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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and analgesic activity of new 1,3,4-oxadiazoles and 1,2,4-triazoles

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Abstract A series of new 1,3,4-oxadiazoles and 1,2,4-triazoles were synthesized in order to obtain new compounds with potential analgesic activity. Compounds were evaluated for their analgesic activities by formalin-induced nociception test. Mefenamic acid (as the reference drug) did not show any activity in the early phase of the formalin test, while compounds **7b**, **7c**, **8c**, and **9a** significantly reduced the nociception in this phase. However in the late phase of formalin test all of the target compounds and mefenamic acid showed analgesic activity in comparison to control.

Keywords 1,3,4-Oxadiazole · 1,2,4-Triazole · Analgesic activity · Formalin test

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Introduction

Arachidonic acid (AA) contained in cellular membranes is released by phospholipase A_2 (PLA_2) and further metabolized by two major enzymatic pathways: 5-lipoxygenase (5-LO) and cyclooxygenase (COX), leading to pro-inflammatory leukotriens (LTs) and prostanoids, respectively. LTB_4 synthesized by 5-LO potently activates cell migration and chemotaxis, as well as superoxide production and lysosomal enzyme secretion in neutrophils. Increased production of this eicosanoid has been involved in hyperalgesic response and related with phathological conditions such as rheumatoid arthritis and inflammatory bowel disease (Nikfar *et al.*, 1997; Hosseini-Tabatabaei and Abdollahi 2008). It is interesting to note that COX inhibition by conventional non-steroidal anti-inflammatory drugs (NSAIDs) or by new COX-2 inhibitors leads to an up-regulation of the 5-LO pathway, yielding various adverse effects such as increase of gastrointestinal damage and asthma (Celotti and Laufer 2001; Rainsford 1999; Fosslien 1998). As a result, a new strategy is to minimize gastric toxicity of NSAIDs considering the dual inhibition of 5-LO and COX enzymes. In this scenario, various structural families of dual inhibitors have been designed and several compounds are currently undergoing preclinical or clinical development (Charlier and Michaux 2003; Moreau *et al.*, 2006; Rao *et al.*, 2005). Among these compounds, some potent NSAIDs, fenamates, resulting from the bioisosteric replacement of the carboxylic acid moiety by a tetrazole, were first reported to inhibit COX and to some extent, 5-LO. Then, several fenamates, among which flufenamic acid **1**, were converted to potent dual COX/5-LOX inhibitors, after substitution of their carboxylate moiety with other acidic heterocycles, namely 1,3,4-oxadizole, 1,3,4-thiadiazole and 1,2,4-triazole. Compound

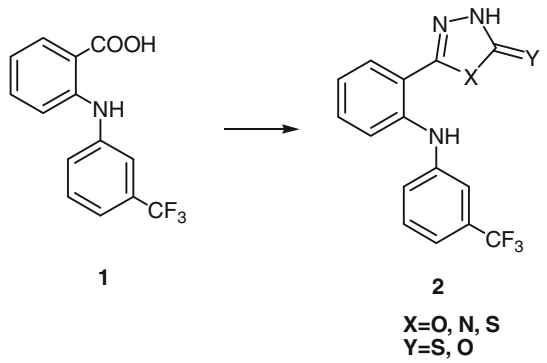


Fig. 1 Dual COX/5-LO inhibitor derived from flufenamic acid

2 (X=O, Y=S) was the most potent synthesized analogue in these series (Fig. 1). (Boschelli *et al.*, 1992; Boschelli *et al.*, 1993).

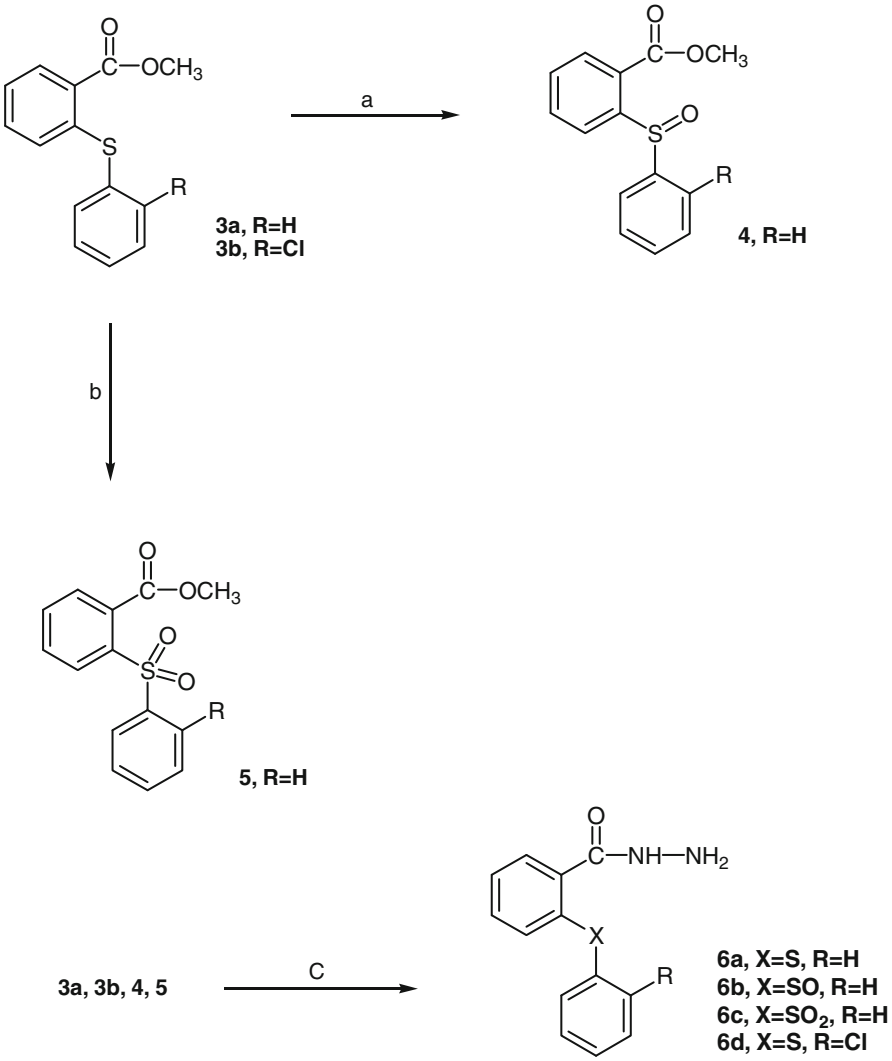
As a part of our ongoing research program to find novel anti-inflammatory, analgesic, and anticonvulsant compounds, herein, we describe the synthesis and analgesic activity of new analogues of compound **2** by bioisosteric replacement of NH with S and oxidation of S to SO and SO_2 in order to make this moiety as a real hydrogen bond acceptor similar to NH in compound **2** and in the hope of obtaining additional inhibitors of AA metabolism (Almasirad *et al.*, 2005; Almasirad *et al.*, 2006; Rineh *et al.*, 2007; Shafiee *et al.*, 2009).

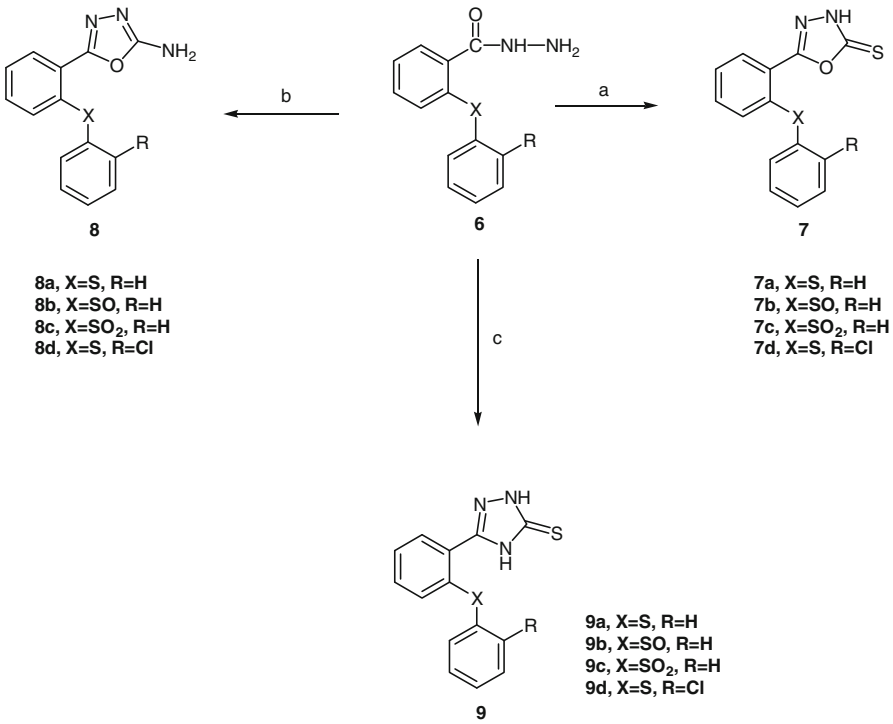
Results and discussion

Chemistry

The designed compounds were synthesized according to Schemes 1 and 2. Compounds **4** and **5** were prepared by oxidation of ester **3a** with hydrogen peroxide (Shafiee *et al.*, 1998; Brannigan *et al.*, 1976). The key intermediate hydrazides **6a–6d** were prepared from the reaction of hydrazine hydrate with compounds **3a**, **3b**, **4**, **5**,

Scheme 1 *a* CH_3COOH, H_2O_2, rt; *b* CH_3COOH, H_2O_2, reflux, and *c* NH_2–$NH_2.H_2O$, EtOH, rt





Scheme 2 *a* CS_2, KOH, reflux; *b* BrCN, Dioxane, rt; and *c* (*1*) KSCN, HCl, H_2O (*2*) NaOH 4%, reflux

respectively. 5-Aryl-1,3,4-oxadiazole-2(3H)-thiones **7a–7d** were prepared by reaction of hydrazides **6a–6d** with KOH and CS_2. Reaction of hydrazides **6a–6d** with cyanogen bromide gave 5-aryl-2-amino-1,3,4-oxadiazoles **8a–8d**. 5-aryl-1,2,4-triazole-3-thiones **9a–9d** were synthesized via reaction of **6a–6d** with potassium thiocyanate and hydrochloric acid followed by cyclization of thiosemicarbazide intermediate with aqueous sodium hydroxide (Almasirad *et al.*, 2007).

Pharmacology

The analgesic effect of target compounds **7a–9c** is shown in Tables 1 and 2. As seen in Table 1, compounds **7b**, **7c**, **8c**, and **9a** showed significant reduction in the early phase of formalin-induced nociception. Mefenamic acid was ineffective on this phase (neurogenic pain). As can be observed from Table 1, oxidation of sulfur in compound **7a** to sulfoxide **7b** or sulfone **7c** increased the activity. However, in triazole series **9**, oxidation of sulfur decreased the activities (compounds **9b** and **9c**). In addition bioisosteric replacement of oxygen atom in compound **7a** with NH increased the activity. In fact, compound **9a** was the most active compound. The effect of synthesized compounds on inflammatory pain is summarized in Table 2. All of the target compounds **7a–9c** as well as mefenamic acid showed significant analgesic activity in the late phase of formalin test whereas compound **7b** was more active than mefenamic acid. The synthesized compounds generally exhibited more activity during the inflammatory phase (57.95–86.12, inhibition range) in comparison to neurogenic phase (13.13–49.38, inhibition range).

Table 1 Analgesic activity of compounds (**7a–9c**) against the early phase (0–10 min) in formalin test

Compound[a]	Nociception[b] (Mean ± SEM)	Inhibition[c] (%)
Control	73.1 ± 6.3	–
Mefenamic acid	55.5 ± 5.9	24.08
7a	63.5 ± 4.7	13.13
7b	43.2 ± 3.4	40.9*
7c	42.5 ± 2.9	41.86*
7d	60.8 ± 4.4	16.83
8a	61 ± 5.9	16.55
8b	51.6 ± 3.6	29.41
8c	43.5 ± 4.7	40.49*
8d	53.6 ± 7.2	26.67
9a	37 ± 7.6	49.38*
9b	50 ± 5.9	31.66
9c	50.3 ± 4.9	31.19

* Differed from control group at $P < 0.01$

[a] All the compounds were tested at the dose of 30 μmol/kg

[b] There were 6 animals in each group

[c] Inhibition percentage was calculated by potential of nociception inhibition of test compounds in comparison to that of control

The pharmacological evaluation of this study showed a good analgesic profile in comparison to control and mefenamic acid. Our results suggest that compounds **7b**, **7c**,

Table 2 Analgesic activity of compounds (**7a–9c**) against the late phase (10–30 min) in the formalin test

Compound[a]	Nociception[b] (mean ± SEM)	Inhibition[c] (%)	Relative activity[d]
Control	193.8 ± 11.4	–	–
Mefenamic acid	48.6 ± 4.8	74.92	1**
7a	55.56 ± 5.1	71.33	0.95**
7b	26.9 ± 6.8	86.12	1.15**
7c	81.5 ± 3.7	57.95	0.77**
7d	37.8 ± 5.2	80.49	1.06**
8a	70.3 ± 4.5	63.73	0.85**
8b	39 ± 3.9	79.88	1.07**
8c	61.5 ± 5.5	68.27	0.91*
8d	63.8 ± 6.7	67.07	0.89*
9a	39.5 ± 3.8	79.62	1.06*
9b	71.1 ± 7.3	63.31	0.85**
9c	68 ± 3.6	64.91	0.87**

*, ** Represent difference from control group at $P < 0.05$ and $P < 0.01$, respectively

[a] All the compounds were tested at the dose of 30 μmol/kg

[b] There were six animals in each group

[c] Inhibition percentage was calculated by potential of nociception inhibition of test compounds in comparison to that of control

[d] Relative activity was calculated by potential of nociception inhibition of test compounds in comparison to that of mefenamic acid

8c and **9a**, unlike mefenamic acid as the reference drug and most of other NSAIDs have an additional and interesting analgesic action, in that these compounds were effective on neurogenic pain (Vaz *et al.*, 1996; Correa and Calixto 1993; Malmberg and Yaksh 1992). The analgesic effect of these compounds during the neurogenic phase of the formalin test suggests that they have a different or additional mechanism of action than classical NSAIDs. However, further studies are needed to determine that mechanism(s) responsible for the analgesic action of compounds **7b**, **7c**, **8c** and **9a**. These four compounds are among the 1,3,4-oxadiazole-2(3H)-thiones, 2-amino-1,3,4-oxadiazoles and 1,3,4-triazole-2-thiones respectively, so all of these scaffolds can be active in the early phase but according to the results, sulfoxide and sulfone moieties are the common functional groups between three of these four compounds. In contrast to the early phase, all of the target compounds and mefenamic acid were active in the late phase that can be explained by inhibition of the arachidonic acid metabolic pathway (Hunskaar *et al.*, 1985).The most active compound **7b** in the late phase, similar to the compound **2** (X=O, Y=S) as a dual COX/5-LO inhibitor, has the 1,3,4-oxadiazole-2-(3H)-thione heterocyclic ring so the dual inhibition of COX/5-LO by these compounds is likely to happen (Boschelli *et al.*, 1992).

Various oxadiazole and triazole derivatives were prepared with the objective of developing new analgesic agents. In summary, synthesized compounds showed analgesic activity in both phases of formalin test indicating involvement of different mechanisms of action. Neurogenic pain generally responds poorly to conventional analgesics and its treatment can be difficult (Davies *et al.*, 1994; Niv and Devor 2006). Therefore, discovery of new drugs for treatment of this kind of pain is clinically relevant.

Experimental

Chemicals were purchased from Merck chemical company (Tehran, Iran), Compounds **3a**, **3b**, **6a**, **7a**, **8a**, **9a** were prepared according to the previously described methods (Almasirad *et al.*, 2007; Bentrude and Martin 1962; Dubuisson and Dennis 1977).

Melting points were taken on a Kofler hot stage apparatus (Reichert, Vienna, Austria) and are uncorrected. ^{1}H-NMR spectra were obtained using a Brucker FT-80 spectrometer (Brucker, Rheinstetten, Germany). Tetramethylsilane was used as an internal standard. Mass spectra were obtained using a Finnigan Mat TSQ-70 spectrometer at 70 eV (Finnigan Mat, Bremen, Germany). The IR spectra were obtained using a Nicolet FT-IR Magna 550 spectrographs (KBr disks) (Nicolet, Madision, WI, USA). The purity of compounds was confirmed by TLC using different mobile phases. The results of the elemental analyses (C, H, N) were within ±0.4% of theoretical values for C, H, and N.

Methyl 2-(phenylsulfinyl)benzoate (**4**, R=H)

To a stirring solution of compound **3a** (7 g, 28.7 mmol) in acetic acid (4 ml) was added 30% hydrogen peroxide

(3 ml) at room temperature. Four additional portions of 30% hydrogen peroxide (3 ml) were added after 2, 4, 6, and 8 h. The stirring was continued for 1 h, and then the mixture was diluted with water and neutralized with 10% aqueous solution of sodium hydroxide. The organic layer was dried (sodium sulfate) and concentrated in vacuo to give 6.39 g (85%) of **4** (R=H), mp 76–77°C; IR (KBr) ν cm^{-1} 1710 (C=O), 1031 (S=O); ^{1}H-NMR($CDCl_3$): 8.78 (dd, 1H, $J = 7.8$, 1.3 Hz, aromatic), 8.09–7.26 (m, 8H, aromatic), 3.87 (s, 3H, CH_3). MS: m/z (%) 260 (M^+, 15), 211(16), 180 (10), 166 (100), 151 (32), 77 (12). Anal. Calcd. for $C_{14}H_{12}O_3S$: C, 64.60; H, 4.65. Found: C, 64.26; H, 4.87.

Methyl 2-(phenylsulfonyl)benzoate (**5**, R=H)

Compound **3a** (4.52 g, 18.5 mmol) was dissolved in 65 ml of acetic acid, and H_2O_2 (12 ml of 30% solution) was added. The solution was heated under reflux for 20 h. The mixture was cooled and diluted with water and worked up similar to compound **4** to give compound (**5**, R=H). The yield was 4.5 g (88%), mp 57–59°C; IR (KBr) ν cm^{-1} 1738 (C=O), 1311, 1158 (SO_2); ^{1}H-NMR ($CDCl_3$): 8.19–7.92 (m, 3H, aromatic), 7.71–7.26 (m, 6H, aromatic), 3.92 (s, 3H, CH_3). MS: m/z (%) 276 (M^+, 10), 244 (93), 212 (100), 182 (95), 152 (85), 104 (30), 77 (34). Anal. Calcd. for $C_{14}H_{12}O_4S$: C, 60.86; H, 4.38. Found: C, 60.98; H, 4.17.

General procedure for the preparation of hyrazides (**6b**–**6d**)

To a stirring solution of compounds **3b**, **4**, or **5** (14 mmol) in 7 ml ethanol at room temperature hydrazine hydrate (14 ml, 280 mmol) was added. After 8 h, the content were poured into water (15 ml), filtered and crystallized from suitable solvents.

2-(Phenylsulfinyl)benzoic acid hydrazide (**6b**)

Yield 92%; mp 145–146°C (ethanol). IR (KBr) ν cm^{-1} 3482, 3380 (NH_2), 3288 (NH), 1623 (C=O), 1024 (S=O). ^{1}H-NMR (DMSO-d_6): 9.95 (bs, 1H, NH), 8.12 (dd, 1H, $J = 7.9$, 1.4 Hz, aromatic), 7.81–7.38 (m, 8H, aromatic), 4.55 (bs, 2H, NH_2). MS: m/z (%) 260 (M^+, 5), 229 (100), 184 (36), 152 (6), 77 (4). Anal. Calcd. for $C_{13}H_{12}N_2O_2S$: C, 59.98; H, 4.65; N, 10.76. Found: C, 60.12; H, 4.52; N, 10.92.

2-(Phenylsulfonyl)benzoic acid hydrazide (**6c**)

Yield 70%; mp 165–167°C (ether). IR (KBr) ν cm^{-1} 3365, 3170 (NH_2, NH), 1631 (C=O), 1324, 1160 (SO_2). ^{1}H-NMR (DMSO-d_6): 9.81 (bs, 1H, NH), 8.20 (dd, 1H, $J = 8.1$, 1.8 Hz, aromatic), 7.92–7.25 (m, 8H, aromatic), 4.65 (bs, 2H, NH_2). MS: m/z (%) 276 (M^+, 18), 245 (99), 181 (10), 152 (100), 77 (41). Anal. Calcd. for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14. Found: C, 56.73; H, 4.52; N, 10.36.

2-(2-Chlorophenylthio)benzoic acid hydrazide (**6d**)

Yield 91%; mp 85–86 (ether). IR (KBr) ν cm^{-1} 3303, 3127 (NH_2, NH), 1665 (C=O). ^{1}H-NMR ($CDCl_3$): 8.78 (bs, 1H, NH), 7.67–7.17 (m, 8H, aromatic), 4.05 (bs, 2H, NH_2). MS: m/z (%) 280 (M^++2, 5) 278 (M^+, 15), 247 (100), 212 (27), 184 (48), 142 (24). Anal. Calcd. for $C_{13}H_{11}ClN_2OS$: C, 56.01; H, 3.98; N, 10.05. Found: C, 56.19; H,3.17; N, 10.26.

General procedure for the preparation of 5-aryl-1,3,4-oxadiazole-2(3H)-thiones (**7b**–**7d**)

A mixtue of hyrazide (**6b**–**6d**) (12.2 mmol), potassium hydroxide (12.2, mmol), carbon disulfide (2.5 ml) and ethanol (14 ml) was heated under reflux for 7 h. The solvent was removed in vacuo and the residue was dissolved in water and acidified with dilute hydrochloric acid. The resulting precipitate was removed by filtration and purified by crystallization or TLC.

5-[2-(Phenylsulfinyl)phenyl]-1,3,4-oxadiazole-2-(3H)-thione (**7b**)

Yield 57%; mp 247–249°C (ethanol). IR (KBr) ν cm^{-1} 3121 (NH), 2530 (weak, SH), 1342 (C=S). ^{1}H-NMR (DMSO-d_6): 14.22 (bs, 1H, NH), 8.22-7.52 (m, 9H, aromatic). MS: m/z (%) 302 (M^+, 11), 213 (100), 184 (31), 152 (10). Anal. Calcd. for $C_{14}H_{10}N_2O_2S_2$: C, 55.61; H, 3.33; N, 9.26. Found: C, 55.42; H, 3.14; N, 9.47.

5-[2-(Phenylsulfonyl)phenyl]-1,3,4-oxadiazole-2(3H)-thione (**7c**)

Yield 86%; mp 140–142°C (ethanol). IR (KBr) ν cm^{-1} 3272 (NH), 1306 (C=S). ^{1}H-NMR (DMSO-d_6): 14.30 (bs, 1H, NH), 8.34 (d, 1H, $J = 7.1$ Hz, aromatic), 7.90–7.60 (m, 8H, aromatic). MS: m/z (%) 318 (M^+, 93), 252 (61), 228 (75), 182 (100), 151 (38), 77 (33). Anal. Calcd. for $C_{14}H_{10}N_2O_3S_2$: C, 52.82; H, 3.17; N, 8.80. Found: C, 52.96; H, 3.07; N, 8.65.

5-[2-(2-Chlorophenylthio)phenyl]-1,3,4-oxadiazole-2-(3H)-thione (**7d**)

Compound **7d** was purified by TLC eluting with petroleum ether-ethyl acetate (1:1) and then crystallized from ethanol,

yield 30%; mp 206–208°C. IR (KBr) ν cm^{-1} 3277 (NH), 1332 (C=S). ^{1}H-NMR (DMSO-d_6): 14.49 (bs, 1H, NH), 7.88–7.37 (m, 7H, aromatic), 6.97–6.85 (m, 1H, aromatic); MS: m/z (%) 322 (M^++2, 40), 320 (M^+, 100), 285 (16), 246 (18), 224 (32), 182 (23). Anal. Calcd. for $C_{14}H_9ClN_2OS_2$: C, 52.41; H, 2.83; N, 8.73. Found: C, 52.60; H, 2.71; N, 8.64.

General procedure for the preparation of 2-amino-5-aryl-1,3,4-oxadiazoles (**8b**–**8d**)

To a stirring solution of hydrazide (**6b**–**6d**) (3.71 mmol) in dioxane (12 ml), sodium bicarbonate (3.71 mmol) in water (4 ml) was added at room temperature. The mixture was stirred at room temperature for 5 min and cyanogen bromide (3.85 mmol) was added. After 4 h, water (30 ml) was added to the mixture and the precipitate was removed by filtration and purified by crystallization or TLC.

2-Amino-5-[2-(phenylsulfinyl)phenyl]-1,3,4-oxadiazole (**8b**)

Yield 45%; mp 228-231°C (ethanol). IR (KBr) ν cm^{-1} 3320, 3275 (NH_2), 1670 (NH_2). ^{1}H-NMR (DMSO-d_6): 8.22 (dd, 1H, $J = 7.4$, 1.7 Hz, aromatic) 7.88–7.40 (m, 8H, aromatic). MS: m/z (%) 285 (M^+, 10), 213 (100), 183 (57), 151 (24). Anal. Calcd. for $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89; N, 14.73. Found: C, 59.12; H, 3.95; N, 14.55.

2-Amino-5-[2-(Phenylsulfonyl)phenyl]-1,3,4-oxadiazole (**8c**)

Yield 50%; mp 166–168°C (ethanol). IR (KBr) ν cm^{-1} 3441, 3344 (NH_2), 1644 (NH_2). ^{1}H-NMR (DMSO-d_6): 8.34–8.23 (m, 1H, aromatic), 7.98–7.52 (m, 8H, aromatic), 7.10 (bs, 2H, NH_2). MS: m/z (%) 301 (M^+, 10), 235 (100), 182 (95), 152 (60), 77 (32). Anal. Calcd. for $C_{14}H_{11}N_3O_3S$: C, 55.80; H, 3.68; N, 13.95. Found: C, 55.96; H, 3.54; N, 13.76.

2-Amino-5-[2-(2-Chlorophenylthio)phenyl]-1,3,4-oxadiazole (**8d**)

Compound **8d** was purified by TLC eluting with petroleum ether-ethyl acetate (1:5) and then crystallized from ethanol, yield 76%; mp 158–159°C; IR(KBr) ν cm^{-1} 3344, 3252 (NH_2), 1640 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.89–7.78 (m, 1H, aromatic), 7.51–6.92 (m, 7H, aromatic), 5.81 (bs, 2H, NH_2). MS: m/z (%) 305 (M^++2, 5), 303 (M^+, 12), 268 (100), 355 (28), 198 (64), 184 (40), 152 (31). Anal. Calcd. for $C_{14}H_{10}ClN_3OS$: C, 55.35; H, 3.32; N, 13.83. Found: C, 55.54; H, 3.26; N, 13.65.

General procedure for the preparation of 5-aryl-1,2,4-triazole-3-thiones (**9b**–**9c**)

A suspension of hydrazide (**6b**–**6d**) (8.2 mmol), potassium thiocyanate (41 mmol), hydrochloric acid (8 ml) and water 100 ml was refluxed for 3 h. After cooling the resulting thiosemicarbazide was removed by filtration and washed with water. It was dissolved in 4% sodium hydroxide solution (5 ml) and refluxed for 4 h. The mixture after charcoal treatment and filtration was acidified with hydrochloric acid to pH 5–6 and the resulting precipitate was filtered and purified by crystallization or TLC.

5-[2-(Phenylsulfinyl)phenyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**9b**)

Yield 47%; mp 247–249°C (ethanol). IR (KBr) ν cm^{-1} 3128 (NH), 2539 (weak, SH), 1320 (C=S). ^{1}H-NMR (DMSO-d_6): 13.72 (bs, 1H, NH), 7.76 (dd, 1H, $J = 7.3$, 2.9 Hz, aromatic), 7.51-7.33 (m, 7H, aromatic), 7.05 (dd, 1H, $J = 7.1$, 2.4 Hz, aromatic). MS: m/z (%) 301 (M^+, 12), 285 (16), 212 (100), 184 (43), 108 (23). Anal. Calcd. for $C_{14}H_{11}N_3OS_2$: C, 55.79; H, 3.68; N, 13.94. Found: C, 55.93; H, 3.79; N, 13.78.

5-[2-(Phenylsulfonyl)phenyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**9c**)

Compound **9c** was purified by TLC eluting with chloroform-ethanol (5:1) and then crystallized from ethanol, yield 30%; mp 133–135°C. IR (KBr) ν cm^{-1} 3132 (NH), 1306 (C=S). ^{1}H-NMR (DMSO-d_6): 13.4 (bs, 1H, NH), 8.26–7.53 (m, 9H, aromatic). MS: m/z (%) 317 (M^+, 45), 252 (100), 220 (20), 165 (17), 77 (10). Anal. Calcd. for $C_{14}H_{11}N_3O_2S_2$: C, 52.98; H, 3.49; N, 13.24. Found: C, 52.76; H, 3.65; N, 13.46.

5-[2-(2-Chlorophenylthio)phenyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**9d**)

Compound **9d** was purified by TLC eluting with chloroform-ethanol (12:1) and then crystallized from ethanol, yield 45%; mp 233–235°C. IR (KBr) ν cm^{-1} 3201, 3172 (NH), 2543 (weak, SH), 1303 (C=S). ^{1}H-NMR (DMSO-d_6): 13.15 (bs, 1H, NH), 7.75–7.21 (m, 8H, aromatic). MS: m/z (%) 321 (M^++2, 35), 319 (M^+, 100) 284 (90), 225 (16), 209 (10). Anal. Calcd. for $C_{14}H_9ClN_2S_2$: C, 55.16; H, 2.98; N, 9.19. Found: C, 55.37; H, 3.14; N, 9.37.

Pharmacology

Male NMRI mice weighing 20–25 g (from the animal house of the Faculty of Pharmacy, TUMS) were used. The

animals were housed in colony cages, condition of constant temperature (22 ± 2°C), a 12 h light/dark schedule, and allowed free access to standard diet and tap water except during the experiment. The animals were allowed to habituate to the laboratory environment for 2 h before the experiments were initiated. All ethical manners for use of laboratory animals were considered carefully and the protocol of study was approved by TUMS ethical committee. The compounds were administered intra-peritoneally (ip), 30 μmol/kg; 0.2 ml/20 g as a suspension in saline and tween 80 (4% w/v) 30 min before formalin injection. Mefenamic acid (Hakim Pharmaceutical Co., Tehran, Iran) (30 μmol/kg, ip) was used as standard drug under the same conditions. The control group received vehicle (0.2 ml/20 g, ip) alone (Rineh *et al.*, 2007).

Formalin-induced test

Final compounds (**7a–9c**) were tested in the formalin-induced pain test. Twenty-five microliters of formalin (0.5%) was injected subcutaneously into the dorsal surface of the right hind paw of the mouse using a micro syringe with a 26-gauge needle. Immediately after the formalin injection, animals were placed individually in glass cylinder (20 cm wide, 25 cm long) and a glass door and a mirror were arranged at a 45° angle under the cylinder to allow clear observation of the paws of the animals. The total time in seconds that the animal spent licking or biting the injected paw during the period of 0–10 min was considered as indicator of neurogenic pain (early phase) and during the 10–30 min (late phase) represented the inflammatory pain (Rineh *et al.*, 2007; Dubuisson and Dennis 1977; Monsef *et al.*, 2004).

Statistics

The results were expressed as the mean ± SEM of 6 animals per group. The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey multi-comparison test. Differences with $P < 0.05$ between experimental groups were considered statistically significant.

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