups four and five animals were administered compounds **14** and **16** doses of 200 mg/kg respectively. While group six animals were given acetyl salicylic acid (200 mg/kg) injected intraperitoneally and served as positive control. All the drugs were administered 30 min before the subplanter injection of the phlogistic agent. The paw volume was measured after every 20 min for a period of 120 min by a volume displacement method (Azuine *et al*, 1996) using a plethysmometer (Table 5). The average inflammation, percentage inflammation and percentage inhibition of oedema were calculated on each dose and recorded (Tables 6,7 and 8). According to the method of Azuine *et al*, 1996, the average inflammation was calculated from the formular: $L_t$-$L_O$. Where $L_t$ is the linear circumference at time t and $L_O$, the linear circumference at zero time (Table 6). The percentage inflammation was calculated as follows:

A/B x100. Where A is the average inflammation of treated group at time t, while B is the average inflammation of control at the same time (Table 7) (Azuine *et al*, 1996). The percentage inhibition of oedema (Table 8) is calculated as follows: 100-Percentage inflammation.




*Scheme 1 :* Synthesis of bridgehead-fused 1,2,3 triazolo [1,5-c] 1,2,4-triazines

## III. Results

Thioureasulphonic acid (2) was produced by reacting thiourea with sodium molybdate dissolved in a mixture of chloroform/methanol. The solution which was added to aqueous H2O2was separated by preparative silica gel thin layer chromatography. Compound (2) when mixed with hydrazine in anhydrous acetonitrile gave a solid hydrazinecarboximidamide **(3)**. Addition of trimethyl orthoformate to compound (3) and heated for 24 h in a sealed vessel afforded 5-amino-1H-1,2,3-triazole **(4)**, which was subsequently added to aqueous HCl and a solution of sodium nitrite. This reaction furnished 1H-1,2,3-triazolo-5-diazonium salt **(5)**. A

solution of compound (5) in aqueous ethanolic solution was separately added to cooled solutions of β-diketone, β- diester and β-keto ester respectively. The crude products were filtered, washed with cold water. Recrystalization from dimethlformamide-water afforeded the corresponding hydrazones **(11), (13)** and **(15)**. Refluxing the hydrazones for several hours and recrystalizing the residue from DMF/$H_2O$ yielded the three novel products: Pure colourless bridgehead-fused-5-methyl-6-methyl ketone-1,2,3-triazolo-[1,5-c]-1,2,4-triazine (12), Light yellow bridgehead-fused-5-methoxy-6-methylester-1,2,3-triazolo-[1,5-c]-1,2,4-triazine (14) and pure colourless bridgehead-fused-5-methyl-6-methylester-1,2,3-triazolo-[1,5--c]-1,2,4-triazine(16). (scheme 1).

The structural assignment of the synthesized compounds is based on the spectral data. In the IR spectrum of compound (7), absorption band at 2990 represents C-N stretching. The $NH_2$ absorption band appeared at 3290 $cm^{-1}$, while 3600 $cm^{-1}$ represents OH aliphatic ring indicating complete oxidation.

$^1H$ and $^{13}C$ NMR studies of this compound confirmed the structure. In $^1H$ NMR spectra data, compound (7) shows a singlet at δ 2.4 due to $NH_2$ proton. The –CH protons in the compound showed a singlet at δ5.26.

In compound **(8)**, the hydrogen bonded N–H stretching appeared at 3351 $cm^{-1}$, while the $NH_2$ absorption band appeared at 3292 $cm^{-1}$, and that at 1650 $cm^{-1}$ is characteristics of C=N stretching. In the 1H NMR spectrum, the singlet for $HN_2$ and NH protons appeared in the region δ4.62 and δ4.65 respectively; while in $^{13}C$ NMR spectrum, a characteristic signal appeared for (CNH) in the range of δ163.

Addition of trimethyl ortho formate to compound (8) yielded compound (9). The IR spectrum of (9) showed broad bands at 33ll and 3293 $cm^{-1}$ for hydrogen bonded N–H and $NH_2$ stretching respectively. The band at 3021 $cm^{-1}$ indicating C–N stretching for NH and $NH_2$ protons were noticed in the regions δ4.0 and δ4.20 respectively. The C–H protons appeared at δ5.8. a characteristic signal for CHN in the $^{13}C$ NMR appeared at δ80.2, while that of (CH) was at δ79.9.

*The three hydrazones:* (11), (13) and (15) were produced when compound (10) was coupled with active methylene compounds: β-diketone, β-diester and β-ketoester respectively.

The IR spectrum of (11) showed a broad band at 3140-3138 $cm^{-1}$ for hydrogen bonded 2NH stretching. The band at 3022 $cm^{-1}$ indicated C-N stretching, while the band at 3252 $cm^{-1}$ appeared for aromatic C-H stretching. The bands at 2929-2861 $cm^{-1}$ were for C-H stretching, while that at 2385 $cm^{-1}$ indicated $CH_3$ groups. The absorption band at 1645 $cm^{-1}$ were located for C=N. The C=O stretching appeared at 1670 $cm^{-1}$. In the $^1H$ NMR spectrum of compound **(11)**, the singlet for –NH protons appeared in the region δ 9.11. The –CHOO protons and $–CH_3$ protons of the compound showed a singlet in the region δ 10.12 and δ 2.116 respectively. A characteristic signal appeared for ($-COCH_3$), (-C=N) and ( $CH_3$) in the range of δ 169.4, δ 138 and δ 88.6 respectively in the $^{13}C$ NMR spectrum.

In compound **13**, 2NH stretching showed at 3159 and 3143 $cm^{-1}$. The band at 1623 $cm^{-1}$ is assigned to C=O stretching, while the absorption bands at 3024, 1644, and 2928- 2918 $cm^{-1}$ are characteristic of C-N, C=N and C-H stretching vibrations. In the $^1H$ NMR spectrum, the singlet for NH and $CH_3$ protons appeared at δ 9.14 and δ 2.14 respectively, while Ar-H protons were noticed at δ 720- 756. The $^{13}C$ NMR studies of this compound confirmed the structure. A characteristic signal appeared for C=O and $COCH_3$ in the range of δ 69.5 and δ149 respectively, while that of C=N were seen in the range of δ139.

In the IR spectrum of compound **15**, the absorption bands at 3154 and 3148 $cm^{-1}$ represent the hydrogen bonded N-H stretching. There were number of peaks at 1620 $cm^{-1}$, 3023 $cm^{-1}$,1644 $cm^{-1}$, and 2927-2867 $cm^{-1}$ representing C=O, C-N, C=N, and CH stretching respectively. In the 1H NMR spectra data, compound 15 showed a singlet at δ 9.16 due to N-H protons. The Ar-H protons appeared in the region of δ 7.21-7.55, while the $–CH_3$ protons in the compound showed a singlet at δ 2.16. In $^{13}C$ NMR spectrum, a characteristic signal appeared for –C=O, $-COCH_3$, and –C=N in the range of δ 69.8. δ148, and δ 138 respectively.

Compounds **12**, **14** and **16** were synthesized from the general procedure by refluxing the solutions of the hydrazones **11**, **13** and **15** respectively in acetic anhydride.

The IR spectrum of compound (12) showed C = C aromatic at 1500 $cm^{-1}$, while that of C–N aromatic appeared at 3020 $cm^{-1}$. The bands at 2929 – 2861 $cm^{-1}$ appeared for C –H stretching while bands at 3250 and 2385 $cm^{-1}$ were for C – H aromatic and $CH_2$ groups. The bands at 2671 $cm^{-1}$ and 1690 $cm^{-1}$ is characteristic of C=O stretching and C=O groups. In the $^1H$ NMR spectrum, the duplet for 2H and 4H protons appeared at δ4.6 and δ 3.7 respectively, while in $^{13}C$ NMR spectrum, a characteristic signal appeared for C=C and C = O in the range of δ111.2 and δ67.0 respectively.

The IR spectrum of compound **(14)**, there were numbers of peaks at 3021$cm^{-1}$ , 1692$cm^{-1}$,3250$cm^{-1}$ and 2691$cm^{-1}$ for C – N aromatic, C = O, CH aromatic and C = O stretching. The absorption band at 2928 – 2860 $cm^{-1}$ were for aromatic C –H stretching.

In the $^1H$ NMR spectra data, compound (14) showed a multiplet at δ6.19 to 5.76 due to $CH_2$ and $CH_3$ protons.

Some characteristic signals appeared for –C – O – O -, -(C=O) in the range of δ 169.4 and δ 67.0 respectively, while in the 13C NMR spectrum, that of $CH_2$, $CH_3$ and CH were located at δ 64.4, δ 55.0 and δ 88.6 respectively.

In the IR spectrum of compound (**16**), the absorption band at 2911 -2818$cm^{-1}$ was for aromatic C – H stretching, while 714$cm^{-1}$ represented C – H out of plane bending and 2928 – 2860 $cm^{-1}$ was for C – H stretching. There were other peaks at 1504cm-1, 3022$cm^{-1}$ and 2693$cm^{-1}$ which were for aromatic C=C, aromatic C – N stretching and C = O stretching. In the $^1$H NMR spectrum, compound (16) showed multiplets for $CH_2$ and $CH_3$ at δ6.19 to δ5.76 respectively.

In the $^{13}$C NMR spectrum, some signals were noticed at δ113.4, δ169.4 and δ67.0 for C=C, -C-O-O- and C=O respectively. That of –$CH_2$ and $CH_3$ were located at δ64.4 and δ16.2 respectively.

The mass spectrophotometric studies performed on the synthesized compounds confirmed the molecular weight values.

The results of the analgesic and anti-inflammatory test are a s presented in figs. 1 and 2, tables 1,2,3,4,5,6,7 &8.

## IV. Discussion

The structural assignment of the synthesized compounds was based on spectra data. The IR spectrum of compound **12** showed bands at 2671 $cm^{-1}$ and 1690 $cm^{-1}$ characteristic of C=O stretching and C=O groups, while that of compound **14** was noticed at 2691 $cm^{-1}$ for C=O stretching. Similarly, the IR spectrum of compound **16** was observed for C=O stretching at 2693 $cm^{-1}$. This confirmed that the three heterocyclic systems have common functional groups.

Differing doses of 10mg/kg to 5000 mg/kg of compound **12**, **14** and **16** 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 signs of toxicity compared with control animals. Similarly, no significant effects were detected in animals treated orally with the compounds up to 5000mg/kg. This high dose only produces intense quietness. This effect was reversed within 3 hours. All the animals survived the test. That is, no death was recorded. This clearly demonstrates that the three synthesized compounds (**12**, **14** & **16**), even at high doses of 5000 mg/kg (5g/kg) were non-toxic to man.

From fig.1, it could be seen that the Bridgehead-fused compounds reduced the acetic acid induced writhing in mice. In control mice treated with 100mg/kg i.p acetic acid, the average writhing movement determined was 31 ± 1 (n = 3) (Table 1).

Pretreatment with 100mg/kg compound 12 30 min, 60 min and 90 min before the administration of the acid reduced the number of writhes to 56.98%, 31.18% and 23.81% of control. This is an indication that the effect of the compound (12) on pain increases with time as shown in fig 1 and table 1.

From fig. 1 and table 2, it shown that, pretreatment with 200mg/kg of the same compound (12) 30 min, 60 min and 90 min before the administration of the acetic acid reduced the number of writhes to 55.56%, 26.67% and 22.22% of control. Similarly, pretreatment with 200mg/kg of compounds **14** and **16**, 30min, 60min and 90min before the administration of acetic acid, reduced the number of writhes to 55.56%, 25.56%, 20.0% and 51.11%, 22.22% and 15.56% of control respectively (Tables 3& 4).

From fig 1, it is seen clearly that the analgesic effect of the Bridgehead-fused compounds (**12**, **14** and **16**) is time and dose dependent. In indomethacin (10mg/kg) treated mice, the number of writhes was reduced to 58.24% of control after 30min (fig. 1).

It is important to draw from here that the potential analgesic values residing in the compounds placed them as very strong drug candidate. This level of potency is highly remarkable.

The effect of the Bridgehead-fused triazolotriazines (compounds **12**, **14** & **16**) on fresh egg albumin-induced oedema in rats are shown in Tables 5, 6, 7 and 8.

From Table 6, it can be seen that in control animals, the sub planter injection of egg-albumin produced a local oedema after 20min.

From fig. 2, it can be said that compound **12** at 100mg/kg and 200mg/kg demonstrated a significant anti-inflammatory effect. The dose dependent effects of this compound in the egg-albumin induced paw oedema showed that the effect was real and not due to counter irritant activity.

In fig.2, it clearly showed that apart from being dose and time dependent, the actions of the Bridgehead-fused compounds on fresh egg albumin induced oedema also depends on substituent effect (compounds **12** **14** & **16**) . The average inflammation was below 0.20mm when the rat was pretreated with compounds **14** and **16** in the first 20min ( Table 6), while that of compound **12** was 0.29 mm. At time 120min, there seems to be a complete inhibition of oedema by 84.48% and 91.38% for compounds **14** and **16** respectively, while compound **12** showed inhibition by 63.79%. The standard drug acetyl ssalicylic acid showed inhibition by 41.38% (Table 8) at the same dose of 200 mg/kg. This significant changes was probably due to the nature of the substituent on the triazine ring. Compound **12** contains one moderately activating group and a weakly deactivating group. Compound 14 has two electron withdrawing groups that deactivate the triazine ring which probably enhanced the percentage inhibition

above that of compound **12**. On the other hand, compound **16** showed an ester group that moderately deactivate and one alkyl group that also moderately activate the ring.

The results of this work as indicated in Tables 5, 6, 7 & 8 and fig. 1 and 2 clearly demonstrate the significant anti-inflammatory properties of the Bridgehead-fused compounds (**12**, **14** & **16**). The suppression of oedema by the compounds may be due to the fused triazolo-triazine rings.

*The mode of action*

The bridge head fused compounds (**12**, **14**, **16**) including Acetyl salicylic acid are among NSAIDs (Non steroidal anti – inflammatory drugs). The mechanisms of action of these systems may be due to their ability to irreversibly inhibit prostaglandin G/H synthesis by acting on the active site of the enzyme. They prevent the formation of products including thromboxane, prostacyclin and other prostaglandins (Laurence, *et al*, 1997).When a tissue is injured or stimulated, prostaglandin 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 (Laurence, *et al*,1997).

The fact is that a drug that prevents the synthesis of prostaglandins is likely to be effective in relieving pain due to inflammation of any kind. This is how acetyl salicylic acid (aspirin) and other non steroidal anti-inflammatory drugs (NSAIDs) act (Laurence, *et al*, 1997). Meaning that NSAIDs act by inhibiting cyclo-oxygenase (prostaglandin G/H synthase). This shows that the synthesized compounds will relieve pain when there is some tissue injuring with consequent inflammation.

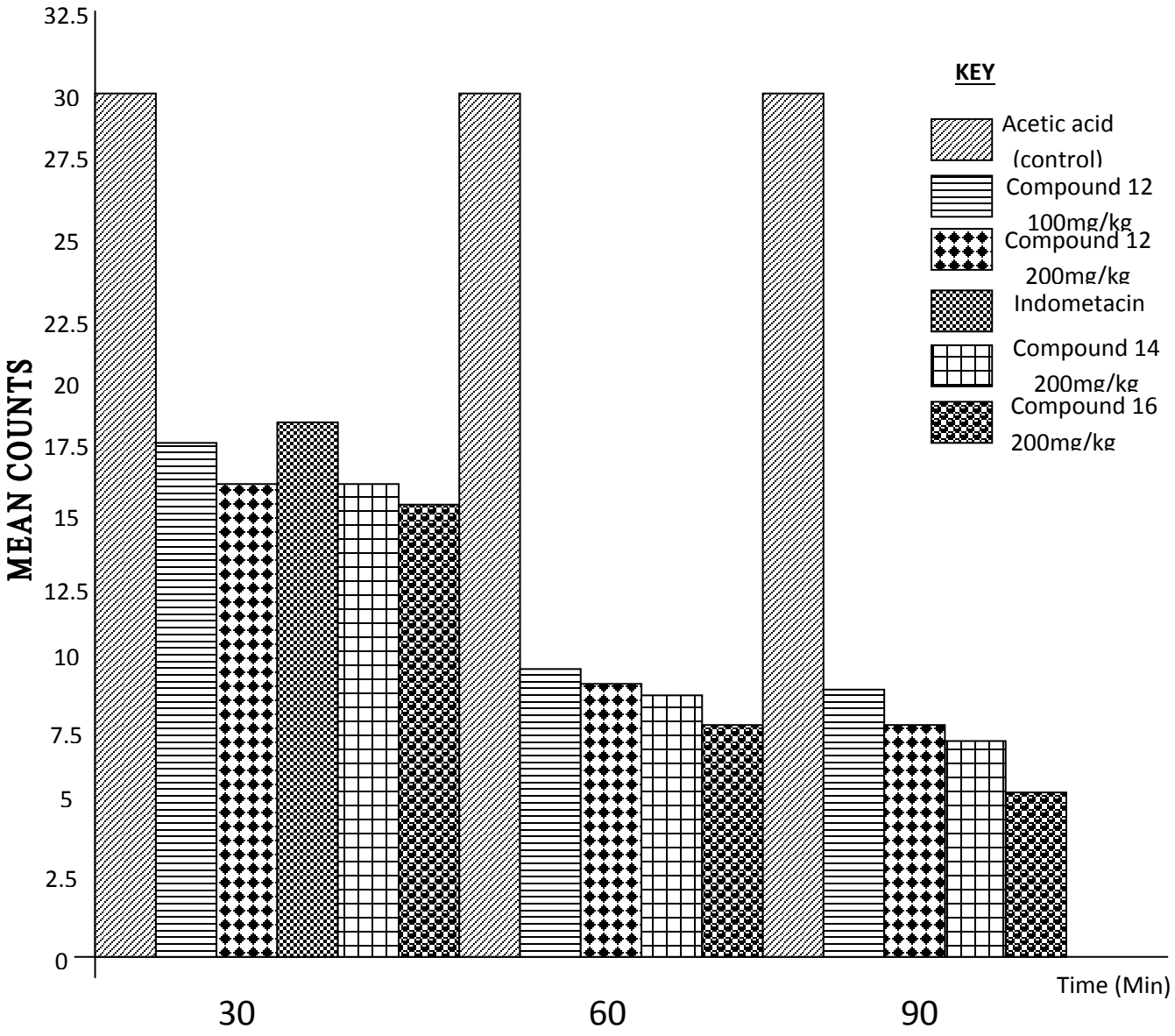



*Figure 2 :* Mean of Analgesic Writhing of 100 / 200 mg/kg

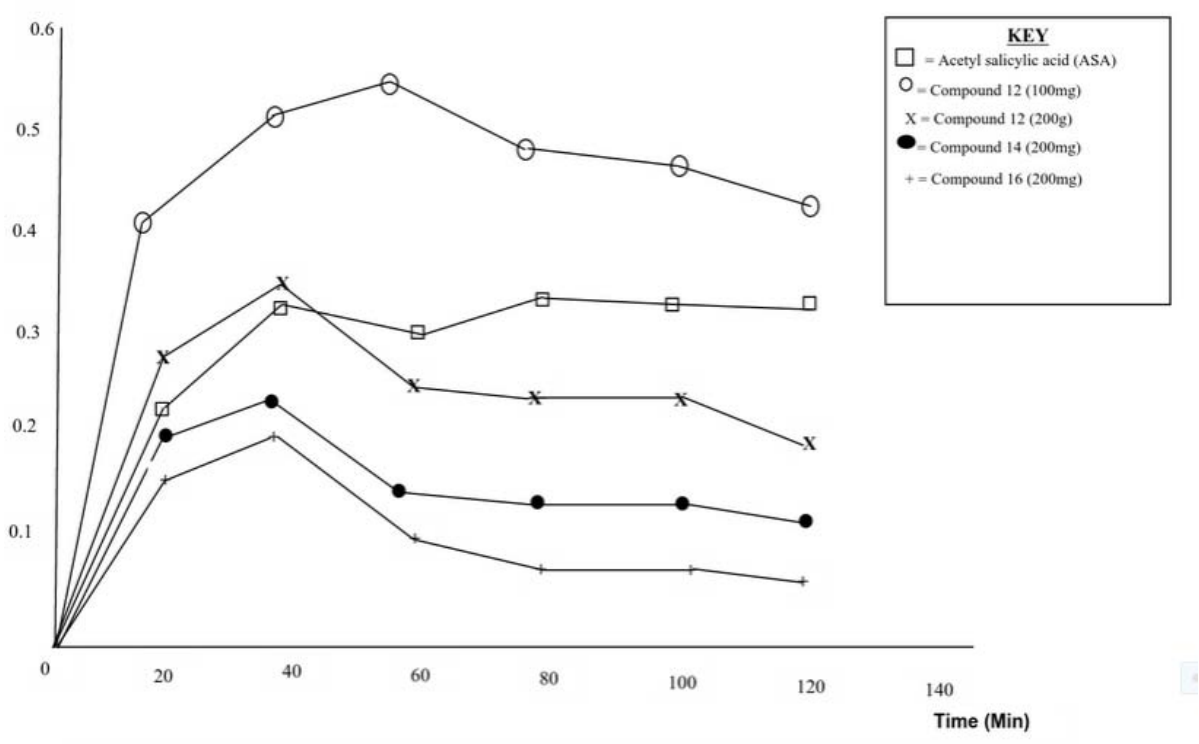



*Figure 3 :* Comparative test of Bridgehead-fused compounds on egg albumin induced paw oedema in rats. N = 5

*Table 1 :* Number of Writhing induced by 0.75% acetic acid in mice Pretreated with 100mg/kg Compound 12

| Animal | Weight (g) | Dose (ml) | Time (min) | No. of Writhes |
|---|---|---|---|---|
| NMT | 38.3 | 0.38 | 0 | 29 Negative |
| HDN | 31.0 | 0.31 | | 31 control (Acetic acid) |
| BKH | 39.5 | 0.20 | | 33 |
| TLN | 36.8 | 0.37 | | 18 |
| TL/BK | 39.3 | 0.39 | 30 | 16 |
| RH/TL | 31.0 | 0.31 | | 19 |
| NM | 37.7 | 0.38 | | 10 |
| HDM | 31.8 | 0.32 | 60 | 11 |
| BKN | 29.1 | 0.29 | | 8 |
| TLM | 30.0 | 0.30 | | 6 |
| HDT | 30.5 | 0.31 | 90 | 9 |
| BK/LE | 30.6 | 0.31 | | 9 |
| RE | 30.4 | 0.30 | | 17 |
| RHM | 39.2 | 0.39 | 30 | 17 |
| LHN | 29.0 | 0.29 | | 19 |
| | | | | Positive control (indomethacic) |

*Table 2 :* Number of Writhing induced by 0.75% acetic acid in mice pretreated with 200 mg/kg Compound 12

| Animal | Weight (g) | Dose (ml) | Ti me (min) | No. of Writhes |
|---|---|---|---|---|
| HDN | 35.5 | 0.18 | 0 | 28 Negative |
| BKH | 34.6 | 0.17 | | 30 control |
| LLT | 40.0 | 0.20 | | 32 |
| RAE | 40.1 | 0.20 | | 17 |
| RL/NT | 37.2 | 0.19 | 30 | 15 |
| LA/TL | 37.5 | 0.19 | | 18 |
| TLE | 39.8 | 0.20 | 8 | |
| REN | 40.1 | 0.21 | 60 | 9 |
| LEN | 34.1 | 0.17 | | 7 |
| HD | 37.4 | 0.19 | | 5 |
| RL | 36.5 | 0.18 | 90 | 10 |
| BK/N | 37.6 | 0.19 | 5 | |

*Table 3 :* Number of Writhing induced by 0.75% acetic acid in mice pretreated with 200 mg/kg Compound 14

| Animal | Weight (g) | Dose (ml) | Time (min) | No. of Writhes |
|---|---|---|---|---|
| HD | 36.5 | 0.18 | 0 | 27 Negative |
| RAE | 35.4 | 0.17 | | 32 control |
| LEN | 39.5 | 0.20 | | 31 |
| RL | 40.1 | 0.20 | | 17 |
| HDN | 39.2 | 0.19 | 30 | 16 |
| RL/NT | 4.5 | 0.19 | | 17 |
| BK/N | 39.8 | 0.20 | | 7 |
| LLT | 40.1 | 0.21 | 60 | 9 |
| TLE | 39.1 | 0.17 | | 7 |
| REN | 37.4 | 0.20 | | 6 |
| BKH | 38.5 | 0.21 | 90 | 5 |
| LA/TL | 37.6 | 0.17 | | 7 |

*Table 4 :* Number of Writhing induced by 0.75% acetic acid in mice pretreated with 200 mg/kg Compound 16

| Animal | Weight (g) | Dose (ml) | Time (min) | No. of Writhes |
|---|---|---|---|---|
| HDN | 35.7 | 0.19 | 0 | 29 Negative |
| BKH | 34.8 | 0.17 | | 28 control |
| LLT | 42.7 | 0.21 | | 33 |
| RAE | 40.3 | 0.20 | | 16 |
| RL/NT | 37.4 | 0.19 | 30 | 16 |
| LA/TL | 37.7 | 0.19 | | 14 |
| TLE | 40.0 | 0.20 | | 6 |
| REN | 40.3 | 0.20 | 60 | 8 |
| LEN | 34.3 | 0.17 | | 6 |
| HD | 37.6 | 0.19 | | 4 |
| RL | 36.7 | 0.18 | 90 | 5 |
| BK/N | 37.8 | 0.19 | | 5 |

*Table 5 :* Paw Volume (mm)

| Treatment Group | 0 min | 20 min | 40 min | 60 min | 80 min | 100 min | 120 min |
|---|---|---|---|---|---|---|---|
| Control Normal Saline 20ml/kg | 0.63 | 1.10±0.12 | 1.24±0.13 | 1.27±0.11 | 1.18±0.06 | 1.19±0.06 | 1.21±0.06 |
| Compound 12 100mg/kg | 0.64 | 1.04±0.04 | 1.18± 0.01 | 1.20±0.04 | 1.11±0.03 | 1.11±0.06 | 1.08±0.04 |
| Compound 12 200mg/kg | 0.65 | 0.94±0.06 | 1.03±0.06 | 0.91±0.06 | 0.90±0.06 | 0.90±0.06 | 0.86±0.05 |
| Acetyl Salicylic Acid 200mg/kg | 0.67 | 0.90±0.03 | 0.99±0.04 | 0.97±0.03 | 1.01±0.05 | 1. 01±0.05 | 1.01±0.04 |
| Compd.14 200mg/kg | 0.68 | 0.86±0.13 | 0.89±0.12 | 0.80±0.07 | 0.79±0.08 | 0.79±0.05 | 0.77±0.03 |
| Compd.16 200mg/kg | 0.68 | 0.83±0.09 | 0.86±0.08 | 0.77±0.04 | 0.74±0.03 | 0.74±0.02 | 0.73±0.02 |

*Table 6 :* Average Inflammation (mm) of the Right Hind Paw

| Treatment Group | 0 min | 20 min | 40 min | 60 min | min | 100 min | 120 min |
|---|---|---|---|---|---|---|---|
| Control Normal Saline 20ml/kg | | 0.57 | 0.61 | 0.64 | 0.55 | 0.56 | 0.58 |
| Compound 12 100mg/kg | | 0.40 | 0.54 | 0.56 | 0.47 | 0.47 | 0.44 |
| Compound 12 200mg/kg | | 0.29 | 0.38 | 0.29 | 0.25 | 0.25 | 0.21 |
| Acetyl Salicylic Acid 200mg/kg | | 0.21 | 0.32 | 0.30 | 0.34 | 0.34 | 0.34 |
| Compound 14 200mg/kg | | 0.18 | 0.21 | 0.12 | 0.11 | 0.11 | 0.09 |
| Compound 16 200mg/kg | | 0.15 | 0.18 | 0.09 | 0.06 | 0.06 | 0.05 |

*Table 7 :* Percentage inflammation (%) of Right Hind Paw

| Treatment Group | 0 min | 20 min | 40 min | 60 min | 80 min | 100 min | 120 min |
|---|---|---|---|---|---|---|---|
| Compound 12 100mg/kg | | 70.18 | 83.53 | 87.50 | 85.45 | 83.9 | 75.9 |
| Compound 12 200mg/kg | | 50.88 | 62.23 | 45.31 | 45.45 | 44.6 | 36.2 |
| Acetyl Salicylic Acid 200mg/kg | | 36.84 | 52.46 | 46.88 | 61.82 | 60.7 | 58.6 |
| Compound 14 200mg/kg | | 31.58 | 34.43 | 18.75 | 20.00 | 19.6 | 15.5 |
| Compound 16 200mg/kg | | 26.32 | 29.51 | 14.06 | 10.91 | 10.71 | 8.62 |

*Table 8 :* Percentage inhibition of Oedema (%)

| Treatment Group | 20 min | 40 min | 60 min | 80 min | 100 min | 120 min |
|---|---|---|---|---|---|---|
| Compound 12 100mg/kg | 9.82 | 11.47 | 12.50 | 14.56 | 16.07 | 24.14 |
| Compound 12 200mg/kg | 36.12. | 37.77 | 54.09 | 54.55 | 55.30 | 63.79 |
| Acetyl Salicylic Acid 200mg/kg | 23.16 | 27.59 | 33.12 | 38.18 | 39.29 | 41.38 |
| Compound 14 200mg/kg | 64.42 | 65.57 | 81.25 | 80.00 | 80.36 | 84.48 |
| Compound 16 200mg/kg | 70.49 | 73.68 | 85.94 | 89.09 | 89.29 | 91.38 |

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