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Original Article

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 befor 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 flumazenile (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.

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 rang 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 anticovulsant 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}HNMR, 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 bezodiazepine 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.).

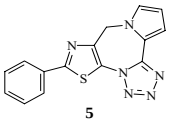
Statistical analysis

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)diazopines (**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 β-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 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).

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



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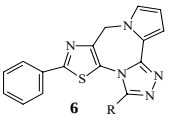

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Comp.	R	mp (°C)	MW
5	-	263-265	306
6a	H	215-217	305
6b	Me	272-274	319
6c	Et	238-239	339
6d	Ph	295-296	381

Table 2. Effects of different doses of tested compounds on PTZ- induced seizures in mice[1]

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

[1]Animals received intraperitoneally each of the soluation 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.* $P<0.001$, ** $P<0.0001$

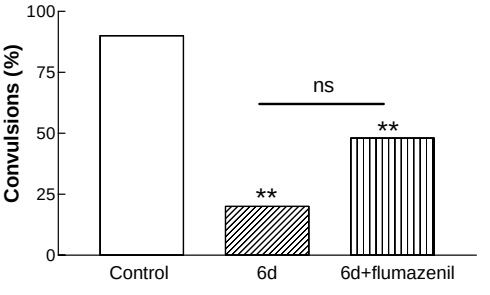
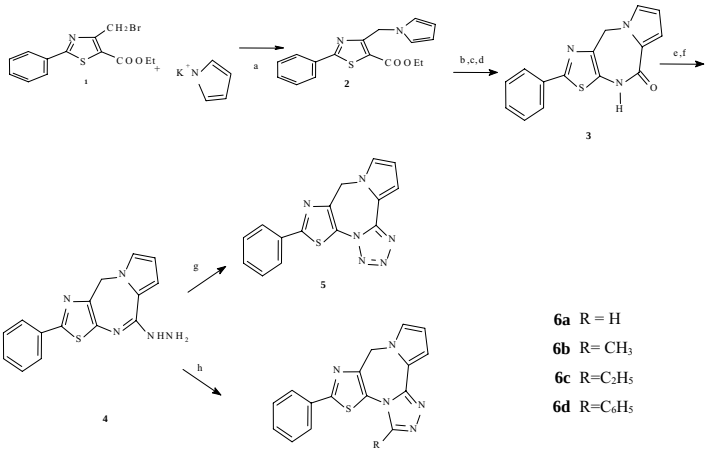


Figure1. Effect of flumazenil on anticonvusantactivity of compound 6d (50mg/kg,i.p.). Pretreatment of animals with flumazenil (10 mg/kg.i.p.). 10min. before test compound did not inhibit anticonvulsant activity induced by compound 6d significantly . Control group received PTZ (80mg/kg.i.p.). ▨ Showed the effect of compound 6d on PTZ - induced convulsions . ▥ Showed the effect of flumazenil on anticovulsant 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(C2H5)3, ClCOOEt, ii: NaN3: d: AcOH, reflux; e: Lawesson′s reagent; f: NH2NH2; g: NaNO2, AcOH; h: RC(OEt)3, 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.

References

(1) Anderson GD. A mechanistic approach to antiepileptic drug interactions. *Ann. Pharmacother.*(1998) 32: 554-563

(2) Cain M, Weber RW, Guzman F, Cook JM, Barker JA, Rice KC, Crawley JN, Paul SN and Skolnick P. Beta-Carbolines: Synthesis and neurochemical and Pharmacological actions on brain benzodiazepine receptors. *J. Med. Chem.* (1982) 25: 1081-1091

(3) Lippa AS, Meyerson LR and Beer B. Molecular substrates of anxiety. Clues from the heterogeneity of benzodiazepine receptors. *Life Sci.* (1982) 31: 1409-1417

(4) Villar HO, Daries MF, Loew GH and Maguire PA. Molecular Models for recognition and activation at the benzodiazepine. *Life Sci.* (1991) 48: 593-602

(5) Chose AK and Crippen CM. Modeling the benzodiazepine receptor binding site by the general three-dimensional structure-directed quantitative structure-activity relationship. *Mol. Pharmacol.* (1990) 37: 725-34

(6) Zhang W, Koehler KF, Harris B, Skolnick P and Cook JM. Synthesis of benzofused benzodiazepines employed as probes of antagonist pharmacophore of benzodiazepine receptors. *J. Med. Chem.* (1994) 37: 745-57

(7) Zang P, Zhang W, Liu R, Harris B and Skolnick P. Synthesis of novel imidazobenzodiazepines as probes of the pharmacophore for diazepam-insensitive "GABA A receptors". *J. Med. Chem.* (1995) 38: 1679-88

(8) Bineshmarvasti M, Sharifzadeh M, jalilian AR, Soltaninejad M and Shafiee A. Synthesis and anticonvulsant activity of N4- substituted triazoly thiazoles. *Daru* (2003) 11: 74-78

(9) Shafiee A, Rastkari N and Sharifzadeh M. Anticonvulsant activity of new 1, 4- dihydropyridine derivatives containing 4- nitroimidazolyl substitutes. *Daru* (2004) 12: 81-86

(10) Shafiee A and Shekarchi M. Synthesis of 7-phenyl-5H-thiazolo[5,4-*e*]pyrrolo[1,2-*a*][1,4]diazepine-10 (9H) one, 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. *J. Heterocyclic Chem.* (2002) 39: 213

(11) Shafiee A, Kiaey G and Vosoghi M. Selenium heterocycles XXXII (1). Synthesis of [1] benzoxepino [3,4-d]thiazole, [1] benzothiepino[3,4-d]thiazole, [1] benzoxepino[3,4-d]selenazole, and [1] Benzothiepino [3,4-d]selenazole [1] Benzothiepino [3,4-d]selenazole. Four novel heterocycles *J. Heterocyclic Chem.* (1981) 18: 789

(12) Weinstock J. A modified Curtius reaction. *J. Org. Chem.* (1961) 29: 3511

(13) Shafiee A, Jalilian AR and Tabatabai-yazdi M. Synthesis & biological activity of thiazolotriazoles as new anticonvulsant agents. *J. Chem & Chem. Eng.* (1998) 17: 14-20

(14) Concas A, Serra M, Sanna E, pepitoni S, Mascia MP and Biggio G. Involvement drugs. In: Avanzini G, Engel J, Fariello R and Heinemann U. (Eds.) *Neurotransmitters in Epilepsy Research* (1992) 77-85

(15) Braestrup T, Honore C, Nielsen M, Petersen EN and Jensen LH. Ligands for benzodiazepine receptor with positive and negative efficacy. *Biochem. Pharmacol.* (1984) 33: 859-862

(16) Squires RF, Saederup E, Crawley JN, Skolnick P and Paul SM. Convulsant Potencies of tetrazole are highly correlated with action on GABA/ Picrotoxin receptor complexes in brain. *Life Sci.* (1984) 35: 1439-1444

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