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Design and Synthesis of 4H-3-(2-Phenoxy)phenyl-1,2,4-triazole Derivatives as Benzodiazepine Receptor Agonists

Tahmineh Akbarzadeh,[a] Sayyed A. Tabatabai,[b] Mohammad J. Khoshnoud,[c] Bijan Shafaghi[c] and Abbas Shafiee[a,*]

[a]*Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[b]*Department of Medicinal Chemistry, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran*
[c]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran*

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Abstract—A series of new 5-substituted analogues of 4H-3-(2-phenoxy)phenyl-1,2,4-triazole and its chlorinated derivatives was designed and prepared. Conformational analysis and superimposition of energy minima conformers of the compounds on estazolam, a known benzodiazepine receptor agonist, revealed that the main proposed benzodiazepine pharmacophores were well matched. Rotarod and pentylenetetrazole-induced lethal convulsion tests showed that the introduction of an amino group in position 5 of 1,2,4-triazole ring especially in chlorinated derivatives had the best effect which was comparable with diazepam.

Introduction

For the past two decades, structure activity relationship of benzodiazepine ligands, have made considerable progress.[1–6] Amongst all models suggested for binding to the benzodiazepine receptor at least two features are common: an aromatic ring and a coplanar proton-accepting group in suitable distance. Also, the presence of a second out-of-plane, aromatic ring could potentiate binding to the receptor.[7,8] On this basis, compounds **1** (Fig. 1), with a simple non-rigid structure were designed which had all the suggested requirements for binding to the benzodiazepine receptors. To clarify whether the designed compounds could mimic the structure of a benzodiazepine agonist, conformational analysis on designed molecules as well as a known benzodiazepine agonist, estazolam (Fig. 1) was performed followed by superimposition of energy minima conformers. As an in vivo model for evaluating benzodiazepine effects, rotarod[9] and pentylenetetrazole (PTZ)-induced lethal convulsion[10] tests were performed on synthesized compounds, and the results were compared with diazepam, a known benzodiazepine agonist.

*Corresponding author. Tel.: +98-21-640-6757; fax: +98-21-646-1178; e-mail: ashafiee@ams.ac.ir

Results

Chemistry

The designed compounds were synthesized according to Scheme 1. Reaction of 2-phenoxybenzoic acids **1**[11,12] with thionyl chloride at 50–55 °C gave corresponding acid chlorides,[13] the key intermediates in the preparation of 1,2,4-triazoles. Acid chlorides were converted to 3-amino-5-(2-phenoxyphenyl)-1,2,4-triazoles **2**, by addition of aminoguanidine hydrogen carbonate followed by cyclization with 5% aqueous solution of sodium hydroxide. Reaction of acid chlorides with thiosemicarbazide followed by treatment with a solution of sodium hydroxide afforded 5-(2-phenoxyphenyl)-1,2,4-triazole-3-thiones **3** in good yield.[14] Sonicating compounds **3** in the presence of suitable alkyl halides in alkaline media afforded 3-alkylthio-5-(2-phenoxyphenyl)-1,2,4-triazoles **4** in 3–5 min. Oxidation of alkylthio derivatives 4 with *m*-chloroperbenzoic acid (MCPBA) gave corresponding 3-alkylsulfonyl-5-(2-phenoxyphenyl)-1,2,4-triazoles **5**.[14]

2-Phenoxybenzoic acid hydrazides were readily prepared via esterification of corresponding benzoic acid derivatives, followed by treatment with hydrazine hydrate in methanol.[14,15] The hydrazides were converted to 2-amino-5-(2-phenoxyphenyl)-1,3,4-oxadiazoles **6** which rearranged to 3-ethoxy-5-(2-phenoxyphenyl)-1,2,4-triazoles **7**

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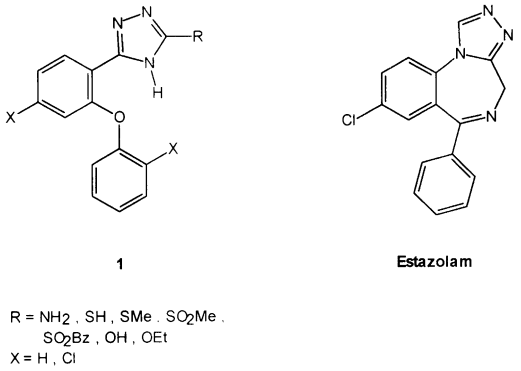


Figure 1. The structure of designed compounds **1** and estazolam.

upon treatment with ethanolic potassium hydroxide. Acid hydrolysis of **7** provided 5-(2-phenoxyphenyl)-1,2,4-triazole-3-ones **8**.[16]

Conformational analysis

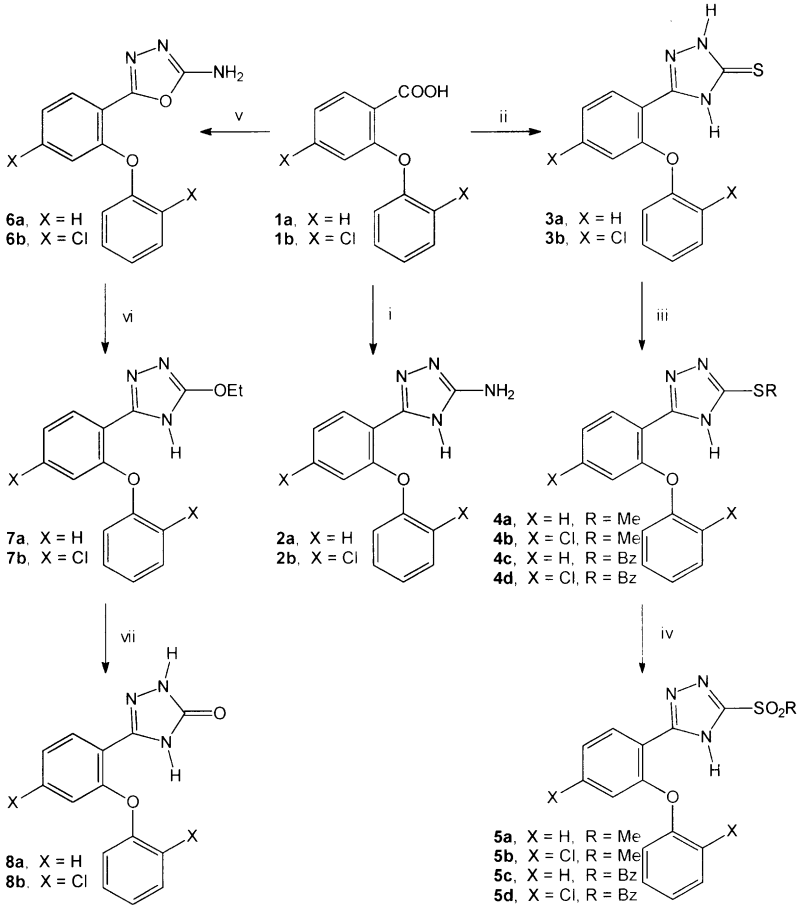
Conformations of the synthesized compounds were analyzed through MMX force field followed by AM1 calculation. The procedure was also performed on a known benzodiazepine agonist, estazolam, as a reference compound. Figure 2 shows energy minima conformers of the compound **2b**, the most potent synthesized analogues, and estazolam, which are superimposed. Obviously, the main benzodiazepine pharmacophores, aromatic rings and proton accepting groups, number 2 nitrogen of the 1,2,4-triazole and triazolobenzodiazepine rings, are well matched.

Pharmacological evaluation

Benzodiazepine activity of the compounds was determined through two routine models, rotarod test and evaluation of the ability of the synthesized compounds to protect mice against a lethal dose of a convulsant agent, pentylenetetrazole (PTZ). Diazepam was considered as a reference benzodiazepine agonist in both models (Table 1). The results show that analogous with chloro substituent on position 2 of phenoxy group and position 4 of phenyl ring are more potent than the corresponding unsubstituted compounds. Amongst substituents that introduced on position 5 of the 1,2,4-triazole ring, amino group (**2b**) had the best effect. The following rank order for the contribution of substituents to the activity of the synthesized compounds was observed:

NH2 > OEt > SMe > SO2Me > SO2Bz > SH, OH.

The activity of the compounds was significantly reduced by flumazenil, a benzodiazepine antagonist.



Scheme 1. Reagents: (i) (1) $SOCl_2$; (2) aminoguanidine hydrogen carbonate; (3) NaOH 5%; (ii) (1) $SOCl_2$; (2) thiosemicarbazide; (3) NaOH 5%; (iii) CH_3I or $PhCH_2Br$, NaOH 10%; (iv) MCPBA; (v) (1) MeOH, H_2SO_4; (2) $NH_2NH_2.H_2O$; (3) BrCN, $NaHCO_3$; (vi) KOH, EtOH; (vii) HCl.

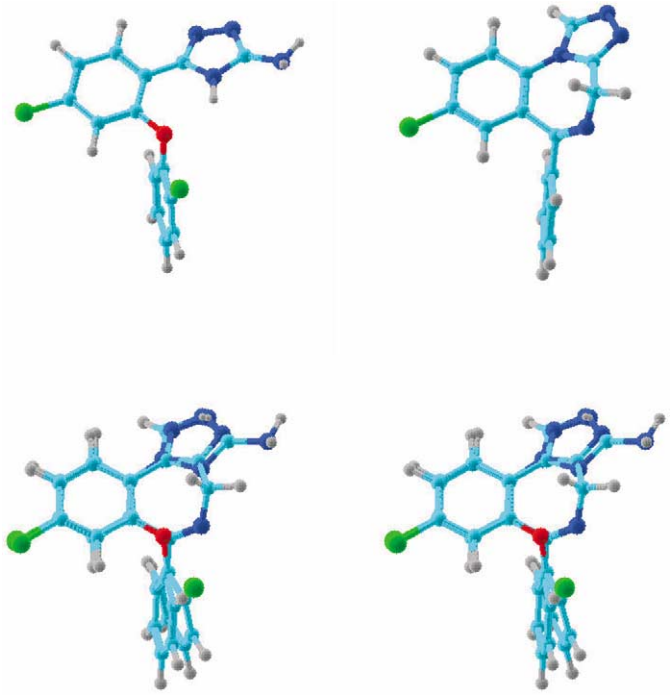
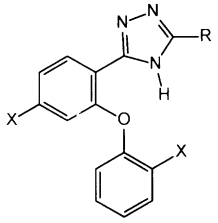


Figure 2. Stereoview of the superimposition of the energy minima conformers of estazolam (top right) and compound **2b** (top left).

Table 1. Pharmacological evaluation of synthesized 1,2,4-triazoles



Compd	R	X	ED_{50} mg/kg[a]	
			Rotarod test	PTZ- induced lethal convulsion test
Diazepam			0.6 (0.3–1.0)[b]	1.0 (0.6–1.5)[b]
2a	NH_2	H	24.0 (15.5–36.3)[b]	100
2b	NH_2	Cl	3.4 (0.1–8.3)[b]	12.0 (8.4–17.0)[b]
3a	SH	H	> 100	100
3b	SH	Cl	> 100	100
4a	SMe	H	89.0 (53.8–192.6)[b]	100
4b	SMe	Cl	22.5 (7.5–39.8)[b]	61.9 (34.2–119.4)[b]
4c	SBz	H	ND[c]	ND
4d	SBz	Cl	> 100	100
5a	SO_2Me	H	89.4 (28.9–> 1000)[b]	100
5b	SO_2Me	Cl	24.7 (4.9–49.1)[b]	87.0 (63.5–127.6)[b]
5c	SO_2Bz	H	> 100	100
5d	SO_2Bz	Cl	25.7 (13.1–47.1)[b]	100
7a	OEt	H	76.1 (5.3–29.6)[b]	100
7b	OEt	Cl	10.6 (2.7–19.8)[b]	35.3 (25.1–50.2)[b]
8a	OH	H	> 100	100
8b	OH	Cl	> 100	100

[a] $n = 10$, 95% confidence limits in parentheses, LD_{50} of all compounds > 300 mg/kg.
[b] ED_{50} significantly increased in the presence of flumazenil 10 mg/kg ($p < 0.05$).
[c] Not determined.

Discussion

The results presented here are part of our efforts to design simple non-rigid structures with benzodiazepine activity based on proposed SAR.

The designed structures have the main benzodiazepine pharmacophores: an aromatic ring and a coplanar proton-accepting group, number 2 nitrogen of 1,2,4-triazole ring. A second out-of-plane aromatic ring, phenoxy group, could potentiate binding to the receptor.[7]

In order to confirm whether the designed compounds could mimic proper conformation for binding to the benzodiazepine receptor, conformational analysis was performed on synthesized compounds and a known benzodiazepine agonists, estazolam.

Superimposition of energy minima conformers shows that the main proposed pharmacofores are well matched.

Chloro substituted analogues on position 2 of phenoxy group and position 4 of phenyl ring are more potent than corresponding unsubstituted compounds. These two positions are well matched to positions 2′ and 7 of the benzodiazepine ring; it has been established that electron withdrawing substituent on these positions enhance the activity.[1] These confirm the results of the superimposition studies. Amino group was the best substituent for the 1,2,4-triazole ring, and compound **2b** was comparable with diazepam especially in rotarod test.

The fact that the activity of the compounds is significantly reduced by flumazenil, a benzodiazepine antagonist, confirms that this effect is mediated through benzodiazepine receptors.

Conclusion

The study indicates that some synthesized 1,2,4-triazoles with a simple non-rigid structure in which the proposed pharmacophores have a proper steric direction could show benzodiazepine activity comparable with diazepam confirming the suggested SAR for benzodiazepine agonists. This could lead us to the new class of benzodiazepine receptor ligands.

Experimental

Chemistry

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The UV spectra were obtained using a Perkin–Elemer Model 550 SE. The IR spectra were obtained using a Nicolet FT-IR Magna 550 spectrographs. The ^1H NMR spectra were obtained using Bruker FT-80 or Varian 400 unity plus spectrometers and chemical shifts (δ) are in ppm relative to internal tetramethylsilane. Mass spectra were obtained using a Finnigan TSQ 70 Mass spectrophotometer at

70 ev. Elemental microanalyses were within ±0.4% of theoretical values for C, H, Cl and N.

3-Amino-5-[4-chloro-2-(2-chlorophenoxy)phenyl]-4H-1,2,4-triazole (2b). To a stirring solution of aminoguanidine hydrogen carbonate (2.14 g) in dry pyridine (26 mL), at −5 °C, a solution of 4-chloro-2-(2-chlorophenoxy)benzoic acid chloride (5 g 16.6 mmol) in dry benzene (26 mL) was added. The stirring was continued for half an h at −5 °C and then overnight at room temperature. The solvent was evaporated. To the residue, water (35 mL) was added. The crude white precipitate was suspended in 5% aqueous solution of sodium hydroxide (140 mL) and heated at reflux for 8 h. After cooling the reaction mixture was filtered. The filtrate was acidified with hydrochloric acid and the precipitate was filtered, washed with ethyl acetate and crystallized from ethanol to give 1 g (20%) of **2b**, mp 265–267 °C; uv (ethanol): λ_{max} 207 nm (log ε = 4.25), 264 nm (log ε = 3.98), 298 nm (log ε = 3.72); ir (potassium bromide): ν 3371 (N–H), 3283, 3227 (NH_2), 3078 (C–H aromatic), 1685 cm^{-1} (NH_2); ms: *m*/*z* (%) 320 (M^+, 27), 287 (32), 285 (100). Anal. calcd for $C_{14}H_{10}Cl_2N_4O$: C, 52.36; H, 3.14; N, 17.45. Found: C, 52.64; H, 3.36; N, 17.30.

5-[4-Chloro-2-(2-chlorophenoxy)phenyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (3b). To a stirring solution of thiosemicarbazide (0.58 g, 6.4 mmol) in dry pyridine (10 mL), at −5 °C a solution of 4-chloro-2-(2-chlorophenoxy)benzoic acid chloride (1.92 g) in dry benzene (10 mL) was added. The stirring was continued for half an hour at −5 °C and then overnight at room temperature. The solvent was evaporated. To the residue water (20 mL) was added. The crude yellow precipitate was suspended in 5% aqueous solution of sodium hydroxide (215 mL) and heated at reflux for 8 h. After cooling the solution was acidified with hydrochloric acid and the precipitate was filtered and crystallized from ethanol to give 1.8 g (84%) of **3b**, mp 250–252 °C; uv (ethanol): λ_{max}, 256 nm (log ε = 4.26), 305 nm (log ε = 3.86); ir (potassium bromide): ν 3441 (N–H), 3083 (C–H aromatic), 1613 cm^{-1} (N–H); ms: *m*/*z* (%) 337 (M^+, 44), 304 (38), 302 (100). Anal. calcd for $C_{14}H_9Cl_2N_3OS$: C, 49.72; H, 2.68; N,12.42. Found: C, 49.49; H, 2.46; N,14.73.

5-[4-Chloro-2-(2-chlorophenoxy)phenyl]-3-methylthio-4H-1,2,4-thiazole (4b). Compound **3b** (2 g, 5.92 mmol) was dissolved in ethanol (3 mL) and 10% aqueous solution of sodium hydroxide (2.4 mL) with sonication. CH_3I (0.84 g 5.92 mmol) was then added and the solution mixture was sonicated for 3 min. The precipitate was filtered and crystallized from methanol to give 1.8 g (87%) of **4b**, mp 146–148 °C; uv (ethanol): λ_{max} 245 nm (log ε = 4.18), 296 nm (log ε = 3.90); ^{1}H NMR ($CDCl_3$): δ 8.32 (d, 1H, H_6, $J_{5,6}$ = 8.8 Hz), 7.13–7.60 (m, 5H, aromatic), 6.69 (d, 1H, H_3, $J_{3,5}$ = 1.6 Hz), 2.65 ppm (s, 3H, SCH_3); ms: *m*/*z* (%) 351 (M^+, 65), 318 (100), 316 (70), 300 (15), 264 (10), 228 (5). Anal. calcd for $C_{15}H_{11}Cl_2N_3OS$: C, 51.15; H, 3.15; N,11.93. Found: C, 50.86; H, 3.23; N, 12.24.

5-[4-Chloro-2-(2-chlorophenoxy)phenyl]-3-methylsulfonyl-4H-1,2,4-triazole (5b). To a stirring solution of **4b** (750 mg, 2.13 mmol) in dichloromethane (30 mL) at 0 °C *m*-chloroperbenzoic acid (2.2 g, 12.78 mmol) was added. The mixture was stirred overnight at room temperature. Saturated sodium carbonate solution (2×20 mL) were added and vigorously stirred for 15 min. The organic layer was dried (sodium sulfate) and evaporated under reduced pressure. The residue was crystallized from ethanol to give 600 mg (73%) of **5b**, mp 178–180 °C; uv (ethanol): λ_{max} 213 nm (log ε = 4.53), 256 nm (log ε = 4.30), 266 nm (log ε = 4.22), 292 nm (log ε = 3.82), 302 nm (log ε = 3.76); ir (potassium bromide): ν 3350, 3284 (N–H), 3074 cm^{-1} (C–H aromatic), ^{1}H NMR ($CDCl_3$): δ 8.38 (d, 1H, H_6, $J_{5.6}$ = 8.6 Hz), 7.10–7.65 (m, 5H, aromatic), 6.70 (d, 1H, H_3), 3.34 ppm (s, 3H, CH_3); ms: *m*/*z* (%) 383 (M^+, 18), 350 (45), 348 (100). Anal. calcd for $C_{15}H_{11}Cl_2N_3O_3S$: C, 46.89; H, 2.89; N, 10.94. Found: C, 46.81; H, 2.86; N, 10.59.

5-[4-Dhloro-2-(2-chlorophenoxy)phenyl]-2-amino-1,3,4-oxadiazole (6b). Sodium bicarbonate (2.25 g, 26.8 mmol) in water (64 mL) was added at room temperature to a solution of 4-chloro-2-(2-chlorophenoxy)benzoic acid hydrazide (7.9 g, 26.6 mmol) in dioxane (85 mL). After the mixture was stirred at room temperature for 5 min, cyanogen bromide (3.5 g, 33.03 mol) was added. The reaction mixture was stirred for 1 h, the precipitate was filtered and crystallized form ethyl acetate to give 8 g. (94%) of **6b**, mp 158–160 °C; uv (ethanol): λ_{max} 279 nm (log ε = 4.13), 302 nm (log ε = 4.06); ir (potassium bromide): ν 3452, 3318 (NH_2), 3124 (C–H aromatic), 1664 cm^{-1} (NH_2); ms: *m*/*z* (%) 321 (M^+, 14), 288 (65), 286 (100). Anal. calcd for $C_{14}H_9Cl_2N_3O_2$: C, 52.20; H, 2.82; N, 13.04. Found: C, 52.01; H, 2.59; N, 13.12.

3-[4-Chloro-2-(2-chlorophenoxy)phenyl]-5-ethoxy-4H-1,2,4-triazole (7b). To a room temperature suspension of **6b** (2 g, 6.21 mmol) in ethanol (50 mL) potassium hydroxide (1.37 g) was added. The solution was heated at reflux for 3 h, and an additional potassium hydroxide (240 mg) was added. After an additional 3 h of heating the reaction mixture was allowed to cool at room temperature and neutralized with acetic acid. The solvent was evaporated under reduced pressure and the residue was crystallized from ethyl acetate to give 1.5 g (69%) of **7b**, mp 167–168 °C; uv (ethanol): λ_{max} 266 nm (log ε = 4.08), 294 nm (log ε = 3.86), 305 nm (log ε = 3.79); ^{1}H NMR ($CDCl_3$): 8.27 (d, 1H, H_6, $J_{5,6}$ = 8.5 Hz), 7.09–7.62 (m, 5H, aromatic), 6.67 (d, 1H, H_3, $J_{3,5}$ = 1.94), 4.39 (q, 2H, CH_2), 1.45 (t, 3H, CH_3); ms: *m*/*z* (%), 349 (M^+, 95), 316 (43), 314 (98), 288 (38), 286 (98). Anal. calcd for $C_{16}H_{13}Cl_2N_3O_2$: C, 54.88; H, 3.74; N, 12.00. Found: C, 55.09; H, 3.55; N, 11.87.

5-[4-Chloro-2-(2-chlorophenoxy)phenyl]-2,4-dihydro-3H-1,2,4-trizole-3-one (8b). Compound **7b** (0.4 g, 1.14 mmol) was suspended in concentrated HCl (10 mL) and heated at reflux for 24 h. The suspension was cooled to room temperature filtered and washed with water to give 0.3 g (82%) of **8b**, mp 286–289 °C; uv (ethanol): λ_{max} 273 nm (log ε = 4.02), 300 nm (log ε = 3.81); ir (potassium bromide), ν 3221 (N–H), 3088 (C–H aromatic), 1700 cm^{-1} (C=O); ms: *m*/*z* (%) 321 (M^+, 60), 288 (32), 286 (100). Anal. calcd for $C_{14}H_9Cl_2N_3O_2$: C,

52.20; H, 2.82; N, 13.04. Found: C, 52.01; H, 3.14; N, 12.77.

Compounds **2a**–**8a**, **4c**, **5c**, **4d** and **5d** were prepared similarly.

Calculations

Conformational analysis of the synthesized compounds and estazolam were preliminarily performed by MMX force field method implemented in PCMODEL 6.0 software.[17] The conformers were optimized further by AM1 calculation using MOPAC 6.0 program.[18] Global energy minima conformers of the designed compounds were superimposed on corresponding conformer of estazolam molecule which was considered as a reference benzodiazepine agonist.

Pharmacological evaluation

Male NMRI mice (Pasteur Institute, Iran) weighting 20–25 g ($n = 10$) were used in the experiments. The animals were kept in the groups of ten in cages under constant temperature ($24 \pm 1\,^{\circ}C$) and 12 h light/dark schedule. They had free access to standard mouse diet and tab water except during the experiment. On the day of the experiment, animals were transferred to individual cases randomly and allowed to acclimatize for 30 min before injection of drug or vehicle. Test compounds, flumazenil (Hoffmann La Rosch) and diazepam (Sigma) were given ip (10 mL/kg) as a freshly prepared solution in 50% DMSO and 50% sterile normal saline. Flumazenil were injected 5 min before administration of vehicle, diazepam or the test compounds.

Rotarod test

Groups of 10 mice were trained to stay for 2 min on rotarod apparatus, 3.0 cm in diameter, at 8 rpm (Tajhiz gostar co.). Twenty-four hours later, the animals were injected with vehicle, diazepam or the synthesized compounds and placed in the apparatus 30 min later. Impairment of coordinated motor movement was defined as the inability of the mice to remain on the rotarod for the 2-min test period.[9]

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 (100 mg/kg, ip) and the dead mice were counted 30 min later.[10]

Statistical analysis

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. Rightward shift of the ED_{50} in logarithmic scale after administration of flumazenil was considered significant if both lower and upper bonds of 95% confidence interval were greater or less than one. All statistical calculations were performed by SPSS for windows (Rel. 10.0.5. 1999. Chicago: SPSS Inc.).

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