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ORIGINAL RESEARCH

Discovery of Fused Triazolo-thiadiazoles as Inhibitors of TNF-alpha: Pharmacophore Hybridization for Treatment of Neuropathic Pain

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ABSTRACT

Introduction: Neuropathic pain is a complex, chronic pain state that is usually accompanied by tissue injury. With neuropathic pain, the nerve fibers themselves may be damaged, dysfunctional, or injured.

Methods: A series of pharmacophoric hybrids of substituted aryl semicarbazides incorporated into a fused triazolo-thiadiazole nucleus were synthesized and evaluated for neuropathic pain activity. After the assessment of neurotoxicity and peripheral analgesic activity, the compounds were evaluated in two peripheral neuropathic pain models, the chronic constriction injury and partial sciatic nerve ligation, to assess their antiallodynic and antihyperalgesic potential.

Results: Selected compounds exhibiting promising efficacies (4b, 6a, and 7e) revealed median effective dose (ED_{50}) values ranging from 7.62–28.71 mg/kg in four behavioral assays of allodynia and hyperalgesia (spontaneous pain, tactile allodynia, cold allodynia, and mechanical hyperalgesia). Studies carried out to assess the underlying mechanism revealed that compounds suppressed the inflammatory component of the neuropathic pain by inhibiting tumor necrosis factor (TNF)-alpha and preventing oxidative and nitrosative stress.

Conclusion: Using a hybrid design approach, the present study identified novel chemical compounds that could be a potential lead for the treatment of neuropathic pain.

Keywords: 2-Diphenyl-1-picrylhydrazyl; Antiallodynic; Antihyperalgesic; Neuropathic pain; Nitric oxide; TNF-alpha; Triazolo-thiadiazole

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INTRODUCTION

Neuropathic pain syndromes characterized by neuronal hyperexcitability in damaged areas of

the peripheral or central nervous system involve complex pathophysiological processes [1]. The development of inflammatory microenvironment at the site of nerve injury and the release of inflammatory mediators, together with their coupled signaling pathways, contribute to the generation and maintenance of neuropathic pain [2, 3]. Following nerve injury, peripheral as well as central sensitization occurs [4, 5] with upregulation of tumor necrosis factor-alpha (TNF-alpha), a pro-inflammatory cytokine, and its receptors in Schwann cells, endothelial cells, and in the dorsal horn of the spinal cord and hippocampus [6–8]. TNF-alpha, a key mediator in the inflammation, activates T cells and macrophages, thereby initiating the innate immune response. Consequently, the release of other inflammatory cytokines, sympathomimetic amines, prostaglandins, and nitric oxide (NO) is stimulated, which are involved in sensitization of primary afferent nociceptors [5–8]. Activated microglia and pro-inflammatory cytokines also induce the generation of free radicals leading to oxidative and nitrosative stresses, which exaggerate pain states [9, 10]. Inhibiting TNF synthesis with thalidomide or treatment with anti-TNF neutralizing antibodies at the time of nerve injury blocks the development of hyperalgesia and allodynia in neuropathic animals [11, 12].

In recent years, the condensed bridgehead nitrogen heterocyclic system of triazolo-thiadiazole, which may be considered as the cyclic analog of thiosemicarbazide and biguanide has received considerable attention. A wide spectrum of biological activities, specifically anticonvulsant [13], analgesic [14], anxiolytic [15], and anti-inflammatory [16] properties has been reported for triazolo-thiadiazole in the literature. The 1,2,4-triazole core of the above fused system has gained considerable attention regarding its action against neuropathic pain acting through various targets. Many triazole-based P_2X_7 antagonists [17, 18] sodium channel blockers [19], and σ-receptor inhibitors [20] have been reported. The literature also reveals many 1,2,4-triazole-based cannabinoid modulators [21, 22] possessing antinociceptive efficacies.

The primary focus of our research has been on the design and synthesis of pharmacophoric hybrids of aryl semicarbazones to develop multifunctional leads useful in the treatment of neurological disorders such as epilepsy and neuropathic pain [23–26]. We have previously reported the cyclization of various aryl semicarbazones to 1,2,4-triazoles, which resulted in improved anticonvulsant activities [27]. Moreover, the literature reveals that acylthiosemicarbazides and their corresponding cyclized 1,3,4-thiadiazole derivatives possess anti-inflammatory [28, 29] and analgesic [30] activities.

In view of the above reports, the design and synthesis of pharmacophoric hybrids of aryl semicarbazides into triazolo-thiadiazole templates was accomplished followed by assessment of their antinociceptive potential and underlying mechanism of action. Various reports on the antinociceptive efficacy of 4-aminobutyric acid (GABA) and gabapentin [31] and our research on N-spiro GABA derivatives [32] prompted us to explore the structure–activity relationships of GABA and N-spiro GABA derivatives in the cyclized triazolo-thiadiazole template.

MATERIALS AND METHODS

Chemistry

General

Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus

(Buchi, Flawil, Switzerland) and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on a Bruker Avance (300 MHz) HMR machine (Bruker, Fällanden, Switzerland). Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. Mass spectra were measured with a Shimadzu GC–MS-QP5000 spectrophotometer. Elemental analyses (C, H, and N) were undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within ±0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silicagel-G (Merck, Darmstadt, Germany) coated aluminum plates, visualized by iodine vapor and UV light.

General Scheme for the Synthesis of Potassium Dithiocarbazinate (2a–2e)

Substituted phenyl semicarbazide, 1a–1e (0.083 mol) was added to a solution of potassium hydroxide (0.125 mol) in methanol (50 mL) at 0–5°C under stirring. To this, carbon disulfide (0.025 mol) was added dropwise with constant stirring. The reaction mixture was stirred continuously for 12 h at room temperature. The precipitated potassium dithiocarbazinate salt was filtered, washed with anhydrous ether, and dried in a vacuum. The potassium salt thus obtained was used in the next step without further purification.

General Scheme for the Synthesis of 4-Amino-5-substituted-3-mercapto-(4H)-1,2,4-triazoles (3a–3e)

Potassium dithiocarbazinate derivatives, 2a–2e (0.02 mol) and hydrazine hydrate (99%, 0.04 mol) in water (25 mL) were refluxed for 10–15 h with occasional shaking. The reaction mixture was cooled to room temperature and diluted with cold water (10 mL). On acidification with dilute hydrochloric acid (HCl), a white precipitate resulted, which was filtered, washed with cold water, dried, and recrystallized from ethanol.

General Scheme for the Synthesis of 3,6-Disubstituted-[1, 2, 4]-triazolo-[3,4-b]-1,3,4-thiadiazoles (4a–4$_3$e, 5a–5e, 6a–6e, 7a–7e, 8a–8e)

An equimolar mixture (0.01 mol) of 4-amino-5-substituted-3-mercapto-(4H)-1,2,4-triazoles (3a–3e) and aromatic acids in phosphorous oxychloride (10 mL) were refluxed for 5 h. After the completion of the reaction, the reaction mixture was slowly poured into crushed ice with vigorous stirring and neutralized with sodium bicarbonate ($NaHCO_3$). The precipitated solid was filtered, washed with cold water, and recrystallized from ethanol.

6-(2-Aminoethyl)-N-(4-bromophenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (4a)

Yield: 64%; melting point (m.p.): 185°C; ^{1}H NMR (DMSO-d_6) δ: 2.01 (p, 2H), 2.63 (t, 2H), 2.84 (t, 2H), 4.78 (br s, 2H, D_2O exchangeable), 6.91 (d, 2H), 7.13 (d, 2H), 8.24 (br s, NH, D_2O exchangeable), ^{13}C NMR δ 34.3, 41.8, 117.2, 119.4, 134.6, 139.2, 159.8, 168.8, 169.1: MS (ESI) 338.99 $(MH)^+$. Anal. $C_{11}H_{11}BrN_6S$ (C, H, N).

6-(2-Aminoethyl)-N-(4-chlorophenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (4b)

Yield: 72%; m.p.: 192°C; ^{1}H NMR (DMSO-d_6) δ: 2.03 (p, 2H), 2.66 (t, 2H), 2.83 (t, 2H), 4.72 (br s, 2H, D_2O exchangeable), 7.01 (d, 2H), 7.22 (d,

2H), 8.63 (br s, NH, D_2O exchangeable), ^{13}C NMR δ 34.2, 41.6, 122.2, 127.4, 129.6, 137.2, 157.8, 167.6, 168.2: MS (ESI) 295.05 $(MH)^+$. Anal. $C_{11}H_{11}ClN_6S$ (C, H, N).

6-(2-Aminoethyl)-N-(2,4-dimethylphenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (4c)

Yield: 71%; m.p.: 178°C; ^1H NMR (DMSO-d_6) δ: 2.02 (p, 2H), 2.14 (s, 3H), 2.37 (s, 3H), 2.68 (t, 2H), 2.84 (t, 2H), 4.84 (br s, 2H, D_2O exchangeable), 6.57 (d, 1H), 6.87–6.91 (m, 2H), 9.10 (br s, NH, D_2O exchangeable), ^{13}C NMR δ 17.2, 21.6, 34.1, 41.6, 116.3, 126.3, 128.1, 132.1, 137.2, 139.4, 157.3, 167.1, 168.2: MS (ESI) 289.12 $(MH)^+$. Anal. $C_{13}H_{16}N_6S$ (C, H, N).

6-(2-Aminoethyl)-N-(2,5-dimethylphenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (4d)

Yield: 68%; m.p.: 152°C; ^1H NMR (DMSO-d_6) δ: 2.04 (p, 2H), 2.13 (s, 6H), 2.38 (s, 3H), 2.66 (t, 2H), 2.86 (t, 2H), 4.59 (br s, 2H, D_2O exchangeable), 6.67–6.69 (m, 2H), 6.94 (d, 1H), 9.10 (br s, NH, D_2O exchangeable), ^{13}C NMR δ 17.2, 21.6, 34.1, 41.6, 115.3, 118.2, 126.1, 129.2, 136.1, 141.2, 157.2, 167.1, 168.1: MS (ESI) 289.12 $(MH)^+$. Anal. $C_{13}H_{16}N_6S$ (C, H, N).

6-(2-Aminoethyl)-N-(2,6-dimethylphenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (4e)

Yield: 61%; m.p.: 144°C; ^1H NMR (DMSO-d_6) δ: 2.01 (p, 2H), 2.14 (s, 6H), 2.66 (t, 2H), 2.88 (t, 2H), 4.71 (br s, 2H, D_2O exchangeable), 6.69 (t, 1H), 6.96 (d, 2H), 9.41 (br s, NH, D_2O exchangeable), ^{13}C NMR δ 17.8, 34.1, 41.6, 121.4, 128.2, 136.4, 139.2, 157.1, 167.2, 168.3: MS (ESI) 289.12 $(MH)^+$. Anal. $C_{13}H_{16}N_6S$ (C, H, N).

N-(4-Bromophenyl)-6-(heptan-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (5a)

Yield: 69%; m.p.: 157°C; ^1H NMR (DMSO-d_6) δ: 0.93–1.10 (t, 6H), 1.35–1.40 (m, 4H), 1.56–1.62 (q, 4H), 2.66–2.68 (t, 1H), 6.94 (d, 2H), 7.16 (d, 2H), 8.43 (br s, 1H, D_2O exchangeable), ^{13}C NMR δ 14.2, 20.3, 38.3, 116.1, 118.2, 132.3, 137.2, 157.2, 167.2, 168.1: MS (ESI) 394.06 $(MH)^+$. Anal. $C_{16}H_{20}BrN_5S$ (C, H, N).

N-(4-Chlorophenyl)-6-(heptan-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (5b)

Yield: 78%; m.p.: 171°C; ^1H NMR (DMSO-d_6) δ: 0.96–1.11 (t, 6H), 1.37–1.41 (m, 4H), 1.54–1.59 (q, 4H), 2.65–2.67 (t, 1H), 7.04 (d, 2H), 7.25 (d, 2H), 8.81 (br s, 1H, D_2O exchangeable), ^{13}C NMR δ 14.2, 20.3, 38.3, 122.7, 127.1, 129.5, 137.1, 157.2, 167.3, 168.2: MS (ESI) 350.11 $(MH)^+$. Anal. $C_{16}H_{20}ClN_5S$ (C, H, N).

N-(2,4-Dimethylphenyl)-6-(heptan-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (5c)

Yield: 73%; m.p.: 153°C; ^1H NMR (DMSO-d_6) δ: 0.97–1.11 (t, 6H), 1.36–1.42 (m, 4H), 1.55–1.60 (q, 4H), 2.14 (s, 3H), 2.37 (s, 3H), 2.66–2.69 (t, 1H), 6.59 (d, 1H), 6.87–6.92 (m, 2H), 9.40 (br s, 1H, D_2O exchangeable), ^{13}C NMR δ 14.2, 17.3, 20.3, 21.1, 38.3, 116.3, 126.3, 128.2, 131.2, 137.2, 139.3, 157.3, 167.4, 168.1: MS (ESI) 344.19 $(MH)^+$. Anal. $C_{18}H_{25}N_5S$ (C, H, N).

N-(2,5-Dimethylphenyl)-6-(heptan-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (5d)

Yield: 75%; m.p.: 138°C; ^1H NMR (DMSO-d_6) δ: 0.96–1.12 (t, 6H), 1.36–1.41 (m, 4H), 1.54–1.60 (q, 4H), 2.13 (s, 3H), 2.36 (s, 3H), 2.67–2.69 (t, 1H), 6.57–6.61 (m, 2H), 6.91 (d, 1H), 8.89 (br s, 1H, D_2O exchangeable), ^{13}C NMR δ 14.2,

17.3, 20.3, 21.1, 38.3, 115.2, 118.3, 126.1, 129.2, 136.2, 141.2, 157.3, 167.3, 168.1: MS (ESI) 344.19 $(MH)^+$. Anal. $C_{18}H_{25}N_5S$ (C, H, N).

N-(2,6-Dimethylphenyl)-6-(heptan-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (5e)

Yield: 80%; m.p.: 129°C; ^1H NMR (DMSO-d_6) δ: 0.95–1.12 (t, 6H), 1.36–1.42 (m, 4H), 1.56–1.61 (q, 4H), 2.15 (s, 6H), 2.64–2.67 (t, 1H), 6.67 (t, 1H), 6.98 (d, 2H), 9.41 (br s, 1H, D_2O exchangeable), ^{13}C NMR δ 14.2, 17.3, 20.3, 38.3, 121.1, 128.3, 136.2, 137.5, 157.2, 167.2, 168.1: MS (ESI) 344.18 $(MH)^+$. Anal. $C_{18}H_{25}N_5S$ (C, H, N).

N-(4-Bromophenyl)-6-(pyridin-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (6a)

Yield: 81%; m.p.: 203°C; ^1H NMR (DMSO-d_6) δ: 6.94 (d, 2H), 7.13 (d, 2H), 7.89 (d, 2H), 8.45 (d, 2H), 8.91 (br s, 1H, D_2O exchangeable, ^{13}C NMR δ 116.3, 118.3, 121.2, 132.4, 137.1, 143.1, 143.6, 149.2, 157.3, 167.4: MS (ESI) 372.98 $(MH)^+$. Anal. $C_{14}H_9BrN_6S$ (C, H, N).

N-(4-chlorophenyl)-6-(pyridin-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (6b)

Yield: 65%; m.p.: 216°C; ^1H NMR (DMSO-d_6) δ: 7.02 (d, 2H), 7.23 (d, 2H), 7.90(d, 2H), 8.46 (d, 2H), 8.95 (br s, 1H, D_2O exchangeable), ^{13}C NMR δ 121.2, 122.1, 127.3, 129.2, 137.1, 143.1, 143.6, 149.2, 157.3, 167.4: MS (ESI) 329.03 $(MH)^+$. Anal. $C_{14}H_9ClN_6S$ (C, H, N).

N-(2,4-Dimethylphenyl)-6-(pyridin-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (6c)

Yield: 62%; m.p.: 192°C; ^1H NMR (DMSO-d_6) δ: 2.14 (s, 3H), 2.36 (s, 3H), 6.58 (d, 1H), 6.88–6.94 (m, 2H), 7.91 (d, 2H), 8.54 (d, 2H), 9.12 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 17.3, 21.1, 116.3, 121.2, 126.1, 128.1, 131.3, 137.2, 139.3, 143.0, 143.5, 149.1, 157.1, 167.2: MS (ESI) 323.10 $(MH)^+$. Anal. $C_{16}H_{14}N_6S$ (C, H, N).

N-(2,5-Dimethylphenyl)-6-(pyridin-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (6d)

Yield: 68%; m.p.: 183°C; ^1H NMR (DMSO-d_6) δ: 2.13 (s, 3H), 2.37 (s, 3H), 6.66–6.71 (m, 2H), 6.90 (d, 2H), 5.64 (br s, D_2O exchangeable, 1H), 7.92 (d, 2H), 8.45 (d, 2H), ^{13}C NMR δ 17.3, 21.1, 115.2, 118.3, 121.4, 126.1, 129.2, 136.2, 141.2, 143.1, 143.6, 149.2, 157.2, 167.1: MS (ESI) 323.10 $(MH)^+$. Anal. $C_{16}H_{14}N_6S$ (C, H, N).

N-(2,6-Dimethylphenyl)-6-(pyridin-4-yl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (6e)

Yield: 70%; m.p.: 161°C; ^1H NMR (DMSO-d_6) δ: 2.15 (s, 6H), 6.65 (t, 1H), 6.96 (d, 2H), 7.91 (d, 2H), 8.47 (d, 2H), 9.21 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 17.3, 121.1, 128.3, 136.2, 137.5, 143.1, 143.4, 149.1, 157.3, 167.4: MS (ESI) 323.10 $(MH)^+$. Anal. $C_{16}H_{14}N_6S$ (C, H, N).

N-(4-Bromophenyl)-6-(4-nitrophenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (7a)

Yield: 68%; m.p.: 199°C; ^1H NMR (DMSO-d_6) δ: 6.93 (d, 2H), 7.14 (d, 2H), 7.81 (d, 2H), 8.08 (d, 2H), 9.71 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 116.1, 118.2, 124.3, 128.1, 132.3, 137.1, 139.2, 143.2, 147.4, 157.3, 167.3: MS (ESI) 416.97 $(MH)^+$. Anal. $C_{15}H_9BrN_6O_2S$ (C, H, N).

N-(4-Chlorophenyl)-6-(4-nitrophenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazole-3-amine (7b)

Yield: 69%; m.p.: 207°C; ^1H NMR (DMSO-d_6) δ: 7.01 (d, 2H), 7.25 (d, 2H), 7.83 (d, 2H), 8.10 (d, 2H), 9.12 (br s, D_2O exchangeable, 1H), ^{13}C

NMR δ 122.7, 124.3, 127.1, 128.1, 129.5, 137.1, 139.2, 143.2, 147.4, 157.0, 167.1: MS (ESI) 373.02 $(MH)^+$. Anal. $C_{15}H_9ClN_6O_2S$ (C, H, N).

N-(2,4-Dimethylphenyl)-6-(4-nitrophenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (7c)

Yield: 77%; m.p.: 176°C; ^{1}H NMR (DMSO-d_6) δ: 2.13 (s, 3H), 2.34 (s, 3H), 6.56 (d, 1H), 6.87–6.92 (m, 2H) 7.81 (d, 2H), 8.10 (d, 2H), 8.76 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 17.3, 21.1, 116.3, 124.2, 126.1, 128.1, 128.6, 131.3, 137.2, 139.3, 139.4, 143.1, 147.1, 157.3, 167.1: MS (ESI) 367.09 $(MH)^+$. Anal. $C_{17}H_{14}N_6O_2S$ (C, H, N).

N-(2,5-Dimethylphenyl)-6-(4-nitrophenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (7d)

Yield: 73%; m.p.: 162°C; ^{1}H NMR (DMSO-d_6) δ: 2.14 (s, 3H), 2.35 (s, 3H), 6.66–6.70 (m, 2H), 6.90 (d, 2H), 7.81 (d, 2H), 8.09 (d, 2H), 9.71 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 17.3, 21.1, 115.1, 118.2, 124.4, 126.3, 128.1, 129.0, 136.1, 139.6, 141.2, 143.1, 147.4, 157.1, 167.2: MS (ESI) 367.09 $(MH)^+$. Anal. $C_{17}H_{14}N_6O_2S$ (C, H, N).

N-(2,6-Dimethylphenyl)-6-(4-nitrophenyl)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-3-amine (7e)

Yield: 71%; m.p.: 151°C; ^{1}H NMR (DMSO-d_6) δ: 2.16 (s, 6H), 6.65 (t, 1H), 6.96 (d, 2H), 7.82 (d, 2H), 8.03 (d, 2H), 9.65 (br s, D_2O exchangeable, 1H), δ 17.3, 121.1, 124.4, 128.3, 136.2, 137.5, 139.2, 143.1, 147.4, 157.3, 167.3: MS (ESI) 367.09 $(MH)^+$. Anal. $C_{17}H_{14}N_6O_2S$ (C, H, N).

3-(3-(3-(4-Bromophenylamino)-[1, 2, 4]triazolo [3,4-b][1, 3, 4]thiadiazol-6-yl)propyl)-3-azaspiro [5.5]undecane-2,4-dione (8a)

Yield: 80%; m.p.: 174°C; ^{1}H NMR (DMSO-d_6) δ: 1.42–1.53 (m, 10H), 2.08–2.17 (m, 6H), 2.91 (t, 2H), 3.01 (t, 2H), 6.92 (d, 2H), 7.13 (d, 2H), 9.32 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 15.3, 20.4, 25.1, 26.2, 39.1, 41.2, 41.4, 116.1, 118.2, 132.3, 137.2, 157.1, 167.1, 168.2, 171.3: MS (ESI) 517.09 $(MH)^+$. Anal. $C_{22}H_{25}BrN_6O_2S$ (C, H, N).

3-(3-(3-(4-Chlorophenylamino)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-6-yl)propyl)-3-aza spiro[5.5]undecane-2,4-dione (8b)

Yield: 75%; m.p.: 183°C; ^{1}H NMR (DMSO-d_6) δ: 1.42–1.53 (m, 10H), 2.08–2.15 (m, 6H), 2.94 (t, 2H), 3.02 (t, 2H), 7.02 (d, 2H), 7.22 (d, 2H), 9.08 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 15.3, 20.4, 25.1, 26.1, 39.3, 41.2, 41.3, 122.0, 127.4, 129.1, 137.2, 157.0, 167.4, 168.1, 171.3: MS (ESI) 473.14 $(MH)^+$. Anal. $C_{22}H_{25}ClN_6O_2S$ (C, H, N).

3-(3-(3-(2,4-Dimethylphenylamino)-[1, 2, 4] triazolo[3,4-b][1, 3, 4]thiadiazol-6-yl)propyl)-3- azaspiro[5.5]undecane-2,4-dione (8c)

Yield: 74%; m.p.: 159°C; ^{1}H NMR (DMSO-d_6) δ: 1.43–1.54 (m, 10H), 2.09–2.17 (m, 9H), 2.35 (t, 3H), 2.92 (t, 2H), 3.02 (t, 2H), 6.59 (d, 1H), 6.88–6.93 (m, 2H), 9.63 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 15.1, 17.5, 20.1, 21.2, 25.3, 26.1, 39.2, 41.1, 41.4, 116.1, 126.2, 128.2, 131.3, 137.1, 139.3, 157.1, 167.2, 168.1, 171.4: MS (ESI) 467.21 $(MH)^+$. Anal. $C_{24}H_{30}N_6O_2S$ (C, H, N).

3-(3-(3-(2,5-Dimethylphenylamino)-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazol-6-yl)propyl)- 3-azaspiro[5.5]undecane-2,4-dione (8d)

Yield: 75%; m.p.: 141°C; ^{1}H NMR (DMSO-d_6) δ: 1.43–1.53 (m, 10H), 2.08–2.15 (m, 9H), 2.35 (s, 3H), 2.91 (t, 2H), 3.03 (t, 2H), 6.65–6.69 (m, 2H), 6.89 (d, 2H), 9.64 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 15.3, 17.4,

20.3, 21.1, 25.1, 26.2, 39.1, 41.2, 41.4, 115.1, 118.2, 126.1, 129.2, 136.3, 141.1, 157.3, 167.1, 168.2, 171.2: MS (ESI) 467.21 $(MH)^+$. Anal. $C_{24}H_{30}N_6O_2S$ (C, H, N).

3-(3-(3-(2,6-Dimethylphenylamino)-[1, 2, 4] triazolo[3,4-b][1, 3, 4]thiadiazol-6-yl)propyl)-3-azaspiro[5.5]undecane-2,4-dione (8e)

Yield: 72%; m.p.: 124°C; ^{1}H NMR (DMSO-d_6) δ: 1.42–1.54 (m, 10H), 2.07–2.17 (m, 6H), 2.25 (s, 6H), 2.93 (t, 2H), 3.02 (t, 2H), 6.66 (t, 1H), 6.97 (d, 2H), 9.62 (br s, D_2O exchangeable, 1H), ^{13}C NMR δ 15.1, 17.3, 20.1, 25.1, 26.1, 39.2, 41.1, 41.5, 121.2, 128.2, 129.1, 136.4, 137.2, 157.2, 167.2, 168.1, 171.1: MS (ESI) 467.21 $(MH)^+$. Anal. $C_{24}H_{30}N_6O_2S$ (C, H, N).

Pharmacology

Swiss albino mice (either sex) with weights ranging from 20–25 g were used for the assessment of neurotoxicity, acetic acid-induced writhing, and formalin-induced flinching model. Wistar rats of either sex (200–250 g) were used for the inflammatory and neuropathic pain models. All experiments were approved by the Institutional Animal Ethics Committee. Animals were housed six (mice) and four (rats) per cage at constant temperature under a 12 h light/dark cycle (lights on at 7:00 AM), with food and water ad libitum.

Motor Impairment

Minimal motor impairment was measured in mice by the rotarod test. The mice were trained to stay on an accelerating rotarod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials [33].

Acetic Acid-Induced Writhing

Writhing was induced in a group of mice by an intraperitoneal injection of 0.1 mL of 2% (v/v) acetic acid. Test group mice received acetic acid 30 min after the administration of test compounds. The number of writhings occurring for a period of 30 min was recorded. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The percentage inhibition of the writhing response was calculated [34].

Formalin-Induced Flinching

The test involved intraplantar injection of 25 μL of 1% formalin into the hind paw of mice, which resulted in flinches in the paw in the early phase (0–5 min) and the late phase (10–30 min) [35]. Time spent in paw licking and biting was monitored in each 5 min and calculated for both the phases. Test compounds were administered 30 min before the experiment.

Unilateral Mononeuropathy: Chronic Constriction Nerve Injury Model

Unilateral mononeuropathy was produced in rats using the chronic constriction injury (CCI) model performed essentially as described by Bennett and Xie [36]. The rats were anesthetized with an intraperitoneal dose of ketamine (55 mg/kg) and xylazine (5 mg/kg) with additional doses of the anesthetic given as needed. Under aseptic conditions, a 3 cm incision was made on the lateral aspect of the left hind limb at the mid-thigh level. The left paraspinal muscles were then separated from the spinous processes and the common left sciatic nerve was exposed just above the trifurcation point. Four loose ligatures were made with a 4-0 braided silk suture around the sciatic nerve with about 1-mm spacing. The

wound was then closed by suturing the muscle using chromic catgut with a continuous suture pattern. Finally, the skin was closed using silk thread with horizontal-mattress suture pattern.

Induction of Peripheral Mononeuropathy: Partial Sciatic Nerve Ligation Model

As described by Seltzer et al. [37], in anesthetized rats, the left sciatic nerve was exposed at mid-thigh level through a small incision, cleared of adhering muscle tissue, and one-half of the nerve thickness was tightly ligated using 7.0 silk suture. The wound was closed and dusted with neomycin powder. The animals were then transferred to their home cages and left for recovery.

Sensory Testing Using Nociceptive Assays

Compounds (100 mg/kg, i.p.) were administered at $t = 0$, in 30% (v/v) PEG 400. The control group of rats received only the vehicle. Gabapentin (100 mg/kg, i.p.) was used as a positive control. Paw withdrawal duration (PWD) was assessed in spontaneous pain and cold allodynia, and paw withdrawal threshold (PWT) was assessed in tactile allodynia and mechanical hyperalgesia. Percentage reversal in spontaneous pain, allodynia, or hyperalgesia was calculated for each animal as defined below [38],

$$\%\,\text{Reversal} = \frac{(\text{post-dose value} - \text{pre-dose value})}{(\text{contralateral paw value} - \text{pre-dose value})} \times 100$$

Carrageenan-Induced Paw Edema and Quantification of TNF-alpha

Paw edema was induced in Wistar rats by intraplantar injection of 50 µl of 2% carrageenan (λ-carrageenan, type IV, Sigma-Aldrich Company Ltd, Dorset, UK) diluted in saline. The volume of the paw edema (mL) was determined at 0, 60, 120, and 180 min using a water plethysmometer (Ugo Basile, Varese, Italy). Indomethacin (10 mg/kg, i.p.) was used as a positive control [39]. The percentage protection against inflammation was calculated as: $V_c - V_d/V_c \times 100$, where V_c is the increase in paw volume in the absence of the test compound (control) and V_d is the increase of paw volume after administration of the test compound.

For the measurement of TNF-alpha, whole right hind paws were collected at the third hour after carrageenan injection. After rinsing with ice-cold normal saline, they were homogenized at 4°C, and the homogenate was centrifuged at 12,000 rpm for 5 min. The supernatant obtained was assayed using a TNF-alpha enzyme-linked immunosorbant assay (ELISA) kit [40].

Estimation of Total Nitrite/Nitrate

On day 9 post-CCI, after 2 h of administration of test compounds, the total nitrate/nitrite in the brain and sciatic nerve was estimated according to the reported procedure [9]. The method involved reduction of nitrate to nitrite followed by calorimetric estimation using Griess's reagent. The concentration of nitrite in the supernatant was calculated using a standard curve and expressed as a percentage of the control.

2,2-Diphenyl-1-picrylhydrazyl Assay

A solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dissolving 5 mg DPPH in 2 mL methanol, and the solution was kept in the dark at 4°C. Varying concentrations of test compounds (200 µL) were taken in a 96-well microplate. Then, 5 µL methanolic DPPH solution (final concentration 300 µM) was added to each well. After 20 min incubation, absorbance of the solution was read using an ELISA plate reader (EL340 Biokinetic reader,

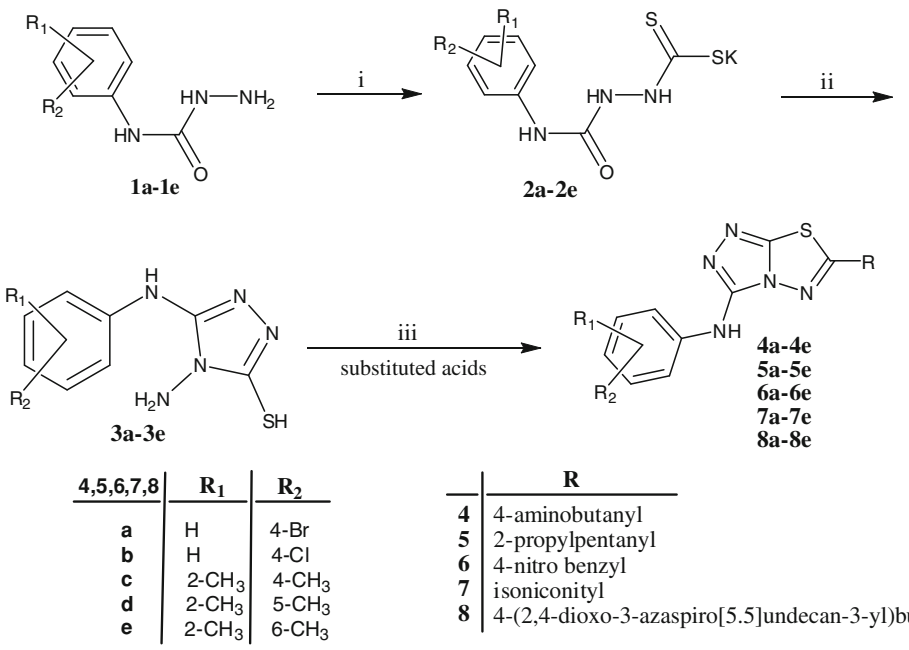
Bio-Tek Instrumentation, CA, USA) at a wavelength of 517 nm. A methanolic solution of DPPH served as a control. A dose–response curve was plotted to determine the half maximal inhibitory concentration (IC_{50}) values. All tests and analyses were run in triplicate and averaged [41]. Percentage scavenging was calculated according to the following equation.

$$\%\,\text{Scavenging} = \frac{\text{Absorbance (DPPH)} - \text{Absorbance (DPPH + compound)}}{\text{Absorbance (DPPH)}} \times 100$$

RESULTS

With the aim of synthesizing pharmacophoric hybrids of chemical entities with proven antinociceptive properties, the synthesis of various substituted phenyl semicarbazides (1a–1e), was accomplished from substituted anilines via urea formation as per our previously reported procedure [26]. Upon treatment with carbon disulfide in the presence of potassium hydroxide in ethanol, substituted phenyl semicarbazides gave the potassium salt of the corresponding 4-dithiocarbamate derivatives (2a–2e), which underwent ring closure with an excess of hydrazine hydrate to give 4-amino-3-substituted-5-mercapto-(4H)-1,2,4-triazoles (3a–3e). Resultant triazoles were further converted to 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (4a–4e, 5a–5e, 6a–6e, 7a–7e, 8a–8e) via a one-pot reaction by condensation with various acids in the presence of phosphorous oxychloride as outlined in Fig. 1. The synthesis of N-spiro GABA (8a–8e) was accomplished as reported in our earlier work [32]. The structures of the synthesized compounds were characterized by both spectral and elemental analysis, and the data were within ±0.4% of the theoretical values.

Following assessment of neurotoxicity of the synthesized compounds by rotarod (data not shown), the non-neurotoxic compounds were further evaluated in acetic acid-induced writhing and formalin-induced flinching models (Table 1). The acetic acid-induced writhing model is a chemical pain test used to evaluate acute antinociceptive function, whereas the formalin model is a tonic inflammatory pain model used to distinguish peripherally and centrally acting compounds. It is characterized by two phases: first phase (0–5 min) occurs due to direct stimulation of nociceptors (C-fibers), whereas the second phase (10–30 min) results following the development of a localized inflammatory response along with activation of N-methyl-D-aspartate (NMDA) and non-NMDA receptors and nitric oxide production [42, 43]. All tested compounds, except 6e, suppressed the acetic acid-induced writhing response significantly ($P < 0.01$) in comparison with the control (Table 1). The standard drug, indomethacin, exhibited the highest percentage inhibition (96.1%). In the formalin-induced flinching model, all of the compounds showed significant suppression in both phases, with the exception of compounds 4a, 4d, 6d, 6e, 7c, and 8d, which were effective only in the second phase of the formalin assay. Indomethacin (72.1%) significantly reversed flinching responses in the second phase of the formalin assay. The triazolo-thiadiazoles were further evaluated in two well-established peripheral neuropathic pain models: the CCI and the partial sciatic nerve ligation (PSNL) model. In the CCI model, the left sciatic nerve proximal to the trifurcation point was constricted with four loose ligatures using 4-0 braided silk thread [36]. In the PSNL model, left sciatic nerve was



4,5,6,7,8	R_1	R_2
a	H	4-Br
b	H	4-Cl
c	2-CH_3	4-CH_3
d	2-CH_3	5-CH_3
e	2-CH_3	6-CH_3

	R
4	4-aminobutanyl
5	2-propylpentanyl
6	4-nitro benzyl
7	isoniconityl
8	4-(2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanyl

[a]Reagents and conditions: **i.** CS_2, KOH, 18h; **ii.** $NH_2NH_2.H_2O$, 12h; **iii.** $POCl_3$, 6h

Fig. 1 Synthetic route to substituted triazolo-thiadiazoles

exposed at mid-thigh level and one-half of the nerve thickness was tightly ligated using 7.0 silk sutures [37]. Four nociceptive assays with the aim of determining the severity of behavioral neuropathic responses, namely allodynia and hyperalgesia, were performed. The assays involved measurement of the degree of spontaneous (ongoing) pain and tests of hind limb withdrawal to cold and mechanical stimuli (tactile allodynia, cold allodynia, and mechanical hyperalgesia). A minimum of 10 min separated the testing procedures to reduce the influence of prior nociceptive testing. All animals included in the study showed altered sensory responses in all the four behavioral nociceptive tests, 9 days post-surgery. The sham-operated animals showed no significant difference from the preoperative baseline sensory response values. Percentage reversal in spontaneous pain, tactile allodynia, cold allodynia, and mechanical hyperalgesia was assessed day 9 post-surgery.

In the CCI model (Fig. 2), compounds 4b, 6a, and 7e completely reversed the spontaneous pain response throughout the time period of testing (0.5–2.0 h) similar to gabapentin. Compounds 7a and 8b were effective only up to 0.5 h, whereas compounds 4a, 4e, 7b, and 8e were effective up to 1 h. The onset of action of compound 7c was at 1 h. Other compounds were ineffective in this test. Three compounds, 4b, 6a, and 7e, were effective in attenuating the tactile allodynia throughout the 2 h experiment similar to gabapentin. Compound 4a was effective only up to 0.5 h of the experiment whereas compounds 4e, 8b, and 8e were effective up to 1 h. All other compounds were ineffective in this test. In the cold allodynia produced in CCI rats, significant reversal of paw withdrawal durations was observed at all timepoints by the administration of compounds 4b and 7e. Gabapentin was also found to be effective at all the timepoints.

Table 1 Acute antinociceptive efficacy of triazolo-thiadiazoles

Compound	Acetic acid-induced writhing % Inhibition	Formalin-induced flinching	
		% Inhibition	
		Phase I	Phase II
Control	–	–	–
4a	93.6*	13.4	68.5*
4b	91.3*	65.5*	90.9*
4c	67.9*	45.2*	69.8*
4d	48.2*	13.4	56.3*
4e	79.3*	43.4*	62.4*
5a	64.6*	41.5*	76.8*
5b	67.9*	47.9*	87.9*
5c	50.9*	47.7*	86.4*
5d	62.2*	43.8*	63.4*
5e	90.2*	50.5*	85.7*
6a	94.0*	63.4*	84.1*
6b	56.6*	73.2*	62.1*
6c	46.2*	65.3*	67.9*
6d	48.2*	23.4	62.3*
6e	25.5	10.9	66.6*
7a	53.8*	40.4*	89.9*
7b	57.5*	56.0*	71.7*
7c	69.8*	22.6	53.6*
7d	58.5*	47.9*	91.5*
7e	92.6*	52.3*	93.5*
8a	51.9*	63.5*	58.3*
8b	62.2*	63.1*	68.1*
8c	68.0*	61.3*	71.0*
8d	44.3*	15.2	60.6*
8e	76.4*	49.1*	74.7*
Indomethacin[a]	96.1	4.35	72.1*

Vehicle treated animals received 30% (v/v) PEG 400 in water

* Represents significance at $P < 0.05$ compared to the vehicle (one way analysis of variance [ANOVA], followed by Dunnet Test, $n = 4$) at a dose of 100 mg/kg

[a] Indomethacin was taken as a positive control at 5 mg/kg

Compound 6b was effective only up to 0.5 h whereas compounds 4a, 6a, 7c, 8c, and 8e were effective up to 1 h of the experiment. Mechanical hyperalgesia was significantly attenuated at all the timepoints by 4b and 6a similar to gabapentin. Compounds 7b and 8e were effective only up to 0.5 h, whereas compounds 4e, 5a, 7e, and 8c were effective until 1 h. Compound 6c exhibited irregular activity pattern.

Overall, it appears that in the CCI model of neuropathic pain compounds that showed promising results include 4b, 6a, 7e, and 8e, which were effective in four tests; 4a and 4e, effective in three tests; and 7b, 7c, 8b, and 8c, effective in two tests.

In the PSNL model (Fig. 3), the paw withdrawal durations due to spontaneous ongoing pain were significantly reduced by compounds 4b, 6a, and 7e throughout the experiment, similar to gabapentin. The compounds 7c and 8e were effective up to 0.5 h of the experiment, whereas compounds 4a, 4e, and 8b exhibited activity up to 1 h. The tactile allodynia produced in the PSNL model was effectively reversed by compounds 4b and 6a at all the timepoints, similar to gabapentin. Compound 4a was effective only up to 0.5 h of the experiment, whereas compounds 4e, 7e, 8b, 8c, and 8e were effective up to 1 h of the experiment. The onset of action for compound 6b was at 1 h. Cold allodynia produced in the PSNL model was completely reversed by compounds 4b, 6a, and 7e. Compounds 6b and 6d were effective only up to 0.5 h of the experiment, whereas compounds 4a, 4e, 6c, 8c, and 8e were effective up to 1 h of the experiment. Compounds 4b, 7b, and 7e significantly reversed mechanical hyperalgesia at all the timepoints, similar to gabapentin. Compounds 6a, 7a, and 8c were effective in first 1 h of the experiment. Compound 7c exhibited

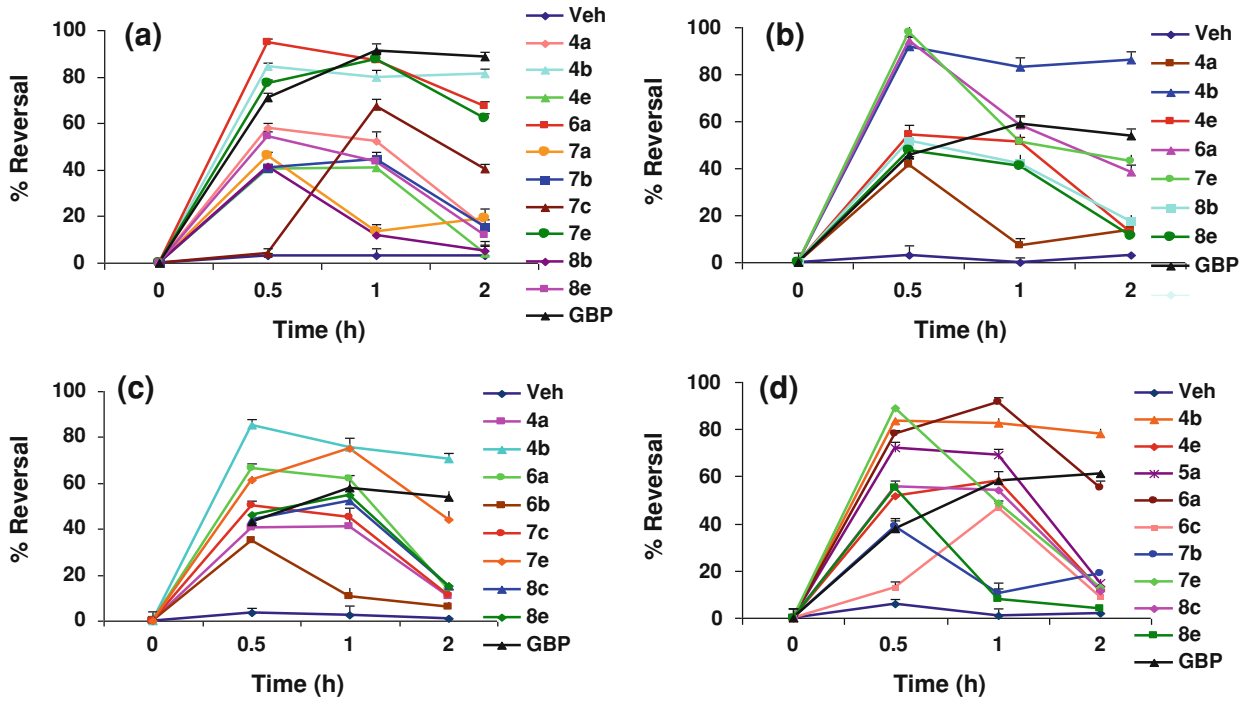


Fig. 2 Efficacy of compounds in spontaneous pain (**a**), tactile allodynia (**b**), cold allodynia (**c**), and mechanical hyperalgesia (**d**) in chronic constriction injury rats. Each value represents the percent reversal (mean ± SEM) in spontaneous pain, tactile allodynia, cold allodynia, and mechanical hyperalgesia of four rats. *Asterisk* denotes a significant value, in comparison to their respective vehicle control at $P < 0.05$ (one-way analysis of variance [ANOVA], followed by post hoc Dunnet test)

irregular activity. Overall, it appears, in the PSNL model, compounds that exhibited promising results included 4b, 6a, and 7e, which were effective in four tests; 4a, 4e, and 8a, effective in three tests; and 6b, 7c, 8b, and 8c, effective in two tests.

Compounds exhibiting more than 90% reversal in one or more of the nociceptive assays (4b, 6a, and 7e) were taken further for ED_{50} studies (Table 2). In the CCI model, compound 6a reversed spontaneous pain with an ED_{50} value of 13.92 mg/kg at 0.5 h. Compound 4b reversed tactile and cold allodynia with an ED_{50} value of 7.62 and 16.92 mg/kg at 0.5 and 1 h, respectively. Compound 7e reversed mechanical hyperalgesia with an ED_{50} value of 12.94 mg/kg at 0.5 h. In the PSNL model, compound 4b reversed spontaneous pain and tactile allodynia with an ED_{50} value of 14.91 and 13.95 mg/kg at 0.5 and 1 h, respectively. In cold allodynia and mechanical hyperalgesia, compound 6a was the most effective compound with an ED_{50} value of 21.91 and 28.10 mg/kg at 0.5 and 1 h, respectively.

The significant reversal exhibited by most of the test compounds in acetic acid-induced writhing model supported their role as peripherally acting analgesics. Significant suppression of flinching observed in both the phases of formalin assay (Table 1) suggested the mediation of anti-inflammatory pathways. The carrageenan-induced paw edema model was used to investigate the probable role of the selective compounds (4b, 6a, and 7e) in the inhibition of inflammatory mediators. A significant reduction in edema was observed for compounds 4b, 6a, and 7e at all the timepoints (Table 3). TNF-α levels quantified in the carrageeenan injected paw were also

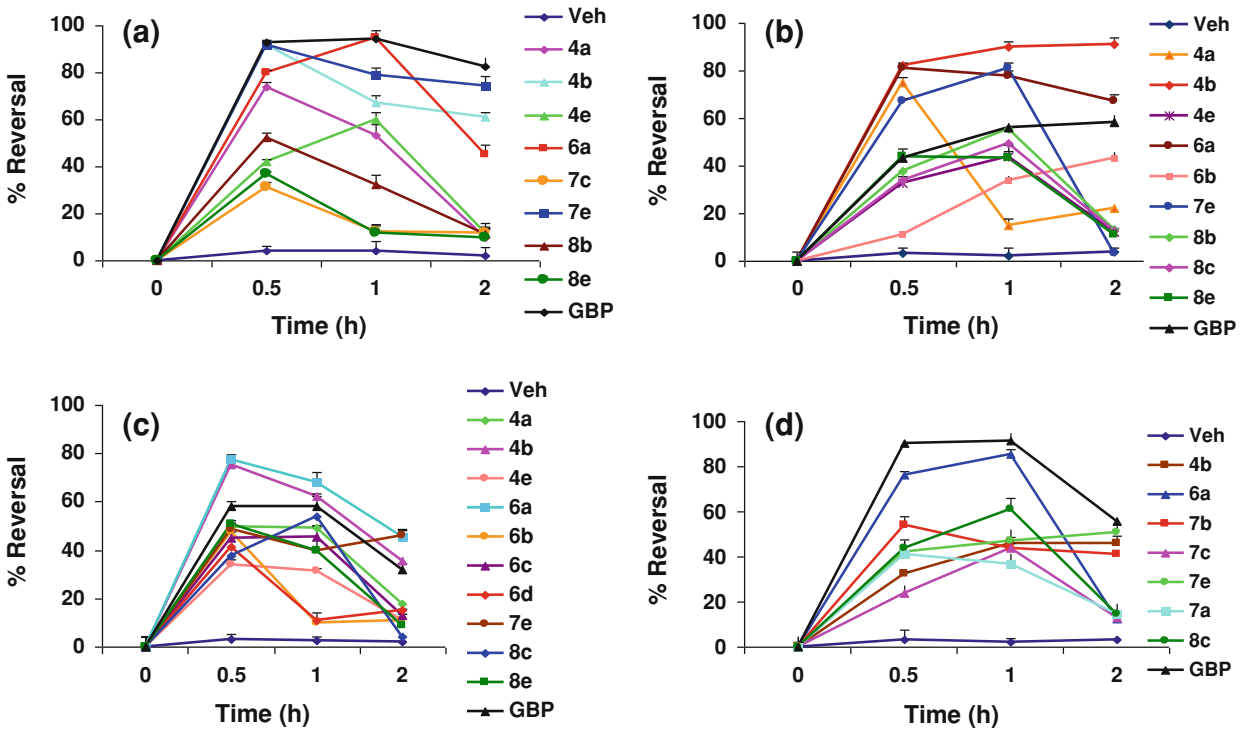


Fig. 3 Efficacy of compounds in spontaneous pain (**a**), tactile allodynia (**b**), cold allodynia (**c**), and mechanical hyperalgesia (**d**) in partial sciatic nerve ligation rats. Each value represents the percent reversal (mean ± SEM) in spontaneous pain, tactile allodynia, cold allodynia, and mechanical hyperalgesia of four rats. *Asterisk* denotes a significant value, in comparison to their respective vehicle control at $P < 0.05$ (one-way analysis of variance [ANOVA], followed by post hoc Dunnet test)

Table 2 Median effective dose (ED_{50}) studies of compounds 4b, 6a, and 7e

Treatment	ED_{50} values (mg/kg) in CCI model (TPE in min)[a]				ED_{50} values (mg/kg) in PSNL model (TPE in min)[a]			
	SP	TA	CA	MH	SP	TA	CA	MH
4b	17.01 (1.0)	7.62 (0.5)	16.92 (1.0)	14.62 (1.0)	14.91 (0.5)	13.95 (1.0)	28.07 (0.5)	–
6a	13.92 (0.5)	8.92 (0.5)	28.71 (0.5)	13.45 (1.0)	18.51 (1.0)	19.53 (0.5)	21.91 (0.5)	28.10 (1.0)
7e	22.90 (1.0)	14.28 (0.5)	21.41 (1.0)	12.94 (0.5)	21.42 (1.0)	22.65 (1.0)	–	–

Each value represents median effective dose in SP, TA, CA, and MH

CA cold allodynia; *CCI* chronic constriction injury; *MH* mechanical hyperalgesia; *PSNL* partial sciatic nerve ligation; *SP* spontaneous pain; *TA* tactile allodynia

[a] TPE represents time of peak effect in hours

found to be inhibited by compounds 4b, 6a, and 7e. As a result, there was inhibition of keratinocyte chemokines (KC), leading to subsequent inhibition of interleukin-1beta, prostanoids, and sympathomimetic amines, as evidenced by reduction of edema [44]. The occurrence of nitrosative stress following nerve injury as evident by the significant increase in

Table 3 Percent protection in the edema and inhibition of tumor necrosis factor (TNF)-alpha in the carrageenan injected paw by compounds 4b, 6a, and 7e

Treatment	% Protection in carrageenan-induced paw edema			% Inhibition of TNF-alpha[a]
	60 min	120 min	180 min	
Vehicle	–	–	–	–
4b	54.5 ± 2.3*	50.4 ± 2.6*	71.4 ± 3.4*	82.6 ± 2.8*
6a	43.3 ± 1.7*	62.6 ± 3.7*	77.2 ± 4.1*	88.8 ± 1.5*
7e	54.7 ± 3.7*	50.1 ± 2.1*	81.7 ± 2.3*	82.0 ± 3.1*
Indomethacin[b]	50.3 ± 2.6*	61.2 ± 3.8*	66.9 ± 4.2*	–

* Represents significance at $P < 0.05$ compared to the vehicle (one way analysis of variance [ANOVA], followed by Dunnet Test, $n = 4$)

[a] Percent inhibition of TNF-alpha in the paw of carrageenan-stimulated mice as compared to vehicle-treated controls

[b] Indomethacin was tested at the dose of 10 mg/kg i.p.

nitrite and nitrate levels in both the brains and sciatic nerves of the rats, led us to estimate the levels of nitrite, a metabolite of nitrate in the brain and sciatic nerve of CCI rats using Griess's reagent. Also, the free-radical scavenging activity of the compounds was assessed spectrophotometrically via the DPPH assay. No significant reduction of nitrite in the brain of CCI rats was found when compared to vehicle-treated animals for compounds 4b, 6a, and 7e. However, a significant reduction was observed in the sciatic nerve of the CCI animals when compared to the vehicle-treated group for 4b, 6a, and 7e. The results indicate the inhibition of local NO at the site of nerve injury. The compounds (4b, 6a, and 7e) were found to exhibit free-radical scavenging abilities in the

Table 4 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and effect of compounds 4b, 6a, and 7e on nitrosative stress

Treatment	% Inhibition of TNF-alpha[a]	DPPH scavenging activity[b] IC_{50} (μM)	% Inhibition of nitrosative stress (nitrite)[a]	
			Brain	Sciatic nerve
Vehicle	–	–	–	–
4b	82.6 ± 2.8*	190	19.4 ± 1.2	48.7 ± 1.8*
6a	88.8 ± 1.5*	201	24.4 ± 2.1	58.3 ± 2.3*
7e	82.0 ± 3.1*	209	22.3 ± 1.6	61.2 ± 3.1*
Indomethacin[c]	–	–	–	–

Asterisk denotes a significant value, in comparison to their respective vehicle control at $P < 0.05$ (one-way analysis of variance [ANOVA], followed by post hoc Dunnet test)

TNF tumor necrosis factor

[a] Percent inhibition of nitric oxide in brain and sciatic nerve of chronic constriction injury rats. Compounds were tested at the respective minimal ED_{50} dose administered i.p.

[b] DPPH radical scavenging activity of the test compounds (values are represented as % scavenging calculated from the average of triplicate experiments)

[c] Indomethacin was tested at the dose of 10 mg/kg i.p.

DPPH assay, thereby acting by reducing oxidative stress (Table 4).

DISCUSSION

The results obtained in the nociceptive assays provide an insight into the structure–activity relationships of the triazolo-thiadiazoles. Functionalization of the aryl ring of the semicarbazide fragment forming triazolo-thiadiazoles with dimethyl substitutions resulted in variable antinociceptive efficacy. Compounds having a 2,4-dimethyl substituted aryl semicarbazide fragment (6c, 7c, and 8c) reversed one or more nociceptive parameters in both CCI and PSNL animals. Introduction of a 2,5-dimethyl substituted aryl ring proved to be detrimental for the antinociceptive efficacy in both CCI and PSNL animals. Only one compound, 6d, was found to be effective against cold allodynia in PSNL animals. The introduction of electron-releasing 2,6-dimethyl substitutions (4e, 7e, and 8e) resulted in significant attenuation of one or more nociceptive parameters in neuropathic animals. Introduction of an electron-withdrawing halogen (bromo) para to the aryl ring resulted in significant activity against one or more nociceptive assays. Compounds 4a, 5a, 6a, and 7a were effective in CCI animals, whereas compounds 4a, 6a, 7a, and 8a were found to be effective in one or more nociceptive parameters in PSNL animals. Compounds with para chloro-substituted aryl rings (4b, 6b, 7b, and 8b) resulted in significant attenuation of one or more nociceptive parameters in neuropathic animals.

Accounting for the nociceptive efficacies observed for triazolo-thiadiazoles in the neuropathic pain models, it can be generalized that unlike 2-propylpentanyl substitution at the R_2 position (5a–5e), 2-aminobutanyl substitution (4a, 4b, and 4e) at the R_2 position was beneficial for nociceptive efficacy. Aryl substitution with a 4-nitobenzyl group (6a–6d) and heteroaryl substitution with an isonicotinyl group (7a, 7b, 7c, and 7e) resulted in significant attenuation of nociceptive parameters. N-spiro GABA substitution (8a, 8b, 8c, and 8e) also proved to be additive for antinociceptive efficacy.

In conclusion, utilizing a pharmacophoric hybrid approach, a series of structurally novel triazolo-thiadiazoles derivatives were synthesized and evaluated for acute antinociceptive, antiallodynic, and antihyperalgesic potential. In the carrageenan-induced paw edema model, in which there is a pronounced local TNF-alpha response, compounds 4b, 6a, and 7e significantly inhibited paw swelling as well as localized TNF-alpha levels in the paw, thereby suppressing the inflammatory component of neuropathic pain. The neuroprotection exhibited by compounds 4b, 6a, and 7e is also associated with their free-radical scavenging abilities and subsequent attenuation of oxidative and nitrosative stresses.

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