SHORT COMMUNICATIONS

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

Cui Xiangshu[1, 2], Guan Liping[3], Li Hailan[3], Piao Huri[3] and Quan Zheshan[1, 3**]

(1. Key Laboratory of Organic Functional Factors of the Changbai Mountain, Ministry of Education, Yanbian University, Yanji 133002, China; 2. College of Basic Medicine, Yanbian University, Yanji 133000, China; 3. College of Pharmacy, Yanbian University, Yanji 133000, China)

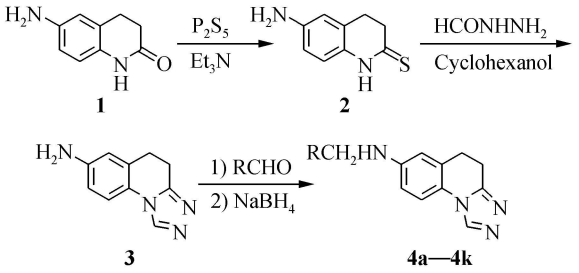
Accepted on January 26, 2007

Abstract A series of 7-substituted-benzylamino-4, 5-dihydro-[1, 2, 4] triazolo[4, 3-a] quinoline derivatives was synthesized and evaluated for their anticonvulsant activity. The subcutaneous pentylenetetrazole test (sc-PTZ) demonstrated that the most effective compound in controlling the sc-PTZ induced seizure was 7-(3-bromine-benzylamino)-4, 5-dihydro-[1, 2, 4] triazolo[4, 3-a] quinoline (**4j**) with an ED_{50} of 5.0 mg/kg and the PI of 20.7, which was also safer than the reference drugs. And the maximal electroshock test (MES) demonstrated that among these derivatives, 7-(3-fluorobenzylamino)-4, 5-dihydro-[1, 2, 4] trizolo[4, 3-a] quinoline (**4i**), with an ED_{50} of 15.3 mg/kg and the PI of 7.2, was the safest in MES test. Furthermore, their neurotoxicities were measured by the rotarod neurotoxicity test, and the results showed that all derivatives possessed lower neurotoxicity.

Keywords: quinoline, triazole, synthesis, anticonvulsant, epilepsia.

In our previous search for the positive inotropic activity of quinolinones[1], we found that 6-alkoxy-3, 4-dihydro-2(1H)-quinoliones derivatives had anticonvulsant activity, among them the compound 6-benzyloxy-3, 4-dihydro-2(1H)-quinolione showed the most potent anticonvulsant activity[2]. Introduction of triazole ring to the first and second position of 6-benzyloxy-3, 4-dihydro-2(1H)-quinolione caused a remarkable increase in the anticonvulsant activity, as seen in 7-benzyloxyl-4, 5-dihydro-[1, 2, 4] triazolo[4, 3-a] quinoline[3].

In order to obtain a novel anticonvulsant agent having more potency and lower neurotoxicity, a series of 7-benzyamine-4, 5-dihydro-[1, 2, 4] triazolo[4, 3-a] quinoline derivatives (compounds **4a—4k**) was designed and synthesized. Here, we report the synthesis and characterization of these compounds by ^{1}H-NMR, MS and elemental analysis. Their anticonvulsant activity was evaluated by the maximal electroshock (MES) test and the subcutaneous pentyenetrezole (sc-PTZ) test, and their neurotoxicity was elicited by the Rotarod test. Target compounds were prepared along the reaction sequence in Fig 1.



4a: R= $CH_2C_6H_5$
4g: R= $CH_2C_6H_4$(*p*-$N(CH_3)_2$)
4b: R= $CH_2C_6H_4$(*p*-CH_3)
4h: R= $CH_2C_6H_4$(*m*-OCH_3)
4c: R= $CH_2C_6H_4$(*p*-Cl)
4i: R= $CH_2C_6H_4$(*m*-F)
4d: R= $CH_2C_6H_4$(*p*-OCH_3)
4j: R= $CH_2C_6H_4$(*m*-Br)
4e: R= $CH_2C_6H_3$(3, 4-Cl_2)
4k: R= $CH_2CH{=}CHC_6H_5$
4f: R= $CH_2C_6H_4$(*p*-F)

Fig. 1. Synthesis of compounds **4a—4k**.

1 Experimental

Melting points were determined in open capillary tubes and were uncorrected. ^{1}H-NMR spectra were measured on a BRUKER AV-300, and all chemical shifts were given in ppm relative to tetramethysilane.

* Supported by National Natural Science Foundation of China (Grant No. 30460151)

** To whom correspondence should be addressed. E-mail: zsquan@ybu.edu.cn

Mass spectra were measured on an HP1100LC. Elemental analysis was performed on a Pekin-Elmer 204Q. All reagents were of analytic or chemical purity grade.

1.1 Synthesis

1.1.1 Synthesis of 6-amino-3, 4-dihydro-2(1*H*) quinolinethione (2)

In a three-necked round-bottomed flask containing 20 mL triethylamine and 30 mL aceonitrile, P_2S_5 1.3 g (5.8 mmol) was added slowly in an ice bath with stirring. After being dissolved, 6-amino-3, 4-dihydro-2(1*H*)quinoline (1) 0.81 g (4.8 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. The solid obtained after filtration was recrystallized in EtOAC-hexane (1∶3) to afford a yellow solid of 0.74 g (84%) with mp 158—160 ℃. ^{1}H-NMR ($CDCl_3$) δ: 2.62 (t, 2H, J = 7.0 Hz, CH_2), 2.83 (t, 2H, J = 7.0 Hz, CH_2), 6.40—6.70 (m, 3H, Ar-H), 11.8 (s, 1H, NH). MS: (M+1): 179. Analytical calculation for $C_9H_{10}N_2S$: C, 60.64; H, 5.65; N, 15.72. Found: C, 60.52; H, 5.79; N, 15.48.

1.1.2 Synthesis of 7-amino-4, 5-dihydro-[1, 2, 4] triazolo[4, 3-a] quinoline (3)

A mixture of 6-amino-3, 4-dihydro-2(1*H*)quinolinethione (2) 0.5 g (2.8 mmol), formic anhydrazine 0.21 g (3.4 mmol) and cyclohexanol 20 mL was refluxed for 6 h in a nitrogen atmosphere. the solvent was removed under reduced pressure and the residue was extracted with dichloromethane. The dichloromethane layer was washed with water three times and dried over with anhydrous $MgSO_4$. After the solvent was removed under reduced pressure, the product was purified by silican gel column chromatography (dichloromethane∶methanol = 10∶1). A yellow solid (0.36 g, 65%) with mp 161—162 ℃ was obtained (decomposed). IR (KBr): cm^{-1} 3337, 1506. ^{1}H-NMR ($CDCl_3$) δ: 2.87 (t, 2H, J = 7.2 Hz, CH_2), 2.97 (t, 2H, J = 7.2 Hz, CH_2), 6.70—7.29 (m, 3H, C_6H_3), 8.52 (s, 1H, N-N=C-H). MS: (M+1) 201.

1.1.3 General procedure for the synthesis of 7-benzylamine-4, 5-dihydro -[1, 2, 4] triazole [4, 3-a] quinolines (**4a—4k**)

7-Amino-4, 5-dihydro-[1, 2, 4] triazolo[4, 3-a] quinoline (3) 2.01 g (0.01 mol) and benzene 30 mL were placed in a three-necked round-bottomed bottle equipped with a dropping funnel and water trap. The mixture was heated to reflux, to which the suitable substituted-benzaldehyde (0.01 mol) was added dropwise with stirring, refluxed until no water came out. Solvents were removed under reduced pressure. The residue was dissolved in methanol, and sodium borohydride (0.012 mol) was added slowly in a ice bath. The mixture was allowed to stir at room temperature for 2h. After evaporation of solvent, the residue was extracted with 30 mL dichloromethane twice, washed with water twice, and dried over with anhydrous $MgSO_4$. After removing the solvent under reduced pressure, the product was purified by silican gel column chromatography (dichloromethane∶methanol = 10∶1), producing compounds **4a—4k**. The data of yield, melting point, elemental analysis, MS and ^{1}H-NMR are listed in Tables 1 and 2.

Table 1. Yield, melting point and elemental analysis data of compounds **4a—4k**

Compd.	R	Yield (%)	m. p. (℃)	Elemental analysis found (Calcd.) %		
				C	H	N
4a	$-CH_2C_6H_5$	71	149—151	73.89 (73.75)	5.84 (6.01)	20.27 (20.06)
4b	$-CH_2C_6H_4$(*p*-CH_3)	64	118—120	74.46 (74.28)	6.25 (6.41)	19.30 (19.03)
4c	$-CH_2C_6H_4$(*p*-Cl)	72	169—171	65.70 (65.93)	4.86 (4.68)	11.41 (11.12)
4d	$-CH_2C_6H_4$(*p*-OCH_3)	54	148—150	70.57 (70.60)	5.92 (5.89)	18.29 (18.03)
4e	$-CH_2C_6H_3$(3, 4-Cl_2)	75	176—180	59.14 (59.19)	4.09 (4.12)	16.23 (16.11)
4f	$-CH_2C_6H_4$(*p*-F)	70	172—174	69.37 (69.15)	5.14 (5.30)	9.04 (9.12)
4g	$-CH_2C_6H_4$(*p*-$N(CH_3)_2$)	53	194—196	71.45 (71.31)	6.63 (6.78)	21.93 (21.79)
4h	$-CH_2C_6H_4$(*m*-OCH_3)	57	88—90	70.57 (70.32)	5.92 (6.21)	18.29 (18.16)
4i	$-CH_2C_6H_4$(*m*-F)	64	102—104	69.37 (69.09)	5.14 (5.37)	9.04 (8.89)
4j	$-CH_2C_6H_4$(*m*-Br)	65	99—101	57.48 (57.56)	4.26 (4.41)	15.77 (15.59)
4k	$-CH_2CH=CHC_6H_5$	72	134—136	75.44 (75.23)	6.96 (7.12)	17.60 (17.42)

Table 2. MS and ^{1}H-NMR data of compounds **4a**—**4k**

Compd.	MS (m/z) $[M+H]^+$	^{1}H-NMR (δ)
4a	277	2.87 (t, 2H, J = 7.2 Hz, CH_2), 3.06 (t, 2H, J = 7.2 Hz, CH_2), 4.29 (s, 2H, $NHCH_2Ar$), 6.50—7.30 (m, 8H, C_6H_3, C_6H_5), 8.43 (s, 1H, N=C-H)
4b	291	2.27 (s, 3H, $ArCH_3$), 2.85 (t, 2H, J = 7.0 Hz, CH_2), 2.99 (t, 2H, J = 7.0 Hz, CH_2), 4.24 (s, 2H, $NHCH_2Ar$), 6.51—7.42 (m, 7H, C_6H_3, C_6H_4), 9.0 (s, 1H, N=C-H)
4c	312	2.27 (s, 3H, $ArCH_3$), 2.85 (t, 2H, J = 7.0 Hz, CH_2), 2.99 (t, 2H, J = 7.0 Hz, CH_2), 4.24 (s, 2H, $NHCH_2Ar$), 6.51—7.42 (m, 7H, C_6H_3, C_6H_4), 9.0 (s, 1H, N=C-H)
4d	307	2.96 (t, 2H, J = 7.2 Hz, CH_2), 3.17 (t, 2H, J = 7.2 Hz, CH_2), 3.82 (s, 3H, m-$ArOCH_3$), 4.30 (s, 2H, $NHCH_2Ar$), 6.56—7.32 (m, 7H, C_6H_3, C_6H_4), 8.51 (s, 1H, N=C-H)
4e	326	2.95 (t, 2H, J = 7.1 Hz, CH_2), 3.17 (t, 2H, J = 7.1 Hz, CH_2), 4.36 (s, 2H, $NHCH_2Ar$), 6.53—7.47 (m, 6H, C_6H_3, C_6H_3), 8.52 (s, 1H, N=C-H)
4f	295	2.91 (t, 2H, J = 7.0 Hz, CH_2), 3.17 (t, 2H, J = 7.0 Hz, CH_2), 4.32 (s, 2H, $NHCH_2Ar$), 6.50—7.34 (m, 7H, C_6H_3, C_6H_4), 8.50 (s, 1H, N=C-H)
4g	319	2.97 (t, 2H, J = 7.1 Hz, CH_2), 3.17 (m, 8H, $(CH_3)_2N$, CH_2), 4.36 (s, 2H, $NHCH_2Ar$), 6.53—7.47 (m, 7H, C_6H_3, C_6H_4), 8.52 (s, 1H, N=C-H)
4h	307	2.96 (t, 2H, J = 7.2 Hz, CH_2), 3.17 (t, 2H, J = 7.2 Hz, CH_2), 3.82 (s, 3H, m-$ArOCH_3$), 4.35 (s, 2H, $NHCH_2Ar$), 6.56—7.32 (m, 7H, C_6H_3, C_6H_4), 8.51 (s, 1H, N=C-H)
4i	295	2.95 (t, 2H, J = 7.3 Hz, CH_2), 3.17 (t, 2H, J = 7.3 Hz, CH_2), 4.39 (s, 2H, $NHCH_2Ar$), 6.54—7.37 (m, 7H, C_6H_3, C_6H_4), 8.51 (s, 1H, N=C-H)
4j	355	2.95 (t, 2H, J = 7.3 Hz, CH_2), 3.16 (t, 2H, J = 7.3 Hz, CH_2), 4.36 (s, 2H, $NHCH_2Ar$), 6.54—7.45 (m, 7H, C_6H_3, C_6H_4), 8.52 (s, 1H, N=C-H)
4k	319	2.95 (t, 2H, J = 7.2 Hz, CH_2), 3.17 (t, 2H, J = 7.2 Hz, CH_2), 3.98 (d, 2H, J = 5.1 Hz, CH_2NH), 6.32 (dt, 1H, J = 15.8, 5.1 Hz, ArCH=C-H), 6.61—7.40 (m, 9H, C_6H_3, C_6H_5CH=), 8.52 (s, 1H, N=C-H)

1.2 Pharmacology

The MES test, sc-PTZ test, and the rotarod test were carried out by the standard procedures described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA)[4,5]. All compounds, dissolved in polyethylene glycol-400, were evaluated for anticonvulsant activities in C57B/6 mice (body weight 18—25 g). In Phase Ⅰ screening (Table 3), each compound was administered at the dose ages of 30, 100, and 300 mg/kg for evaluating the anticonvulsant activity, and its neurotoxicity was measured at 30 min and 4 h after administration. Anticonvulsant efficacy was measured in the MES test and the sc-PTZ test. In the MES test, seizures were elicited with a 60 Hz alternating current of 50 mA 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 sc-PTZ test was carried out by the subcutaneous injection of a convulsant dose (CD_{97}) of pentylenetetrazol (85 mg/kg in mice). The elevation of the pentylenetrazol-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 3. Phase Ⅰ anticonvulsant and toxicity data in mice

Compd.	scPTZ		MES		Rotarod toxicity	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
4a	30[a)]	—	30	—	100	—
4b	30	—	30	—	100	—
4c	300	—	300	—	—	—
4d	30	—	30	—	300	—
4e	300	—	300	—	—	—
4f	30	—	30	—	100	—
4g	300	—	300	—	—	—
4h	100	—	100	—	300	—
4i	30	—	30	—	100	—
4j	30	—	30	—	100	—
4k	30	—	30	—	100	—

a) Dose is in mg/kg; — means no activity at 300 mg/kg

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 tested compound until at least three points were established in the range of 10%—90% seizure protection or minimal observed neurotoxicity. From the plot of this 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 National Institute of Neurological Disorders and Stroke. These data are shown in Table 4, which also includes the control data with marketed antiepileptic drugs such as phenytoin, carbamazepine, phenobarbital, and valproate.

Table 4. Phase Ⅱ quantitative anticonvulsant date in mice

Compd.	ED_{50} [a]		TD_{50}	PI [b]	
	scPTZ	MES		scPTZ	MES
Ⅱ	24.0 (21.6—26.7)	17.3 (14.8—20.4)	61.4 (51.4—73.3)	2.6	3.5
4a	11.2 (10.3—12.1)	19.7 (18.0—21.6)	81.8 (75.4—88.8)	7.3	4.1
4d	11.4 (10.4—12.5)	29.3 (26.7—32.1)	106.4 (101.8—111.3)	9.3	3.6
4f	16.2 (14.9—17.6)	28.3 (25.8—31.0)	102.4 (98.2—106.9)	6.3	3.6
4i	9.9 (9.0—10.9)	15.3 (24.3—9.6)	109.8 (102.5—117.5)	11.0	7.2
4j	5.0 (4.5—5.4)	20.5 (18.8—22.3)	102.3 (93.9—111.4)	20.7	5.0
4k	20.5 (18.6—22.4)	32.9 (30.2—35.8)	122.8 (117.0—128.9)	6.0	3.7
Phenytoin	＞300	9.5 (8.1—10.4)	65.5 (52.5—72.9)	—	6.9
Carbamazepin	＞100	8.8 (5.5—14.1)	71.6 (45.9—135)	—	8.1
Phenobarbital	13.2 (5.8—15.9)	21.8 (21.8—25.5)	69.0 (62.8—72.9)	5.2	3.2
Valproate	149 (123—177)	272 (247—338)	426 (369—450)	2.9	1.6

a) Doses was in mg/kg; b) $PI = TD_{50}/ED_{50}$

2 Result and discussion

Compounds were prepared according to Scheme 1. Compound **1** 6-amino-3, 4-dihydro-2(1*H*)quinoline was prepared from aniline and 3-chloropropanoyl chloride using the method described previously[6, 7]. Compound **2** was prepared by reacting of compound **1** with phosphorous pentasulfide in acetonitrile in the presence of triethylamine, which reacted further with formic anhydrazine in cyclohexanol to afford compound **3**[8, 9]. 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**—**4k** were obtained through the reduction-alkylation reaction of compound **3** with suitable benzaldehyde in benzene and further reduced by sodium borohydride in methanol at room temperature in moderate yields.

The designed target compounds contain the attract electron, which were 4-Cl, 3, 4-2Cl, 4-F, 3-F, 3-Br and confess electron, which were 4-CH_3, 4-OCH_3, 3-OCH_3, Ph-CH=CH at the phenyl ring and so on. The initial evaluation (Phase Ⅰ) of the anticonvulsant activity is presented in Table 1, which shows that most compounds at the doses of 30 mg/kg or below have the activity. The structure-activity relationships were analyzed, which were the confess electron of p-OCH_3, p-CH_3 and the attract electron of p-F, m-F, m-Br, which were the weakest for 3, 4-2Cl, 4-Cl and 4-N$(CH_3)_2$ in the MES-induced seizure. The results of the subcutaneous pentylenetetrazole test are presented in Table 2. The compounds with benzylamine into the position seven of 4, 5-dihydro-[1, 2, 4] trizolo[4, 3, -a] quinoline have an increased anticonvulsant activity when compared with the leading compound in sc-PTZ. Among these, 7-(3-Bromobenzyl-amine)-4, 5-dihydro-[1, 2, 4] trizolo [4, 3, -a] quinoline (compound **4j**) was the most effective one and the leading compound Ⅱ, with an ED_{50} value of 5.0 mg/kg and the protective index ($PI = ED_{50}/TD_{50}$) value of 20.7 in the sc-PTZ test, and was the safest. In general, the sc-PTZ-induced seizure is the model of petitmal seizure. If the compound has obvious activity against the sc-PTZ, it is an effective drug of healing petitmal seizure[10]. Compared with the positive control drugs such as phenytion, carbamazepin, phenobarbital and valproate, compound **4j** was not only more effective but safer.

The results of quantitative anticonvulsant evaluation (phase II) showed that the compounds **4a**, **4i** and **4j**, with the ED_{50} values of about 19.7 mg/kg, 15.3 mg/kg, 20.5 mg /kg respectively in the MES test, were more effective than the others. Their anticonvulsant activity was superior to that of valproate (one of the control drugs in our present work) and comparable to that of phenobarbital in the MES test. 7-(3-Fluorobenzylamine)-4, 5-dihydro-[1, 2, 4] trizolo[4, 3, -a] quinoline (compound **4i**), with an ED_{50} value of 15.3 mg/kg and the protective index (PI = ED_{50}/ TD_{50}) value of 7.2 (the greatest PI values), was the safest among the synthesized compounds in the MES test. When 4-fluoro was substituted at the benzyl of compound **4i**, its anticonvulsant activity was comparable to trizolo compound on the structure-activity relationships in our previous search. A fluorine atom into the phenyl of the derivatives can exhibit the most anticonvulsant activity[11], which changes the electronic distribution and increases their receptor binding, leading to an activity of an organic compound.

The neurotoxicity of the target compounds was weaker than that of the leading compound Ⅱ and phenytion, carbamazepin, Phenobarbital, with the TD_{50} values of 81.8—122.8 mg/kg and the protective index (PI) value of 3.5 in the sc-Met test, which was superior to the leading compound Ⅱ with PI of 3.6—5.6.

In conclusion, compound Ⅱ is the leading compound used in this study. The anticonvulsant activity of its derivatives 7-substituted-benzylamine)-4, 5-dihydro-[1, 2, 4] trizolo[4, 3, -a] quinoline (compound **4i**) was analyzed. The target compound was superior to the leading compound and the control drugs in the sc-PTZ test, and its neurotoxicity was weaker.

References

1 Piao H R, Quan Z S, Xu M X, et al. Synthesis and positive inotropic activities of 3, 4-dihydro-6-(4-substituted-1-piperazinylacetamin)-2(1*H*)-quinolinone derivatives. Chin J Med Chem, 1999, 9: 79—83

2 Quan Z S, Wang C S and Jun W Y. Synthesis of 6-alkyloxyl-3, 4-dihydro-2(1H)-quinoliones and their anticonvulsant activities. Bull Kor Chem Soc, 2005, 26: 1757—1760

3 Cui L J, Xie Z F, Piao H R, et al. Synthesis and anticonvulsant activity of 1-substituted-7-benzyloxy-4, 5-dihydro-[1, 2, 4] triazolo [4, 3-a] quinoline. Biol Pharm Bull, 2005, 28: 1216—1220

4 Krall R J, Penry J K, White B G, et al. Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia, 1978, 19: 409—428

5 Poter R J, Cereghino J J and Gladding G D. Antiepileptic drug development program. Cleveland Clin Q, 1984, 51: 293—305

6 Wu C X, Dai L Y, Chen Y Q, et al. A new method for synthsis of 6-hydroxy-3, 4-dihydro-2(1*H*) quinolinone. Hua Xue Tong Bao, 2003, 66: 337—339

7 Wu C X, Dai L Y, Chen Y Q, et al. Synthesis of 6-amino-3, 4-dihydro-2(1*H*)-quinolinone. Chin J Pharm, 2004, 35, 522—523

8 Hestrer J B, Rudzik A D and Kamdar B. 6-Phenyl-4H-s-triazolo[4, 3-a][1, 4] benzodiazepines which have central nervous system depressant activity. J Med Chem, 1971, 14: 1078—1081

9 Chen F E and Liu X Q. Synthesis of alprazolam. Chin J Pharm, 1990, 21: 345—346

10 Xu S Y, Bian R L and Chen X. The Empirical Method of the Pharmacology (the Second Edition). Beijing: The People Health Publishing Company, 1991, 676—677

11 Wang J M, Jun C S, Chai K Y, et al. Synthesis and anticonvulsant activity of 1-substituted benzyl-*N*-substituted-1, 2, 3-triazole-4-formamides. Progress in Natural Science, 2006, 16(9): 925—929