

## SYNTHESES AND ANTICONVULSANT ACTIVITY OF N<sub>4</sub>-SUBSTITUTED TRIAZOLYLTHIAZOLES

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### ABSTRACT

Due to the good anticonvulsant activity of various 1,2,4-triazoles, several new N<sub>4</sub>-substituted triazolylthiazoles were prepared by the general method for 1,2,4-triazole ring closure. Anticonvulsant activity of compounds was measured against pentylenetetrazole-induced seizures in mice by intraperitoneal injections of different doses of the test compounds. Pretreatment of animals with flumazenil (10 mg/kg, i.p.) as a benzodiazepine receptors antagonist did not have any significant effect on anticonvulsant activity of the test compounds. These results demonstrate that the anticonvulsant activity of N<sub>4</sub>-substituted triazolylthiazole agents is not probably mediated by direct interaction with benzodiazepine receptor complex.

**Keywords:** 1,2,4-Triazoles, Anticonvulsant, Thiazoles, Flumazenil, Pentylenetetrazole

### INTRODUCTION

Recent studies on the structure-activity relationship of benzodiazepine have shown the necessity of at least two common features for binding of an agonist; 1; an aromatic ring, 2; a coplanar proton-accepting group at a suitable distance. Moreover, the presence of a second out-of-plane aromatic ring could potentiate binding to the receptor (1). In continuation of preparation of new triazole compounds (2), the possible anticonvulsant activity of some new compounds according to the above structure-activity relationship were investigated. It was concluded that N<sub>4</sub>-substituted triazolylthiazoles could be good candidates for anticonvulsant agents. Conformation analysis followed by superimposition of energy minimal conformers of compounds on the known benzodiazepine agonist, estazolam, was performed to clarify whether these compounds could mimic agonist structure of benzodiazepine ligands. Anticonvulsant activity of the new compounds was determined by an *in vivo* model for evaluation of benzodiazepine effects. In the present study effects of N<sub>4</sub>-substituted triazolylthiazoles on pentylenetetrazole-induced convulsions and their mechanisms were investigated.

### MATERIALS AND METHODS

#### Chemistry

The synthesis of the title compounds was accomplished as shown in Scheme 1. Reaction

of hydrazide **1** with different alkyl or phenyl isothiocyanates in ethanol and aqueous sodium hydroxide at room temperature (**3**) gave compound **2**. Refluxing the latter compound with aqueous sodium bicarbonate afforded 4-alkyl(or phenyl)-5-(2,4-dimethyl-5-thiazolyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione **3**.

Alkylation of the thione functional group (**3**) by appropriate alkyl halides gave compound **4**. Oxidation of **4** by *m*-chloroperbenzoic acid (MCPBA) afforded the appropriate sulfones **5**. The spectroscopic data of compound **3c**, **3d**, **4b**, **4c**, **5a**, **5b** and **6** have been reported elsewhere (4). The newly synthesized compounds (**3a**, **3b** and **4a**) were characterized by <sup>1</sup>H NMR, MS and IR spectroscopy. The purity of all products was determined by thin-layer chromatography using several solvent systems of different polarity. The physical constants of the final compounds are summarized in Table 1.

#### 5-(2,4-Dimethylthiazole-5-carboxyl)-4-methylthiosemicarbazide (**2a**, R=R<sub>1</sub>=CH<sub>3</sub>)

To a stirring mixture of recrystallized 2,4-dimethyl-thiazole-5-carboxylic acid hydrazine **1a** (**3**) (1.27 g, 10 mmol), sodium hydroxide solution (10 mmol, as a 2N solution) at 25°C, was added re-distilled methyl isothiocyanate (730 mg, 10 mmol) through a dropping funnel. After addition, the flask was kept at above

temperature and the mixture was stirred vigorously for 24 hr until a homogenous red clear solution was formed. The final mixture was filtered and the filtrate was neutralized by addition of hydrochloric acid while the flask temperature was maintained at room temperature by an ice-water bath. After neutralization, the mixture was filtered and the precipitate was crystallized from ethanol-water to give **2a** (85%); mp: 243-245°C.

$^1\text{H NMR}$  (DMSO)  $\delta$  (ppm) 11.74 (s, 1H, NH), 10.33 (s, 1H, NH), 9.43 (s, 1H, NH), 3.31 (s, 3H,  $\text{CH}_3\text{-N}$ ), 2.77 (s, 3H, thiazole- $\text{CH}_3$ ) and 2.74 (s, 3H, thiazole- $\text{CH}_3$ ). MS: m/z (%), 244 ( $\text{M}^+$ , 34), 212 (100), 186 (44), 63(23); IR  $\nu_{\text{max}}$  3270, 3450, 1545, 1234; UV( $\text{CH}_3\text{OH}$ )  $\lambda_{\text{max}}$  264 nm,  $\log \epsilon = 3.5$ .

**5-(2, 4-Dimethylthiazole -5-carboxyl)-4-cyclohexylthiosemicarbazide (2b, R=CH<sub>3</sub>, R<sup>c</sup>=cyclohexyl)**

Compound **2b** was prepared according to the above procedure in 88% yield; using **1a** (1.27 g, 10 mmol), sodium hydroxide solution (10 mmol, as a 2N solution) and re-distilled cyclohexyl isothiocyanate (1.42 g, 10 mmol); mp: 154-157°C.

$^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm) 11.27(s, 1H, NH), 10.54(s, 1H, NH), 9.73 (s, 1H, NH), 3.27-2.89 (m, 11H, cyclohexyl), 2.76 (s, 3H, thiazole- $\text{CH}_3$ ) and 2.71 (s, 3H, thiazole- $\text{CH}_3$ ). MS: m/z(%), 312 ( $\text{M}^+$ , 64), 289 (44), 245 (100), 163(54). IR  $\nu_{\text{max}}$  3260, 3475, 1530, 1225; UV( $\text{CH}_3\text{OH}$ )  $\lambda_{\text{max}}$  267 nm,  $\log \epsilon = 3.5$ .

**5-(2,4-Dimethyl-5-thiazolyl)-4-methyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (3a, R=R<sup>c</sup>=CH<sub>3</sub>, R<sup>e</sup>=SH)**

A re-crystallized portion of **2a** (244 mg, 1 mmole) and sodium bicarbonate (840 mg, 10 mmol) in water (10 ml) was refluxed for 12 h. until a clear solution was formed. The final mixture was cooled to room temperature, filtered and neutralized by addition of hydrochloric acid while the flask temperature was maintained at room temperature with an ice-water bath. After neutralization, the mixture was filtered and the precipitate was crystallized from ethanol-water to give **3a** (79%); mp: 267-270°C.

$^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm) 8.1(s, 1H, NH), 3.57(s, 3H, N- $\text{CH}_3$ ), 2.76 (s, 3H, thiazole- $\text{CH}_3$ ) and 2.71 (s, 3H, thiazole- $\text{CH}_3$ ). MS: m/z(%),

226 ( $\text{M}^+$ , 57), 199 (54), 163(100). IR  $\nu_{\text{max}}$  3258, 1552, 1211; UV( $\text{CH}_3\text{OH}$ )  $\lambda_{\text{max}}$  252 nm,  $\log \epsilon = 3.5$ .

**5-(2,4-Dimethyl-5-thiazolyl)-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (3b, R=CH<sub>3</sub>, R<sup>c</sup>=cyclohexyl, R<sup>e</sup>=SH)**

Compound **3b** was prepared according to the above procedure in 79% yield; using **2b** (312 mg, 1 mmole) and sodium bicarbonate (840 mg, 10 mmol) in water (10 ml); mp: 143-146°C.

$^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm) 8.2(s, 1H, NH), 3.57-3.21(m, 11H, N-cyclohexyl), 2.71 (s, 3H, thiazole- $\text{CH}_3$ ) and 2.63 (s, 3H, thiazole- $\text{CH}_3$ ). MS: m/z(%), 294 ( $\text{M}^+$ , 25), 256 (43), 197(100), 163(80). IR  $\nu_{\text{max}}$  3276, 1571, 1222; UV( $\text{CH}_3\text{OH}$ )  $\lambda_{\text{max}}$  251 nm,  $\log \epsilon = 3.5$ .

**3-(2,4-Dimethyl-5-thiazolyl)-4-phenyl-5-isopropylthio-4H-1,2,4-triazole (4a, R=CH<sub>3</sub>, R<sup>c</sup>=Ph, R<sup>e</sup>=(CH<sub>3</sub>)<sub>2</sub>CH-S)**

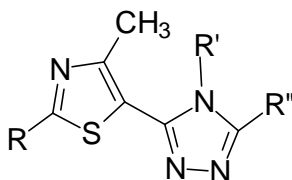
To a stirring solution of 5-(2,4-Dimethyl-5-thiazolyl)-4-phenyl-2,4-dihydro-3H-1, 2, 4-triazole-3-thione (**3**) (288 mg, 1 mmole) in sodium hydroxide (80 mg, 2 mmol) in water (10 ml) and ethanol (2 ml), was added 2-propyl bromide (123 mg, 1 mmol) dropwise. After the addition was completed, the mixture was stirred at room temperature for 24 h and it was then diluted with water (5 ml). The mixture was filtered and the precipitate was crystallized from ethyl acetate to give **4a** (68%); mp: 113-115°C.

$^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm) 7.98 (m, 2H, aromatic), 7.43 (m, 3H, aromatic), 2.65 (s, 3H, thiazole- $\text{CH}_3$ ) and 2.57 (s, 3H, thiazole- $\text{CH}_3$ ), 2.23 (m, 1H,  $\text{CH}(\text{CH}_3)_2$ ), 1.96 (d, 6H,  $\text{CH}(\text{CH}_3)_2$ ); MS: m/z(%), 294 ( $\text{M}^+$ , 25), 256 (43), 197(100), 163(80). IR  $\nu_{\text{max}}$  3276, 1571, 1222; UV( $\text{CH}_3\text{OH}$ )  $\lambda_{\text{max}}$  251 nm,  $\log \epsilon = 3.5$ .

**Pharmacology**

**Animals:** Male albino mice (supplied from Pasteur Institute of Iran) weighting 20-30 g were used for pharmacological study. Animals were allowed free access to food and water except during the experiment and housed at controlled room temperature with 12h light 12h dark cycle. Animals were placed individually in glass cylinder (25 cm width, 25 cm length) and allowed to habituate for 30 min before the drug administration according to previously reported methods (3). For induction of convulsions pentylenetetrazole (PTZ, Sigma, USA) (80

Table 1. Physical constants of compounds 3-5



Compound	R	R	R	MW	Mp (°C)
3a	CH <sub>3</sub>	CH <sub>3</sub>	SH	226	210-212
3b	CH <sub>3</sub>	Cyclohexyl	SH	295	140-143
3c	CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>	SH	529	278-280
3d	C <sub>6</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	SH	543	214-216
4a	CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>	<i>S-i</i> -C <sub>3</sub> H <sub>7</sub>	330	113-115
4b	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	SCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	441	172-175
4c	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	<i>S-n</i> -C <sub>3</sub> H <sub>7</sub>	392	98-100
5a	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	SO <sub>2</sub> - <i>n</i> -C <sub>3</sub> H <sub>7</sub>	438	278-280
5b	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	SO <sub>2</sub> C <sub>2</sub> H <sub>5</sub>	442	217-219

Table 2. Effects of different doses of tested compounds on PTZ-induced seizures in mice<sup>1</sup>.

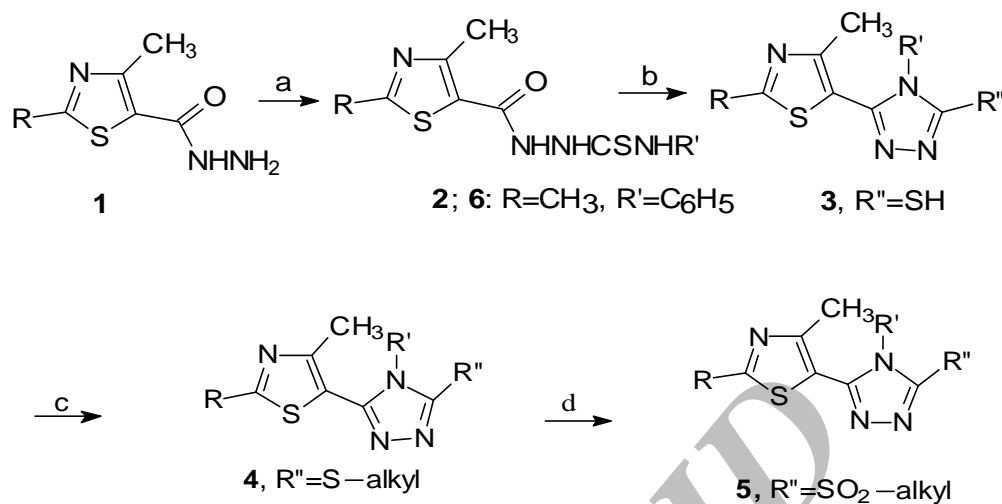
Treatment (mg/kg)	Convulsions (%)	Mortality (%)	Treatment (mg/kg)	Convulsions (%)	Mortality (%)
Control	90	40	4b (50)	60	10
Diazepam (0.5)	40	30	4b (100)	60	10
3a (50)**	20	0	4b (200)	60	0
3a (100)**	40	0	4c (50)	60	10
3a (200)**	30	20	4c (100)**	50	10
3b (25)**	20	0	4c (200)**	50	10
3b (50)**	30	10	5a (50)*	70	10
3b (100)**	0	0	5a (100)*	70	0
3c (50)	80	0	5a (200)*	70	10
3c (100)**	40	0	5a (25)**	50	0
3c (100)**	40	0	5b (50)	60	0
3c (200)	80	0	5b (100)**	50	0
3d (50)	80	0	5b (200)**	40	0
3d (100)	80	0	6 (25)**	50	0
3d (200)	60	0	6 (50)**	20	0
4a (50)**	20	0	6 (100)**	50	0
4a (100)**	50	0			

<sup>1</sup> Animals were administered intraperitoneally vehicle, diazepam, or a compound (3-6) 30 min. before PTZ (80 mg/kg, i.p.) injection. Frequency of seizures and mortality were recorded during 30 min. after PTZ injection. \*p<0.001, \*\*p<0.0001.

Table 3. Effect of flumazenil on anticonvulsant activity of compounds 3a, 3b, 4a and 6\*

Treatment (mg/kg)	Convulsions (%)	Convulsions (%) by compounds+Flumazenil
3a (50)	20	20
3b (100)	0	10
4a (50)	20	40
6 (50)	20	40

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**Scheme 1.** Synthetic steps for the production of the title compounds; a.  $R-N=C=S$ , NaOH; b.  $NaHCO_3$ , reflux; c. alkyl halide, NaOH, EtOH; d. MCP,  $CH_2Cl_2$ , RT.

mg/kg) was injected intraperitoneally (5). Immediately after PTZ injection, each animal was placed into the cylinder and its behavior was observed directly. PTZ was dissolved in 0.9% saline and the test compounds were suspended in CMC (1%) and tween 80 (5%). The test compounds were injected intraperitoneally to groups of 10 mice 30 min before PTZ injection. PTZ-induced convulsions and mortality were evaluated for 30 min. after drug injection based on seizure latency of tonic-clonic convulsions. Control groups received vehicle including water with CMC/tween 80. The anticonvulsant activity of compounds was also compared with diazepam as benzodiazepine agonist (Merck, Germany) (0.5 mg/kg, i.p.). For evaluation of benzodiazepine receptors involvement in the anticonvulsant activity of the test compounds, flumazenil (10 mg/kg, i.p.) as a benzodiazepine receptors antagonist was used 10 min. before injection of the test compounds.

**Statistical analysis:** The data was analyzed by Fisher's exact probability test. Differences with  $p < 0.001$  were considered statistically significant.

## RESULTS AND DISCUSSIONS

$N_4$ -substituted triazolylthiazole systems were tested for assessment of their anticonvulsant activity against PTZ-induced acute seizure. Several lines of evidence have suggested that in the mammalian brain, GABA receptor complex

is involved in the pharmacology of anti-convulsant drugs and in the pathophysiology of seizures and epilepsy. Drugs that facilitate GABA-ergic transmission have a potential anticonvulsant action (6) whereas administration of negative modulators of GABA receptors such as  $\beta$ -carboline derivatives (7) or the chloride-channel blocker pentylenetetrazole (8) induces convulsion. To investigate the possible involvement of a GABA-ergic system in the action of the test compounds, the effects of the test compounds on seizure induced by PTZ were studied. As shown in Table 2, administration of compounds reduced seizures and mortality induced by PTZ significantly.

To clarify involvement of benzodiazepine receptors in the anticonvulsant activity of the test compounds animals were pretreated with flumazenil (10 mg/kg, i.p.) as a benzodiazepine receptor antagonist 5 min. before injection of the test compounds.

Flumazenil did not alter the anticonvulsant activity of the test compounds significantly (Table 3). These results suggest that the anticonvulsant activity of these agents is not probably mediated by activation of benzodiazepine receptors. However many other mechanisms such as interaction with different endogenous substances may be involved in the action of these compounds, which require more experiments and different seizure models to be clarified.

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