Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4803–4805
Bioorganic & Medicinal Chemistry Letters

Synthesis and anticonvulsant activity of 7-alkoxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinolines

Zhi-Feng Xie,[a] Kyu-Yun Chai,[b] Hu-Ri Piao,[a] Kyung-Chell Kwak[b] and Zhe-Shan Quan[a,*]

[a]*College of Pharmacy, Yanbian University, Yanji, Jilin 133000, PR China*
[b]*Department of Chemistry, Wonkwang University, Iksan 570-749, Republic of Korea*

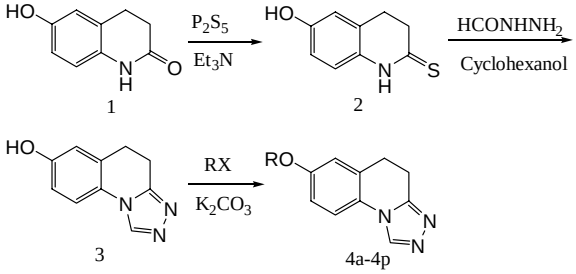
Received 24 May 2005; accepted 15 July 2005
Available online 31 August 2005

Abstract—A series of 7-alkoxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline derivatives was synthesized using 6-hydroxy-3,4-dihydro-1*H*-quinolin-2-one as a starting material. Their anticonvulsant activities were evaluated by the maximal electroshock test (MES test) and the subcutaneous (sc) pentylenetetrazol test (scMet test), and their neurotoxicity was evaluated by the rotarod neurotoxicity test (Tox). MES and scMet tests show that 7-(4-fluorobenzyloxy)-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline **4l** was found to be the most potent with ED_{50} value of 11.8 and 6.7 mg kg^{-1} and protective index ($PI = TD_{50}/ED_{50}$) value of 4.6 and 8.1, respectively.

The derivatives of triazole exhibit a variety of biological activities, such as antitumor,[1] antiinflammatory,[2] antimicrobial,[3,4] antithrombotic,[5] antiplatelet,[6] antiviral,[7] and anticonvulsant activities.[8] In our former work,[9] a series of 1-substituted-7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline derivatives was synthesized and tested for anticonvulsant activity, with the compound 7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline showing the most potent anticonvulsant activity with ED_{50} of 17.3 and 24.0 mg kg^{-1} in MES and scMet tests, respectively.

In the present study, we report the synthesis and anticonvulsant activities of 7-alkoxyl-4,5-dihydro[1,2,4]triazolo-[4,3-*a*]quinolines to investigate the contribution of different alkoxy groups at position 7 of the 4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinoline to anticonvulsant activity. The compounds synthesized were characterized by IR, ^1H NMR, MS, and elemental analysis. The anticonvulsant activity was evaluated by using the maximal electroshock (MES) test and the subcutaneous pentylenetetrazol (sc-Met) test. Neurotoxicity was evaluated by using the rotarod test.

Compounds were prepared according to Scheme 1. Compound **2** was prepared by the reaction of compound **1** with phosphorous pentasulfide in acetonitrile in the presence of triethylamine,[10] which reacted further with formic anhydrazine in cyclohexanol to afford compound **3**.[11] In the preparation of compound **3**, the reaction should be carried out under nitrogen atmosphere and low boiling point solvents should not be used. Compounds **4a–4p** were obtained through a nucleophilic



R:

4a = n-C_2H_5	4g = $CH_2C_6H_5$	4l = $CH_2C_6H_4$(p-F)
4b = n-C_3H_7	4h = $CH_2C_6H_4$(p-CH_3)	4m = $CH_2C_6H_4$(o-F)
4c = n-C_4H_9	4i = $CH_2C_6H_4$(p-Cl)	4n = $CH_2C_6H_3$(2,6-F_2)
4d= n-C_6H_{13}	4j = $CH_2C_6H_4$(p-OCH_3)	4o = $CH_2C_6H_3$(3,4-OCH_2O)
4e = n-C_8H_{17}	4k = $CH_2C_6H_3$(3,4-Cl_2)	4p = $CH_2C_6H_4$(m-Br)
4f = n-$C_{12}H_{25}$		

Scheme 1. Synthesis of compound **4a–4p**.

Keywords: [1,2,4]Triazolo[4,3-*a*]quinolines; Anticonvulsant; Maximal electroshock; Pentylenetetrazol; Neurotoxicity.
* Corresponding author. Tel.: +86 433 2660606; fax: +86 433 2660568; e-mail: zsquan@ybu.edu.cn

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.07.051

substitution reaction of compound **3** with alkyl halide in ethanol in the presence of potassium carbonate in moderate yield.[12]

The MES test, scMet test, and rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA).[13,14] All compounds, which were dissolved in polyethylene glycol-400, were evaluated for anticonvulsant activities with C57B/6 mice in the 18–25 g weight range.

In Phase I screening (Table 1), each compound was administered at dose levels of 30, 100, and 300 mg/kg for evaluating the anticonvulsant activity, and its neurotoxicity was measured at 30 min and 4-h intervals after administration. Anticonvulsant efficacy was measured in the MES and scMet tests. In the MES test, seizures were elicited with a 60-Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. The scMet test was carried out by the subcutaneous injection of a convulsant dose (CD_{97}) of pentylenetetrazol (85 mg/kg in mice). The elevation of pentylenetetrazol-induced seizure threshold was indicated by the absence of clonic spasms for at least 5-s duration over a 30-min period, following the administration of the testing compound. An anticonvulsant drug-induced neurologic deficit was detected in mice by using the rotorod ataxia test.

Table 1. Phase I anticonvulsant and toxicity data in mice (ip)[a]

Compound	MES[b]		ScMet[c]		Rotarod toxicity	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
4a	—	—[e]	—	—	—	—
4b	100[d]	—	100	—	100	—
4c	100	—	100	—	100	—
4d	30	—	30	—	100	—
4e	100	—	100	—	—	—
4f	—	—	—	—	—	—
4g	30	—	100	—	100	—
4h	30	—	30	—	100	—
4i	300	—	300	—	—	—
4j	100	—	100	—	300	—
4k	—	—	—	—	—	—
4l	30	30	30	—	100	—
4m	300	—	300	—	—	—
4n	—	—	—	—	—	—
4o	100	—	100	—	300	—
4p	30	—	30	—	100	—

[a] All tested compounds were dissolved in polyethylene glycol-400.
[b] The maximal electroshock test was induced after 30 min past administration of the tested compounds.
[c] Subcutaneous pentylenetetrazol (85 mg kg^{-1}) 30 min after the tested compounds were administered for 30 min.
[d] Doses were denoted in mg kg^{-1}.
[e] —, no activity at 300 mg kg^{-1}.

The pharmacologic parameters estimated in phase I screening were quantified for compounds **4d**, **4g**, **4h**, **4l**, and **4p** in phase II screening (Table 2). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50} values, groups of 10 mice were given a range of intraperitoneal doses of the testing compound until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plot of those data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of a computer program written at the National Institute of Neurological Disorders and Stroke.

As a result of preliminary screening, compounds **4d**, **4g**, **4h**, **4l**, and **4p** were subjected to phase II trials for quantification of their anticonvulsant activities and neurotoxicities in mice. This phase provides an evaluation of the median effective dose (ED_{50}) and median neurotoxic dose (TD_{50}). The 95% confidence interval, slope of the regression line, and the SE of the slope were then calculated. These data are shown in Table 2, which also

Table 2. Phase II quantitative anticonvulsant data in mice (test drug administered ip)

Compound	ED_{50}[a]			PI[b]	
	MES	scMet	Tox, TD_{50}[c]	MES	scMet
4d	13.5 (11.5–15.8)[e]	17.7 (12.5–20.6)	30.3 (25.5–36.0)	2.2	1.7
4g	17.3 (14.8–20.4)	24.0 (21.6–26.7)	61.4 (51.4–73.3)	3.5	2.6
4h	20.5 (17.0–24.5)	11.1 (9.3–13.1)	65.8 (55.6–77.7)	3.2	5.9
4l	11.8 (12.9–10.9)	6.7 (5.7–7.9)	54.5 (46.1–64.5)	4.6	8.1
4p	23.3 (19.5–28.0)	24.5 (21.4–28.2)	98.6 (87.3–111.3)	4.2	4.0
Phenytoin[d]	9.5 (8.1–10.4)	>300	65.5 (52.5–72.9)	6.9	<0.22
Carbamazepine[d]	8.8 (5.5–14.1)	>100	71.6 (45.9–135)	8.1	<0.22
Phenobarbital[d]	21.8 (21.8–25.5)	13.2 (5.8–15.9)	69 (62.8–72.9)	3.2	5.2
Valproate[d]	272 (247–338)	149 (123–177)	426 (369–450)	1.6	2.9

[a] Dose measured in mg kg^{-1}.
[b] PI = TD_{50}/ED_{50}.
[c] Minimal neurotoxicity was determined by the rotarod test 30 min after the tested compounds were administered.
[d] Data from U. Huseyin et al., 1998.[15]
[e] Data in parentheses are the 95% confidence limits.

includes the control data with marketed antiepileptic drugs, such as phenytoin, carbamazepine, phenobarbital, and valproate. Some of these derivatives showed a high degree of protection against MES and scMet-induced seizures.

The result of the initial evaluation (phase I) indicated that the lengthening of the alkyl chain at position 7 led to an increase of anticonvulsant activity, in which the *n*-hexyl-substituted compound was found to be the most active, while a decreased activity was found if there was an alkyl chain having more than six carbon numbers. In this series, compounds containing alkyl groups bearing three to six carbon atoms at position 7 showed a higher neurotoxicity than others. Among 7-aryloxyl derivatives, benzyloxy-substituted compounds, such as 4-methyl benzyloxy, 4-fluorobenzyloxy, and 3-bromobenzyloxy derivatives, exhibited high activities against seizure induced by both MES and scMet tests.

The result of phase II showed that compound **4d** was the only compound possessing anti-MES activity under a dose of 30 mg/kg among those compounds bearing alkoxyl groups. Its ED_{50} value was 13.5 and 17.3 mg/kg in MES and scMet tests, respectively. Furthermore, it was more potent than phenobarbital (ED_{50} = 21.8 mg/kg) and valproate (ED_{50} = 272 mg/kg), although it had high neurotoxicity (TD_{50} = 30.3 mg/kg). Compounds **4g**, **4h**, **4l**, and **4p** exhibited anti-MES activity with ED_{50} of 17.3, 20.5, 11.8, and 23.3 mg/kg, respectively. And compound **4l**, a 4-fluorobenzyloxy derivative, was found to be the best, which was better than phenobarbital and valproate, much closer to phenytoin (ED_{50} = 9.5 mg/kg). Its PI value in the MES test was 4.6, superior to that of phenobarbital (3.2), but inferior to those of phenytoin (6.9) and carbamazepine (8.1). However, compounds **4m** and **4n**, containing the substituents of 2-fluoro and 2,6-difluoro groups, exhibited little or even no anticonvulsant activities.

4d, **4g**, **4h**, **4l**, and **4p** exhibited moderate anti-Met activities with ED_{50} of 17.7, 24.0, 11.1, 6.7, and 24.5 mg/kg, respectively, in which **4l** was shown to be more potent than phenobarbital, carbamazepine, phenobarbital, and valproate. Its PI (8.1) was obviously higher than those of the control drugs.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No. 30460151).

References and notes

1. Al-Soud, Y. A.; Al-Dweri, M. N.; Al-Masoudi, N. A. *Farmaco* **2004**, *59*, 775.
2. Labanauskas, L.; Udrenaite, E.; Gaidelis, P.; Brukstus, A. *Farmaco* **2004**, *59*, 255.
3. Abak, K.; Sezer, O.; Akar, A.; Anac, O. *Eur. J. Med. Chem.* **2003**, *38*, 215.
4. Gulerman, N. N.; Dogan, H. N.; Rollas, S.; Johansson, C.; Celik, C. *Farmaco* **2001**, *56*, 953.
5. Collin, X.; Sauleau, A.; Coulon, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2601.
6. Cwiklicki, A.; Rehse, K. *Arch. Pharm. (Weinheim, Ger.)* **2004**, *337*, 156.
7. Cunha, A. C.; Figueiredo, J. M.; Tributino, J. L.; Miranda, A. L.; Castro, H. C.; Zingali, R. B.; Fraga, C. A.; Souza, M. C.; Ferreira, V. F.; Barreiro, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2051.
8. Kane, J. M.; Baron, B. M.; Dudley, M. W.; Sorensen, S. M.; Staeger, M. A.; Miller, F. P. *J. Med. Chem.* **1990**, *33*, 2772.
9. Cui, L. J.; Jin, H. G.; Xie, Z. F.; Piao, H. R.; Chai, K. Y.; Quan, Z. S. *Biol. Pharm. Bull.* **2005**, *28*, 1216.
10. Preparation of **2**: In a three-necked round-bottomed flask containing 30 ml acetonitrile and 20 ml triethylamine, P_2S_5 6.13 g (28 mol) was added slowly under an ice bath with stirring. After dissolving, 3.26 g of compound **1** (20 mmol) was added. Then, the mixture was refluxed for 4 h in a nitrogen atmosphere. The solvents were removed under reduced pressure. The residue was poured into 100 ml ice water and stirred for 10 min. The solid obtained after filtration was recrystallized in EtOAc/MeOH to afford a yellow solid 2.94 g (82%), mp 225 °C (decomposed). ^{1}H NMR ($CDCl_3$, 300 MHz) 2.79 (t, 2H, J = 6.6 Hz), 2.95 (t, 2H, J = 6.6 Hz), 6.69–7.04 (m, 3H), 8.32 (s, 1H), 10.93 (s, 1H). MS *m/z* 179 (M^+). Anal. Calcd for C_9H_9NOS: C, 60.31; H, 5.06; N, 7.81. Found: C, 60.23; H, 4.94; N, 7.62.
11. Preparation of **3**: A mixture of compound **2** 3.58 g (20 mmol) and formic hydrazine 1.44 g (24 mmol) in 50 ml cyclohexanol was refluxed for 6 h under a nitrogen atmosphere. After the solvent was removed under reduced pressure, the residue was recrystallized in EtOAc/MeOH (2:1) to afford a pale yellow solid 2.54 g (68%), mp 235 °C (decomposed). ^{1}H NMR ($CDCl_3$, 300 MHz) 2.93 (t, 2H, J = 7.7 Hz), 3.01 (t, 2H, J = 7.7 Hz), 6.74–7.56 (m, 3H), 9.11 (s, 1H), 9.2 (s, 1H). MS *m/z* 187 (M^+). Anal. Calcd for $C_{10}H_9N_3O$: C, 64.16; H, 4.85; N, 22.45. Found: C, 63.92; H, 4.98; N, 22.24.
12. Preparation of **4l**: A mixture of compound **3** 0.94 g (5 mmol) and 4-fluorobenzyl chloride 0.72 g (5 mmol) in 30 ml anhydrous ethanol was refluxed for 5 h in the presence of K_2CO_3 1.44 g (10 mmol). After the solvent was removed, the residue was extracted with dichloromethane (30 ml × 3). The combined dichloromethane layer was washed with water (30 ml × 2), dried over $MgSO_4$, and then the solvent was removed under reduced pressure. A pale yellow solid was obtained (0.83 g, 89%) after being purified by silica gel chromatography (dichloromethane–methanol 10:1), mp 138–140 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): 3.03 (t, 2H, J = 7.7 Hz), 3.20 (t, 2H, J = 7.7 Hz), 5.06 (s, 2H), 6.91–7.44 (m, 7H), 8.58 (s, 1H). MS *m/z* 295 (M^+). IR (KBr) 1606, 1262, 1020, 1226 cm^{-1}. Anal. Calcd for $C_{17}H_{14}FN_3O$: C, 69.14; H, 4.78; N, 14.23. Found: C, 68.97; H, 4.92; N, 14.04.
13. Krall, R. J.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *9*, 409.
14. Poter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B. *Cleveland Clin. Q.* **1984**, *51*, 293.
15. Huseyin, U.; Kim, V. D.; Silvia, C.; Santi, S.; James, P. S.; Paul, D.; Majed, I.; Bernard, M.; Jacques, H. P. *J. Med. Chem.* **1998**, *41*, 1138.