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Short communication

Synthesis and analgesic evaluation of some 5-[β-(10-phenothiazinyl)ethyl]-1-(acyl)-1,2,3,4-tetrazoles

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Abstract

A series of novel 5[β-(phenothiazinyl-10-yl)ethyl]-1-(acyl)-1,2,3,4-tetrazoles (**3–14**) have been synthesized via condensation of 5-[β-(phenothiazinyl-10-yl)ethyl]-1-2,3,4-tetrazole (**2**) with various acylating/sulphonating reagents. 5-[β-(phenothiazinyl-10-yl)ethyl]-1-2,3,4-tetrazole was synthesized by cyanoethylation of phenothiazine with acrylonitrile and Triton B, followed by the cycloaddition of 3-(phenothiazin-10-yl)-propionitrile (**1**) with sodium azide and ammonium chloride. The compounds were screened for analgesic activity, anti-inflammatory activity and ulcerogenicity index. Out of the 12 compounds synthesized, compound (**5**) 5[β-(phenothiazinyl-10-yl)ethyl]-1-(benzoyl)-1,2,3,4-tetrazole, compound (**11**) 5[β-(phenothiazinyl-10-yl)ethyl]-1-(*p*-tolyl)-1,2,3,4-tetrazole showed promising analgesic activity and compound (**6**) 5[β-(phenothiazinyl-10-yl)ethyl]-1-(*p*-chlorobenzoyl)-1,2,3,4-tetrazole and compound (**8**) 5[β-(phenothiazinyl-10-yl)ethyl]-1-(*p*-nitrobenzoyl)-1,2,3,4-tetrazole showed promising anti-inflammatory activity.

Keywords: 5[β-(Phenothiazinyl-10-yl)ethyl]-1(acyl)-1,2,3,4-tetrazoles; Analgesic activity; Anti-inflammatory activity; Ulcerogenicity index

1. Introduction

5-Substituted 1,2,3,4-tetrazoles are reported to possess antibacterial [1–3], antifungal [4], antiviral [5–7], analgesic [8–12], anti-inflammatory [13–16], antiulcer [17–19] and antihypertensive [20,21] activities. The tetrazole function is metabolically stable [22,23] this feature and a close similarity between the acidic character of the tetrazole group and carboxylic acid group [24] have inspired medicinal chemists to synthesize substituted tetrazoles as potential medicinal agents. Tetrazoles with phenothiazine ring attached to fifth position through an ethylidene group are not reported in the literature. Based on these findings, some new 5-[(phenothiazin-10-yl) ethyl]-1-(acyl)-1,2,3,4-tetrazoles were synthesized and screened for analgesic activity, anti-inflammatory activity and ulcerogenicity index.

2. Chemistry

Compounds were prepared as shown in Fig. 1. Cyanoethylation of phenothiazine with acrylonitrile in the presence of Triton B (trimethyl benzyl ammonium hydroxide) gave 3-(phenothiazin-10-yl)-propionitrile (**1**). The requisite compound **1** was totally obtained in 82% yield. 1,5-disubstituted tetrazoles can be synthesized by number of methods, viz. reaction of hydrazoic acid or its salts with imidoyl chloride or imino ethers or diazo coupling of heterocyclic hydrazines or hydrocyanic acid. Most of these methods have limited use in preparative organic chemistry because the use of hydrazoic acid [25] presents considerable experimental difficulties due its toxicity and tendency to explode. However, the simple route reported by Finnegan et al. [26] was adopted for the preparation of 5[β-(phenothiazinyl-10-yl)ethyl]-1-(acyl)-1,2,3,4-tetrazoles. This route replaces the toxic hydrazoic acid by inorganic azide to afford the titled compounds in good yield (59–88%). Compound **1** was cyclized using sodium azide and ammonium chloride to yield compound **2**. Twelve substituted tetrazoles were synthesized from **2** by acylation and tosylation reaction.

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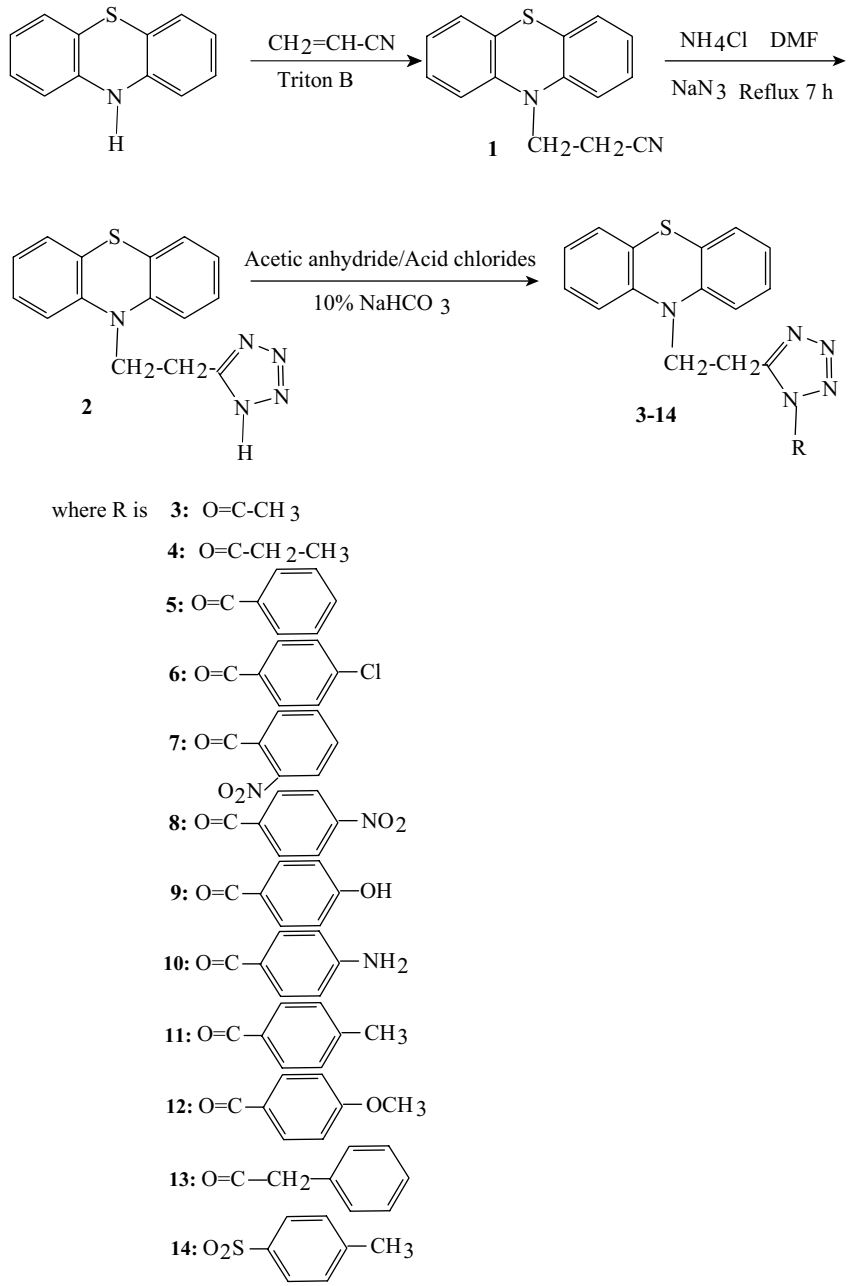

Figure 1. Synthesis of titled compounds.

3. Results and discussion

3.1. Chemistry

All secondary amine undergo cyanoethylation reaction with acrylonitrile and a base. Phenothiazine being a secondary amine was cyanoethylated to **1** by acrylonitrile and Triton B. The yield of the compound **1** was found to be quantitative and it was readily converted to 1,2,3,4-tetrazole by treating them with sodium azide and ammonium chloride in dimethylformamide. The secondary amino group of tetrazole at position 1 of tetrazole is free and hence 12 different derivatives are synthesized using various acyl chlorides.

Infra red spectrum of compound **1** showed a sharp absorption band at 2249 cm^{-1} which is attributed to nitrile group. The synthesized compounds (**2–14**) showed absorption bands at 1038, 1108, 1238, 1286 and 1591 cm^{-1} which are attributed to tetrazole ring. An absorption band at 3448 cm^{-1} is attributed to N–H stretching of the tetrazole ring. The synthesized compounds (**1–14**) showed absorption bands at 1570 and 1596 cm^{-1} which are attributed to the phenothiazine ring. Characteristic absorption bands were observed for carbonyl group, nitro group, hydroxyl group, amino group, methyl group, methoxyl group and aromatic region of the synthesized compounds.

^{1}H-NMR spectra of the synthesized compounds showed two triplets at δ 2.8 and δ 4.2. A triplet at δ 2.8 is due to two protons which are attached to the carbon atom of the nitrile function. The triplet at δ 4.2 is due to the two protons attached

Table 1
Effect of synthesized compounds on Rota rod test for mice

Behaviour	Effect after 30 min of administration (Mean ± S.E.M.) (i.p.)					
	10% v/v Tween 80 suspension	5 mg/kg	15 mg/kg	25 mg/kg	35 mg/kg	45 mg/kg
Grip test	No effect	No effect	No effect	No effect	No effect	1 ± 0.22 **
Motor activity	No effect	No effect	No effect	No effect	No effect	1 ± 0.18 **

** $P < 0.01$ represent significant difference when compared with control groups.

to the carbon atom of nitrile function. 1-H (NH) proton of the tetrazole is undetectable in NMR spectra. Aromatic protons showed multiplets in the range of δ 6.8–7.3. The expected signals with appropriate multiplicities for different types of protons were observed for the derivatives.

3.1.1. Analgesic activity

3.1.1.1. Acetic acid induced writhing method [27]. All compounds tested exhibited activity in a dose of 25 mg/kg with the exception of compounds **7** and **8** that are devoid of analgesic activity. Behavioural parameters such as motor coordination and spontaneous motility were not altered at a dose 25 mg/kg of the test compounds, which has been confirmed from the Rota Rod (Techno, 20 rpm) test (Tables 1 and 2). The analgesic activity of compounds **5** and **11** is found to be superior compared to other synthesized compounds. Introduction of acetyl, propionyl, 4-hydroxybenzoyl and *p*-toluenesulphonyl group at position 1 of tetrazole exhibited moderate analgesic activity. Introduction of Cl, NH_2 and OCH_3 group at 4-position of benzoyl group (attached to position 1 of tetrazole) decreases the analgesic activity. Introduction of NO_2 group in ortho and para position of benzoyl group (attached to position 1 of tetrazole) produces no analgesic activity.

3.1.1.2. Hot plate method. All compounds tested by Eddy's hot plate method [28] exhibited activity in a dose of 25 mg/kg. The analgesic activity of compounds **5** and **11** is found to be superior compared to other synthesized compounds. Acetyl, propionyl, 4-chlorobenzoyl, 2-nitrobenzoyl, 4-aminobenzoyl and 4-methoxybenzoyl analogs exhibited moderate analgesic activity.

3.1.2. Anti-inflammatory activity [29]

It is apparent from Table 5 that compounds **5, 6, 7, 8, 9, 10, 11, 12** and **14** afforded 6–55% protection against carageenin induced edema, whereas the standard drug diclofenac sodium under similar conditions showed 62.5% inhibition. Among the compounds tested, compound **6** and **8** were found to be most potent compounds as they exhibited 55% and 52.5% inhibition, respectively. Compounds **3, 4** and **13** did not reduce the paw volume and hence they were devoid of anti-inflammatory activity. It was found that the introduction of CH_3, OH, OCH_3 and NH_2 groups at C-4 of phenyl ring caused marked decrease in the anti-inflammatory activity.

3.1.3. Evaluation of ulcerogenicity index [30–32]

The ulcer index for compounds **5, 6, 7, 8, 9, 12** and **14** was found to be higher than the other synthesized compounds. The high ulcer index score for the above compounds may be due to the suppression of prostaglandin synthesis by Nimesulide.

4. Conclusions

We prepared a series of 5[β-(phenothiazinyl-10-yl)ethyl]-1-(acyl)-1,2,3,4-tetrazoles and demonstrated that these compounds possessed good analgesic activity tested both by acetic acid induced writhing method and hot plate method and anti-inflammatory activity tested by carrageenin induced paw edema method. The most promising compounds having analgesic activity were 5[β-(phenothiazinyl-10-yl)ethyl]-1-(benzoyl)-1,2,3,4-tetrazole (**5**) and 5[β-(phenothiazinyl-10-yl)ethyl]-1-(*p*-tolyl)-1,2,3,4-tetrazole (**11**) and anti-inflammatory activity were 5[β-(phenothiazinyl-10-yl)ethyl]-1-(*p*-chlorobenzoyl)-1,2,3,4-tetrazole (**6**) and 5[β-(phenothiazinyl-10-yl)ethyl]-1-(*p*-nitrobenzoyl)-1,2,3,4-tetrazole (**8**).

5. Experimental protocols

5.1. Chemistry

Melting points were determined by Veego melting point apparatus and are not corrected. Infrared spectra were obtained on a Perkin Elmer FTIR spectrophotometer using potassium bromide discs. Nuclear magnetic resonance spectra were recorded on Brucker 400 MHz spectrophotemeter. Chemical shifts are reported in parts per million (δ) units relative to internal standard tetramethylsilane. Elemental

Table 2
Effect of synthesized compounds on Rota rod test for rats

Behaviour	Effect after 30 min of administration (Mean ± S.E.M.) (i.p.)					
	10% v/v Tween 80 suspension	5 mg/kg	15 mg/kg	25 mg/kg	35 mg/kg	45 mg/kg
Grip test	No effect	No effect	No effect	No effect	1 ± 0.35 **	2 ± 0.62 **
Motor activity	No effect	No effect	No effect	No effect	1 ± 0.43 **	2 ± 0.98 **

** $P < 0.01$ represent significant difference when compared with control groups.

analysis were performed on Heraeus Carlo Erba 1108 and the analyses indicted by the symbols of the elements were within ±0.4% of theoretical values.

*5.1.1. 3-(Phenothiazin-10-yl)-propionitrile (**1**)*

Phenothiazine (9.95 g, 50 mmol) was mixed with acrylonitrile (12.5 ml) and cooled in ice bath. A crystal of resorcinol was added to prevent polymerization. Triton B (2 ml, 40% v/v) was added dropwise with shaking. A vigorous reaction was set in. It was allowed to subside and then the mixture was heated to reflux on a steam bath for 2 h. The solution was cooled, extracted with ethylene dichloride and dried over anhydrous sodium sulphate. The dried nitrile was recrystallized from ethanol. The desired phenothiazine propionitrile (**1**) was totally obtained as a light yellow solid in 82% overall yield: m.p. 159–160 °C. Smith [33] reported 158–159 °C. Kichifujii [34] reported 160–161 °C. IR: 2926 (C–H), 2853 (C–H), 2249 (C≡N), 1596 and 1570 (phenothiazine ring), 1456 (C–H) $cm^{-1;}$ ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–7.3 (8H, m, Ar–H). Anal. Calcd. for $C_{15}H_{12}N_2S$: C, 71.40; H, 4.79; N, 11.10. Found: C, 71.39; H, 4.64; N, 11.02.

*5.1.2. 5-[β-(Phenothiazinyl-10-yl))ethyl-1,2,3,4-tetrazole (**2**)*

The method described by Finnegan et al. [26] was followed to synthesize the tetrazole. A mixture of compound **1** (3.3 g, 10 mmol), sodium azide (0.65 g, 10 mmol) dimethylformamide (10 ml) and ammonium chloride (5.3 g, 10 mmol) was heated in a oil bath for 7 h at 125 °C. The solvent was removed under reduced pressure. The residue was dissolved in 100 ml of water and carefully acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to 5 °C in ice bath. Compound **2** recrystallized from aqueous methanol (yield 77%) as dark grey solid: m.p. 148–149 °C; IR: 3448 (N–H), 2926 (C–H), 2853 (C–H), 1591 (C=N), 1458 (C–H), 1286 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–7.3 (8H, m, Ar–H). Anal. Calcd. for $C_{15}H_{13}N_5S$: C, 61.00; H, 4.44; N, 23.71. Found: C, 60.89; H, 4.14; N, 23.52.

*5.1.3. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(acetyl)-1,2,3,4-tetrazole (**3**)*

Compound **2** (1 g, 2.5 mmol) was refluxed under a short condenser with acetic anhydride (3 g, 30 mmol) for 15 min. The reaction mixture was then cooled and poured into 20 ml of cold water. The contents were then boiled to decompose the excess acetic anhydride. Compound **3** was recrystallized from aqueous ethanol (yield 88%). The pure compound melted at 134–135 °C. IR: 2930 (C–H), 1774 (C=O), 1596 and 1570 (phenothiazine ring), 1457 (C–H), 1285 (N–N=N–), 1108 and 1138 cm^{-1} (tetrazole ring). ^{1}H-NMR ($CDCl_3$) δ 2.1 (3H, s, CH_3), 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–7.3 (8H, m, Ar–H). Anal. Calcd. for $C_{17}H_{15}N_5OS$: C, 60.52; H, 4.48; N, 20.76. Found: C, 60.34; H, 4.45; N, 20.68.

*5.1.4. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(propionyl)-1,2,3,4-tetrazole (**4**)*

Compound **2** was treated with an equimolar amount of propionyl chloride in 10 ml of 10% w/v sodium bicarbonate solution. The mixture was shaken vigorously in a stoppered test tube. When the odour of propionyl chloride has disappeared, the contents were acidified with dilute hydrochloric acid to congo red and filtered. The dried compound **4** was recrystallized from aqueous ethanol (yield 54%) as a brown solid: m.p. 125–126 °C. IR: 2930 (C–H), 1774 (C=O), 1596 and 1570 (phenothiazine ring), 1457 (C–H), 1285 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1:.}$ ^{1}H-NMR ($CDCl_3$) δ 1.3 (3H, t, J = 7.1 Hz, CH_3), 2.4 (2H, q, J = 7.1 Hz, CH_2), 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–7.3 (8H, m, Ar–H). Anal. Calcd. for $C_{18}H_{17}N_5OS$: C, 60.52; H, 4.48; N, 20.76. Found: C, 60.34; H, 4.45; N, 20.68.

*5.1.5. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(benzoyl)-1,2,3,4-tetrazole (**5**)*

Compound **5** was prepared using the same procedure as for **4**, and was obtained in 59% yield as a grey solid; m.p. 106–107 °C. IR: 2964 (C–H), 1686 (C=O), 1596 and 1570 (phenothiazine ring), 1455 (C–H), 1285 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–8.1 (13H, m, Ar–H). Anal. Calcd. for $C_{22}H_{17}N_5OS$: C, 66.15; H, 4.29; N, 17.53. Found: C, 65.96; H, 4.27; N, 17.50.

*5.1.6.5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*p*-chlorobenzoyl)-1,2,3,4-tetrazole (**6**)*

Compound **6** was prepared using the same procedure as for **4**, and was obtained in 60% yield as a light yellow solid: m.p. 168–169 °C. IR: 2836 (C–H), 1686 (C=O), 1596 and 1570 (phenothiazine ring), 1455 (C–H), 1285 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–8.1 (12H, m, Ar–H). Anal. Calcd. for $C_{22}H_{16}ClN_5OS$: C, 60.89; H, 3.72; N, 16.14. Found: C, 60.65; H,3.70; N, 16.08.

*5.1.7. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*o*-nitrobenzoyl)-1,2,3,4-tetrazole (**7**)*

Compound **7** was prepared using the same procedure as for **4**, and was obtained in 71% yield as a white solid: m.p. 109–110 °C. IR: 3084 (C–H), 1735 (C=O), 1596 and 1570 (phenothiazine ring), 1541 (N=O), 1455 (C–H), 1348 and 1208 (N=O), 1285 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.7–9 (12H, m, Ar–H). Anal. Calcd. for $C_{22}H_{16}N_6O_3S$: C, 59.45; H, 3.63; N, 18.91. Found: C, 59.55; H, 3.61; N, 18.84.

*5.1.8. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*p*-nitrobenzoyl)-1,2,3,4-tetrazole (**8**)*

Compound **8** was prepared using the same procedure as for **4**, and was obtained in 69% yield as a white solid: m.p.

171–172 °C. IR: 2864 (C–H), 1692 (C=O), 1596 and 1570 (phenothiazine ring), 1458 (C–H), 1348 and 1127 (N=O), 1283 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR (DMSO-d_6) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.9–8.3 (12H, m, Ar–H). Anal. Calcd. for $C_{22}H_{16}N_6O_3S$: C, 59.45; H, 3.63; N, 18.91. Found: C, 59.25; H, 3.61; N, 18.82.

*5.1.9. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*p*-hydroxybenzoyl)-1,2,3,4-tetrazole (**9**)*

Compound **9** was prepared using the same procedure as for **4**, and was obtained in 62% yield as a light brown solid: m.p. >210 °C. IR: 3390 (O–H), 1744 (C=O), 1596 and 1570 (phenothiazine ring), 1458 (C–H), 1283 (N–N=N–), 1108 and 1138 (tetrazole ring), 885 (O–H) $cm^{-1.}$ ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 5 (1H, s, OH), 6.85–7.37 (12H, m, Ar–H). Anal. Calcd. for $C_{22}H_{17}N_5O_2S$: C, 63.60; H, 4.12; N, 16.86. Found: C, 63.54; H, 4.11; N, 16.83.

*5.1.10. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*p*-aminobenzoyl)-1,2,3,4-tetrazole (**10**)*

Compound **10** was prepared using the same procedure as for **4**, and was obtained in 62% yield as a dark brown solid: m.p. >210 °C. IR: 3346 (N–H), 2880 (C–H), 1703 (C=O), 1596 and 1570 (phenothiazine ring), 1458 (C–H), 1320 (C–N), 1285 (N–N=N) 1108 and 1138 (tetrazole ring) $cm^{-1.}$ $_1$H-NMR (DMSO-d_6) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 3.63 (2H, s, NH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.6–7.8 (12H, m, Ar–H). Anal. Calcd. for $C_{22}H_{18}N_6OS$: C, 63.75; H, 4.38; N, 20.28. Found: C, 63.68; H, 4.13; N, 20.18.

*5.1.11. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*p*-methylbenzoyl)-1,2,3,4-tetrazole (**11**)*

Compound **11** was prepared using the same procedure as for **4**, and was obtained in 64% yield as a light grey solid: m.p. 121–122 °C. IR: 2972 (C–H), 1675 (C=O), 1611(C=C), 1596 and 1570 (phenothiazine ring), 1457 (C–H), 1283 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR (DMSO-d_6) δ 2.5 (3H, s, CH_3), 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–7.9 (12H, m, Ar–H). Anal. Calcd. for $C_{23}H_{19}N_5OS$: C, 66.81; H, 4.63; N, 16.94. Found: C, 66.58; H, 4.61; N, 16.89.

*5.1.12. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*p*-methoxybenzoyl)-1,2,3,4-tetrazole (**12**)*

Compound **12** was prepared using the same procedure as for **4**, and was obtained in 68% yield as a light brown solid: m.p.158–159 °C. IR: 2985 (C–H), 1686 (C=O), 1604 (C=C), 1596 and 1570 (phenothiazine ring), 1457 (C–H), 1283 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, J = 7.1 Hz, CH_2), 3.9 (3H, s,CH_3), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.4–8.1 (12H, m, Ar–H). Anal. Calcd. for $C_{23}H_{19}N_5O_2S$: C, 64.32; H, 4.46; N, 16.31. Found: C, 64.15; H, 4.04; N, 16.27.

*5.1.13. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(phenyl acetyl)-1,2,3,4-tetrazole (**13**)*

Compound **13** was prepared using the same procedure as for **4**, and was obtained in 64% yield as a light brown solid: m.p. 127–128 °C. IR: 2928 (C–H), 2853 (C–H), 1687 (C=O), 1596 and 1570 (phenothiazine ring), 1583 (C=C), 1457 (C–H), 1283 (N–N=N–), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR ($CDCl_3$) δ 2.3 (2H, s, CH_2), 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–7.3 (13H, m, Ar–H). Anal. Calcd. for $C_{23}H_{19}N_5OS$: C, 66.81; H, 4.63; N, 16.94. Found: C, 66.72; H, 4.61; N, 16.86.

*5.1.14. 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(*p*-toluenesulphonyl)-1,2,3,4-tetrazole (**14**)*

Compound **14** was prepared using the same procedure as for **4**, and was obtained in 60% yield as a grayish black solid: m.p. 97–98 °C. IR: 2954 (C–H), 1596 and 1570 (phenothiazine ring), 1457 (C–H), 1283 (N–N=N–), 1380 and 1173 (S=O), 1108 and 1138 (tetrazole ring) $cm^{-1.}$ ^{1}H-NMR (DMSO-d_6) δ 2.5 (3H, s, CH_3), 2.8 (2H, t, J = 7.1 Hz, CH_2), 4.3 (2H, t, J = 7.1 Hz, CH_2), 6.8–7.9 (12H, m, Ar–H). Anal. Calcd. for $C_{22}H_{19}N_5O_2S_2$: C, 58.78; H, 4.26; N, 16.94. Found: C, 58.57; H, 4.23; N, 16.88.

5.2. Evaluation of analgesic activity

Swiss strain albino mice of either sex weighing 25–30 g were used for this study. The test compounds were administered intraperitoneally in 1/10th of the dose of 250 mg/kg (LD_{50}) in 10% v/v Tween 80 suspensions. LD_{50} of the newly synthesized compounds were determined by Miller and Tainter method [35] administering the compounds intraperitoneally.

5.2.1. Acetic acid induced writhing method [27]

The animals were divided into 15 groups of 10 mice each. The control group of animals was administered with 10% v/v Tween 80 (0.5 ml) suspension. The standard drug was administered with Nimesulide (Dr. Reddy's Laboratories), intraperitoneally in a dose of 10 mg/kg. After 20 min of the administration the test compounds, all the groups of mice were given with the writhing agent 3% v/v aqueous acetic acid in a dose of 2 ml/kg intraperitoneally. The writhings produced in these animals were counted visually for 15 min and the numbers of writhings produced in treated groups were compared with those in the control group. The results are analyzed statistically by student "*t*" test and recorded in Table 3.

5.2.2. Hot plate method [28]

The method suggested by Eddy and Leimbach [28] was adopted for the study. The pain threshold of the animals was measured on a hot plate before treatment of the test and reference compounds, and the animals that showed more than 10 s of reaction time were rejected. The reference compound morphine sulphate (Dr. Reddy's Laboratories)

Table 3
Evaluation of analgesic activity by acetic acid induced writhing method

Treatment	Writhing episodes in 15 min (Mean ± S.E.M.)
Control	30.7 ± 0.5580
Nimesulide	17.1 ± 0.5229 *
Compound **2**	22.4 ± 0.5476 *
Compound **3**	19.5 ± 0.7379 *
Compound **4**	19.7 ± 0.4726 *
Compound **5**	18.2 ± 0.6798 *
Compound **6**	23.6 ± 0.6532 *
Compound **7**	27.6 ± 0.9213 **
Compound **8**	28.6 ± 0.6699 **
Compound **9**	18.3 ± 0.5174 *
Compound **10**	21.5 ± 0.4013 *
Compound **11**	17.8 ± 0.5330 *
Compound **12**	20.7 ± 0.4229 *
Compound **13**	22.7 ± 0.5972 *
Compound **14**	19.7 ± 0.4726 *

Dose: 25 mg/kg for test compounds and 10 mg/kg for Nimesulide.
* $P < 0.001$ and ** $P < 0.01$ represent significant difference when compared with control groups.

was administered intraperitoneally in a dose of 5 mg/kg. After the treatment of test and reference compounds, the pain threshold of the animals was measured at 15 min and 30 min of time interval. The results are analyzed statistically by student "*t*" test and recorded in Table 4.

5.2.3. Anti-inflammatory activity [29]

The anti-inflammatory activity was evaluated by carrageenin induced rat paw edema method [29]. Albino rats of wistar strain weighing 100–200 g of either sex were divided into 15 groups each of six animals. Tween 80 suspensions (10% v/v) of the test compounds were administered intraperitoneally in a dose of 25 mg/kg. The control group was given only 10% v/v Tween 80 (0.5 ml) suspension. One group was administered with diclofenac sodium (Novartis Laboratories) as standard, intraperitoneally in a dose of 2 mg/kg. After 30 min of the administration of test compounds paw edema was induced in albino rats by injecting 0.1 ml of carrageenin (1% v/v suspension in normal saline) into subplantar region of the left hind paw. After 3 h the increase in rat paw volume was recorded. The anti-inflammatory activity was measured in terms of percentage inhibition of edema of each group was calculated against control group using the following formula

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100$$

where C and T represent the average percentage increase in paw volume of the control and test groups, respectively. The results are analysed statistically by student "*t*" test and recorded in Table 5.

5.2.4. Evaluation of ulcerogenicity index

Ulceration in rats was induced as described by Goel et al. [30]. Albino rats of wistar strain weighing 100–200 g of either sex were divided into 15 groups each of six animals. Control group of animals were administered only with 10% v/v Tween 80 suspension intraperitoneally. One group was administered with Aspirin (German Remedies) intraperitoneally in a dose of 200 mg/kg once daily for three days. The remaining group of animals was administered with test compounds intraperitoneally in a dose of 25 mg/kg. On fourth day, pylorus was ligated as per the method of Shay et al. [31]. Animals were fasted for 36 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar [32] and recorded in Table 6.

Table 4
Evaluation of analgesic activity by hot plate method

Treatment	Average reaction time in seconds before treatment	Reaction time in (seconds) after	
		15 min	30 min
Control	4.95 ± 1.202	4.91 ± 1.234	4.95 ± 1.062
Morphine sulphate	4.50 ± 0.578 *	6.86 ± 0.332 *	11.06 ± 0.739 *
Compound **2**	5.05 ± 0.907 *	6.64 ± 0.819 *	7.62 ± 0.655 *
Compound **3**	4.80 ± 0.651 *	7.98 ± 0.719 *	8.67 ± 0.789 *
Compound **4**	4.69 ± 0.953 *	7.64 ± 0.606 *	8.78 ± 0.803 *
Compound **5**	4.91 ± 0.847	9.20 ± 0.684 *	10.91 ± 0.674 *
Compound **6**	4.62 ± 0.905	7.43 ± 0.923 *	8.78 ± 0.585 *
Compound **7**	4.81 ± 0.703	7.43 ± 0.923 *	7.99 ± 0.636 *
Compound **8**	4.55 ± 0.957	6.64 ± 0.798 *	6.50 ± 0.697 *
Compound **9**	4.78 ± 0.883	6.63 ± 0.514 *	6.43 ± 0.796 *
Compound **10**	4.83 ± 0.739	6.42 ± 0.769 *	8.67 ± 0.571 *
Compound **11**	4.63 ± 1.054	8.71 ± 0.839 *	10.87 ± 0.864 *
Compound **12**	5.01 ± 1.198	6.82 ± 0.748 *	7.83 ± 0.807 *
Compound **13**	4.82 ± 0.884	6.24 ± 0.850 *	7.13 ± 0.704 *
Compound **14**	4.74 ± 0.747	6.65 ± 0.543 *	7.50 ± 0.876 *

Dose: 25 mg/kg for test compounds and 5 mg/kg for morphine sulphate.
Comparison with control * $P < 0.001$.

Table 5
Evaluation of anti-inflammatory activity by carrageenin induced paw edema method

Treatment	Paw volume Mean ± S.E.M. (ml)	(%) Decrease in paw volume
Control	0.80 ± 0.003	00.0
Diclofenac sodium	0.30 ± 0.02 *	62.50
Compound **2**	0.80 ± 0.0013	00.0
Compound **3**	0.80 ± 0.0003	00.0
Compound **4**	0.80 ± 0.0047	00.0
Compound **5**	0.68 ± 0.30 **	15.00
Compound **6**	0.36 ± 0.0453 *	55.00
Compound **7**	0.64 ± 0.25 **	20.00
Compound **8**	0.38 ± 0.022 *	52.50
Compound **9**	0.72 ± 0.0016	10.00
Compound **10**	0.75 ± 0.0046	06.25
Compound **11**	0.74 ± 0.0111	07.50
Compound **12**	0.64 ± 0.24 **	20.00
Compound **13**	0.80 ± 0.081	00.0
Compound **14**	0.59 ± 0.20 **	26.25

Dose: 25 mg/kg for all the test compounds and 2 mg/kg for diclofenac sodium.
* $P < 0.001$ and ** $P < 0.01$ represent significant difference when compared with control groups.

Table 6
Evaluation of ulcerogenicity index

Treatment	Ulcer index (Mean ± S.E.M.)
Control	1.01 ± 0.21
Aspirin	1.61 ± 0.36
Compound **2**	0.94 ± 0.04 *
Compound **3**	0.85 ± 0.07 *
Compound **4**	0.97 ± 0.06 *
Compound **5**	1.02 ± 0.19 **
Compound **6**	1.46 ± 0.32 **
Compound **7**	1.06 ± 0.21 **
Compound **8**	1.28 ± 0.66 **
Compound **9**	1.08 ± 0.14 **
Compound **10**	0.95 ± 0.13 *
Compound **11**	0.98 ± 0.53 *
Compound **12**	1.20 ± 0.29 **
Compound **13**	0.87 ± 0.07 *
Compound **14**	1.17 ± 0.26 **

Dose: 25 mg/kg for test compounds and 200 mg/kg for aspirin.
* $P < 0.05$ and ** $P < 0.01$ represent significant difference when compared with control groups.

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