Arch. Pharm. Res.
DOI 10.1007/s12272-016-0881-y

RESEARCH ARTICLE

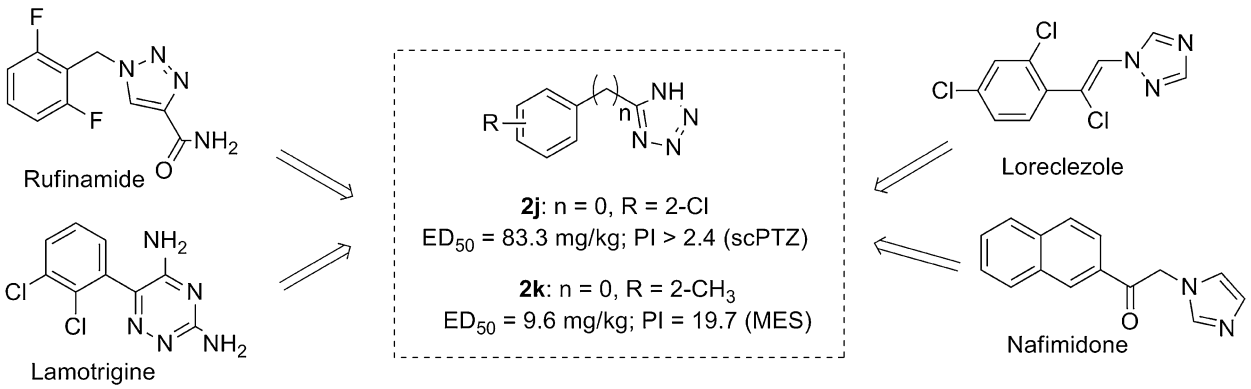
Design, synthesis and evaluation of 5-substituted 1-*H*-tetrazoles as potent anticonvulsant agents

Ai-Mei Liao[1,4] · Tiantian Wang[2,3] · Bangrong Cai[1] · Yi Jin[2,3] · Seunghoon Cheon[1] · ChangJu Chun[1] · Zengtao Wang[2]

Received: 4 July 2016 / Accepted: 21 December 2016

Abstract A series of 5-substituted 1-*H*-tetrazoles were designed and synthesized as potent anticonvulsant agents. Their preliminary anticonvulsant activities were evaluated using maximal electroshock and subcutaneous pentylenetetrazole (scPTZ) seizure tests. Neurotoxicity was determined using rotarod test. The results indicated that the compound **2j** in scPTZ model exhibited the ED_{50} values of 83.3 mg/kg, superior to the standard drug ethosuximide with the maximum activity. In addition, compound **2k** showed the most potent activity in MES model with ED_{50} value of 9.6 mg/kg and TD_{50} value of 189.5 mg/kg after intraperitoneal injection in mice, and displayed a high protective index (TD_{50}/ED_{50}) of 19.7 compared to reference antiepileptic drugs.

Graphical Abstract



Keywords Synthesis · 5-Substituted 1-*H*-tetrazoles · Anticonvulsant

✉ ChangJu Chun
cchun1130@jnu.ac.kr

✉ Zengtao Wang
zengtaowang@126.com

[1] Research Institute of Drug Development, College of Pharmacy, Chonnam National University, 77 Yongbong-ro, Buk-Gu, Gwangju 61186, Republic of Korea

[2] Department of Medicinal Chemistry, College of Pharmacy, JiangXi University of Traditional Chinese Medicine, Nanchang 330004, China

[3] The National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine, Nanchang 330006, China

[4] School of life Science, Hefei Normal University, Hefei 230601, China

Published online: 31 December 2016

Introduction

Epilepsy defined as a brain disorder is characterized by a long-term predisposition to epileptic seizures and has neurobiological, cognitive, psychological, and social consequences (Eadie 1984). As a major neurological disorder, epilepsy is an emerging medical and socioeconomic threat that affects more than 50 million people, approximately 1 and 2% of the world's population. The number is steadily increasing as the modern lifestyle is faster and more stressful (Bialer and White 2010). Currently available antiepileptic drugs (AEDs), the only medical choice of epilepsy, effectively reduce the severity and number of seizures in only 60% of patients. Around 40% of patients suffer from types of seizure which can't be adequately controlled by AEDs even are resistant to them, leading to lower quality of life and higher death ratio (Kwan and Brodie 2000; French 2007; Hirsch and Arzimanoglou 2004). Moreover, the recent antiepileptic therapy is often limited by severe adverse effects and occupying with other dose related toxicities (Greenwood 2000). Consequently, safe and effective drugs to protect and treat epilepsy are in great urgent demand.

Studies have greatly expanded the discovery of antiepileptic drugs from screening processes to development of drugs. However, design of new compounds with medical activity is a major challenge in pharmaceutical industry. Furthermore, due to the incomplete and uncertain pathogenesis of human epilepsy, also the complex and variable acting mechanism of AEDs, it is more difficult to develop new antiepileptic drugs using rational methodologies (Barkmeier and Loeb 2009). Molecular modification is considered to be an effective source for new biological active agents (Kraus 1983). Thus, an important and most useful strategy for developing new anticonvulsants is the ligand-based approach that establishes and utilizes various pharmacophores by analyzing structural characteristics of existing clinically effective AEDs and typical anticonvulsant compounds. Accordingly, this method has been applied mainly for introducing structural modifications into existing AEDs to obtain more efficacious drugs that can suppress seizures and/or drugs with minimal or no adverse effects compared to the parental compounds.

In previous decades, many approaches have been employed to identify the structural features that are essential for anticonvulsant activity in various compounds. As a result, it was well established that the crucial core fragment of anticonvulsant agents contains an aromatic functional group joined to the heterocyclic ring system, which is considered as 'drug-like'. This common template is present in the structures of AEDs such as rufinamide, lamotrigine, loreclezole, and nafimidone (Fig. 1).

Based on these observations, in order to develop new and therapeutic anticonvulsants, we designed and synthesized a series of 5-substituted 1-*H*-tetrazoles based on the structural characteristics analysis of current AEDs, and evaluated their anticonvulsant activities in comparison to those of phenytoin, phenobarbital, and ethosuximide as reference drugs.

Materials and methods

Chemistry

Melting points were determined in open capillary tubes and were uncorrected. Infrared spectra (vs = very strong, s = strong, m = medium, w = weak) were measured on a PerkinElmer Fourier transform infrared spectroscopy instrument (Waltham, MA, USA) over a wavelength range of 4000–450 cm^{-1}, where ν = stretching vibrations and δ = bending vibrations. ^{1}H NMR spectra were measured on a Bruker Avance 600 MHz NMR spectrometer

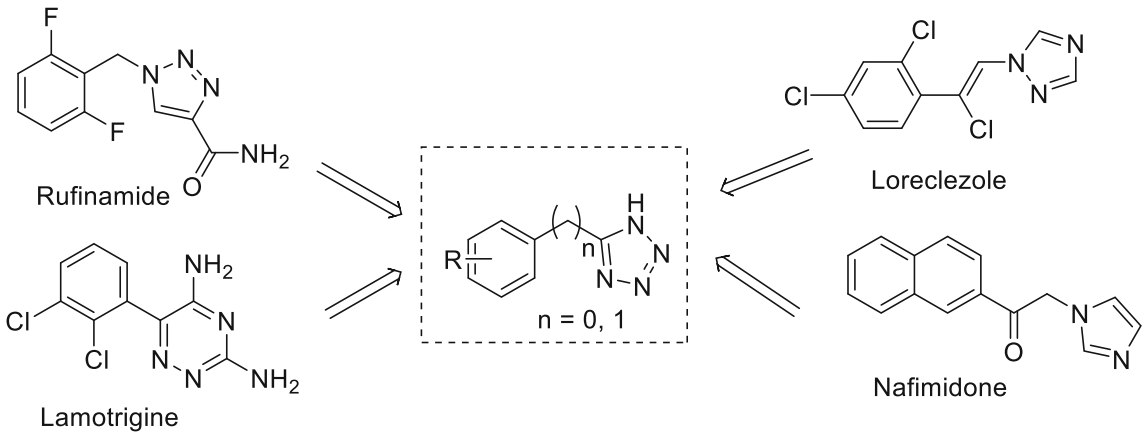


Fig. 1 Reported and designed nitrogen hetero atomic system as anticonvulsants

(Billerica, MA, USA), with all chemical shifts given in ppm relative to tetramethylsilane. Chemical shift values are in hertz. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets for ^{1}H-NMR data. Thin layer chromatography (TLC) was performed using Merck silica gel GF254 aluminum plates (Darmstadt, Germany). Reagents were purchased from Xiya Reagent Co., Ltd. (Chengdou, China).

General procedure for preparation of 5-substituted 1-*H*-tetrazoles (2a–2m)

Nitriles (1 mmol) were added to a solution of NaN_3 (3 mmol), PhMe (50 mL), and $Et_3N{\cdot}HCl$ (3 mmol) in a round-bottomed flask. The reaction mixture was stirred at 110 °C. After completion of the reaction (as indicated by TLC), the product was cooled and extracted with water. Next, 36% HCl was added dropwise to the aqueous layer to precipitate the tetrazoles. After filtration, the solid was dried under reduced pressure and recrystallized from EtOAc/Et_2O to yield the 5-substituted-1-*H*-tetrazoles.

5-Benzyl-1-*H*-tetrazole (2a)

White solid (yield 56%); mp: 123–125 °C (123–124 °C (Cantillo et al. 2011)); FT-IR: 3200–2400 (w, ν(N–H)), 3103 (w, ν(C–H)), 2951 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1603 (s, ν(C=C)), 1531 (s, δ(N–H)), 1493 (s, ν(C=C)), 1458 (s, δ(CH_2)), 733 (s, δ(C–H, Ph)), 695 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.33–7.36 (m, 2H, ArH), 7.26–7.29 (m, 2H, ArH), 4.29 (s, 2H, CH_2). ESI-HRMS calcd for $C_8H_8N_4$ ($[M+H]^+$): 161.0749; found: 161.0823.

5-(4-Methoxybenzyl)-1-*H*-tetrazole (2b)

White solid (yield 43%); mp: 158–161 °C (156–158 °C (Rama et al. 2011)); FT-IR: 3200–2300 (w, ν(N–H)), 3015 (w, ν(C–H)), 2970 (w, ν(CH_3)), 2954 (s, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1612 (s, ν(C=C)), 1555 (s, δ(N–H)), 1513 (s, ν(C=C)), 1463 (s, δ(CH_2)), 1246 (s, ν(C–O, Ph)), 1026 (s, ν(C–O, OCH_3)), 835 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.19 (d, 1H, ArH, J = 8.4 Hz), 6.89 (d, 1H, ArH, J = 8.4 Hz), 4.20 (s, 2H, CH_2), 3.72 (s, 3H, CH_3). ESI-HRMS calcd for $C_9H_{10}N_4O$ ($[M+H]^+$): 191.0855; found: 191.0929.

5-(4-Methylbenzyl)-1-*H*-tetrazole (2c)

White solid (yield 46%); mp: 158–160 °C (161 °C (Wehman and Popov 1966)); FT-IR: 3100–2300 (w, ν(N–H)), 3004 (w, ν(C–H)), 2917 (w, ν(CH_3)), 2860 (w, ν(CH_3)), 1900–1600 (w, overtones δ(C–H, Ph)), 1575 (s, δ(N–H)), 1514 (s, ν(C=C)), 813 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.13–7.16 (m, 4H, ArH), 4.23 (s, 2H, CH_2), 2.27 (s, 3H, CH_3). ESI-HRMS calcd for $C_9H_{10}N_4$ ($[M+H]^+$): 175.0905; found: 175.0980.

5-(4-(Trifluoromethyl)benzyl)-1-*H*-tetrazole (2d)

White solid (yield 68.9%); mp: 174–176 °C; FT-IR: 3100–2300 (w, ν(N–H)), 3008 (w, ν(C–H)), 2956 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1623 (s, ν(C=C)), 1576 (s, δ(N–H)), 1325 (vs, ν(C–F)), 844 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.72 (d, 1H, ArH, J = 8.4 Hz), 7.52 (d, 1H, ArH, J = 8.4 Hz), 4.42 (s, 2H, CH_2). ESI-HRMS calcd for $C_9H_7F_3N_4$ ($[M+H]^+$): 229.0623; found: 229.0697.

5-(4-Fluorobenzyl)-1-*H*-tetrazole (2e)

White solid (yield 53%); mp: 147–150 °C; FT-IR: 3100–2300 (w, ν(N–H)), 3007 (w, ν(C–H)), 2971 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1601 (s, ν(C=C)), 1580 (s, δ(N–H)), 1508 (s, ν(C=C)), 1224 (vs, ν(C–F)), 833 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.31–7.33 (m, 2H, ArH), 7.16–7.19 (m, 2H, ArH), 4.29 (s, 2H, CH_2). ESI-HRMS calcd for $C_8H_7FN_4$ ($[M+H]^+$): 179.0655; found: 179.0731.

5-(3-Nitrobenzyl)-1-*H*-tetrazole (2f)

White solid (yield 61%); mp: 159–161 °C; FT-IR: 3100–2400 (w, ν(N–H)), 3007 (w, ν(C–H)), 2955 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1573 (s, δ(N–H)), 1520 (s, ν(C=C)), 1351 (s, ν(NO_2)), 775 (s, δ(C–H, Ph)), 727 (s, δ(C–NO_2)), 697 (s, δ(C–H, Ph)); ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.23 (s, 1H, ArH), 8.16 (d, 1H, ArH, J = 7.8 Hz), 7.77 (d, 1H, ArH, J = 7.8 Hz), 7.66 (t, 1H, ArH, J = 7.8 Hz), 4.49 (s, 2H, CH_2). ESI-HRMS calcd for $C_8H_7N_5O_2$ ($[M+H]^+$): 206.0600; found: 206.0668.

5-(3-Methylbenzyl)-1-*H*-tetrazole (2g)

White solid (yield 63%); mp: 130–132 °C; FT-IR: 3120 (w, ν(C–H)), 3100–2300 (w, ν(N–H)), 2989 (w, ν(CH_3)), 2921 (s, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1610 (s, ν(C=C)), 157 7(s, δ(N–H)), 1493 (s, ν(C=C)), 758(s, δ(C–H, Ph)), 688 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.22 (t, 1H, ArH, J = 7.8 Hz), 7.08–7.09 (m, 2H, ArH), 7.05–7.06 (m, 1H, ArH), 4.24 (s, 2H, CH_2), 2.28 (s, 3H, CH_3). ESI-HRMS calcd for $C_9H_{10}N_4$ ($[M+H]^+$): 175.0905; found: 175.0976.

5-(2-Chlorobenzyl)-1-*H*-tetrazole (2h)

White solid (yield 62%); mp: 124–126 °C; FT-IR: 3000–2400 (w, ν(N–H)), 2993 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1581 (s, δ(N–H)), 1492 (s, ν(C=C)), 1474 (s, ν(C=C)), 755 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 7.47–7.50 (m, 1H, ArH), 7.39–7.42 (m, 1H, ArH), 7.34–7.38 (m, 2H, ArH), 4.39 (s, 2H, CH_2). ESI-HRMS calcd for $C_8H_7ClN_4$ ($[M+H]^+$): 195.0359; found: 195.0433.

5-(2-Methylbenzyl)-1-*H*-tetrazole (2i)

White solid (yield 55%); mp: 156–158 °C; FT-IR: 3100–2100 (w, ν(N–H)), 2962 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1607 (s, ν(C=C)), 1562 (s, δ(N–H)), 1485 (s, ν(C=C)), 1463 (s, ν(C=C)), 744 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.13–7.21 (m, 4H, ArH), 4.26 (s, 2H, CH_2), 2.27 (s, 3H, CH_3). ESI-HRMS calcd for $C_9H_{10}N_4$ ($[M+H]^+$): 175.0905; found: 175.0976.

5-(2-Chlorophenyl)-1-*H*-tetrazole (2j)

White solid (yield 58%); mp: 178–180 °C (176–177 °C (Obushak et al. 2008); FT-IR: 3200–2300 (w, ν(NH)), 3060 (w, ν(C–H)), 2971 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1601 (s, ν(C=C)), 1562 (s, δ(N–H)), 1469 (s, ν(C=C)), 776 (s, ν(C–Cl)), 745 (s, δ(CH, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.47–7.50 (m, 1H, ArH), 7.39–7.42 (m, 1H, ArH), 7.34–7.38 (m, 2H, ArH), 4.39 (s, 2H, CH_2). ESI-HRMS calcd for $C_7H_5ClN_4$ ($[M+H]^+$): 181.0203; found: 181.0277.

5-(*o*-Tolyl)-1-*H*-tetrazole (2k)

White solid (yield 52%); mp: 153–155 °C (155–156 °C (Butler and Garvin 1981)). FT-IR: 3200–2300 (w, ν(N–H)), 3029 (w, ν(C–H)), 2970 (w, ν(CH_3)), 1900–1600 (w, overtones δ(C–H, Ph)), 1607 (s, ν(C=C)), 1562 (s, δ(N–H)), 1485 (s, ν(C=C)), 1463 (s, δ(CH_3)), 744 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.69 (d, 1H, ArH, J = 7.8 Hz), 7.49 (td, 1H, ArH, J = 1.2, 7.2 Hz), 7.44 (d, 1H, ArH, J = 7.8 Hz), 7.40 (dt, 1H, ArH, J = 1.2, 7.2 Hz), 2.48 (s, 3H, CH_3). ESI-HRMS calcd for $C_8H_8N_4$ ($[M+H]^+$): 161.0749; found: 161.0825.

5-(Naphthalen-2-ylmethyl)-1-*H*-tetrazole (2l)

White solid (yield 66%); mp: 159–161 °C; FT-IR: 3200–2500 (w, ν(N–H)), 3130 (w, ν(C–H)), 3051 (w, ν(C–H)), 2953 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1599 (s, ν(C=C)), 1539 (s, δ(N–H)), 1505 (s, ν(C=C)), 863 (s, δ(C–H, Ph)), 813 (s, δ(C–H, Ph)), 762 (s, δ(C-H, Ph)), cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.88–7.91 (m, 3H, ArH), 7.80 (s, 1H, ArH), 7.49–7.54 (m, 2H, ArH), 7.42 (dd, 1H, ArH, J = 1.8, 8.4 Hz), 4.47 (s, 2H, CH_2). ESI-HRMS calcd for $C_{12}H_{10}N_4$ ($[M+H]^+$): 211.0905; found: 211.0979.

5-Benzhydryl-1-*H*-tetrazole (2m)

White solid (yield 70%); mp: 166–168 °C (165–166 °C (Gutmann et al. 2010)); FT-IR: 3100–2400 (w, ν(N–H)), 3090 (w, ν(C–H)), 3058 (w, ν(C–H)), 3003 (w, ν(C–H)), 2949 (w, ν(CH_2)), 1900–1600 (w, overtones δ(C–H, Ph)), 1599 (s, ν(C=C)), 1565 (s, δ(N–H)), 1496 (s, ν(C=C)), 1455 (s, δ(CH_2)), 743 (s, δ(C–H, Ph)), 692 (s, δ(C–H, Ph)) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.34–7.37 (m, 4H, ArH), 7.27–7.31 (m, 6H, ArH), 5.96 (s, 1H, CH). ESI-HRMS calcd for $C_{14}H_{12}N_4$ ($[M+H]^+$): 237.1062; found: 237.1131.

Pharmacology

Maximal electroshock seizure test (MES)

The maximal electroshock seizure test was carried out according to the standard protocol (Krall et al. 1978). Male mice (Kunming, China), weighing 18–22 g, were used as experimental animals purchased from the Laboratory of Animal Research, JiangXi University of Traditional Chinese Medicine (Nanchang, JiangXi Province, China). The animals were allowed to acclimatize with free access to food and water for a 24 h period before testing except during the experiment. Abolition of hind limb tonic extension spasm was recorded as anticonvulsant activity. The test compounds were dissolved in an aqueous solution of 50% polyethylene glycol. In preliminary screening, each compound was administered as an ip injection at three dose levels (30, 100, 300 mg/kg body mass) and the anticonvulsant activity assessed after 0.5, 1, 2, and 4 h intervals of administration.

Subcutaneous pentylenetetrazole seizure test (scPTZ)

This test involved treating the mice with metrazol (pentylenetetrazole, 85 mg/kg in mice). This produced clonic seizures lasting for a period of at least 5 s in 97% of the animals tested. At the anticipated time of testing, the convulsant was subcutaneously administered. The test compounds were intraperitoneally administered in mice and the animals were observed over a 30 min period. Mice

were tested 0.5 and 4 h after doses of 100 and 300 mg/kg of the test compound were administered. The absence of clonic spasms over the period of observation indicated the compound's ability to abolish the effect of pentylenetetrazol on the seizure threshold.

Neurotoxicity screening

The rotarod test was used to evaluate neurotoxicity. The mice were trained to stay on an accelerating rotarod that rotated at 6 revolutions per minute. The rod diameter was 3.2 cm. Trained animals were given ip injection of the test compounds in doses of 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

Quantification studies

The median effective (ED_{50}) was calculated using the Bliss method. The ED_{50} values were presented as the mean with 95% confidence intervals. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials (Dunham et al. 1957). Neurotoxicity was expressed as the median toxic dose (TD_{50} in mg/kg) eliciting minimal neurological toxicity in 50% of animals. The quantitative determination of ED_{50} and TD_{50} values was performed at the previously estimated time of peak effect (1 h) after ip injection into mice. Groups of six animals received various doses of compound **2k** until at least three points were established in the range of 10–90% seizure protection. The results are shown in Table 3.

Calculation of drug-likeness and ADME properties

A computational study of titled compounds was performed to predict ADME properties. Polar surface area (TPSA) (Ertl et al. 2000), number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms, and violations of Lipinski's rule of five (Lipinski et al. 2001) were calculated using the Molinspiration online property calculation toolkit (Molinspiration Cheminformatics 2010). Absorption (%ABS) was calculated as: $\%ABS = 109 - (0.345 \times TPSA)$ (Zhao et al. 2002).

Results and discussion

The synthetic strategy used to prepare the target compounds **2a**–**2m** is depicted in Scheme 1. Substituted benzonitriles **1a**–**1m** were reacted with sodium azide (NaN_3) in the presence of toluene (PhMe) and triethylamine hydrochloride ($Et_3N{\cdot}HCl$) at a refluxing temperature to afford 5-substituted 1-*H*-tetrazoles (Lenda et al. 2005). All products are known compounds and their structures were established by FT-IR, ^1H NMR, LC–MS and melting point analysis and confirmed by comparing their physical and spectra data with those in the literature. The physicochemical properties and structures of **2a**–**2m** are summarized in Table 1. Since lipophilicity is important feature of central nervous system drugs, enabling them to pass the blood brain barrier; the clog P values of the compounds were calculated using ChemDrawUltra 12.0 program. As shown in Table 1, clog P values of compound **2d** (clog P: 2.85) is higher than those of other compounds (clog P:1.80 for compound **2b**, clog P: 2.42 for compound **2c**, clog P: 2.49 for **2h**, and clog P: 2.04 for **2k**).

The preclinical discovery and development of a new bioactive chemical entity for the treatment of epilepsy rely mainly on the use of predictable animal models. The obtained compounds **2a**–**2m** were submitted to in vivo evaluation using the methods described in the Antiepileptic Drug Development Program of the National Institutes of Health according to previously described testing procedures (Krall et al. 1978; Porter et al. 1984). Generally, primary studies of anticonvulsants in mice involve maximal electroshock (MES), and subcutaneous pentylenetetrazole (scPTZ). MES and scPTZ are regarded as the 'gold standard' of the measurement of new anticonvulsants. The first test detects compounds possessing the therapeutic activity for human generalized tonic–clonic seizures induced by an electrical stimulus. The second test is capable to recognize the agents raising the threshold of chemically-induced generalized absence seizures (Farghaly and El-Kashef 2005). In the study, antiepileptic property of synthesized compounds was detected by these methods. Further, their neurotoxicity profiles was primarily evaluated by Rotarod screening. For the most promising derivatives, ED_{50} and TD_{50} values were further determined.

The test compounds were administered intraperitoneally (ip) into mice at doses of 30, 100, and 300 mg/kg (at 0.5, 1,

Scheme 1 Synthetic protocol of the target compounds **2a**–**2m**

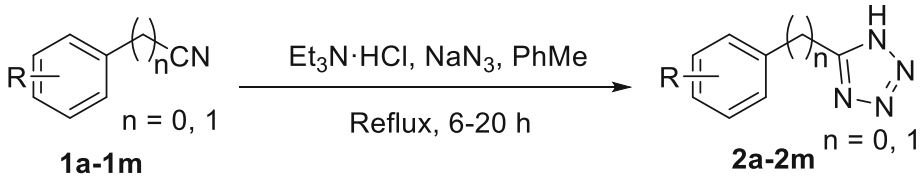
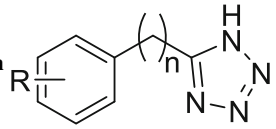
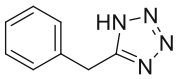
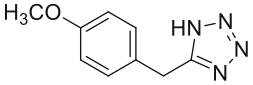
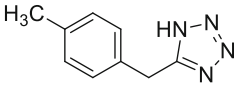
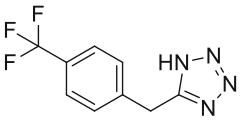
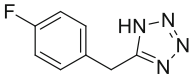
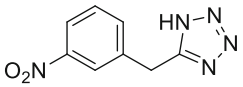
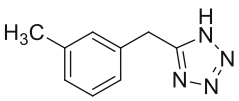
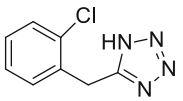
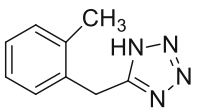
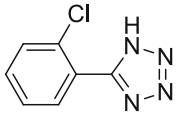
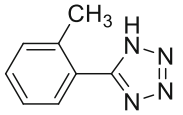
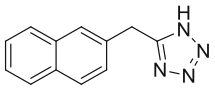
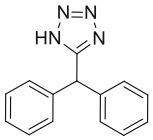


Table 1 Physicochemical properties and structures of all synthesized compounds **2a–2m**



Compounds	R	N	Structures	Clog P[a]
2a	H	1		1.93
2b	4-OCH_3	1		1.80
2c	4-CH_3	1		2.42
2d	4-CF_3	1		2.85
2e	4-F	1		2.09
2f	3-NO_2	1		1.96
2g	3-CH_3	1		2.42
2h	2-Cl	1		2.49
2i	2-CH_3	1		2.42
2j	2-Cl	0		2.11
2k	2-CH_3	0		2.04
2l		1		2.93
2m		1		3.60

[a] Calculated using Chemdraw Ultra 12.0 program (Cambridge Software)

2, and 4 h) for MES screening, whereas 100 and 300 mg/kg were used for scPTZ screening (at 0.5 and 4 h) and neurotoxicity testing (at 0.5, 1, 2, and 4 h). Phenytoin and phenobarbital were selected as standard drugs for the MES and minimal motor impairment test, whereas ethosuximide was used for scPTZ screening. The screening results for anticonvulsant activity and neurotoxicity studies are summarized in Table 2.

In this test, the activities of the synthesized compounds in the MES screening in mice were highest for *ortho*-substituted derivatives. 2-Chloro derivative **2h** displayed high protection at doses of 30 mg/kg for 1 h, but with 300 mg/kg for 0.5 h. The possible reason is that **2h** with an unfavorable lipophilicity enabled the longer time of peak concentration. Replacement of the Cl of 2h with a CH_3 group gave **2i**, which, interestingly, showed protective effect at a dose of 30 mg/kg with markedly slow absorption at 4 h. A similar trend was observed in *meta*-substituted compounds **2f** and **2g**, of which only the 3-CH_3 derivative **2g** revealed notable activity at a dose of 30 mg/kg at 4 h in the MES test. Another nitro-derivative **2f** was moderately active at a dose of 100 mg/kg and 1 h of testing. Except for **2b** and **2c** at doses of 100 mg/kg (at 4 and 2 h post-administration, respectively), the other *para*-substituted compounds **2d** and **2e** were low or inactive in the preliminary MES screening, which showed a similar potency as unsubstituted derivative **2a**. High hydrophobic naphthalene derivative **2l** showed moderate activity at a dose of 100 mg/kg (at 1 and 2 h post-administration), which had lower activity than diphenylmethane derivative **2m** (at a dose of 30 mg/kg at 1 h post-administration).

Increased clog P values of compound **2d** resulted in decreased and lost MES activity, respectively, when compared to compounds (**2b** and **2c**) which are more active with lower clog P values. Similar trend was observed when comparison to **2h** and **2k**. The reason for this appearance was the fact that increased lipophilicity does not necessarily enhance anticonvulsant properties; conversely, it may sometimes lead to reduced or lost activity. Therefore, lipophilicity is not the only important parameter for anticonvulsant ability, but some additional properties and action mechanisms of the compounds also account for the anticonvulsant activity.

Preliminary structure–activity relationship (SAR) studies indicated that *ortho*-substituted derivatives **2h** and **2i** displayed remarkable activity. Consequently, further structural modifications of **2h** and **2i** are necessary. We prepared compounds **2j** and **2k** with one less carbon atom chain than that in **2h** and **2i**. Interestingly, these prepared compounds showed significantly increased anticonvulsant activity. Compound **2k** showed the highest activity in this series according to the MES screening results. Additionally, **2j** exhibited high activity in the scPTZ model at a dose of 100 mg/kg at 0.5 and 4 h. Pentylenetetrazole destroys the balance between glutamic acid and γ-aminobutyric acid, which are an excitatory neurotransmitter and an

Table 2 Anticonvulsant activity and neurotoxocity of compounds **2a–2m** administered intraperitoneally to mice

Compound	Intraperitoneal injection in mice[a]									
	MES[b]				scPTZ[c]		NT[d]			
	0.5 h	1 h	2 h	4 h	0.5 h	4 h	0.5 h	1 h	2 h	4 h
2a	300	300	300	300	–	–	300	300	300	300
2b	300	300	300	100	300	300	300	300	300	300
2c	300	–	100	300	300	–	300	300	300	300
2d	–	300	300	–	300	300	300	300	300	300
2e	300	–	–	–	–	–	300	300	300	300
2f	–	100	–	300	–	–	300	300	300	300
2g	–	100	100	30	–	–	300	300	300	300
2h	300	30	100	100	–	–	300	300	300	300
2i	–	100	–	30	–	–	300	300	300	300
2j	100	100	–	300	100	100	300	300	300	300
2k	30	30	–	300	–	–	300	300	300	300
2l	300	100	100	–	–	–	300	300	300	300
2m	300	30	100	–	–	–	300	300	300	300
Phenytoin	30			30	–	–	100			100
Phenobarbital	100			30	30	300	100			300
Ethosuximide	–	–	–	–	100	300	–	–	–	–

[a] Doses of 30, 100, and 300 mg/kg were administered in MES screening, whereas doses of 100 and 300 mg/kg were used in scPTZ and NT screening. The animals were examined at 0.5, 1, 2, and 4.0 h after injection for MES and NT and 0.5 and 4.0 h for scPTZ. The dash (–) indicates the absence of activity at the maximum dose administered (300 mg/kg)

[b] Maximal electroshock test

[c] Subcutaneous pentylenetetrazole test

[d] Neurotoxicity screening (rotarod test)

Table 3 Quantitative anticonvulsant evaluation in mice (ip)

Compounds	TPE (h)[a]	ED_{50} (MES)[b]	ED_{50} (scPTZ)[b]	TD_{50} (NT)[c]	PI[d]
2j	1	nd	83.3 (51.0–125.4)[e]	>200	>2.4 (scPTZ)
2k	1	9.6 (7.5–26.7)	nd	189.5 (137.1–276.5)	19.7 (MES)
Phenytoin	2	9.5 (8.1–10.4)	>300	65.5 (52.5–72.9)	6.9 (MES)
Phenobarbital	1	21.8 (21.8–25.5)	13.2 (5.8–15.9)	69 (62.8–72.9)	3.2 (MES) 5.2 (scPTZ)
Ethosuximide	2	–	167.0 (116.0–237.0)	>500	>3.0 (scPTZ)

[a] Time to peak effect

[b] ED_{50} median effective dose affording anticonvulsant protection in 50% of animals, the dose is measured in mg/kg

[c] TD_{50} median toxic dose eliciting minimal neurological toxicity in 50% of animals, the dose is measured in mg/kg

[d] *PI* protective index (TD_{50}/ED_{50})

[e] 95% confidence intervals given in parentheses

nd not determined

inhititory one, respectively, leading to the tonic convulsion (Baughman and Gilbert 1980). Increase and accumulating glutamic acid damages the hippocampus which is responsible for sensation, cognition, learning and memory (Wang et al. 2006). Therefore, these active compounds especially **2j** and **2k** may prevent CNS excitation or stress, since they were demonstrated to protect seizures in scPTZ test (Nugroho et al. 2013).

Acute neurological toxicity of synthesized compounds study was carried out by rotarod test (Sun et al. 2006). The results indicated that all compounds showed neurotoxicity at the highest dose (300 mg/kg) with a wide range of safety. The toxicity of compound **2f** is highest, which maybe because of the presence of nitro group.

Based on the results of preliminary screening, **2j** and **2k** were further examined in the second phase to quantify their

Table 4 Pharmacokinetic parameters important for good oral bioavailability of semicarbazones compounds **2a**–**2m**

Compounds	MW	HBA	HBD	NVio	N-ROTB	Volume	TPSA	% ABS
Rule	<500	<10	<5	≤1	–	–	–	–
2a	160.18	4	1	0	2	144.77	54.47	90.21
2b	190.21	5	1	0	3	170.32	63.70	87.02
2c	174.21	4	1	0	2	161.33	54.47	90.21
2d	228.18	4	1	0	3	176.07	54.47	90.21
2e	178.17	4	1	0	2	149.70	54.47	90.21
2f	205.18	7	1	0	3	168.10	100.29	74.40
2g	174.21	4	1	0	2	161.33	54.47	90.21
2h	194.62	4	1	0	2	158.31	54.47	90.21
2i	174.21	4	1	0	2	161.33	54.47	90.21
2j	180.60	4	1	0	1	141.50	54.47	90.21
2k	160.18	4	1	0	1	144.53	54.47	90.21
2l	210.24	4	1	0	2	188.76	54.47	90.21
2m	236.28	4	1	0	3	216.21	54.47	90.21

MW molecular weight, *HBA* number of hydrogen bond acceptors, *HBD* number of hydrogen bond donors, *nVio* number of Lipinski's violations, *n-ROTB* number of rotatable bonds, *volume* molecular volume, *TPSA* topological polar surface area, *%ABS* percentage of absorption

anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) in mice. Their protective index (PI) fixed as the ratio of TD_{50}–ED_{50} were calculated. For compound **2j** (ED_{50} = 83.3) in the scPTZ model, although the ED_{50} was much lower than that of ethosuximide (ED_{50} = 167.0), its PI was lower than that of the standard due to the lower TD_{50} value for the drug. Compound **2k** showed an ED_{50} value of 9.6 mg/kg in the MES model and a TD_{50} value of 189.5 mg/kg. Consequently, the PI value of **2k** (19.7) was higher than those of the reference drugs, 6.9 for phenytoin and 3.2 for phenobarbital (Table 3). **2k** was the most potent anticonvulsants of all tested compounds in this study since the PI value is considered to be an index representing the margin of safety and tolerability between anticonvulsant doses and doses of anticonvulsant drugs exerting acute adverse effects (Loscher and Nolting 1991).

A computational study to predict the absorption, distribution, metabolism and excretion (ADME) properties of the compounds was performed. Topological polar surface area (TPSA) is a descriptor that is strongly correlated with passive molecular transport through membranes, and accordingly predicts the transport properties of drugs in the intestines and blood brain barrier crossing (Ertl et al. 2000). The percentage of absorption (%ABS) was calculated using TPSA. Based on these parameters, all titled compounds exhibited a high %ABS ranging from 74.4 to 90.21% (Table 4). None of the compounds violated Lipinski's parameters, indicating that they are promising agents for use in epilepsy therapy.

Conclusion

In summary, we designed and synthesized a series of 5-substituted 1-*H*-tetrazoles as anticonvulsant agents. The anticonvulsant activity and neurotoxicity of all synthesized compounds were evaluated using MES- and scPTZ-induced seizure testing and rotarod test. The SARs and ADME predictions of these compounds were also covered. The anticonvulsant potency of these compounds probably attributed to the favorable structural combination of *ortho*-substituted derivatives. In current study, the most potent anticonvulsant agent in scPTZ screen was **2j** with an ED_{50} value of 83.3 mg/kg, which is much lower than that of the standard drug ethosuximide, showing maximum activity. In addition, the compound **2k** showed the highest activity in MES model, with an ED_{50} value of 9.6 mg/kg and TD_{50} value of 189.5 mg/kg, after ip injection in mice, and displayed a higher PI value of 19.7 compared to reference antiepileptic drugs. This research shed light on the useful information for developing new anticonvulsant agents with high activity and low toxicity and further investigation on the precise mechanism of action of novel anticonvulsants in future.

Acknowledgements This work was supported by the Science and Technology Research Projects of Jiangxi Provincial Education Department (No. GJJ150845), Traditional Chinese Medicine Research Project of Jiangxi Provincial Health and Family Planning Commission (No. 2016A035) and PhD Start-up Fund of Jiangxi University of Traditional Chinese Medicine (No. 2014BS016).

References

Barkmeier DT, Loeb JA (2009) An animal model to study the clinical significance of interictal spiking. Clin EEG Neurosci 40:234–238

Baughman RW, Gilbert CD (1980) Aspartate and glutamate as possible neurotransmitters of cells in layer 6 of the visual cortex. Nature 287:848–850

Bialer M, White HS (2010) Key factors in the discovery and development of new antiepileptic drugs. Nat Rev Drug Discov 9:68–82

Butler RN, Garvin VC (1981) A study of annular tautomerism, interannular conjugation, and methylation reactions of ortho-substituted 5-aryltetrazoles using carbon-13 and hydrogen NMR spectroscopy. J Chem Soc 1:390–393

Cantillo D, Gutmann B, Kappe CO (2011) Mechanistic insights on azide-nitrile cycloadditions: on the dialkyltin oxide-trimethylsilyl azide route and a new Vilsmeier-Haack-type organocatalyst. J Am Chem Soc 133:4465–4475

Dunham NW, Miya TS, Edwards LD (1957) The pharmacological activity of a series of basic esters of mono- and dialkylmalonic acids. J. Am. Pharm. Assoc 46:64–66

Eadie MJ (1984) Anticonvulsant drugs. An update. Drugs 27:328–363

Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 43:3714–3717

Farghaly A-R, El-Kashef H (2005) Pyrazoles and pyrazolo[4,3-e]pyrrolo[1,2-a]pyrazines, I. Synthesis and antimicrobial activity. Chem Mon 136:217–227

French JA (2007) Refractory epilepsy: clinical overview. Epilepsia 48(Suppl 1):3–7

Greenwood RS (2000) Adverse effects of antiepileptic drugs. Epilepsia 41:S42–S52

Gutmann B, Roduit J-P, Roberge D, Kappe CO (2010) Synthesis of 5-substituted 1H-tetrazoles from nitriles and hydrazoic acid by using a safe and scalable high-temperature microreactor approach. Angew Chem Int Ed 49:7101–7105

Hirsch E, Arzimanoglou A (2004) Children with drug-resistant partial epilepsy: criteria for the identification of surgical candidates. Revue Neurologique 160:5s210–5s219

Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA (1978) Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia 19:409–428

Kraus JL (1983) Isosterism and molecular modification in drug design: tetrazole analogue of GABA: effects on enzymes of the gamma-aminobutyrate system. Pharmacol Res Commun 15:183–189

Kwan P, Brodie MJ (2000) Early identification of refractory epilepsy. N Engl J Med 342:314–319

Lenda F, Guenoun F, Tazi B, Ben Larbi N, Allouchi H, Martinez J, Lamaty F (2005) Synthesis of new tetrazole-substituted pyroaminoadipic and pipecolic acid derivatives. Eur J Org Chem 2005:326–333

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26

Loscher W, Nolting B (1991) The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. IV. protective indices. Epilepsy Res 9:1–10

Molinspiration Cheminformatics B, Slovakrepublic, (2010) http://www.molinspiration.com/services/properties.html Accessed 16 Aug 2010

Nugroho A, Lim SC, Choi J, Park HJ (2013) Identification and quantification of the sedative and anticonvulsant flavone glycoside from *Chrysanthemum boreale*. Arch Pharmacal Res 36:51–60

Obushak ND, Pokhodylo NT, Pidlypnyi NI, Matiichuk VS (2008) Synthesis of 1,2,4- and 1,3,4-oxadiazoles from 1-aryl-5-methyl-1H-1,2,3-triazole-4-carbonyl chlorides. Rus J Organ Chem 44:1522–1527

Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Scoville B, White BG (1984) Antiepileptic drug development program. Clevel Clin Q 51:293–305

Rama V, Kanagaraj K, Pitchumani K (2011) Syntheses of 5-substituted 1H-tetrazoles catalyzed by reusable CoY zeolite. J Org Chem 76:9090–9095

Sun XY, Jin YZ, Li FN, Li G, Chai KY, Quan ZS (2006) Synthesis of 8-alkoxy-4,5-dihydro-[1,2,4]triazole[4,3-a]quinoline-1-ones and evaluation of their anticonvulsant properties. Arch Pharmacal Res 29:1080–1085

Wang WP, Iyo AH, Miguel-Hidalgo J, Regunathan S, Zhu MY (2006) Agmatine protects against cell damage induced by NMDA and glutamate in cultured hippocampal neurons. Brain Res 1084:210–216

Wehman TC, Popov AI (1966) Charge-transfer complexes of mono- and disubstituted tetrazoles with π-electron acceptors. J Phys Chem 70:3688–3693

Zhao YH, Abraham MH, Le J, Hersey A, Luscombe CN, Beck G, Sherborne B, Cooper I (2002) Rate-limited steps of human oral absorption and QSAR studies. Pharm Res 19:1446–1457