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Evaluation of anticonvulsant activity of QUAN-0806 in various murine experimental seizure models

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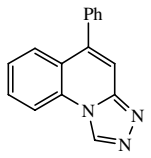
A new quinoline derivative, QUAN-0806 (7-hexyloxy-5-phenyl-1,2,4-triazolo[4,3-α]quinoline) was tested for anticonvulsant activity using the maximal electroshock seizure (MES) and the rotarod neurotoxicity (Tox) tests in mice. The MES test showed that QUAN-0806 exhibited higher activity ($ED_{50} = 6.5$ mg/kg) and lower toxicity ($TD_{50} = 228.2$ mg/kg), resulting in a higher protective index ($PI = 35.1$) than the reference drugs phenytoin, carbamazepin, phenobarbital, and valproate. In addition, QUAN-0806 was found to exhibit significant oral activity against MES-induced seizures with low oral neurotoxicity in mice. QUAN-0806 was tested in chemically induced models (pentylenetetrazole, PTZ; isoniazid, ISO; 3-mercaptopropionic acid, 3-MPA; and strychnine, STRYC) to further investigate the anticonvulsant activity. QUAN-0806 produced significant antagonistic activity against seizures induced by PTZ, 3-MPA, and ISO, suggesting that QUAN-0806 influences GABAergic neurotransmission by activating glutamate decarboxylase (GAD) or inhibiting (GABA)-α-oxoglutarate aminotransferase (GABA-T) in the brain.

1. Introduction

Epilepsy, an ubiquitous disease characterized by recurrent seizures, inflicts more than 60 million people worldwide according to epidemiological studies (Loscher 1998). For epilepsy treatment, nearly 95% of clinically available drugs were approved before 1985 and they could provide satisfactory seizure control for 60–70% of the patients. These drugs, however, also cause notable adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anaemia (Leppik 1994; Perucca 1996; Lin and Kadaba 1997) and even life-threatening conditions (Al-Soud et al. 2003). Therefore, the continued search for safer and more effective new antiepileptic drugs (AEDs) is imperative and challenging in medicinal chemistry.



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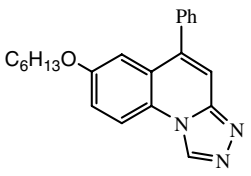

QUAN-0806

Compound **I** (5-phenyl-[1,2,4]-triazolo[4,3-α]quinoline) was first identified to exhibit anticonvulsant activity in our previous study (Guan et al. 2008), though the activity was weaker than that of the reference drug (phenytoin) in the maximal electroshock seizure (MES) test. In our attempts to obtain compounds with better anticonvulsant activity, using the information on structure-activity relationships (SAR) and the findings of our recent investigation (Jin et al. 2006; Xie et al. 2005) on the SAR of anticonvulsant activity, we noted that the introduction of a different substitution group in the 7th position on the phenyl ring resulted in the compound QUAN-0806 with a hexyloxy group substitution at the 7th position. This compound exhibited the strongest anticonvulsant activity ($ED_{50} = 6.5$ mg/kg) and lower toxicity ($TD_{50} = 228.2$ mg/kg), resulting in a protective index ($PI = 35.1$) greater than the reference drugs phenytoin, carbamazepin, phenobarbital, and valproate in MES tests.

The anticonvulsant activity of QUAN-0806 was further investigated by testing it in chemically induced models (pentylenetetrazole, PTZ; isoniazid, ISO; 3-mercaptopropionic acid, 3-MPA; and strychnine, STRYC). The results of these studies showed that QUAN-0806 not only had stronger anticonvulsant activity, but also exhibited markedly lower neurotoxicity than the reference drugs phenytoin, carbamazepin, phenobarbital, and valproate; therefore, a larger PI was observed for this compound in the MES test. In addition, QUAN-0806 significantly antagonised seizures induced by PTZ, 3-MPA, and ISO, but was ineffective against STRYC-induced seizures. These results suggest that QUAN-0806 might influence GABAergic neurotransmission by activating glutamate decarboxylase (GAD) or inhibiting (GABA)-α-oxoglutarate aminotransferase (GABA-T) in the brain.

2. Investigations and results

2.1. Effects on MES

Table 1 describes the anticonvulsant activities of QUAN-0806 and the reference standard in the MES test performed in our laboratory. QUAN-0806 showed dose-dependent anticonvulsant activity with an ED_{50} value of 6.5 mg/kg and median toxicity dose (TD_{50}) of 228.2 mg/kg, resulting in a PI of 35.1, which is much greater than the PI of the reference drugs phenytoin, carbamazepin, phenobarbital, and valproate.

2.2. Time-course of MES anticonvulsant activity of QUAN-0806

The peak effect of an oral dose of 100 mg/kg QUAN-0806 was exhibited between 0.5 and 5 h after administration (Fig.). The peak of protection was observed 2 h after the p.o. injection. Then, QUAN-0806 was evaluated for its oral activity against MES-induced seizures and oral neurotoxicity in mice (Table 2) with phenytoin as the reference. The time-to-peak effect (TPE) was maximal at 2 h, which was comparable with that of phenytoin. The data in Table 2 clearly indicate an increase in anticonvulsant potency and neurotoxicity of QUAN-0806 when administered orally compared with that on intraperitoneal (i.p.) administration. Nevertheless, the PI values were comparable in the two modes of drug delivery. The oral ED_{50}/i.p. ED_{50} ratios for QUAN-0806 were less than 9.76 and 6.88 for the rotarod and MES tests, respectively. These ratios suggested that QUAN-0806 was adequately absorbed in mice after oral administration. No neurotoxicity was found after oral administration at the very high dose of 2000 mg/kg; therefore, the PI in oral administration was 44.7, which was higher than that in i.p. administration and better than that of any of the prototype drugs.

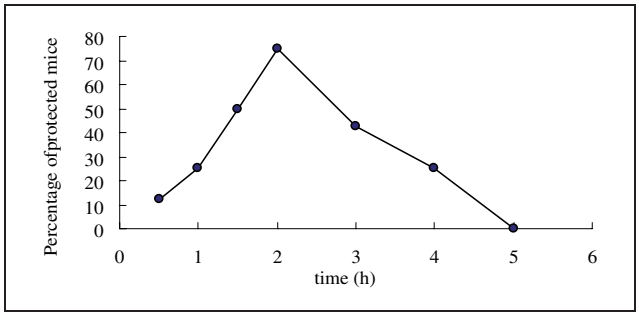

Fig.: Time-course of QUAN-0806 (100 mg/kg) in the maximal electroshock seizure test (the number of animals at each point was 7 to 8)

2.3. Chemically induced seizures

QUAN-0806 blocked tonic-extension seizures induced by the chemical convulsants tested, as shown in Table 3. QUAN-0806 was also effective against seizures induced by PTZ, ISO, and 3-MPA, with ED_{50} values of 25.0, 39.4, and 19.7 mg/kg, respectively. However, it failed to protect animals from seizures induced by STRYC at a dose of 100 mg/kg.

3. Discussion

The results of the present study showed that QUAN-0806 not only possesses higher anticonvulsant activity but also

Table 1: Quantitative anticonvulsant data in mice (test drug administered i.p.)

Compound	MES, ED_{50}[a]	TD_{50}[b]	PI[c] (TD_{50}/ED_{50})
I	28.4 (21.1–38.5)[d]	126.8 (105.5–152.4)	4.5
QUAN-0806	6.5 (4.6–9.2)[c]	228.2 (189.7–274.2)	35.1
Phenytoin	9.5 (8.1–10.4)	65.5 (52.5–72.9)	6.9
Carbamazepine	8.8 (5.5–14.1)	71.6 (45.9–135)	8.1
Phenobarbital	21.8 (21.8–25.5)	69 (62.8–72.9)	3.2
Valproate	272 (247–338)	426 (369–450)	1.6

[a] ED_{50}-median effective dose affording anticonvulsant protection in 50% animals
[b] TD_{50}-median toxic dose eliciting minimal neurological toxicity in 50% animals
[c] PI protective index (TD_{50}/ED_{50})
[d] 95% confidence limits given in parentheses

Table 2: Pharmacological evaluation of QUAN-0806, phenytoin, and carbamazepin administered orally to mice

Compound	TPE (h)	MES, ED_{50}[a]	TD_{50}[b]	PI[c] (TD_{50}/TD_{50})
QUAN-0806	2	44.7 (31.0–64.4)[d]	> 2000	> 44.7
Phenytoin	2	9.0 (7.39–10.6)	86.7 (80.4–96.1)	9.6
Carbamazepin	2	15.4 (12.4–17.3)	217 (131.5–270.1)	14.1

[a] ED_{50}-median effective dose affording anticonvulsant protection in 50% animals
[b] TD_{50}-median toxic dose eliciting minimal neurological toxicity in 50% animals
[c] PI protective index (TD_{50}/ED_{50})
[d] 95% confidence limits given in parentheses

Table 3: Anticonvulsant activity of QUAN-0806 in chemically induced seizure tests

Compound	Pentylenetetrazole	Isoniazid	3-Mercaptopropionic acid	Strychnine
QUAN-0806	25.0 (17.3–36.1)	39.4 (32.8–47.4)	19.7 (16.5–23.5)	100 >[a]

[a] QUAN-0806 failed to control the seizure induced by strychnine at a dose of 100 mg/kg

lower toxicity than the reference drugs in the MES test. As shown in Table 1, QUAN-0806 had an ED_{50} value of 6.5 mg/kg and median toxicity dose (TD_{50}) of 228.2 mg/kg, resulting in a PI of 35.1, which is much greater than the PI of the prototype drugs phenytoin, carbamazepin, phenobarbital, and valproate.

The anticonvulsant activity of QUAN-0806 was further investigated against seizures induced by PTZ, 3-MPA, ISO, and STRYC for confirmation and to investigate the possible mechanisms behind this activity. As shown in Table 3, QUAN-0806 was effective against seizures induced by PTZ, ISO, and 3-MPA with ED_{50} values of 25.0, 39.4, and 19.7 mg/kg, respectively.

The anticonvulsant profile shows that QUAN-0806 has positive activity in both tests, namely, chemical and electrical tests. These animal models are usually considered suitable to identify anticonvulsant activity and have been used for all the compounds currently used for the treatment of a wide variety of seizures. They may predict clinical efficacy and the mechanism of drug action (Kupferberg 1992; Wieland et al. 1997; Bourgeois 1998).

The chemical models of seizure, e.g., PTZ-induced seizure, are considered as experimental models for 'generalized absence seizure'. They produce both clonic and tonic seizures when administered parenterally. A progression of symptoms is observed, depending on the dose and the route of administration. These symptoms follow a pattern of focal activation of the CNS, such as myoclonic jerks and clonic spasms, followed by a generalized tonic seizure. The MES test, which was most frequently used as an animal model, was employed for the identification of anticonvulsant activity of drugs for the 'grand mal'.

Pentylenetetrazole and isoniazid have been reported to produce seizures by inhibiting aminobutyric acid (GABA) neurotransmission (Okada et al. 1989; Olsen et al. 1981). GABA is the main inhibitory neurotransmitter substance in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures (Gale 1992), while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that compound QUAN-0806 might have inhibited or attenuated pentylenetetazole-induced and isoniazid-induced seizures in mice by enhancing GABAergic neurotransmission.

3-Mercaptopropionic acid was a competitive inhibitor of GABA synthesis enzyme glutamate decarboxylase (GAD), and it inhibits the synthesis of GABA resulting in decreased GABA level in the brain (Loscher, 1979). The QUAN-0806 showed moderate antagonism to 3-mercaptopropionic acid-induced seizures, suggesting that it might activate GAD or inhibit aminotransferase (GABA-T) in the brain.

As shown in Table 3, QUAN-0806 failed to protect animals from seizures induced by STRYC at a dose of 100 mg/kg. It is known that STRYC directly antagonizes the inhibitory spinal reflexes of glycine (Sayin et al. 1993), therefore, the results suggest that QUAN-0806 do not influence the glycine system.

In conclusion, QUAN-0806 showed stronger anticonvulsant activity, considerably lower neurotoxicity, and a larger PI than the prototype drugs phenytoin, carbamazepin, phenobarbital, and valproate in the MES test. In addition, QUAN-0806 produced significant antagonism against the seizures induced by PTZ, 3-MPA, and ISO, but was ineffective against strychnine-induced seizures. These results suggest that QUAN-0806 influences GABAergic neurotransmission by activating glutamate decarboxylase (GAD) or inhibiting (GABA)-α-oxoglutarate aminotransferase (GABA-T) in the brain.

4. Experimental

4.1. Animals

C57B/6 mice of either sex (obtained from the Laboratory of Animal Research, College of Pharmacy, Yanbian University) weighing from 18 to 25 g were used in these studies. The ratio of male and female mice in the control and drug-treated groups was kept same to avoid variation in responses due to sex differences. The animals were housed in plastic cages and were maintained on a 12 : 12-h light-dark (7 : 00 a.m. to 7 : 00 p.m.) schedule in a temperature-controlled (21 ± 2 °C) animal room and relative humidity (about 40–50%). The animals had free access to standard rodent chow and water and were acclimatized to their environment for one week prior to experimentation. The animals were randomly distributed into different groups. Each experimental group comprised of a minimum of 10 animals. Mice were moved from the animal care facility to the testing laboratory room; each animal was caged separately after recording its body weight and was randomized to receive the treatments according to a random number table. Each mouse was used only once. The MES test and the rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD), Epilepsy Branch, National Institutes of Health, Bethesda, MD, U.S.A. (Hester 1979a, 1980b). All animal procedures conform to the Provision and General Recommendations of Chinese Experimental Animal Administration Legislation.

4.2. Drugs

QUAN-0806 was synthesized in our laboratory (China). Its structure was characterized using IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis techniques. QUAN-0806 was dissolved in polyethylene glycol-400 (Sigma, USA) and injected intraperitoneally (i.p.), and injection volume (0.05 ml/20 g) was kept constant. PTZ, ISO, and 3-MPA (Sigma, USA) were dissolved in sodium chloride. PTZ and STRYC (Sigma, USA) were administered subcutaneously (s.c.) in the scruff of the neck of the animals, while ISO and 3-MPA were administered intraperitoneally (i.p.). All drugs were freshly prepared prior to sue and injection volume (0.1 ml/20 g) was kept constant. The dosage selection, route of drug administration and time-scheduling of different compounds was based on preliminary experiments and pharmacokinetic considerations.

4.3. Convulsing tests

4.3.1. Maximal electroshock seizure

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. At 30 min after the administration of the compounds, the activities were evaluated in MES test (Hester 1979a, 1980b).

4.3.2. Oral administration on Maximal electroshock seizure

The time-course effect of QUAN-0806 in the MES test was determined. A 100 mg/kg dose of QUAN-0806 suspension in a mixture containing 0.5% methylcellulose was injected periocularly (p.o.) in mice. The mice were divided into 7 groups (n = 7 or 8). Subsequently, the animals were subjected to the MES test at various times: 0.5, 1, 1.5, 2, 3, 4, and 5 h. The peak of protection was observed 2 h after the p.o. injection. Then, QUAN-0806 was evaluated for its oral activity against MES-induced seizures and oral neurotoxicity; phenytoin was used as the reference. The time-to-peak effect (TPE) was maximal at 2 h, which was comparable with that of phenytoin and carbamazepin.

4.3.3. Protective index

Protective index (PI) for QUAN-0806 was calculated by dividing a given TD_{50} value, evaluated in the chimney test, by the respective ED_{50} value determined in the MES test. The PI is considered an index of the margin of safety and tolerability between anticonvulsant doses and doses of QUAN-0806 exerting acute adverse effects in preclinical studies (Löscher and Nolting 1991).

4.3.4. Chemically induced seizures

Mice were given doses of convulsant drugs that could induce seizures at least 97% of animals. The doses used were: PTZ, 85 mg/kg; ISO, 250 mg/kg; 3-MPA, 40 mg/kg; and STRYC, 1.2 mg/kg; 30 mg/kg. The test compounds were administered i.p. to groups of 10 mice 30 min before i.p.

administration of ISO and s.c. injection of PTZ, 3-MPA and STRYC in a dose at which 100% of the animals showed convulsive reactions. The mice were placed in individual cages and observed for 1 h. The dose which prevented 50% of the treated animals from tonic convulsions (ED_{50}) was calculated (Krall et al. 1978; Porter et al. 1984; Bernasconi et al. 1988; Arnoldi et al. 1990; Geurts et al. 1998).

4.3.5. Rotarod test

30 min after the administration of the compounds, the animals were tested on a 1-inch diameter, knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in three trials (Sun et al. 2006).

4.4. Statistics

Groups of 10 mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plots of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slopes of the regression line and the standard error of the slopes were calculated by a computer program written by the National Institute of Neurological Disorders and Stroke.

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