Scientific paper

Synthesis, Anticonvulsant and Muscle Relaxant Activities of Substituted 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole

Ali Almasirad[a], Nasim Vousooghi[b], Sayyed Abbas Tabatabai[c], Abbas Kebriaeezadeh[d] and Abbas Shafiee[e]*

[a] *Department of Medicinal Chemistry, Faculty of Pharmacy, Islamic Azad University, Tehran Iran.*

[b] *Department of Pharmacology, Faculty of Medicine, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.*

[c] *Department of Medicinal Chemistry, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran*

[d] *Department of Pharmacology and toxicology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran.*

** Corresponding author: [e] Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran. Tel: 0098-21-66406757; Fax: 0098-21-66461178; E.mail: ashafiee@ams.ac.ir*

Received: 01-02-2007

Abstract

A series of 5-[2-(phenylthio)phenyl]-1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole derivatives were synthesized. Compounds were evaluated *in vivo* for their anticonvulsant and muscle relaxant activities using PTZ and rotarod tests, respectively. Only compound 3-amino-5-[2-(phenylthio)phenyl]-4*H*-1,2,4-triazole (**5**) showed weak anticonvulsant activity. However, most of the compounds were active in rotarod test and the most effective compound was 5-[2-(phenylthio)phenyl]-1,3,4-oxadiazole-2(3*H*)-one (**13**) which had comparable activity with diazepam.

Keywords: 1,3,4-Oxadiazole, 1,2,4-Triazole, benzodiazepine, anticonvulsant activity, muscle relaxant activity

1. Introduction

Benzodiazepines (BZs), which allosterically modulate the actions of GABA by GABA-A receptors have found widespread use as anxyolitics, sedative/hypnotics, muscle relaxants and anticonvulsants. Despite their widespread clinical use, the classical 1,4-BZs such as diazepam are also known to produce unwanted side effects including sedation, development of tolerance and dependence, rebound symptoms at withdrawal and amnesic

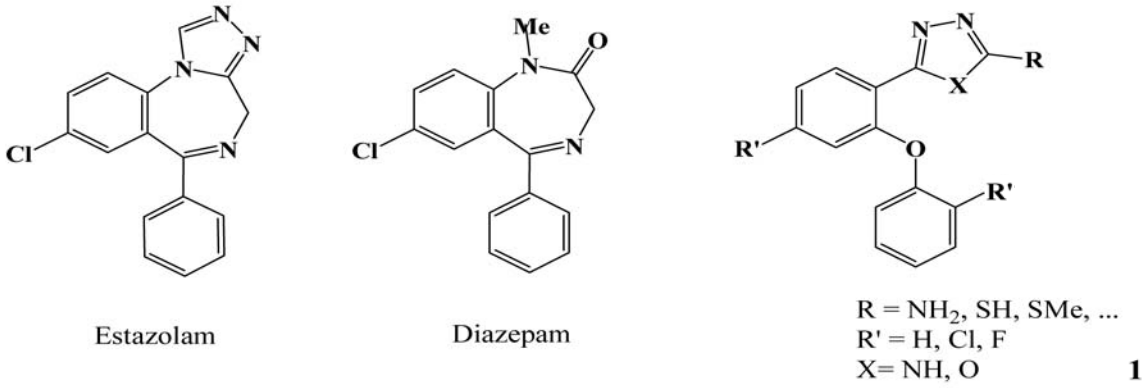
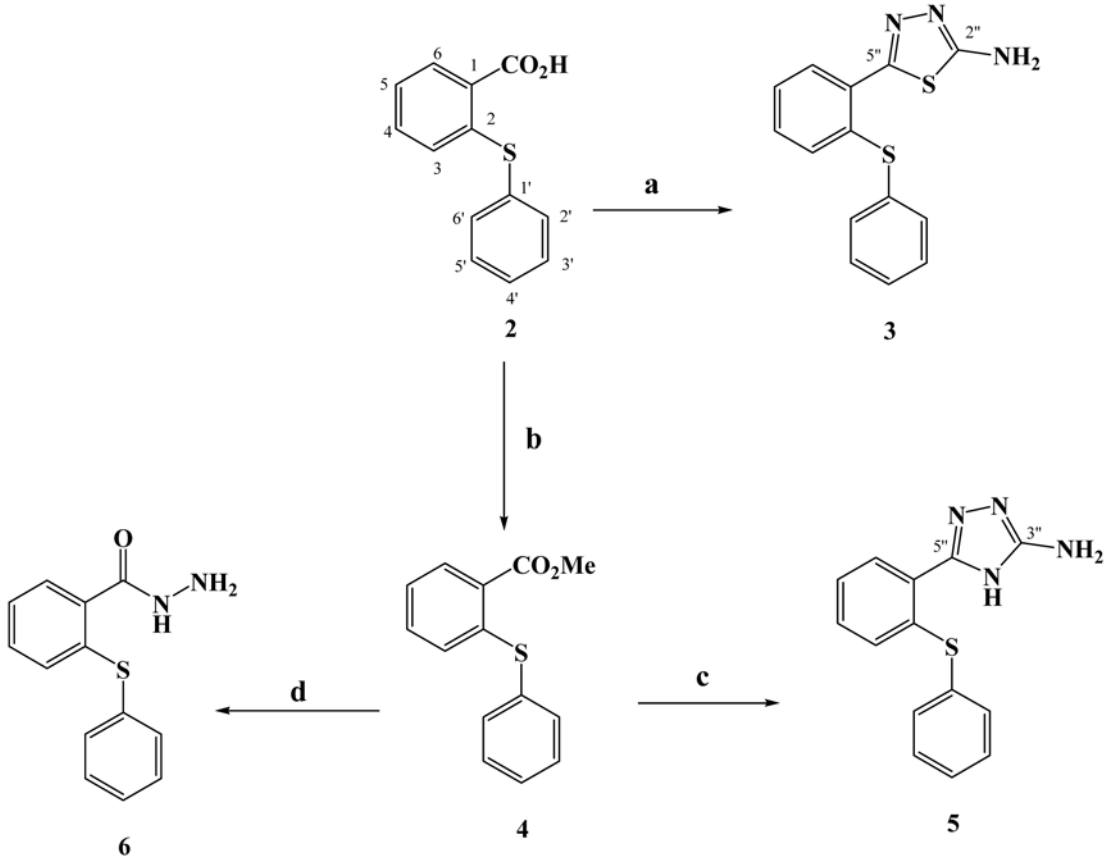


Figure 1. Structures of estazolam, diazepam and compounds **1**.

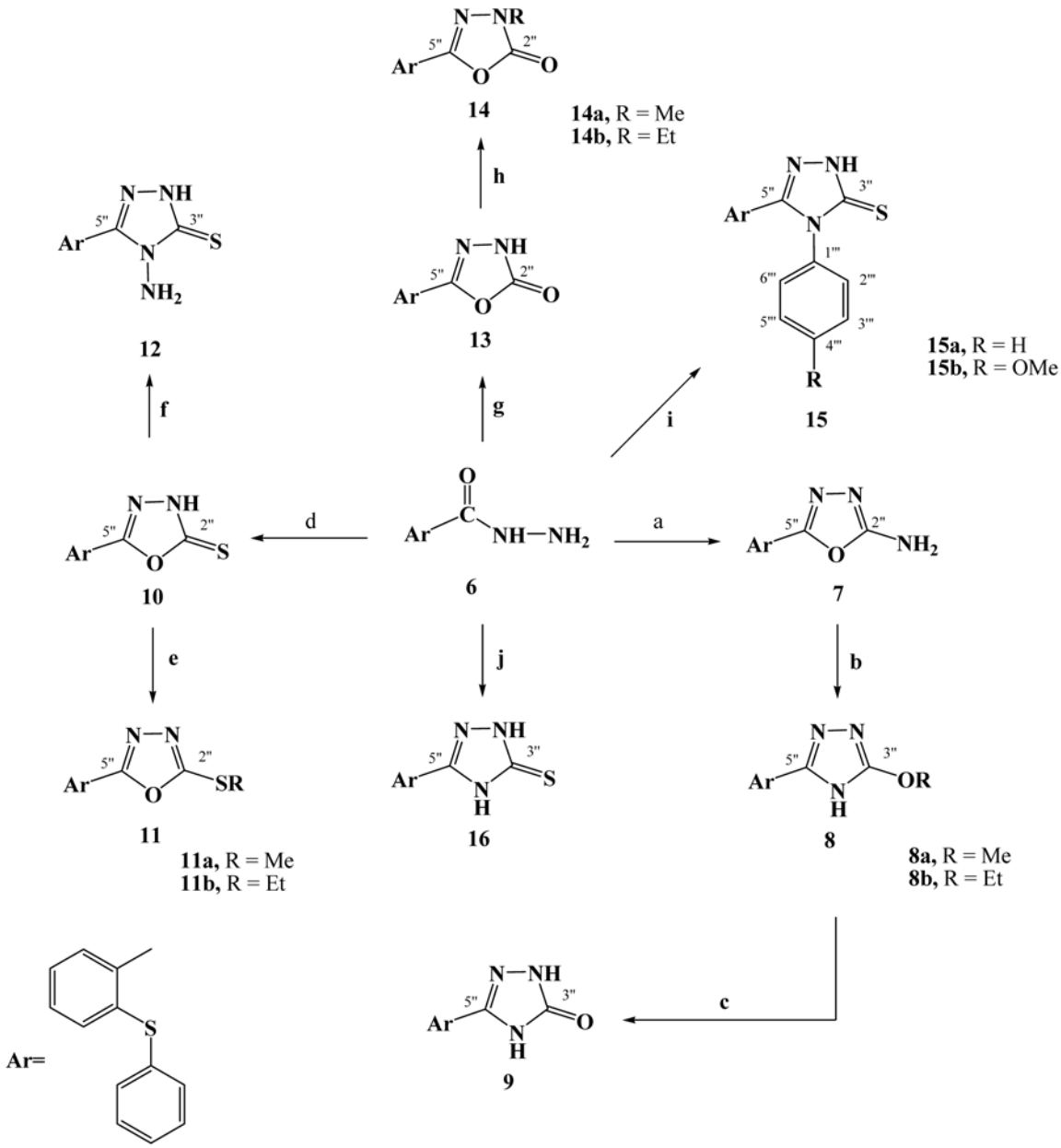
effects.[1] Thus, the search continues for new BZ-receptor ligands with enhanced selectivity, safety and efficacy. Moreover, an impressive number of structurally novel agents, which do not contain the 1,4-BZ nucleus, have been shown to interact with the BZ-receptor.[2,3] In previous studies we designed and synthesized a new group of flexible BZ agonists with 1,2,4-triazole and 1,3,4-oxadiazole rings, compounds **1** (Figure 1), on the basis of known triazolobenzodiazepines such as estazolam (Figure 1).[4,5] The designed sructure had the main BZ pharmacophores: an aromatic ring and a coplanar proton-accepting group, number 2 or 3 nitrogen of 1,2,4-triazole or 1,3,4-oxadiazole rings. A second out-of-plane aromatic ring, phenoxy group, could potentiate binding to the receptor.[6] Some of the synthesized compounds showed Bz activity comparable with diazepam.[4] For the purpose of evaluating the effects of different substitutents on pharmacological activity a new series of 1,2,4-triazole, 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives by bioisosteric replacement of oxygen with sulfur in phenoxy group were synthesized. Rotarod and pentylenetetrazole (PTZ) induced lethal convulsion tests were performed on these compounds, and the results were compared with diazepam, a known BZ agonist.[7,8]

2. Results and Discussion

The designed compounds were synthesized according to Schemes 1 and 2. Reaction of 2-(phenylthio)benzoic acid (**2**) with thiosemicarbazide and sulfuric acid afforded 2-amino-5-[2-(phenylthio)phenyl]-1,3,4-thiadiazole (**3**).[9,10] Methyl 2-(phenylthio)benzoate (**4**) which was readily prepared via esterification of compound **2**, was converted to 3-amino-5-[2-(phenylthio)phenyl]-4*H*-1,2,4-triazole (**5**), through reaction with aminoguanidine nitrate in presence of sodium methoxide.[11,12] The key intermediate 2-(phenylthio)benzoic acid hydrazide (**6**) was prepared from the reaction of hydrazine hydrate with compound **4**.[11] Reaction of hydrazide **6** with cyanogen bromide gave 2-amino-5-[2-(phenylthio)phenyl]-1,3,4-oxadiazole (**7**) which rearranged to 3-alkoxy-5-[2-(phenylthio)phenyl]-1,3,4-triazoles **8** upon treatment with methanolic or ethanolic postassium hydroxide. Acid hydrolysis of **8** provided 5-[2-(phenylthio)phenyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**9**).[4,11] 5-[2-(Phenylthio)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (**10**) was prepared by reaction of hydrazide **6** with KOH and CS_2.[13] Sonication of compound **10** in the presence of methyl or ethyl iodide in alkaline media afforded 2-alkylthio-5-[2-(phenylthio) phenyl]-1,3,4-oxadiazoles



Scheme 1. a: thiosemicarbazide, H_2SO_4, 120 C; b: MeOH, H_2SO_4, reflux; c: aminoguanidine nitrate, MeONa, MeOH, reflux,; d: $N_2H_4 \times H_2O$, EtOH, rt



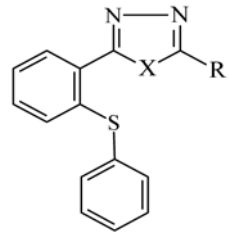
Scheme 2. a: BrCN, Dioxane, rt; b: KOH, MeOH or EtOH, reflux; c: HCl, reflux; d: CS_2, KOH, EtOH, reflux; e: MeI or EtI, NaOH 10%, EtOH, sonication, f: $N_2H_4 \times H_2O$, reflux; g: 1,1¢-CDI, triethylamine, THF, rt; h: MeI or EtI, KOH 4%, EtOH, rt; i: (1) phenylisothiocyanate or *p*-methoxyphenylisothiocyanate, EtOH, reflux; (2) NaOH, H_2O, reflux; j: (1) KSCN, HCl, H_2O, reflux; (2) NaOH 4%, reflux

11.[4] The 4-amino-5-[2-(phenylthio)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**12**) was synthesized by the reaction of **10** with hydrazine hydrate.[14] The 1,3,4-oxadiazol-2-one **13** was prepared by treating **6** with 1,1'-carbonyldiimidazole in the presence of triethylamine.[12] Reaction of compound **13** with methyl or ethyl iodide under basic conditions afforded 3-alkyl-5-[2-(phenylthio) phenyl]-1,3,4-oxadiazol-2-ones **14**.[15] Reaction of compound **6** with phenyl or *p*-methoxyphenylisothiocyanate yielded thiosemicarbazides as intermediates which were converted to 4-aryl-1,2,4-triazole-3-thiones **15** in aqueous sodium hydroxide.[16] 5-[2-(Phenylthio)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**16**) was readily prepared via reaction of **6** with potassium thiocyanate and hydrochloric acid followed by cyclization of thiosemicarbazide intermediate with aqueous sodium hydroxide.[14]

BZ activity of the compounds was determined through two routine models, rotarod test and evaluation of the ability of the synthesized compounds in protection of mice against a lethal dose of a convulsant agent PTZ.

Diazepam was considered as a reference BZ agonist (Table 1). The results showed that anticonvulsant effect of

Table 1: Pharmacological evaluation of synthesized compounds



Compound No. [a]	X	R	ED50 mg/kg [b,c]	
			Rotarod test	PTZ-induced lethal convulsion test
3	S	NH_2	ND[d]	>100
5	NH	NH_2	ND[d]	73.7 (59.3–92)[e]
7	O	NH_2	42.5 (28.1–58.8)[e]	>100
8a	NH	OMe	35.9 (22.9–48.2)[e]	>100
8b	NH	OEt	31.6 (20.7–50.3)[e]	>100
9	NH	OH	71.1 (59.2–85.5)[e]	>100
10	O	SH	28.9 (22.7–36.7)[e]	>100
11a	O	SMe	32.7 (23.1–45.5)[e]	>100
13	O	OH	5.6 (2.7–9.6)[e]	>100
15a	N-Ph	SH	66.4 (51.3–81.1)[e]	>100
15b	N-(4-OMe)C_6H_4	SH	ND[d]	>100
16	NH	SH	70.0 (53.1–87.4)[e]	>100
Diazepam			0.7 (0.4–1.1)[f]	1.7 (1.2-2.5)[f]

[a] compounds 11b, 12, 14a and 14b were not evaluated
[b] n = 10, 95% confidence limits in parentheses
[c] ED_{50} values and 95% confidence limits were determined using probit-log(dose) model with flumazenil and the test compounds as a categorical covariate and forcing through parallel dose response.
[d] Not determined
[e] ED50 did not significantly increase in the presence of flumazenil 10 mg/kg ($P > 0.05$)
[f] ED50 significantly increased in the presence of flumazenil 10 mg/kg ($P < 0.05$)

the synthesized compounds is weaker than our previously synthesized structures.[4,5] However, similar to the previous results, introduction of amino group on position 3 of the 1,2,4-triazole ring (compound **5**) resulted in better activity. None of target compounds, except **5** showed anticonvulsant effect in doses lower than 100 mg/kg. However, most of the compounds were effective in rotarod test and the results showed that oxadiazole ring is more effective than triazole ring in muscle relaxant activity.

Hydroxy, thiole and methylthio groups on position 3 of 1,3,4-oxadiazole ring and alkoxy on position 3 of 1,2,4-triazole ring are the best substituents in this test and compound **13** had comparable activity with diazepam in rotarod test. Compound **13** was more potent than the best compound in our previous study.[4] There was a major difference between pharmacological results of the present and our previous studies. The activity of the compounds was not significantly reduced by flumazenil, a benzodiazepine antagonist in both tests. This means that replacement of phenoxy group with phenylthio may cause a change in the mechanism of action.

Therefore it can be concluded that the newly synthesized compounds mediate their activity through an unknown mechanism. Further work is necessary to clarify the mechanism of their muscle relaxant and antiepileptic activity.

3. Experimental

Chemicals were purchased from Merck Chemical company (Darmstadt, Germany). Melting points were taken on a Kofler hot stage apparatus (Reichert, Vienna, Austria) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker FT-500 spectrometer (Bruker, 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). Elemental analyses were crarried out with a Perkin-Elmer Model 240-c apparatus (Perkin Elmer, Norwalk, CT, USA). The results of the elemental analyses (C, H, N) were within ±0.4% of the calculated amounts.

2-Amino-5-[2-(phenylthio)phenyl]-1,3,4-thiadiazole (3). To a stirring solution of compound **2** (2 g, 8.7 mmol)

in sulfuric acid (4 mL) at 0 C thiosemicarbazide (0.76 g, 8.3 mmol) was added in small amounts. After the addition was completed, the solution was heated at 120 C for 7 h. It was cooled, mixed with ice and concentrated ammonium hydroxide solution, thereafter the precipitate was filtered. The resulting precipitate was purified by column chromatography, eluting with chloroform and then crystallized from ethanol to provide 1.37 g (55%) of **3**, mp 209–211 C. IR (KBr): ν 3472–3340 (NH_2), 3056 (CH, aromatic), 1640 cm^{-1} (NH_2). ^{1}HNMR ($CDCl_3$): δ 7.69 (dd, *J* = 7.4, 1.9 Hz, 1H, aromatic), 7.53–7.44 (m, 5H, aromatic), 7.38–7.32 (m, 2H, aromatic), 6.90 (dd, *J* = 7.9, 1.5 Hz, 1H, aromatic), 6.30 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$): δ 174.5 (C-5″), 160.6 (C-2″), 138.4 (C-1), 134.9 (C-1′), 133.2 (C-2), 131.6 (C-3), 129.8 (C-2′ and C-6′), 129.9 (C-4), 129.5 (C-3′ and C-5′), 129.6 (C-6), 126.3 (C-5), 123.1 (C-4′). MS: *m/z* (%) 285 (M^+, 13), 257 (16), 212 (100), 184 (50), 152 (15), 139 (20). Anal. Calcd. for $C_{14}H_{11}N_3S_2$: C, 58.92; H, 3.89; N,14.72. Found: C, 58.98; H, 3.75; N, 14.65.

3-Amino-5-[2-(phenylthio)phenyl]-4*H*-1,2,4-triazole (5). A solution of sodium methoxide (0.19 g, 8.26 mmol) in anhydrous methanol (10 mL) was treated with aminoguanidine nitrate (1.125 g, 8.2 mmol) and **4** (0.5 g, 2.04 mmol) respectively. The reaction was heated at reflux for 30 h, poured into ice water (60 mL) brought to pH 7 with aqueous 3N HCl and filtered. The resulting precipitate was purified by TLC, eluting with chloroform-ethanol (12:1) and then crystallized from ethanol to provide 230 mg (38%) of **5**, mp 267–268 C. IR (KBr): ν 3416–3326 (NH_2), 3226 (NH), 3052 cm^{-1} (CH, aromatic). ^{1}H NMR (DMSO-d_6): δ 12.43 (bs, 1H, NH), 7.83 (d, *J* = 5.4 Hz, 1H, aromatic), 7.48–7.41 (m, 5H, aromatic), 7.20-7.17 (m, 2H, aromatic), 6.80 (d, *J* = 6.9, 1H, aromatic), 6.15 (bs, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ 158.9 (C-5″), 157.5 (C-2″), 137.6 (C-1′), 135.0 (C-2), 134.7 (C-3), 131.0 (C-1), 130.7 (C-2′ and C-6′), 130.1 (C-4), 129.4 (C-3′ and C-5′), 129.3 (C-6), 128.4 (C-5), 125.8 (C-4′). MS: *m/z* (%) 268 (M^+, 100), 234 (56), 225 (76), 212 (51), 191 (65), 184 (57), 151 (43), 134 (53), 91 (72), 77 (41). Anal. Calcd. for $C_{14}H_{12}N_4S$: C, 62.66; H, 4.51; N, 20.88. Found: C, 62.91; H, 4.67; N, 20.70.

2-(Phenylthio)benzoic acid hydrazide (6). To a stirring solution of compound **4** (10 g, 41 mmol) in 21 mL of ethanol at room temperature hydrazine hydrate (41 mL, 820 mmol) was added. After 5 h the contents were poured into water (50 mL) and filtered. The precipitate was crystallized from ethanol to give 9.5 g (95%) of **6**; mp 132–133 C. IR (KBr): ν 3317, 3125 (NH_2, NH), 1644 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ 8.43 (bs, 1H, NH), 8.21 (dd, *J* = 7.6, 1.9 Hz, 1H, aromatic), 7.61–7.56 (m, 5H, aromatic), 7.46–7.40 (m, 2H, aromatic), 7.05 (dd, *J* = 7.7, 1.4 Hz, 1H, aromatic), 4.05 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$): δ 165.1 (C=O), 137.1 (C-1), 135.1 (C-1′), 134.2 (C-2), 131.5 (C-4), 130.8 (C-3), 129.8 (C-2′ and C-6′), 129.5 (C-3′ and C-5′), 129.3 (C-6), 126.8 (C-5), 123.2 (C-4′). MS: *m/z* (%), 244 (M^+, 100), 213 (98), 183 (95), 152 (74), 139 (52), 107 (96), 76 (28). Anal. Calcd. for $C_{13}H_{12}N_2OS$: C, 63.91; H, 4.95; N, 11.47. Found: C, 64.06; H, 4.88; N, 11.51.

2-Amino-5-[2-(phenylthio)phenyl]-1,3,4-oxadiazole (7). To a stirring solution of compound **6** (1.81 g, 7.42 mmol) in dioxane (24 mL) sodium bicarbonate (0.623 g, 7.42 mmol) in water (8 mL) was added at room temperature. The mixture was stirred at room temperature for 5 min and cyanogen bromide (0.816 g, 7.70 mmol) was added. After 4 h water (60 mL) was added to the mixture and the precipitate was removed by filtration and crystallized from ethanol to give 1.85 g (93%) of **7**; mp 188–190 C. IR (KBr): ν 3308-3195 (NH_2), 1673 cm^{-1} (NH_2). ^{1}H NMR (DMSO-d_6): δ 7.73 (dd, *J* = 7.5, 1.8 Hz, 1H, aromatic), 7.51–7.45 (m, 5H, aromatic), 7.36–7.30 (m, 2H, aromatic), 6.92 (dd, *J* = 7.8, 1.4 Hz, 1H, aromatic), 6.27 (bs, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ 164.6 (C-5″), 156.9 (C-2″), 138.3 (C-1), 135.0 (C-1′), 133.1 (C-2), 131.4 (C-3), 130.9 (C-2′ and C-6′), 129.9 (C-4), 129.4 (C-3′ and C-5′), 129.2 (C-6), 126.7 (C-5), 123.1 (C-4′). MS: *m/z* (%) 269 (M^+, 100), 245 (92), 197 (81), 184 (78), 165 (41), 152 (38). Anal. Calcd. for $C_{14}H_{11}N_3OS$: C, 62.43; H, 4.12; N,15.60. Found: C, 62.24; H, 4.29; N, 15.64.

3-Methoxy-5-[2-(phenylthio)phenyl]-4*H*-1,2,4-triazole (8a). To a suspension of compound **7** (1.99 g, 7.4 mmol) in methanol (20 mL) at room temperature potassium hydroxide (2 g, 35.70 mmol) was added. The solution was heated at reflux for 16 h. After cooling the reaction mixture was neutralized with acetic acid. The solvent was evaporated under reduced pressure and the residue was purified by TLC, eluting with chloroform-ethanol (20:1) and then crystallized from ethyl acetate to provide 0.88 g (42%) of **8a**, mp 128–130 C. IR (KBr): ν 3229 (NH), 3054 cm^{-1} (aromatic). ^{1}H NMR ($CDCl_3$) δ 11.52 (bs, 1H, NH), 8.31 (dd, *J* = 7.9, 1.3 Hz, 1H, aromatic), 7.47–7.44 (m, 2H, aromatic), 7.39–7.35 (m, 1H, aromatic), 7.33–7.29 (m, 2H, aromatic), 7.28–7.25 (m, 3H, aromatic), 4.08 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 160.05 (C-3″), 154 (C-5″), 135.5 (C-1′), 135 (C-2), 132.2 (C-3), 131.7 (C-1), 130.8 (C-2′ and C-6′), 130.3 (C-4), 129.7 (C-3′ and C-5′), 129.7 (C-6), 129.2 (C-5), 127.8 (C-4′), 57.4 (CH_3). MS: *m/z* (%) 283 (M^+, 100), 250 (17), 225 (30), 184 (15). Anal. Calcd. for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N,14.83. Found: C, 63.79; H, 4.69; N, 14.58.

3-Ethoxy-5-[2-(phenylthio)phenyl]-4*H*-1,2,4-triazole (8b). This compound was prepared similarly as **8a** in 52% yield, mp 93–94 C (ethyl acetate). IR (KBr): ν 3247 (NH), 3075 cm^{-1} (CH, aromatic). ^{1}H NMR ($CDCl_3$) δ 11.52 (bs, 1H, NH), 8.31 (dd, *J* = 8.0, 1.4 Hz, 1H, aromatic), 7.48–7.45 (m, 2H, aromatic), 7.41–7.38 (m, 1H, aromatic), 7.34–7.30 (m, 2H, aromatic), 7.29–7.26 (m, 3H,

aromatic), 4.44 (q, 2H, CH_2), 1.48 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 167.4 (C-3″), 154.4 (C-5″), 135.5 (C-1′), 135.1 (C-2), 132.4 (C-3), 131.2 (C-1), 130.9 (C-2′ and C-6′), 130.3 (C-4), 129.9 (C-3′ and C-5′), 129.8 (C-6), 129.1 (C-5), 127.8 (C-4′), 66.2 (CH_2), 15.1 (CH_3). MS: *m/z* (%) 297 (M^+, 100), 267 (51), 225 (73), 212 (27), 183 (45), 148 (30), 133 (62), 117 (81). Anal. Calcd. for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N,14.13. Found: C, 64.51; H, 5.15; N, 14.26.

5-[2-(Phenylthio)phenyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (9). Compound **8b** (0.42 g, 1.4 mmol) was suspended in concentrated hydrochloric acid (10 mL) and heated at reflux for 4 h. The suspension was cooled to room temperature and the precipitate was filtered and crystallized from ethanol to give 0.24 g (63%) of **9**; mp 263–265 C. IR (KBr): ν 3324 (NH), 3602 (CH, aromatic), 1685 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6): δ 11.72 (bs, 1H, NH), 11.62 (bs, 1H, NH), 8.43 (dd, *J* = 8.1, 1.5 Hz, 1H, aromatic), 7.54–7.51 (m, 2H, aromatic), 7.47–7.44 (m, 1H, aromatic), 7.38–7.34 (m, 2H, aromatic), 7.33–7.30 (m, 3H, aromatic). ^{13}C NMR (DMSO-d_6): δ 158.0 (C-3″), 151.0 (C-5″), 136.5 (C-1′), 136.0 (C-1), 133.5 (C-2), 132.9 (C-3), 131.3 (C-2′ and C-6′), 131.0 (C-4), 129.9 (C-3′ and C-5′), 129.6 (C-5), 125.5 (C-4′), 125.9 (C-6). MS: *m/z* (%) 269 (M^+, 100), 224 (47), 183 (21), 133 (74), 90 (28). Anal. Calcd. for $C_{14}H_{11}N_3OS$: C, 62.43; H, 4.12; N,15.60. Found: C, 62.66; H, 4.20; N, 15.76.

5-[2-(Phenylthio)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (10). A mixture of hydrazide **6** (1.49 g, 6.10 mmol), potassium hydroxide (0.34 g), carbon disulfide (1.25 mL) and ethanol (7 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 crystallized from methanol to give 1.66 g (95%) of **10**; mp 207–209 C. IR (KBr): ν 3283 (NH), 1337 cm^{-1} (C=S). ^{1}H NMR ($CDCl_3$): δ 14.16 (bs, 1H, NH), 7.88 (dd, *J* = 7.7, 1.5 Hz, 1H, aromatic), 7.51–7.49 (m, 2H, aromatic), 7.44–7.40 (m, 3H, aromatic), 7.28–7.21 (m, 2H, aromatic), 6.94 (d, *J* = 7.9 Hz, 1H, aromatic). ^{13}C NMR ($CDCl_3$): δ 160.5 (C-2″), 160.2 (C-5″), 140.3 (C-2), 135.3 (C-1′), 132.4 (C-1), 131.9 (C-4), 130.2 (C-2′ and C-6′), 129.7 (C-3), 129.6 (C-6), 128.9 (C-3′ and C-5′), 125.7 (C-5), 120.3 (C-4′). MS: *m/z* (%) 286 (M^+, 100), 225 (98), 197 (57), 184 (45), 136 (38). Anal. Calcd. for $C_{14}H_{10}N_2OS_2$: C, 58.72; H, 3.52; N, 9.78. Found: C, 58.46; H, 3.28; N, 9.94.

2-(Methylthio)-5-[2-(phenylthio)phenyl]-1,3,4-oxadiazole (11a). Compound **10** (1.58 g, 6.1 mmol) was dissolved in mixture of ethanol (4 mL) and 10% aqueous sodium hydroxide solution (2.75 mL). Methyl iodide (0.44 mL, 7 mmol) was added and the solution was sonicated for 20 min. Water (20 mL) was added to the reaction mixture and extracted with ether. The organic layer was washed with water and dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography eluting with petroleum ether-ethyl acetate (4:1) and then crystallized from methanol to give 1.41 g (85%) of **11a**; mp 44–46 C. IR (KBr): ν 3073 cm^{-1} (aromatic). ^{1}H NMR ($CDCl_3$): δ 7.90 (dd, *J* = 7.7, 1.6 Hz, 1H, aromatic), 7.53–7.51 (m, 2H, aromatic), 7.40–7.37 (m, 3H, aromatic), 7.30–7.23 (m, 2H, aromatic), 7.04 (dd, *J* = 7.0, 1.2 Hz, 1H, aromatic), 2.77 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 164.9 (C-2″), 164.2 (C-5″), 139.7 (C-1), 134.6 (C-1′), 133.1 (C-2), 131.8 (C-3), 130.0 (C-2′ and C-6′), 129.8 (C-4), 129.6 (C-3′ and C-5′), 129.0 (C-6), 125.8 (C-5), 122.0 (C-4′), 15.7 (CH_3). MS: *m/z* (%) 300 (M^+, 55), 213 (100), 183 (90), 152 (20). Anal. Calcd. for $C_{15}H_{12}N_2OS_2$: C, 59.97; H, 4.03; N, 9.33. Found: C, 59.89; H, 4.12; N, 9.40.

2-(Ethylthio)-5-[2-(phenylthio)]phenyl]-1,3,4-oxadiazole (11b). Compound **11b** was prepared similarly as **11a** in 80% yield; mp 66–67 C (methanol). IR (KBr): ν 3067 cm^{-1} (aromatic). ^{1}H NMR ($CDCl_3$): δ 7.92 (dd, *J* = 7.9, 1.5 Hz, 1H, aromatic), 7.55–7.53 (m, 2H, aromatic), 7.54–7.42 (m, 3H, aromatic), 7.34–7.26 (m, 2H, aromatic), 7.07 (dd, *J* = 7.98, 1.1 Hz, 1H, aromatic), 3.35 (q, *J* = 7.4 Hz, 2H, CH_2), 1.56 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 165.1 (C-2″), 164.8 (C-5″), 139.9 (C-1), 134.8 (C-1′), 133.2 (C-2), 131.7 (C-3), 130.1 (C-2′ and C-6′), 130.0 (C-4), 129.6 (C-3′ and C-5′), 129.2 (C-6), 125.9 (C-5), 122.1 (C-4′), 27.5 (CH_2), 15.3 (CH_3). MS: *m/z* (%) 314 (M^+, 52), 298 (71), 270 (50), 252 (26), 213 (100), 84 (184), 152 (17). Anal. Calcd. for $C_{16}H_{14}N_2OS_2$: C, 61.12; H, 4.49; N, 8.91. Found: C, 60.96; H, 4.61; N, 8.68.

4-Amino-5-[2-(phenylthio)phenyl]-2,4-dihydro-3*H*-triazole-3-thione (12). To a suspension of **10** (0.4 g, 1.4 mmol) in ethanol (2 mL), hydrazine hydrate (0.14 mL, 2.8 mmol) was added. The reaction was heated at reflux for 20 h, cooled and acidified with cold aqueous 3N hydrochloric acid. The mixture was extracted with ether and the organic layer was washed with water and dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by TLC eluting with chloroform-ethanol (22:1) and then crystallized from ethanol to give 50 mg (11%) of **12**, mp 138–140 C. IR (KBr): ν 3432, 3336 (NH_2), 3250 (NH), 1325 (C=S). ^{1}H NMR ($CDCl_3$): δ 13.40 (bs, 1H, NH), 7.64 (dd, *J* = 7.5, 1.3 Hz, 1H, aromatic), 7.46–7.40 (m, 6H, aromatic), 7.39–7.36 (m, 1H, aromatic), 7.07 (d, *J* = 7.4 Hz, 1H, aromatic), 4.90 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$): δ 167.0 (C-2″), 149.8 (C-5″), 137.5 (C-1′), 134.0 (C-1), 133.8 (C-2), 131.7 (C-3), 131.6 (C-2′ and C-6′), 130.5 (C-4), 130.3 (C-3′ and C-5′), 129.2 (C-5), 127.3 (C-4), 125.9 (C-6). MS: *m/z* (%) 300 (M^+, 100), 267 (28), 212 (50), 208 (52), 197 (38), 150 (31), 109 (28), 93 (30), 76 (33). Anal. Calcd. for $C_{14}H_{12}N_4S_2$: C, 55.97; H, 4.03; N, 18.65. Found: C, 55.84; H, 3.95; N, 18.44.

5-[2-(Phenylthio)phenyl]-1,3,4-oxadiazol-2(3*H*)-one (13). To a solution of compound **6** (1 g, 4.10 mmol) and triethyl amine (0.60 mL, 4.30 mmol) in THF (10 mL), 1,1'-carbonyldiimidazole (0.84 g, 5.1 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 18 h. The volatiles were removed in vacuo, and the residue was dissolved in ether. The ether solution was washed with 1N hydrochloric acid and dried over sodium sulfate, filtered, and concentrated in vacuo. The solid was recrystallized from ethanol to provide 0.98 g (89%) of **13**; mp 160–163 C. IR (KBr): ν 3503 (weak, OH), 3172 (NH), 1792 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ 9.65 (bs, 1H, NH), 7.80 (dd, *J* = 7.7, 1.7 Hz, 1H, aromatic), 7.53–7.41 (m, 5H, aromatic), 7.27–7.21 (m, 2H, aromatic), 7.00 (s, 1H, aromatic). ^{13}C NMR ($CDCl_3$): δ 152.2 (C-2″), 151.9 (C-5″), 139.4 (C-2), 135.1 (C-1′), 132.5 (C-1), 131.4 (C-4), 130.2 (C-2′ and C-6′), 129.4 (C-3), 129.0 (C-6), 128.8 (C-3′ and C-5′), 125.6 (C-5), 121.2 (C-4′). MS: *m/z* (%) 270 (M^+, 100), 228 (36), 213 (80), 184 (76), 136 (97). Anal. Calcd. for $C_{14}H_{10}N_2O_2S$: C, 62.21; H, 3.73; N.10.36. Found: C, 62.46; H, 3.78; N, 10.20.

3-Methyl-5-[2-(phenylthio)phenyl]-1,3,4-oxadiazol-2(3*H*)-one (14a). To a stirring solution of **13** (0.50 g, 1.85 mmol) in 4% aqueous KOH solution (5.5 mL) and ethanol (5.5 mL) at 0 C, methyl iodide (0.23 mL, 3.70 mmol) was added. After stirring for 0.5 h at 0 C, the reaction mixture was stirred at room temperature for 6 h. The precipitate was filtered, washed with 4% solution of KOH and crystallized from ethanol to give 0.34 g (65%) of **14a**, mp 127–128 C. IR (KBr): ν 1777 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$) δ 7.81–7.79 (dd, *J* = 7.6, 1.7Hz, 1H, aromatic), 7.52–7.40 (m, 5H, aromatic), 7.26–7.20 (m, 2H, aromatic), 7.10 (s, 1H, aromatic), 3.60 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 153.0 (C-2″), 152.1 (C-5″), 139.5 (C-2), 135.4 (C-1′), 132.6 (C-1), 131.3 (C-4), 130.1 (C-2′ and C-6′), 129.3 (C-3), 129.2 (C-6), 128.7 (C-3′ and C-5′), 125.8 (C-5), 121.4 (C-4′), 43.7 (CH_3). MS: *m/z* (%) 284 (M^+, 100), 226 (23), 213 (25), 197 (46), 184 (74), 152 (17). Anal. Calcd. for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.24; H, 4.31; N, 9.63.

3-Ethyl-5-[2-(phenylthio)phenyl]-1,3,4-oxadiazol-2(3*H*)-one (14b). Compound **14b** was prepared similarly as **14a** in 67% yield, mp 109–111 C (ethanol). IR (KBr): ν 1751 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ 7.82 (dd, *J* = 7.3, 1.6 Hz, 1H, aromatic), 7.56–7.45 (m, 5H, aromatic), 7.31–7.25 (m, 2H, aromatic), 7.03 (s, 1H, aromatic), 3.94 (q, 2H, CH_2), 1.48 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 153.4 (C-2″), 152.5 (C-5″), 139.6 (C-2), 135.3 (C-1′), 132.6 (C-1), 131.5 (C-4), 130.2 (C-2′ and C-6′), 129.5 (C-3), 129.1 (C-6), 128.9 (C-3′ and C-5′), 125.7 (C-5), 121.4 (C-4′), 41.6 (CH_2), 13.9 (CH_3). MS: *m/z* (%) 298 (100), 270 (55), 252 (28), 211 (35), 184 (38). Anal. Calcd. for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73; N, 9.39. Found: C, 64.57; H, 4.68; N, 9.25.

4-Phenyl-5-[2-(phenylthio)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (15a). A mixture of compound **6** (1.90 g, 7.80 mmol), phenyl isothiocyanate (0.93 mL, 7.80 mmol) and absolute ethanol (10 mL) was refluxed for 5 h. After cooling to room temperature, water was added to the reaction mixture and the resulting semicarbazide was filtered and washed with water then added to 2N sodium hydroxide solution (85 mL) and refluxed for 6 h. The resulting solution was treated with charcoal and filtered. The filtrate was acidified with hydrochloric acid to pH 5–6. The precipitate was filtered, dried and recrystallized from ethanol to give 1.97 g (70%) of **15a**, mp 220–222 C. IR (KBr): ν 3142 (NH), 2556 (weak, SH), 1327 cm^{-1} (C=S). ^{1}H NMR (DMSO-d_6): δ 14.19 (s, 1H, NH), 7.59 (d, *J* = 6.9 Hz, 1H, aromatic), 7.40–7.29 (m, 10H, aromatic), 7.11–7.09 (m, 2H, aromatic), 7.05 (d, *J* = 7.9, 1H, aromatic). ^{13}C NMR (DMSO-d_6): δ 168.6 (C-3″), 150.5 (C-5″), 137.7 (C-1′), 134.5 (C-1‴), 134.3 (C-1), 132.9 (C-2), 132.6 (C-3), 132.4 (C2′ and C6′), 131.8 (C-4), 130.5 (C-3′ and C-5′), 129.9 (C-3‴ and C-5‴), 129.7 (C-5), 129.1 (C-4′), 128.9 (C-6), 128.0 (C-2‴ and C-6‴), 127.9 (C-4‴). MS: *m/z* (%) 361 (M^+, 100), 284 (32), 251 (27), 225 (26), 210 (22), 148 (20). Anal. Calcd. for $C_{20}H_{15}N_3S_2$: C, 66.45; H, 4.18; N,11.62. Found: C, 66.58; H, 3.96; N, 11.75.

4-(4-Methoxyphenyl)-5-[2-(phenylthio)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (15b). Compound **15b** was prepared similarly as **15a** in 85% yield, mp 184–185 C (ethanol). IR (KBr): ν 3114 (NH), 2530 (weak, SH), 1332 cm^{-1} (C=S). ^{1}H NMR ($CDCl_3$) δ 14.15 (s, 1H, NH), 7.59 (dd, *J* = 7.6, 1.3 Hz, 1H, aromatic), 7.40–7.30 (m, 5H, aromatic), 7.21 (d, *J* = 8.9 Hz, 2H, aromatic), 7.12 (dd, *J* = 7.9, 2.1 Hz, 2H, aromatic), 7.08 (d, *J* = 7.8 Hz, 1H, aromatic), 6.90 (d, *J* = 8.9 Hz, 2H, aromatic), 3.74 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 168.8 (C-3″), 160.2 (C-4‴), 150.8 (C-5″), 137.6 (C-1′), 134.5 (C-1), 132.9 (C-2), 132.6 (C-3), 132.2 (C-2′ and C-6′), 132.0 (C-4), 130.5 (C-3′ and C-5′), 130.3 (C-2‴ and C-6‴), 128.8 (C-5), 128.2 (C-4′), 128.0 (C-6), 127.1 (C-1‴), 114.9 (C-3‴ and C-5‴), 56.2 (CH_3). MS: *m/z* (%) 391 (M^+, 100), 252 (26), 225 (24), 184 (11), 139 (36), 77 (29). Anal. Calcd. for $C_{21}H_{17}N_3OS_2$: C, 64.42; H, 4.38; N, 10.73. Found: C, 64.13; H, 4.25; N, 10.54.

5-[2-(Phenylthio)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (16). A suspension of compound **6** (2 g, 8.20 mmol), potassium thiocyanate (3.98 g, 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 obtained after charcoal treatment and filtration was acidified with hydrochloric acid to pH 5–6 and the resulting precipitate filtered and crystallized from ethanol to give 1.61 g (69%) of **16**; mp

247–248 C; IR (KBr): ν 3126 (NH), 2521 (weak, SH), 1322 cm^{-1} (C=S). ^{1}H NMR (DMSO-d_6): δ 13.75 (s, 1H, NH), 13.73 (s, 1H, NH), 7.67 (dd, *J* = 7.6, 1.4 Hz, 1H, aromatic), 7.45–7.38 (m, 6H, aromatic), 7.37–7.34 (m, 1H, aromatic), 7.06 (d, *J* = 7.5 Hz, 1H, aromatic). ^{13}C NMR (DMSO-d_6): δ 167.4 (C-3″), 150.1 (C-5″), 137.9 (C-1′), 134.0 (C-1), 133.9 (C-2), 131.9 (C-3), 131.2 (C-2′ and C-6′), 130.8 (C-4), 130.7 (C-3′ and C-5′), 129.4 (C-5), 127.3 (C-4′), 125.9 (C-6). MS: *m/z* (%) 285 (M^+,100), 225 (98), 210 (23), 184 (17), 150 (11). Anal. Calcd. for $C_{14}H_{11}N_3S_2$: C, 58.92; H, 3.89; N, 14.72. Found: C, 59.24; H, 4.14; N, 14.91

Pharmacology. Male NMRI mice (Pasteur Institute of Iran) weighing 20–25 g were used. The animals were housed in groups of 10 in cages at conditions of constant temperature (24 ± 1 C) and a 12 h light/dark schedule and allowed free access to standard mouse diet and tap water except during the experiment. Test compounds, flumazenil and diazepam were given ip (10 mL/kg) as a freshly prepared solution in 50% DMSO and 50% sterile normal saline. Flumazenil was injected 5 min before administration of vehicle, diazepam or the test compounds.

Rotarod test. The apparatus for the rotarod test consist of a base platform and a rotating rod is placed at a height of 15 cm from the base. The 30 cm long rod is divided into five equal sections by six disks. Groups of 10 mice were trained to stay for 2 min on rotarod apparatus at 8 rpm. Twenty-four hours later, the animals were injected with vehicle, diazepam or the synthesized compounds and placed in the apparatus 30 min later. Muscle relaxant activity was defined as the inability of the mice to remain on the rotarod for the 2-min test period.[4,7]

PTZ-induced lethal convulsion test. The test compounds, diazepam and vehicle were administered to groups of 10 mice 30 min before the injection of PTZ (110 mg/kg, ip) and the dead mice were counted 30 min later.[4,8]

4. Acknowledgement

This work was supported by grants from the Research Council of Tehran University of Medical Sciences and by INSF (Iran National Science Foundation).

5. References

1. R. W. DeSimone, C. A. Blum, *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2723–2726.
2. H. Dumoulin, M. Boulouard, M. Daoust, S. Rault, *Eur. J. Med. Chem*. **1998**, *33*, 201–207.
3. G. Trapani, M. Franco, A. Latrofa, A. Carotti, G. Gerichi, M. Serra, G. Biggio, G. Liso, *Eur. J. Med. Chem*. **1996**, *31*, 575–587.
4. T. Akbarzadeh, S. A. Tabatabai, M. J. Khoshnoud, B. Shafaghi, A. Shafiee, *Bioorg. Med. Chem*. **2003**, *11*, 769–773.
5. A. Almasirad, S.A. Tabatabai, M. Faizi, A. Shafiee, *Bioorg. Med. Chem. Lett*. **2004,** *14*, 6057–6059
6. R. I. Fryer, in: C. Hansch, P. G. Sammes, J. B. Taylor (Ed.): Comprehensive Medicinal Chemistry, *Pergamon press*, New York, **1990**, pp 539–565.
7. S. Grasso, G. De Sarro, A. De Sarro, N. Micale, M. Zappala, G. Puia, M. Baraldi, C. De Micheli, *J. Med. Chem.* **1999**, *42*, 4414–4421.
8. C. Morpurgo, *Arzneim.-Forsch./Drug Res*. **1971**, *21*, 1727–1734.
9. K. Kameo, Y. Asami, K. Ogawa, T. Matsunaga, S. Saito, K. Tomisawa, K. Sota, *Chem. Pharm. Bull*. **1989**, 37, 1260–1267.
10. K. T. Potts, R. M. Huseby, *J. Org. Chem*. **1966**, *31*, 3528–3531.
11. A. Almasirad, M, Sheikhha, R. Hosseini, S. A. Tabatabai, A. Shafiee, *Arch. Pharm. Pharm. Med. Chem.* **2004**, *337*, 193–200.
12. M. D. Mullican, M. W. Wilson, D. T. Connor, C. R. Kostlan, D. J. Schrier, R. D. Dyer, *J. Med. Chem*. **1993**, *36*, 1090–1099.
13. A. Shafiee, E. Naimi, P. Mansobi, A. Foroumadi, M. Shekari, *J. Heterocylic Chem*. **1995**, *32*, 1235–1239.
14. B. N. Goswami, J. C. S. Kataky, J. N. Baruah, *J. Heterocyclic. Chem*. **1984**, *21*, 1225–1229.
15. P. C. Gogoi, J. C. S. Kataky, *Indian J. Chem*. **1990**, *29B*, 1159–1160.
16. B. S. Vashi, D. S. Mehta, V. H. Shah, *Indian J. Chem.* **1996**, *35B,* 111–115.

Povzetek

Predstavljena je sinteza serije derivatov 5-[2-(feniltio)fenil]-1,3,4-oksadiazolov, 1,3,4-tiadiazolov in 1,2,4-triazolov. Spojine so bile s PTZ in rotarod testom *in vivo* preizkušene za morebitno antikonvulzivno aktivnost in kot sredstvo za mišično sproščanje. Samo ena spojina, 3-amino-5-[2-(feniltio)fenil]-4*H*-1,2,4-triazol (**5**) je pokazala nizko antikonvulzivno aktivnost. Vendar pa je bila večina spojin aktivnih na rotarod testu; najbolj učinkovit je bila 5-[2-(feniltio)fenil]-1,3,4-oksadiazol-2(3*H*)-on (**13**), katerega aktivnost je primerljiva z aktivnostjo diazepama.