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Research Article

Synthesis and Anticonvulsant Activity of 5-Substituted 1*h*-Tetrazoles against Pentylenetetrazole-Induced Seizures

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ABSTRACT

Nine 5-Substituted 1*H*-tetrazole derivatives ***(T1-T9)*** in two categories (phenyl tetrazole and phenylmethyl tetrazole) were synthesized by cycloaddition of a nitrile compounds with sodium azide using nano-$SnCl_4$-SiO_2 as a mild catalyst in good yield.

The subcutaneous pentylenetetrazole (PTZ) seizure models were used for screening the anticonvulsant activity of the compounds. Male BALB/c mice received increasing doses of tetrazole derivatives (10, 20 and 30 mg/kg). After 30 minutes, the myoclonic and generalized seizures latencies induced by pentylenetetrazole (85 mg/kg) was measured and compared to diazepam as a positive control. Our results showed, compounds ***T1***, ***T3,*** and ***T7*** revealed significant anticonvulsant activity in the PTZ test.

KEYWORDS: Nano-$SnCl_4$-SiO_2, 5-Substituted 1*H*-tetrazoles, Anticonvulsant, PTZ

INTRODUCTION

Epilepsy, one of the most frequent neurological afflictions in men characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsion, inflicts more than 60 million people worldwide (1). About 95% of the drugs currently available to treat epilepsy were approved before 1985, and they provide satisfactory seizure control in most of the patients. However, these drugs are associated with remarkable adverse reactions, such as depression, anxiety, psychosis, and cognitive deficits which largely affect the life quality of the patients with epilepsy and their familie (2). Accordingly, the discovery of new anticonvulsant agents with superior efficacy and improved safety profile is of fundamental importance. In the two past decades many attempts have established to identify the structural features of compounds crucial for anticonvulsant activity (3). Tetrazoles and their derivatives are an important class of heterocycles in a wide range of applications (4-6), such as, organocatalysis and transition metal catalysis, anti microbial, anti-inflammatory, and antihypertensive activities (7). Generally, their based-on-drugs are classified depending on substituent pattern in the tetrazole nucleus. Tetrazole nucleus is a vital

heterocycle that is present in a variety of therapeutical agents, including those with anticonvulsant and antidepressant activities (8-11). The common protocols for synthesis of 5-substituted- 1*H*-tetrazoles has been reported to proceed *via* [3 + 2] cycloaddition of azide with nitriles In the presence of a suitable catalyst. In our previous studies (12-16), we synthesized some organic compounds in the presence of nano-$TiCl_4.SiO_2$ as an efficient and reusable acidic catalyst and antibacterial and antifungal activities of them were also determined (12,13,16). In this work, our investigation is based on the development of another eco-friendly and low-cost heterogeneous catalyst (nano-$SnCl_4.SiO_2$) for the synthesis of some 5-substituted 1*H*-tetrazoles. Here we are going to synthesize two different categories of tetrazole (phenyl and phenylmethyl tetrazole) using nano-$SnCl_4.SiO_2$ and evaluate their possible anticonvulsant properties. Short reaction time, high yield, the simplicity of operation and easy work-up are some advantages of these types of catalysts.

MATERIALS AND METHODS

All chemicals were purchased from Merck and used without any additional purification. The products were characterized by FT-IR (ATR), ^{1}H-NMR, and a comparison of their physical properties with those reported in the literature. FT-IR (ATR) spectra were acquired on a Bruker, Eqinox 55 spectrometer. A Bruker (DRX-400 Avance) NMR was used to record the ^{1}H NMR spectra. Spectrophotometer (UV/Vis biotek model UVIKONXL), Melting points were determined with a Thermo Scientific Electrothermal digital apparatus (Thermo Fisher Scientific Inc.). Pentylenetetrazole (PTZ) was obtained from Sigma (St. Louis, USA).

General Procedure for the Synthesis of Tetrazole Derivatives

Sodium azide (2 mmol) and Nano-$SnCl_4$-SiO_2 (0.05 g) in DMF (5 mL) were reacted with different substituted benzonitrile (1 mmol) for compounds ***T1, T2, T3, T9***, with isonicotine nitrile for compound ***T5*** and with different substituted phenyl acetonitrile for compounds ***T4, T6, T7, T8*** at 120 °C for 1 h (Table 1). After completion the reaction (as monitored by TLC) the mixture was allowed to cool to room temperature and the catalyst was removed by filtration. Then a white solid was obtained by adding ice-water and 4N HCl (5 mL) to the residue. Washing the crude product with cold chloroform gave us the final pure tetrazole compound with reasonable yield.

The chemical structure of all the products was confirmed by their physical and spectral data. The synthesis results and spectroscopic data of the final products have been shown in Table 1.

Animals

Adult male NMRI mice (25-30 g) were purchased from the Animal House of Shiraz University of Medical Sciences Shiraz, Iran. The animal house temperature was maintained at 22±2ºC with a 12 h light/dark cycle. All animals were kept for one week prior to experimentation and were given free access to food and water. Each animal was tested once. All animal experiments were carried out in agreement with recommendations of the Declaration of Helsinki and internationally established principles for the use of experimental animals (Grant number 94-01-103-10843).

Pentylenetetrazole Seizure Model

Behavioral tests were performed on groups consisting 10 mice. In order to study on anticonvulsant activity, three different concentrations of the synthesized compounds and diazepam as positive control were prepared freshly. Pentylenetetrazol (PTZ) was dissolved in normal saline while diazepam (DZP), and all compounds were dissolved in 40% dimethyl sulfoxide (DMSO). Control groups received normal saline or DMSO. All control and the synthesized compounds were administrated intraperitoneally (IP) in the volume of 10 mL/kg animal body weight. 2–3 min after the administration of 85 mg/kg of PTZ, the convulsive response was appeared and the animals were observed for 30 min. During this time, we recorded the latency to myoclonic jerks, latency to generalized seizure, and the duration of the first generalized seizure.

The majority of the mice died due to the seizure after 30 minutes (17).

Statistical Analysis

Data obtained from delay convulsion behavior were expressed as Mean ± SEM and were analyzed by One-way ANOVA along with Dennett's post-test. $p<0.05$ was considered significant.

RESULTS

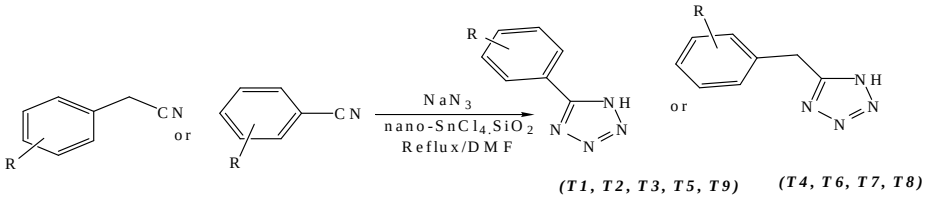
Under the optimized reaction conditions, we chose a variety of structurally divergent nitriles to understand the scope and generality of the nano-$SnCl_4.SiO_2$ promoted [2+3] cycloaddition reaction to form 5-substituted 1*H*-tetrazoles. PTZ administration resulted in seizure occurrence in all PTZ-injected animals. Diazepam (1 and 2 mg/kg) protected all the animals (100%) from PTZ-induced seizure. Our results showed even pretreatment with the compounds ***T1, T2, T4, T9*** at doses of 10, 20 and 30 mg/kg did not prevent the development of myoclonic seizure, but compound ***T3*** at dose of 30 mg/kg significantly protected the mice from myoclonic seizure ($P<0.05$) (Figure 1).

Also as shown in Figure 2, pretreatment with the compounds ***T1*** (30mg/kg), ***T3*** (30mg/kg), ***T7*** (20mg/kg) and ***T9*** (20mg/kg), significantly increased the latency time to the onset of a clonic seizure in comparison to the control group.

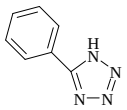
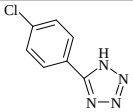
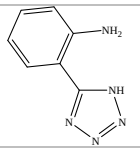
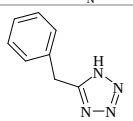
Figure 3, in addition,, shows Compounds ***T3*** (30mg/kg) and ***T7*** (30mg/kg) significantly protected the animals from tonic seizure.

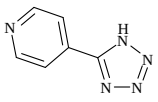
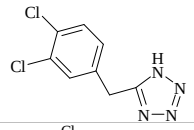
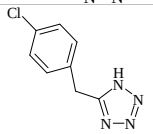
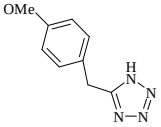
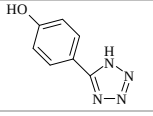
Statistical analysis revealed that pretreatment with the dose of 30mg/kg of ***T1***, ***T3*** and ***T7*** compounds increased the latency for the first myoclonic and generalized tonic–clonic seizure compared to vehicle-treated mice (Figure 4). No mortality was observed in animals treated with the tetrazole derivatives at all doses before PTZ administration, but only during several minutes after ***T3*** and ***T8*** administration at dose 30, mg/kg mortality was observed (3 animals of 10, data not shown).

Table 1 Synthesis results and spectral data of 5-Substituted 1*H*-tetrazole compounds in the presence of nano-$SnCl_4.SiO_2$[a]



(T1, T2, T3, T5, T9) *(T4, T6, T7, T8)*

Compound	Spectral data	Chemical structure	Yield (%)[b]
T1	FT-IR: □ (KBr) = 2600-300, 1608, 1563, 1485, 1465, 1409, 1480, 726, 687 cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): 8.01 (brs, 2H), 7.51 (brs, 3H) ppm.		98
T2	FT-IR: □ (KBr) = 2500-3000, 1654, 1610, 1561, 1487, 1434, 831 cm^{-1}, ^1H NMR (500 MHz, DMSO-*d*6): 8.10 (d, *J*= 10.53, 2H), 7.69 (d, J= 8.41, 2H) ppm.		92
T3	FT-IR: □ (KBr)=2910-3000, 1662, 1620, 1558, 1492, 1448, 823, ^{1}HNMR (300 MHz, DMSO-*d*6) =3.91 (S, NH_2), 6.57-6.62 (m, 1H), 6.79 (d, *J*= 8.4, 1H), 7.27-7.32 (m, 1H), 7.35 (dd, *J*= 7.8, *J*= 1.4, 1H) ppm.		85
T4	FT-IR: □ (KBr) = 2400-300, 1592, 1549, 1496, 1248, 775 cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): 4.26 (s, 2H, CH_2), 7.25-1.31 (m, 5H) ppm.		88

T5	FT-IR: (KBr) =2500-3000, 1631, 1529, 1440, 1338, 1292, 1042, 990, 846, 751 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$): 8.00 (d, *J*= 7.89, 2H), 7.40 (d, *J*= 7.86, 2H) ppm, ^{13}C-NMR (125 MHz, DMSO-d_6) δ = 127.9, 130.7 ppm.		98
T6	FT-IR: (KBr) = 2500-300, 1560, 1472, 1440, 1260, 1210, 827, 766, 706, 674 cm^{-1}, ^1H NMR (500 MHz, CDCl3): 7.61 (d, J= 8.45, 2H, 2H), 7.28 (d, J= 8.2, 1H), 4.32 (s, 2H) ppm, ^{13}C-NMR (125 MHz, DMSO) δ = 130.2, 131.6, 131.8, 174.8 ppm.		96
T7	FT-IR: (KBr) = 2600-300, 1538, 1492, 1407, 1263, 1207, 834 cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): 7.40 (m, 2H), 7.31 (d, J= 8, 2H), 4.30 (s, 2H) ppm.		95
T8	FT-IR: (KBr) = 2600-3400, 1636, 1514, 1124, 848 cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): 7.19 (brs, 2H), 6.86 (brs, 2H), 4.18 (s, 2H), 3.69 (s, 3H) ppm.		86
T9	FT-IR: (KBr) = 2500-3400, 1648, 1600, 1515, 1470, 1435, 1080, 842 cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): 16.5 (brs, NH), 10.17 (s, 1H), 7.84 (d, *J*= 8, 2H), 6.93 (d, *J*= 7.6, 2H) ppm.		83

[a]Reaction conditions: nitrile (1 mmol), NaN_3 (2 mmol), (0.05 g), nano-$SnCl_4.SiO_2$,DMF (5 mL) at reflux

[b]Isolated yields

DISCUSSION

Our investigation is based on the development of a heterogeneous catalyst for reducing risks to human and the environment. In order to prepare 5-substituted 1*H*-tetrazole in our previous work (12), We have demonstrated another nanocatalyst for the synthesis of preparation of -substituted1*H*-tetrazole derivatives.

Nano-$SnCl_4.SiO_2$ as an efficient catalyst that has been developed in this project. Then, the cycloaddition reaction a wide range of structurally various benzonitriles was chosen and the results are presented in Table 1. Also table 1 shows the substitution effect on the final yield of each product.

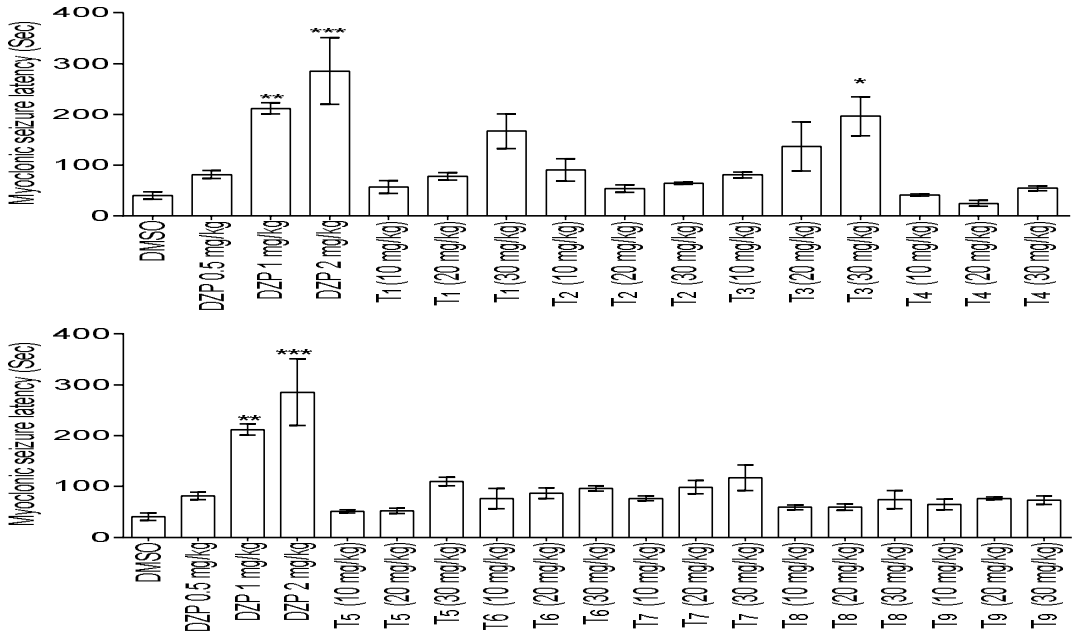


Figure 1. Effect of intraperitoneal injection of different doses of diazepam (0.5, 1 and 2mg/kg) and synthesized compounds T1-T9 (10, 20 and 30 mg/kg) on myoclonic seizure onset time (sec) induced by pentylenetetrazole 80 mg/kg. (n = 10) Data are mean±standard error of the mean of the latency time. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

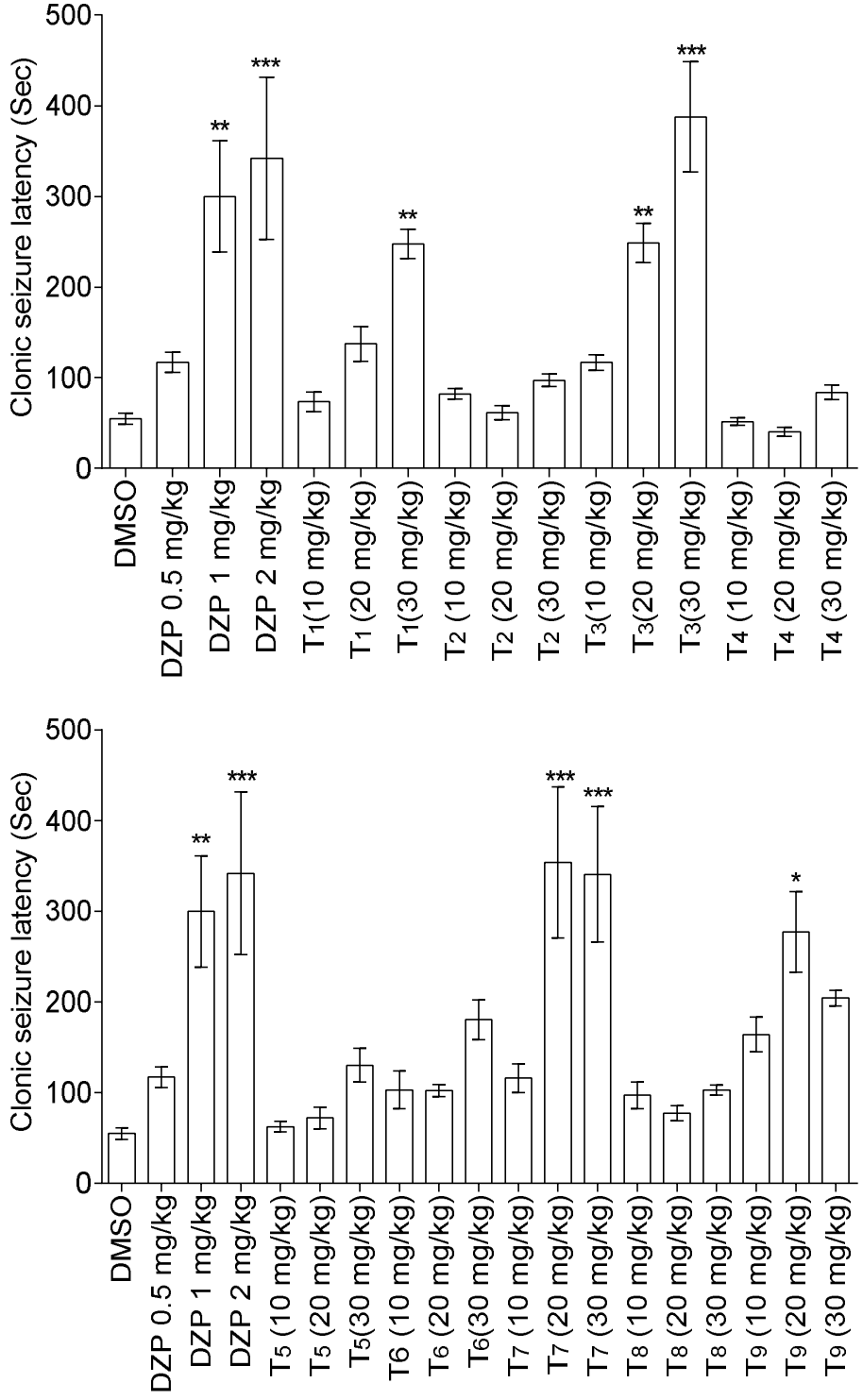


Figure 2. Effect of intraperitoneal injection of different doses of diazepam (0.5, 1 and 2mg/kg) and synthesized compounds T1-T9 (10, 20 and 30 mg/kg) on clonic seizure latency (sec) induced by pentylenetetrazole 80 mg/kg. (n = 10) Data are mean±standard error of the mean of the latency time. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

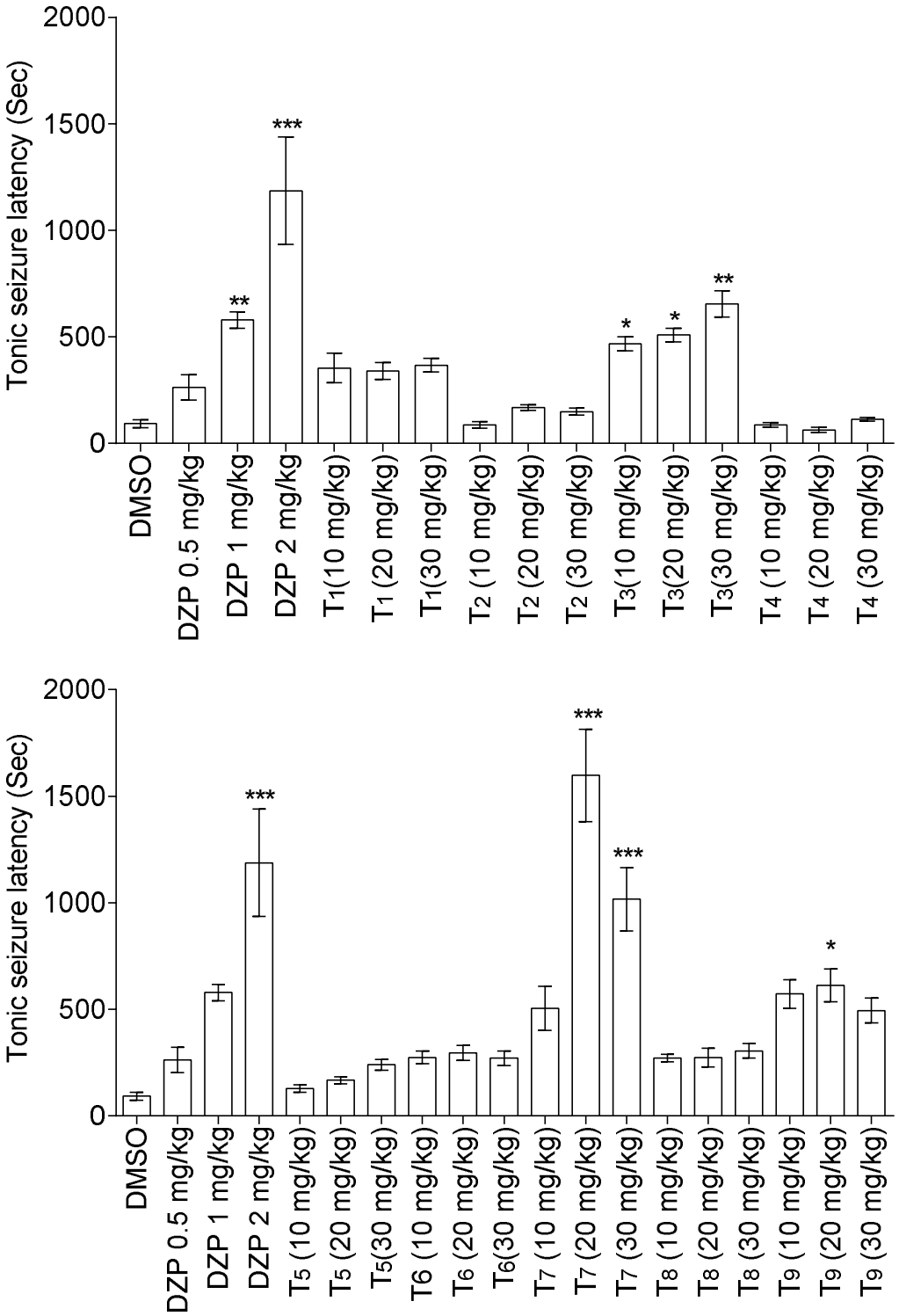


Figure 3. Effect of intraperitoneal injection of different doses of diazepam (0.5, 1 and 2mg/kg) and synthesized compounds T1-T9 (10, 20 and 30 mg/kg) on tonic seizure latency (sec) induced by pentylenetetrazole 80 mg/kg. (n = 10) Data are mean±standard error of the mean of the latency time. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

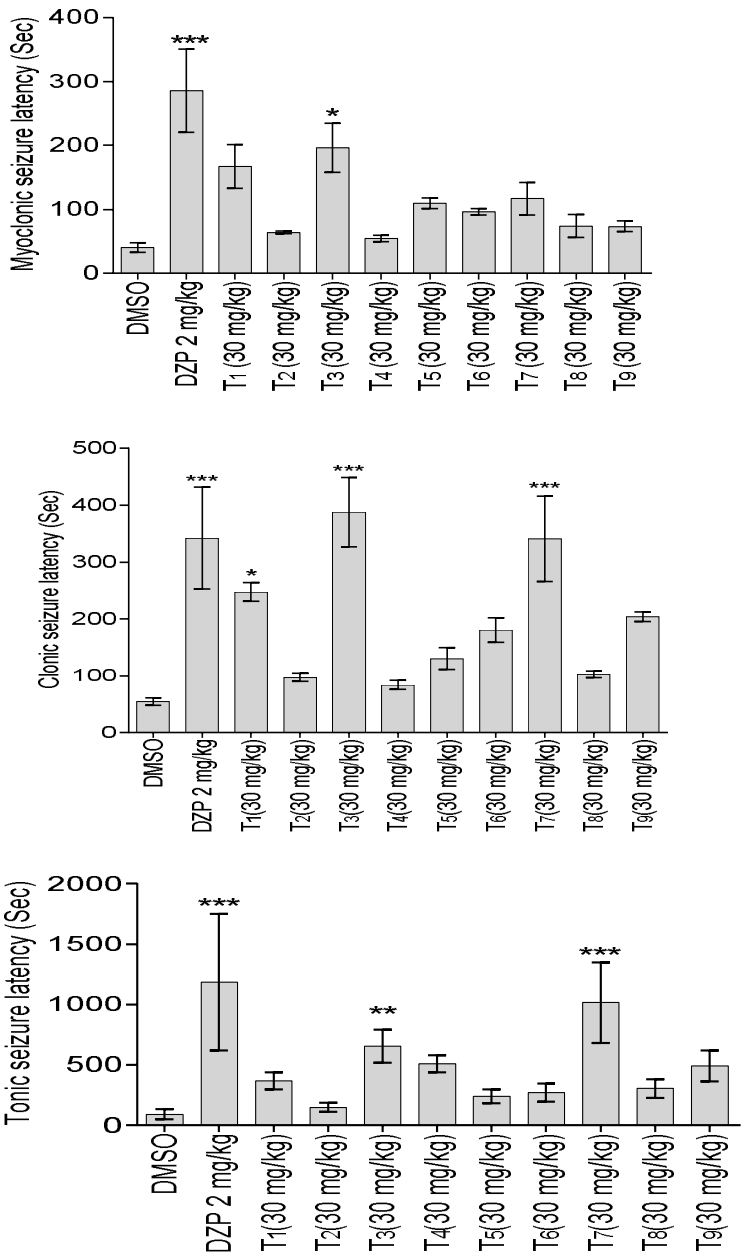
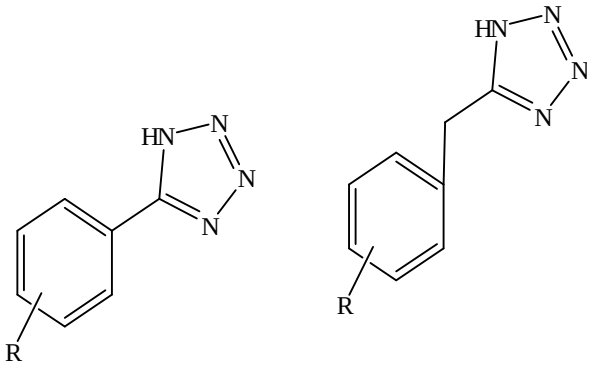


Figure 4 The comparison between diazepam and synthesized compounds T1-T9 (30mg/kg) on the onset of pentylenetetrazole-induced convulsion in mice. (n = 10) Data are mean±standard error of the mean of the latency time.* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

The preclinical development of new compounds for the treatment of epilepsy is based on the use of predictable animal seizure models. The PTZ model is the most frequently used in the early stages of testing. The PTZ test uses chemically induced clonic seizures and is proposed to identify the agents raising the seizure threshold. This test is related to human generalized absence seizures (18). Tetrazole and its derivatives are present in many of the bioactive heterocyclic compounds that are of wide interest because of their diverse biological, pharmaceutical and clinical applications.

In this study, we have synthesized two series of tetrazole derivatives (series ***I*** and series ***II***). In series ***I (T1***, ***T2***, ***T3***, ***T9)*** a phenyl ring is attached directly to the C5 of the tetrazole ring and in series ***II (T4***, ***T6***, ***T7***, ***T8)*** a phenyl ring is attached to the C5 of the tetrazole ring *via* a CH_2 group and also a pyridine tetrazole analogue ***(T5)*** (Figure 5).



Series ***I*** Series ***II***

Figure 5. Two series of tetrazole derivatives (series ***I*** and series ***II***).

The results which presented in Fig. 1-4 reveal that, out of the tested compounds, three compounds namely; ***T1***, ***T3,*** and ***T7*** were able to display noticeable anticonvulsant activity in the PTZ test. It could be obviously recognized that the prominently active compounds ***T1***, ***T3*** and ***T7*** exerted their anticonvulsant activity at 30 mg/kg dose level, however, no activity was observed at dose of 10 mg/kg. Among these compounds, ***T3*** was proved to be the most active members in this class. Furthermore, at a dose level of 30 mg/kg, compound ***T7*** showed the same activity in the PTZ test with more protection against mortality compared to compound ***T3***.

The rest of the tested compounds namely; ***T1, T2***, ***T4, T5, T6*** and ***T8*** lacked significant anticonvulsant activity in PTZ test at the same dose levels.

In comparison of the anticonvulsant activities of the synthetic compounds with diazepam compounds ***T3*** which contains NH_2 residue in *ortho*-position of the phenyl ring exhibited better activity. Maybe NH_2 substitution plays as an electron donor group and increase the hydrophilic properties of the compound.

In comparison of the anticonvulsant activities of ***T9*** and ***T2***, we found OH substitution at the *para*-position of the phenyl ring is more useful for anticonvulsant activity rather than Cl residue in the same position.

Furthermore in series ***II***, the results showed substitution of Cl group at the *para*-position of the phenyl ring increased pharmacological activity.

Comparison between ***T1*** from series ***I*** and ***T4*** from series ***II***, indicated that insertion of a CH_2 group between phenyl and tetrazole rings decreased the pharmacological activity.

Our results also showed substitution of phenyl ring with pyridine at 5 position of tetrazole ring decreases anticonvulsant activity.

CONCLUSION

Firstly we have demonstrated a simple method for the synthesis of tetrazole derivatives by nano-$SnCl_4.SiO_2$ as efficient catalyst under solvent-free condition. In the current study, nine compounds were selected to be screened for their preliminary anticonvulsant activity against subcutaneous PTZ induced seizures in mice. Secondly our results revealed that compounds ***T1, T3,*** and ***T7*** were proved to be the most active anticonvulsant members in this study with special high activity. These compounds displayed noticeable anticonvulsant activity at 30 mg/kg dose level.

Therefore they represent new scaffolds that could be further optimized for future development of more effective anticonvulsant agents.

ACKNOWLEDGEMENTS

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