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Short communication

Synthesis and antinociceptive activity of some substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones

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Abstract

Twelve different derivatives of substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones (**3**–**14**) were synthesized by reacting 9-[2-(1*H*-tetrazol-5-yl)-ethyl]-2,3,4,9-tetrahydro-1*H*-carbazole (**2**) and the appropriate acid chlorides. 9-[2-(1*H*-tetrazol-5-yl) ethyl]-2,3,4,9-tetrahydro-1*H*-carbazole (**2**) was synthesized by reacting 3-(1,2,3,4-tetrahydrocarbazol-9-yl) propionitrile (**1**) with sodium azide and ammonium chloride. The chemical structures were confirmed by means of IR, ^{1}H-NMR, mass spectra and elemental analysis. The compounds were screened for antinociceptive activity by acetic acid induced writhing method and hot plate method. 1-Phenyl-2-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}ethanone (**13**) was found to be the most active compound of the series.
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Keywords: Synthesis; Substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones; Antinociceptive activity

1. Introduction

Tetrazoles are an increasingly popular skeleton [1] with wide ranging applications. They have found use in pharmaceuticals as lipophilic spacers and carboxylic acid surrogates, which improves oral absorption [2]. Substituted 1,2,3,4-tetrazoles were reported to possess antinociceptive activity [3–7], antibacterial [8], antifungal [9–11], antiviral [12], anti-inflammatory [13,14] and antiulcer [15,16] activities. Further, antinociceptive activity of some novel 1-(9′-acridinyl)-5-substituted phenyl tetrazoles [17] and benzimidazole tetrazolyl derivatives [18] were reported in the literature. Therefore, it was envisaged that a new series of tetrazoles containing various acyl moiety would possess antinociceptive activity due to its structural resemblance with reported heterocycles. In continuation of our work on some pharmacologically active tetrazoles [6,14] we hereby report the synthesis and antinociceptive activity of some tetrazoles. In the present study, some new substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones was synthesized and screened for antinociceptive activity.

2. Chemistry

Compounds were prepared as shown in Fig. 1. Cyanoethylation of tetrahydrocarbazole with acrylonitrile in the presence of Triton B (trimethylbenzyl ammonium hydroxide) gave 3-(1,2,3,4-tetrahydrocarbazol-9-yl) propionitrile (**1**). The requisite compound **1** was 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 are of limited use in preparative organic chemistry because; the use of hydrazoic acid [19] presents considerable experimental difficulties due to its toxicity and tendency to explode. However, the simple route reported by Finnegan et al. [20] was adopted for the preparation of substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones. This route replaces the toxic hydrazoic acid by inorganic azide to afford the titled compounds in good yield (54–89%). Compound **1** was cyclized using sodium azide and ammonium chloride to yield com-

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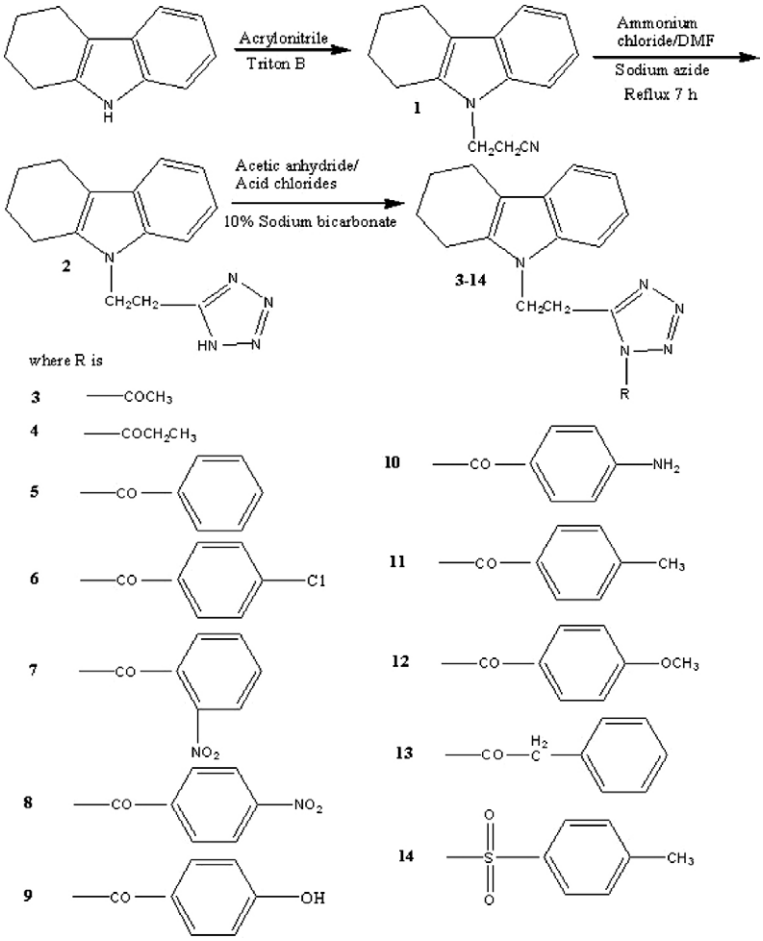


Fig. 1. Synthetic scheme of 5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl) ethyl]thetrazol-1-yl) alkanones.

pound **2**. Twelve substituted tetrazoles were synthesized from **2** by acylation and tosylation reaction.

3. Results and discussion

3.1. Chemistry

Structure activity relationship studies of 5-substituted 1-*H* tetrazole focused initially on the length of the carbon chain between tetrazole and tetrahydrocarbazole ring. Studies on variation of the polymethylene units [$(CH_2)_n N = 1–3$] showed that two carbon unit spacer was the best spacer [24].

All secondary amine undergo cyanoethylation reaction with acrylonitrile and a base. 1,2,3,4-Tetrahydrocarbazole 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.

Infrared spectrum of compound **1** showed sharp absorption band at 2245 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. 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 δ 2.8 and δ 4.3. A triplet at δ 2.8 is due to the methylene protons close to cyano group. The triplet at δ 4.3 is due to deshielding of two protons present near the aromatic nitrogen atom. The chemical shift of NH proton of the tetrazole is undetectable in NMR spectra. Aromatic protons showed multiplets in the range of δ 6.7–7.3. The expected signals with appropriate multiplicities for different types of protons were observed for the derivatives (Scheme 1).

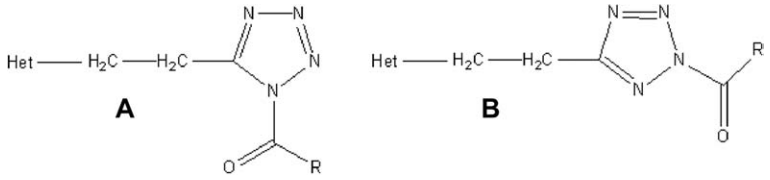
The synthesized tetrazoles **3**–**14**, could exist as two isomers **A** and **B**.

The structure was attributed on the basis of the literature [25,26] to isomers A, because they were synthesized in solution and because isomer B would only be stable in gas phase.

3.2. Antinociceptive activity

3.2.1. Behavioral test

Minimum motor impairment was measured in mouse by Rota rod test showed that all the synthesized compounds have



Scheme 1.

no effect which was indicated by their ability to maintain the equilibrium on the Rota rod for more than 1 min.

3.2.2. Acetic acid induced writhing method [22]

All compounds tested exhibited activity in a dose of 30 mg kg^{-1} with the exception of compounds **6**–**9** that are devoid of antinociceptive activity. Behavioral parameters such as motor coordination and spontaneous motility were not altered at dose of 30 mg kg^{-1} of test compounds, which has been confirmed from Rota rod (Techno, 20 rpm) test (Table 1). The antinociceptive activity of compound **13** was found to be superior compared to other synthesized compounds. Introduction of propionyl, 4-aminobenzoyl, 4-methoxybenzoyl and 4-toluenesulfonyl group at position 1 of 9-[2-(1*H*-tetrazol-5-yl)ethyl]-2,3,4,9-tetrahydro-1*H*-carbazole showed almost equivalent antinociceptive activity as that of nimesulide. 9-[2-(1*H*-tetrazol-5-yl)ethyl]-2,3,4,9-tetrahydro-1*H*-carbazole with acetyl, benzoyl and 4-methylbenzoyl group at position 1 of tetrazole showed moderate antinociceptive activity. Introduction of 4-chlorobenzoyl, 2-nitrobenzoyl, 4-nitrobenzoyl and 4-hydroxybenzoyl group in 9-[2-(1*H*-tetrazol-5-yl)ethyl]-2,3,4,9-tetrahydro-1*H*-carbazole have shown minimum or no antinociceptive activity.

3.2.3. Hot plate method

All compounds tested by Eddy and Leimbach [23] hot plate method exhibited activity in a dose of 30 mg kg^{-1}. The antinociceptive activity exhibited by compounds **8**, **9**, **13**, **14** were found to be superior and the other analogs exhibited moderate antinociceptive activity.

4. Conclusions

We prepared a series of some substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}alkanones and demonstrated that these compounds possessed good antinociceptive activity tested both by acetic acid writhing method and hot plate method. Acetic acid induced writhing method and hot plate method were adopted to assess the peripheral (non-narcotic) and centrally (narcotic) mediated antinociceptive activities. The most promising compound having antinociceptive activity was found to be *1*-phenyl-2-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}ethanone **13**. The results indicate that compounds **8** and **9** found to exhibit peripherally mediated antinociceptive activity and not by centrally mediated antinociceptive activity.

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 spectrophotometer. Chemical shifts are reported in parts per million (δ) units relative to internal standard tetramethyl silane. Mass spectra were recorded on Joel JMS-DX 303 Mass Spectrometer. Elemental analysis was performed on Heraeus Carlo Erba 1108 and the analysis indicated by the symbols of the elements was within ± 0.4% of theoretical values.

*5.1.1. 3-(1,2,3,4-Tetrahydrocarbazol-9-yl) propionitrile (**1**)*

1,2,3,4-Tetrahydrocarbazole (8.56 g, 50 mmol) was mixed with 12.5 ml of acrylonitrile in a 100 ml round bottomed flask. Triton B (2 ml of 40% v/v) was added drop-wise with shaking. A vigorous reaction was set in. It was allowed to subside and then the contents of the flask were refluxed on a steam bath for 2 h. The solution was cooled, extracted with ethylene dichloride and dried over anhydrous sodium sulfate. The dried nitrile recrystallized from ethanol was obtained as a yellow amorphous solid in 82% yield: m.p. 110–111 °C. IR: 2245 (C≡N), 2917 (C–H), 1613 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.9 (s, 4H, tetrahydrophenyl), 2.0 (s, 4H, tetrahydrophenyl), 2.8 (t, $J = 7.1$ Hz, 2H, CH_2), 4.3 (t, $J = 7.1$ Hz, 2H, CH_2), 6.7–7.3 (m, 4H, Ar-H). Anal. $C_{15}H_{16}N_2$.

*5.1.2. 9-[2-(1-*H*-tetrazol-5-yl)ethyl]-2,3,4,9-tetrahydro-1*H*-carbazole (**2**)*

The method described by Finnegan et al. [32] was followed to synthesize the tetrazole. A mixture of 3-tetrahydro-

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

Behavior	Effect after 30 min of administration (mean ± S.E.M.) (i.p.)					
	10% Tween 80 suspension	5 mg kg^{-1}	10 mg kg^{-1}	20 mg kg^{-1}	30 mg kg^{-1}	40 mg kg^{-1}
Grip test	No effect	No effect	No effect	No effect	No effect	1 ± 0.23* *
Motor activity	No effect	No effect	No effect	No effect	No effect	1 ± 0.12* *

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

carbazole-9-yl-propionitrile (2.44 g, 10 mmol), sodium azide (1 g, mmol) dimethylformamide (10 ml) and ammonium chloride (5.3 g, 10 mmol) were taken in a 100 ml round bottomed flask. The contents were heated in an oil bath for 7 h at 125 °C. The solvent was removed under reduced pressure. The residue was dissolved in 100 ml of distilled water and carefully acidified with hydrochloric acid to pH 2. The solution was cooled to 5 °C in ice bath. The product was removed by filtration, washed with several portions of water and dried. The crude product recrystallized from aqueous methanol (yield 89%) was obtained as a yellow solid: m.p. 145–146 °C. IR: 3448 (N–H), 2926 (C–H), 2917 (C–H), 1613 (C=C), 1591 (C=N), 1286 (–N–N=N–), 1308 and 1109 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.9 (s, 4H, tetrahydrophenyl), 2.0 (s, 4H, tetrahydrophenyl), 2.8 (t, J = 7.1 Hz, 2H, CH_2), 3.5 (s, 1H, NH), 4.3 (t, J = 7.1 Hz, 2H, CH_2), 6.7–7.3 (m, 4H, Ar-H). Anal. for $C_{15}H_{17}N_5$.

*5.1.3. 1-{5-[2-(1,2,3,4-Tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}ethanone (**3**)*

Compound **2** (1 g, 4 mmol) was refluxed in acetic anhydride (3 g, 30 mmol) for 15 min. The reaction mixture was cooled and poured into 20 ml of cold water. The contents were the boiled to decompose the excess acetic anhydride. Compound **3** was recrystallized from aqueous ethanol as a brown amorphous solid (yield 59%). The analytical sample melted at 114–115 °C. IR: 2917 (C–H), 1614 (C=C), 1567 (C=C), 1470 (C–H), 1286 (–N–N=N–), 1308 and 1109 (tetrazole ring), cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.2–1.4 (s, 3H, $CO\underline{CH_3}$), 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.7–2.9 (t, J = 6.4 Hz, 2H, $–CH_2$), 4.2–4.4 (t, J = 6.4 Hz, 2H, $N–CH_2$), 7.1–7.3 (m, 4H, Ar-H). Anal. Calcd. for $C_{17}H_{19}N_5O$: C, 66.00; H, 6.19; N, 22.64. Found: C, 66.29; H, 6.16; N, 22.48.

*5.1.4. 1-{5-[2-(1,2,3,4-Tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}propan-1-one (**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. The contents were acidified with 20% v/v hydrochloric acid to and the product obtained was filtered. The dried compound was recrystallized from aqueous ethanol as a yellow amorphous solid (yield 56%). m.p. 141–142 °C. IR: 2926 (C–H), 2853 (C–H), 1710 (C=O), 1567 (C=C), 1475 (C–H), 1370 (C–H), 1286 (–N–N=N–), 1109 and 1308 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.1–1.3 (t, J = 6.4 Hz, 3H, $O=C–CH_2\,\underline{CH_3}$), 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.2–2.4 (q, J = 6.7 Hz, 2H, $O=C–CH_2CH_3$), 2.7–2.9 (t, J = 6.4 Hz, 2H, CH_2), 4.2–4.4 (t, J = 6.4 Hz, 2H, $N–CH_2$), 7.1–7.3 (m, 4H, Ar-H). Anal. for $C_{18}H_{21}N_5O$.

*5.1.5. Phenyl-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]-tetrazol-1-yl}methanone (**5**)*

Compound **5** was prepared using the same procedure as for **4**, and was obtained in 69% yield as white crystalline solid: m.p. 106–107 °C. IR: 2926 (C–H), 2853 (C–H), 1676 (C=O), 1567 (C=C), 1475 (C–H), 1670 (C–H), 1286 (–N–N=N–), 1109 and 1308 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.7–2.9 (t, J = 6.4 Hz, 2H, CH_2), 4.2–4.4 (t, J = 6.4 Hz, 2H, $N–CH_2$), 7.1–8.1 (m, 9H, Ar-H). Anal. for $C_{22}H_{21}N_5O$.

*5.1.6. (4-Chlorophenyl)-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl} methanone (**6**)*

Compound **6** was prepared using the same procedure as for **4**, and was obtained in 59% yield as a white amorphous solid: m.p. 167–168 °C. IR: 2911(C–H), 1692 (C=O), 1592 (C=C), 1470 (C–H), 1109 and 1138 (tetrazole ring), 762 and 681 (C–C1) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 4.3 (t, J = 6.4 Hz, 2H, $N–CH_2$), 7.1–8.4 (m, 8H, Ar-H). Anal. for $C_{22}H_{20}ClN_5O$.

*5.1.7. (2-Nitrophenyl)-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl} methanone (**7**)*

Compound **7** was prepared using the same procedure as for **4**, and was obtained in 79% yield as light a yellow amorphous solid: m.p. 122–123 °C. IR: 2922 (C–H), 1674 (C=O), 1567 (C=C) 1528 (NO_2), 1470 (C–H), 1314 (NO_2), 1109 and 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 4.3 (t, J = 6.4 Hz, 2H, $N–CH_2$), 7.1–8.9 (m, 8H, Ar-H). Anal. for $C_{22}H_{20}N_6O_3$.

*5.1.8. (4-Nitrophenyl)-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl} methanone (**8**)*

Compound **8** was prepared using the same procedure as for **4**, and was obtained in 84% yield as brownish gray solid: m.p. 210–211 °C. IR: 2910 (C–H), 1688 (C=O), 1606 (C=C) 1542 and 1350 (NO_2), 1138 and 1108 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 4.3 (s, J = 6.4 Hz, 2H, $N–CH_2$), 7.1–8.3 (m, 8H, Ar-H). Anal. for $C_{22}H_{20}N_6O_3$.

*5.1.9. (4-Hydroxyphenyl)-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl} methanone (**9**)*

Compound **9** was prepared using the same procedure as for **4**, and was obtained in 72% yield as a white amorphous solid: m.p. 216–217 °C. IR: 3650 (O–H), 2911 (C–H), 1738 (C=O), 1601 (C=C), 1470 (C–H), 1138 and 1108 (tetrazole ring), 806 (C–H out of plane bending) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 4.3 (t, J = 6.4 Hz, 2H, $N–CH_2$), 6.9–7.5 (m, 8H, Ar-H), 7.3 (s, 1H, OH). Anal. for $C_{22}H_{21}N_5O_2$.

*5.1.10. (4-Aminophenyl)-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl} methanone (**10**)*

Compound **10** was prepared using the same procedure as for **4**, and was obtained in 54% yield as a yellow amorphous

solid: m.p. 214–215 °C. IR: 3335 (N–H), 2926(C–H), 1672 (C=O), 1596 (C=C), 1505 (C=C), 1470 (C–H), 1138 and 1108 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.5 (s, 1H, NH_2), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 4.3 (t, J = 6.4 Hz, 2H, N–CH_2), 7.1–7.8 (m, 8H, Ar-H). Anal. for $C_{22}H_{22}N_6O$.

*5.1.11. {5-[2-(1,2,3,4-Tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}-p-tolylmethanone (**11**)*

Compound **11** was prepared using the same procedure as for **4**, and was obtained as a white solid in 68% yield: m.p. 157–158 °C. IR: 2934 (C–H), 1668 (C=O), 1611 (C=C), 1470 (C–H), 1138 and 1108 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.4 (s, 3H, CO–C_6H_4–$\underline{CH_3}$), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 4.3 (t, J = 6.4 Hz, 2H, N–CH_2), 7–8 (m, 8H, Ar-H). Anal. for $C_{23}H_{23}N_5O$.

*5.1.12. (4-Methoxyphenyl)-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl} methanone (**12**)*

Compound **12** was prepared using the same procedure as for **4**, and was obtained in 68% yield as a white solid: m.p. 131–132 °C. IR: 2940 (C–H), 1687 (C=O), 1605 (C=C), 1467 (C–H), 1138 and 1108 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 3.9 (s, 3H, OCH_3), 4.3 (t, J = 6.4 Hz, 2H, N–CH_2), 7.1–8.1 (m, 8H, Ar-H). Anal. for $C_{23}H_{23}N_5O_2$.

*5.1.13. 1-Phenyl-2-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-yl}ethanone (**13**)*

Compound **13** was prepared using the same procedure as for **4**, and was obtained in 58% yield as light yellow solid: m.p. 133–134 °C. IR: 2978 (C–H), 1710 (C=O), 1598 (C=C), 1470 (C–H), 1138 and 1108 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 3.5 (s, 2H, CH_2), 4.3 (t, J = 6.4 Hz, 2H, N–CH_2), 7.1–7.5 (m, 9H, Ar-H). EI-MS *m/z*: 385.32 (Calcd. for $C_{23}H_{23}N_5O$: 385.47). Anal. for $C_{23}H_{23}N_5O$.

*5.1.14. 9-{2-[1-(Toluene-4-sulfonyl-1H-tetrazol-5-yl]ethyl}-2,3,4,9-tetrahydro-1H-carbazole (**14**)*

Compound **14** was prepared using the same procedure as for **4**, and was obtained in 64% yield as grayish black solid: m.p. 142–143 °C. IR: 2922 (C–H), 1614 (C=O), 1426 (C–H), 1375 and 1178 (SO_2), 1138 and 1108 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7–1.9 (d, J = 7.2 Hz, 4H, 1 and 4 tetrahydrophenyl), 1.9–2.1 (d, J = 7.2 Hz, 4H, 2 and 3 tetrahydrophenyl), 2.5 (s, 3H, CH_3), 2.8 (t, J = 6.4 Hz, 2H, CH_2), 4.3 (t, J = 6.4 Hz, 2H, N–CH_2), 7.1–7.9 (m, 12H, Ar-H). EI-MS *m/z*: 421.46 (Calcd. for $C_{22}H_{23}N_5O_2S$: 421.51). Anal. for $C_{23}H_{23}N_5O$.

5.2. Evaluation of antinociceptive activity

Swiss strain albino mice of either sex weighing 25–30 g were used for this study. The test compounds (in 1/10th dose of the average LD_{50} values of titled compounds) were administered intraperitoneally in 10% v/v Tween 80 suspensions. LD_{50} of the newly synthesized compounds were determined by Miller and Tainter [21] method administering the compounds intraperitoneally.

5.2.1. Acetic acid induced writhing method

The method suggested by Witkin et al. [22] was adopted for the study. The animals were divided into 14 groups of mice each. The control group of animals was administered with 10% v/v Tween 80 (0.5 ml) suspension. The standard drug nimesulide (Dr. Reddy's Laboratories), was administered intraperitoneally in a dose of 10 mg kg^{-1} to other group of animals. 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^{-1} 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's *t*-test and recorded in Table 2.

5.2.2. Hot plate method

The method of Eddy and Leimbach [23] 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 sulfate (Dr. Reddy's Laboratories) was administered intraperitoneally in a dose of 5 mg kg^{-1}. After the treatment of test and reference compounds, the pain threshold of the animals

Table 2
Evaluation of antinociceptive activity by acetic acid induced writhing method

Compound	Writhing episodes in 15 min (mean ± S.E.M.)	% Protection	LD_{50} (mg kg^{-1})
Control	30.7 ± 0.5580	00.00	–
Nimesulide	17.1 ± 0.5229*	44.30	–
3	23.8 ± 0.6110**	22.48	285
4	19.7 ± 0.5783*	35.83	283
5	24.2 ± 0.6960**	21.17	286
6	26.0 ± 1.0110***	15.31	282
7	28.5 ± 1.1660***	07.17	287
8	30.4 ± 0.6000***	00.98	289
9	31.3 ± 0.5587***	–	283
10	20.0 ± 0.8299*	34.85	282
11	23.9 ± 1.0260**	22.15	286
12	20.0 ± 0.3944*	34.85	292
13	17.3 ± 0.7895*	43.65	283
14	20.2 ± 0.7424*	34.21	286

Dose: 30 mg kg^{-1} for all the test compounds and 10 mg kg^{-1} for nimesulide. Comparison with control. * Highly significant $P < 0.001$, ** Significant $P < 0.01$, *** Insignificant $P < 0.5$, $N = 6$.

Table 3
Evaluation of antinociceptive activity by hot plate method

Compound	Average reaction time before treatment	Reaction time (in s) after			
		15 min	30 min	1 h	2 h
Control	4.95 ± 1.202	4.91 ± 1.234	4.95 ± 1.062	4.92 ± 0.331	4.95 ± 0.974
Morphine	4.50 ± 0.578*	6.86 ± 0.332*	13.06 ± 0.739*	11.64 ± 0.505*	6.16 ± 0.439*
3	5.50 ± 0.820*	9.85 ± 0.134*	11.82 ± 0.192*	11.83 ± 0.745*	11.16 ± 0.341*
4	4.85 ± 0.970*	10.28 ± 0.779*	11.18 ± 0.242*	11.86 ± 0.392*	11.29 ± 0.435*
5	5.65 ± 0.620*	10.84 ± 0.737*	11.81 ± 0.498*	11.95 ± 0.397*	11.43 ± 0.523*
6	4.76 ± 0.670*	10.27 ± 0.459*	11.14 ± 0.178*	11.42 ± 0.545*	11.11 ± 0.310*
7	4.95 ± 0.980*	10.76 ± 0.735*	10.62 ± 0.254*	10.18 ± 0.188*	10.13 ± 0.571*
8	6.10 ± 0.440*	12.07 ± 0.562*	10.12 ± 0.555*	10.18 ± 0.585*	10.27 ± 0.656*
9	5.85 ± 0.530*	11.13 ± 0.174*	11.60 ± 0.185*	11.99 ± 0.274*	12.01 ± 0.305*
10	4.88 ± 0.950*	9.08 ± 0.399*	10.50 ± 0.247*	11.84 ± 0.223*	11.90 ± 0.214*
11	4.75 ± 0.680*	10.30 ± 0.204*	11.15 ± 0.196*	11.90 ± 0.175*	12.50 ± 0.209*
12	4.65 ± 0.530*	9.56 ± 0.259*	11.25 ± 0.204*	11.50 ± 0.194*	11.60 ± 0.194*
13	5.85 ± 0.630*	10.60 ± 0.197*	12.85 ± 0.222*	11.90 ± 0.214*	11.98 ± 0.175*
14	4.90 ± 0.300*	11.13 ± 0.225*	11.50 ± 0.137*	11.80 ± 0.227*	12.61 ± 0.215*

Dose: 30 mg kg^{-1} for all the test compounds and 10 mg kg^{-1} for morphine. Comparison with control. * Highly significant $P < 0.001$, $N = 6$.

was measured at 15 and 30 min of time interval. The results are analyzed statistically by Student's *t*-test and recorded in Table 3.

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