

## Anticonvulsant Activities of 7-phenyl-5H-thiazolo[5,4-*e*][1,2,3,4]tetrazolo[5,1-*c*]pyrrolo[1,2-*a*][1,4]diazepine and 7-phenyl-5H-thiazolo[5,4-*e*][1,3,4]triazolo[5,1-*c*]pyrrolo[1,2-*a*][1,4]diazepines

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### Abstract

Anticonvulsant activity of 7-phenyl-5H-thiazolo[5,4-*e*][1,2,3,4]tetrazolo[5,1-*c*]pyrrolo[1,2-*a*][1,4]diazepine (**5**) and 7-phenyl-5H-thiazolo[5,4-*e*][1,3,4]triazolo[5,1-*c*]pyrrolo[1,2-*a*][1,4]diazepines (**6a-d**) was measured against pentylenetetrazole (PTZ)-induced seizures in mice. Intraperitoneal injections of different doses (12.5, 25 and 50 mg/kg, i.p.) of test compounds decreased PTZ-induced seizure significantly in a dose-dependent manner. The test compounds were administered 30 min before PTZ (80 mg/kg, i.p.) injection. The maximum response was obtained with 50 mg/kg of compound **6d** that showed more anticonvulsant activity compared to diazepam (0.5 mg/kg). The frequency of mortality was also decreased by all listed compounds. Pretreatment of animals with flumazenil (as a benzodiazepine, BDZ receptor antagonist) decreased, but not completely inhibited the anticonvulsant activity of compound **6d** (50 mg/kg). These results indicate that besides BZD receptors, other neurotransmitter systems may be involved in anticonvulsant activity of the tested compounds.

**Keywords:** benzodiazepine; anticonvulsant; thiazole.

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### Introduction

The epilepsies are common and frequently devastating disorders. Epileptic seizures often cause transient impairment of consciousness, leaving the individual at risk of physical harm and often interfering with education and employment. Therapy is symptomatic with the available antiseizure drugs. Compliance with medicine is a major problem because of the long-term therapy together with unwanted effects of many drugs. The mechanisms of action of

antiseizure drugs fall into two major categories. One is to limit the sustained, repetitive firing of a neuron. A second mechanism appears to involve enhanced gamma-aminobutyric acid (GABA)-mediated synaptic inhibition. Although many treatments are available, much more effort is being developed to novel approaches. The ideal antiseizure drug would suppress all seizures without causing any unwanted effects. Unfortunately, the drugs used currently not only fail to control seizure activity in some patients, but also they frequently cause unwanted effects that range in severity from minimal impairment of CNS to death from aplastic anemia or hepatic failure(1). A wide variety of agents have the

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capacity to depress the function of the central nervous system. One of them are benzodiazepins which have only a limited capacity to produce profound and fatal CNS depression. Because of this measure of safety, benzodiazepins and their newer analogs are largely replaced older agents for the treatment of seizure or anxiety. Benzodiazepine receptor (BZR) ligands do not just belong to the benzodiazepine series and a wide variety of various structural classes of agonists or inverse agonists has been described (2-7). Not all the molecular features responsible for receptor binding have been completely well established. Our previous works also showed that  $N_4$ -substituted triazolyl thiazoles and 1,4-dihydropyridine derivatives have anticonvulsant activity that are mediated by BZR receptors (8,9). In this context, we have recently undertaken the systematic biological evaluation of BZR ligands and related pharmacological studies of various new compounds namely 7-phenyl-5H-thiazolo[5,4-*e*](1,2,3,4)tetrazole [5,1-*c*]pyrrolo[1,2-*a*](1,4)diazepines (**5**) and 7-phenyl-5H-thiazolo[5,4-*e*](1,3,4)triazolo[5,1-*c*]pyrrolo[1,2-*a*](1,4)diazepines (**6a-d**) by an *in vitro* model for evaluation of benzodiazepine effect. For this purpose effect of compounds **5**, **6a-d** on pentylenetetrazole-induced convulsions and their possible mechanisms were investigated.

## Experimental

### Chemistry

The title compounds were synthesized according to the published method (10) starting from ethyl 4-bromomethyl-2-phenylthiazole-5-carboxylate (**1**) (11) (Scheme 1). Alkaline hydrolysis of compound **2** afforded 2-phenyl-4-(1-pyrrolylmethyl)thiazole-5-carboxylic acid which was treated successively with triethyl amine, ethyl chloroformate and sodium azide (12) to give carbonyl azide. Heating the carbonyl azide in a large excess of acetic acid gave 7-phenyl-5H-thiazolo[5,4-*e*]pyrrolo[1,2-*a*](1,4)diazepine-10 (9H) one (**3**). Reaction of 1 equivalent of compound **3** in the presence of 1.1 equivalent of Lawesson's reagent gave thiolactam. Reaction of thiolactam with hydrazine hydrate in ethanol under ultrasonic irradiation for 12 min. gave compound **4**. Compounds **5**, **6a-d** were

synthesized by reacting 10-hydrazino-7-phenyl-5H-thiazolo[5,4-*e*]pyrrolo[1,2-*a*](1,4)diazepine (**4**) with sodium nitrite in acetic acid and with different derivatives of triethyl orthoformate in ethyl acetate, respectively. The structures of all compounds were confirmed by  $^1\text{H}$ NMR, Mass spectral and Elemental analysis. The purity of all compounds were confirmed by TLC using different mobile phases. The physical constants of the final compounds are summarized in Table 1.

### Pharmacology

#### Animals

Male albino mice (supplied from Pasteur Institute of Iran) weighting 20-30 g were used for pharmacological study. Animals had free access to food and water, except during the experiment and housed at controlled room temperature with 12 h light 12 h dark cycle.

#### Anticonvulsant activity

Animals were placed individually in a glass cylinder (25 cm width, 25 cm length) and allowed to habituate for 30 min. before administration of the drug (13). For induction of convulsions, pentylenetetrazole (PTZ, Sigma USA) (80 mg/kg) was injected intraperitoneally. 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, mortality and seizure latency were evaluated for 30 min. after injecting the drug based on seizure occurrence of tonic-clonic convulsions. Control groups received vehicle having water with CMC/tween 80. The anticonvulsant activity of compounds was also compared with diazepam (Merck, Germany) (0.5 mg/kg, i.p.) as benzodiazepine receptor agonist. For evaluation of benzodiazepine receptors involvement in the anticonvulsant activity of the most effective compound (**6d**), flumazenil (10 mg/kg, i.p.) as a benzodiazepine receptor antagonist was used 10 min before injection of the tested compound (**6d** 50 mg/kg, i.p.).

### Statistical analysis

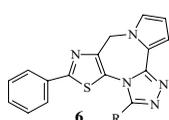
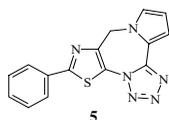
The statistical significance of differences was estimated by one-way analysis of variance (ANOVA), followed by Newman Keuls post hoc test. Differences with  $p < 0.05$  were considered statistically significant.

### Results and discussions

7-Phenyl-5H-thiazolo[5,4-*e*](1,2,3,4)tetrazolo[5,1-*c*]pyrrolo[1,2-*a*](1,4)diazepine (**5**) and 7-phenyl-5H-thiazolo[5,4-*e*](1,3,4)triazolo[5,1-*c*]pyrrolo[1,2-*a*](1,4)diazepines (**6a-d**) 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 anticonvulsant drugs and GABA-ergic transmission has a potential role in the pathophysiology of seizures (14). Administration of negative modulators of GABA receptors such as  $\beta$ -carboline derivatives or the chloride channel blocker pentylenetetrazole, is associated with seizure attacks (15,16). 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. Results were expressed as percent of convulsions and mortality (Table 2). As shown in table 2 all the tested compounds reduced seizures and mortality induced by PTZ significantly compared to control groups. In addition, comparing of compounds **6a-d** with different moieties at  $R_2$  position of triazole ring reveals that the presence of phenyl group at the  $R_2$  position which gives a more lipophile compound, increased the anticonvulsant

**Table 1.** Physical constants of compounds **5**, **6a-d**

Comp.	R	mp (°C)	MW
<b>5</b>	-	263-265	306
<b>6a</b>	H	215-217	305
<b>6b</b>	Me	272-274	319
<b>6c</b>	Et	238-239	339
<b>6d</b>	Ph	295-296	381

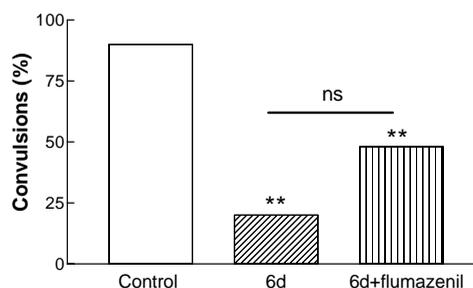


**Table 2.** Effects of different doses of tested compounds on PTZ-induced seizures in mice<sup>1</sup>

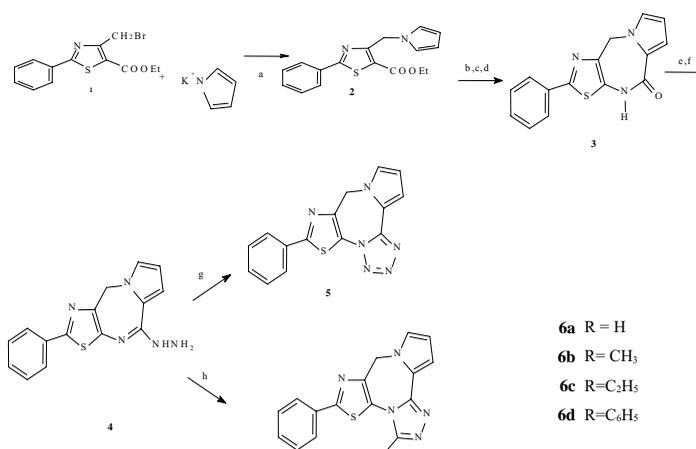
Treatment (mg/kg)	convulsions (%)	Mortality (%)
<b>5</b> (12.5)*	60	0
<b>5</b> (25)**	40	0
<b>5</b> (50)**	30	0
<b>6a</b> (12.5)*	60	0
<b>6a</b> (25)**	50	0
<b>6a</b> (50)**	40	0
<b>6b</b> (12.5)*	60	0
<b>6b</b> (25)**	50	0
<b>6b</b> (50)**	40	0
<b>6c</b> (12.5)**	50	0
<b>6c</b> (25)**	40	0
<b>6c</b> (50)**	40	0
<b>6d</b> (12.5)*	60	0
<b>6d</b> (25)**	50	0
<b>6d</b> (50)**	20	0
control	90	40
Diazepam (0.5)	40	30

<sup>1</sup>Animals received intraperitoneally each of the solution vehicle, diazepam, or compounds **5**, **6a-d**, 30 min. before PTZ (80 mg/kg, i.p.) injection. Frequency of seizures and mortality were recorded during 30 min. after PTZ injection. \*  $P < 0.001$ , \*\*  $P < 0.0001$

activity of compound **6d**. The seizure latency was also increased non-significantly by the tested compounds (data not shown). To clarify involvement of the benzodiazepine receptors in the anticonvulsant activity of the compound **6d**, animals were pretreated with flumazenil (10 mg/kg, i.p.) as a benzodiazepine receptor antagonist 10 min. before injection of the compound **6d** (50 mg/kg, i.p.). Flumazenil decreased the anticonvulsant activity of compound **6d** but this inhibitory effect was not complete (Fig 1).



**Figure 1.** Effect of flumazenil on anticonvulsant activity of compound **6d** (50mg/kg,i.p.). Pretreatment of animals with flumazenil (10 mg/kg,i.p.), 10min. before test compound did not inhibit anticonvulsant activity induced by compound **6d** significantly. Control group received PTZ (80mg/kg,i.p.). ▨▨▨▨ Showed the effect of compound **6d** on PTZ - induced convulsions. ▤▤▤▤ Showed the effect of flumazenil on anticonvulsant activity of the compound **6d**. No significant (ns) difference was observed between animals received **6d** and animals pretreated with flumazenil before test compound. Each group includes 10 animals



**Scheme 1.** a: i: 25°C, ii: 60°C, 4 hours; b: KOH, MeOH; c: i: N(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>, ClCOOEt, ii: NaN<sub>3</sub>; d: AcOH, reflux; e: Lawesson's reagent; f: NH<sub>2</sub>NH<sub>2</sub>; g: NaNO<sub>2</sub>, AcOH; h: RC(OEt)<sub>3</sub>, PTSA

These results indicate that beside GABA-ergic system, other neurotransmission mechanisms or endogenous substances are probably involved in anticonvulsant activity of the tested compounds. However further experiments with different seizure models are required to clarify the exact mechanisms of these agents.

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