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Original article

Synthesis of novel 7-benzylamino-2*H*-1,4-benzoxazin-3(4*H*)-ones as anticonvulsant agents

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Abstract

A series of 7-benzylamino-2*H*-1,4-benzoxazin-3(4*H*)-ones were synthesized using 2-amino-5-nitrophenol as a starting material. Their anticonvulsant activities were evaluated by the maximal electroshock test (MES test) and their neurotoxicity was evaluated by the rotarod neurotoxicity test (Tox.). The MES test showed that 7-(4-fluorobenzylamino)-2*H*-1,4-benzoxazin-3(4*H*)-one **4b** was the most potent with ED_{50} value of 31.7 mg/kg and protective index ($PI = TD_{50}/ED_{50}$) value of 7.2. To explain the possible mechanism of anticonvulsant activity, the compound **4b** was tested in sc-PTZ test, isoniazid test and strychnine test.

Keywords: 1,4-Benzoxazin-3(4*H*)-one; Anticonvulsant; MES; sc-PTZ; Isoniazid; Strychnine

1. Introduction

1,4-Benzoxazin-3(4*H*)-one derivatives have shown various biological activities, such as anti-inflammatory [1], antiulcer [2], antiplatelet [3], antihypertensive [4], and antifungal [5], antimicrobial [6], neuropeptide Y (NPY) Y5 receptor antagonists [7], serotonin reuptake inhibitors [8], prostacyclin receptor agonists, etc. [9]. The synthesis and anticonvulsant activity of compounds **4**–**7** in this paper, however, has not been reported.

In our search for new compounds with anticonvulsant activity, 2*H*-1,4-benzoxazin-3(4*H*)-one (compound **I**) showed a slight positive anticonvulsant activity with an effective dose of 300 mg/kg in the anti-MES test. In order to obtain compounds with better anticonvulsant activity, we synthesized 7-benzylamino-2*H*-1,4-benzoxazin-3(4*H*)-ones (**4a**–**4n**) using 2*H*-1,4-benzoxazin-3(4*H*)-one (**I**) as the lead compound. The hypothesis was that the introduction of a substituted benzylamino into the 7th position of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one would increase the lipophilic property of the compounds and increase their permeability to the blood–brain barrier thus probably enhances their anticonvulsant activity. The new compounds were evaluated as anticonvulsant agents in experimental epilepsy models, i.e., maximal electroshock test (MES), the most active compound (**4b**) was tested in pentylenetetrazole (sc-PTZ) test, isoniazid test and strychnine test, and the possible mechanism of action was conjectured.

2. Result and discussion

2.1. Chemistry

Target compounds **4a**–**4n** were synthesized according to Scheme 1. 7-Nitro-2*H*-1,4-benzoxazin-3(4*H*)-one (compound **2**) was prepared using 2-amino-5-nitrophenol (compound **1**) as starting material, which reacted further with 2-chloroacetyl chloride, reacting time was 4 h with yield of 75% [15]. Based on the reference, TEBA was added as

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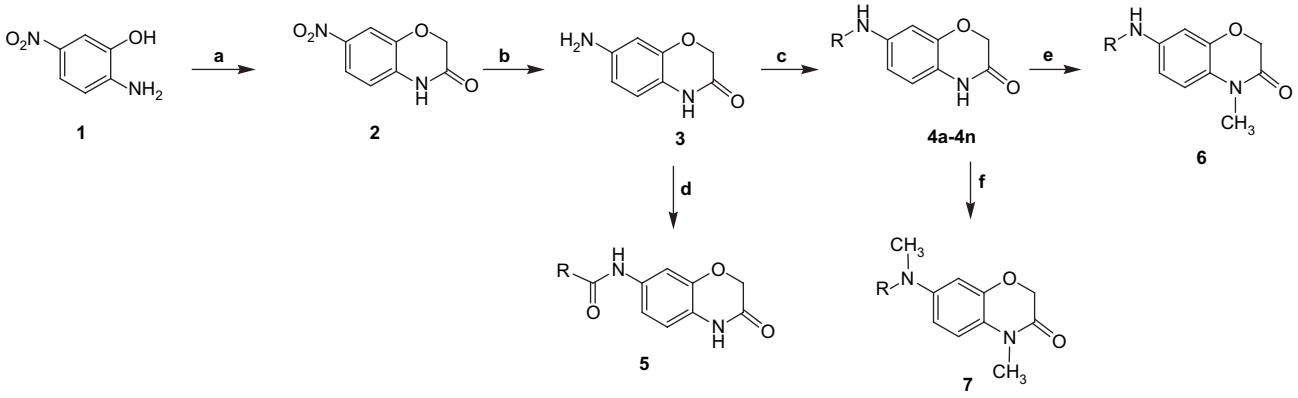


Scheme 1. The synthesis route of target compounds.

activator and refluxing for 2 h, the yield was increased to 87% which was consonant with the reported method [16].

7-Nitro-2*H*-1,4-benzoxazin-3(4*H*)-one (compound **2**) was reduced by iron powder in hydrochloride condition to product 7-amino-2*H*-1,4-benzoxazin-3(4*H*)-one (compound **3**). Compounds **4a**–**4n** were obtained through the condensation reaction of compound **3** with suitable benzaldehyde in benzene and further reduced by sodium borohydride in methanol at room temperature in moderate yields.

Compound **5** was synthesized by 7-amino-2*H*-1,4-benzoxazin-3(4*H*)-one (compound **3**) and benzoyl chloride. Synthesis of compounds **6** and **7** was achieved by reacting compound **4b** with dimethyl sulfate [17]. These, however, were reacted in different temperature. Compounds **6** and **7** were obtained at 35–40 °C and 110–115 °C, respectively.

2.2. Pharmacology

The results of pharmacology test of all synthesized compound and reference drugs were shown in Table 1. Most of compounds showed remarkable anticonvulsant activity. Compound **I** exhibited anti-MES effect only under the high dose of 300 mg/kg, whereas compounds **4a**–**4e**, **4g**, **4i**, **4j**, **4m** and **4n** showed anti-MES effect at the dose of <150 mg/kg. We reason that the increase in anticonvulsant activity might be due to their easier passage across biological membranes after the introduction of the alkylamino at the 7th position of compound **I**.

Analyzing the activities of synthesized compounds **4a**–**4n**, the following SAR was gained.

Generally, the anticonvulsant activity of organic compound may be increased remarkably after the introduction of a halogen atom. So, some halogen substituted derivatives were designed and synthesized in this paper. Comparison of the halogen substituted derivatives indicated that different halogen atoms contributed to the anticonvulsant activity in the order of F > Cl > Br; the introduction of atom F on the benzyl ring gained stronger activity.

Compared the derivatives with different F-substituted position on the benzyl ring, their activity order was *p*-F > *o*-F > *m*-F. *p*-F substituted derivative **4b** (7-(4-fluorobenzylamino)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one) was the strongest in all tested compounds with ED_{50} value of 31.7 mg/kg, and exhibited the lowest neurotoxicity with TD_{50} value of 228.2 mg/kg, a higher protective index ($PI = TD_{50}/ED_{50}$, 7.2) was gained than reference drug Phenytoin Sodium. And activity order of the atom Cl substituted derivatives was consistent with atom F substituted derivatives, it was *p*-Cl > *o*-Cl > , *m*-1Cl, 2,4-Cl_2.

Five electron-donor derivatives were also designed and prepared, containing *p*-CH_3, *p*-OH, *p*-OCH_3, *p*-OC_2H_5, 3,4-OCH_2O–. The pharmacology test revealed that their activities were lower than halogen substituted derivatives and the activity order was 3,4-OCH_2O– > *p*-OC_2H_5 > *p*-CH_3 > *p*-OH, *p*-OCH_3.

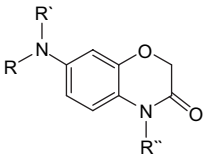
7-Benzoylamino derivative **5** showed distinctly lower anticonvulsant activity than 7-benzylamino derivative **4a** with ED_{50} value higher than 150 mg/kg. This may be due to the lipophilic property that was decreased for the existence of carbon group, decreased their permeability to the blood–brain barrier thus probably weakened their anticonvulsant activity.

Compound **4b** was methylized to products 4-methyl derivatives **6** and **7**, which were tested as potent anticonvulsant agents. But the anticonvulsant activity decreased obviously after this change. 4-Methyl blocked the combination of amide and receptor caused a decreased activity. The result proved that the amide structure in benzoxazin-3(4*H*)-one may be the active center combined with the receptor, which was essential group for the anticonvulsant.

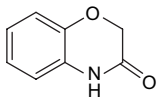
Furthermore, the most active compound **4b** was tested against the convulsant induced by the chemical substances, containing PTZ, Isoniazid and Strychnine. Compound **4b** was administered i.p. at dose of 100 mg/kg, which was between the ED_{50} and TD_{50} value. Reference drug Carbamazepine was administered i.p. at dose of 50 mg/kg.

As shown in Table 2, compound **4b** completely inhibited the clonic seizures induced by sc-PTZ, but the reference drug did not show any inhibition activity. The results in the

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



4a-4n, 5-7



I

Compound	R	R′	R″	MES,[a] ED_{50} (mg/kg)	TD_{50}[b] (mg/kg)	PI[c]
I	—	—	—	300	>300	—
4a	$-CH_2C_6H_5$	−H	−H	63.4 (39.78−101.01)	182.5 (114.53−290.81)	2.9
4b	$-CH_2C_6H_4$(*p*-F)	−H	−H	31.7 (19.89−50.50)	228.2 (143.21−363.63)	7.2
4c	$-CH_2C_6H_4$(*m*-F)	−H	−H	76.1 (47.74−121.22)	228.2 (143.21−363.63)	3.0
4d	$-CH_2C_6H_4$(*o*-F)	−H	−H	54.8 (34.37−87.28)	182.6(114.25−290.11)	3.3
4e	$-CH_2C_6H_4$(*p*-Cl)	−H	−H	65.7 (41.23−104.69)	>300	>4.6
4f	$-CH_2C_6H_4$(*m*-Cl)	−H	−H	>150	—[d]	—
4g	$-CH_2C_6H_4$(*o*-Cl)	−H	−H	91.3 (57.29−145.47)	>300	>3.3
4h	$-CH_2C_6H_3$(2,4-Cl_2)	−H	−H	>150	—[d]	—
4i	$-CH_2C_6H_4$(*p*-Br)	−H	−H	131.5 (82.49−209.46)	>300	>2.3
4j	$-CH_2C_6H_4$(*p*-CH_3)	−H	−H	131.5 (82.49−209.46)	>300	>2.3
4k	$-CH_2C_6H_4$(*p*-OH)	−H	−H	>150	—[d]	—
4l	$-CH_2C_6H_4$(*p*-OCH_3)	−H	−H	>150	—[d]	—
4m	$-CH_2C_6H_4$(*p*-OC_2H_5)	−H	−H	91.3 (57.29−145.47)	>300	3.3
4n	$-CH_2C_6H_4$(3,4-OCH_2O−)	−H	−H	63.4 (39.79−101.03)	>300	>4.7
5	$-COC_6H_5$	−H	−H	>150	—[d]	—
6	$-CH_2C_6H_4$(*p*-F)	−H	$-CH_3$	>150	—[d]	—
7	$-CH_2C_6H_4$(*p*-F)	$-CH_3$	$-CH_3$	91.3 (57.29−145.47)	>300	>3.3
Phenytoin				9.5 (8.1−10.4)	65.5 (52.5−72.9)	6.9
Carbamazepine				9.2 (6.9−11.7)	74.4 (59.1−87.5)	8.1

[a] Maximal electroshock test.
[b] Minimal meurotoxicity was determined by the rotarod test after the tested compounds were administrated 30 min.
[c] PI = TD_{50}/ED_{50}.
[d] Minimal meurotoxicity was not tested.

Table 3 demonstrated that reference drug Carbamazepine inhibited the clonic seizures, tonic seizures and death induced by Isoniazid at the rate of 10%, 10% and 0%, respectively. But compound **4b** completely controlled the clonic seizures, tonic seizures and death induced by Isoniazid.

As PTZ and Isoniazid have been shown to interact with the GABA neurotransmitter and the GABA receptor complex [18−20], antagonism of PTZ and Isoniazid-induced seizures suggests that the compound **4b** might have effects on GABA-ergic neurotransmission.

On the other hand, in the strychnine-induced seizure model (Table 4), the control mice were administered vehicle only and all animals died caused by strychnine. Compound **4b** inhibited the clonic seizures, tonic seizures and death by 40%, 30% and 10%, respectively, and this was consistent with reference drug Carbamazepine. It is known that strychnine directly antagonize the inhibitory spinal reflexes of glycine [21]. Compound **4b** therefore, might cause seizure suppression by acting on glycine inhibitory mechanisms.

In conclusion, in the present study, through the introduction of substituted benzylamino to the 7th position of 2*H*-1,4-benzoxazin-3(4*H*)-one, we found that 7-(4-fluorobenzylamino)-2*H*-1,4-benzoxazin-3(4*H*)-one (**4b**) possessed the most potent anticonvulsant acitivity with ED_{50} value of 31.7 mg/kg and PI value of 7.2. Compound **4b** showed antagonism of PTZ and Isoniazid-induced seizures suggests that the compound **4b** might have effects on GABA-ergic neurotransmission and glycine system.

3. Experimental section

3.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730. ^{1}H NMR spectra were measured on a AV-300 (Bruker, Switzerland), and all chemical shifts were given in parts per million relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). Elemental analyses were performed on a 204Q CHN (Perkin−Elmer, USA). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer. The major chemicals were purchased from Alderich Chemical Corporation. All other chemicals were of analytical grade.

Table 2
Effect of compound **4b** on PTZ-induced convulsant in mice

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO		0.5	100	20	10
Carbamazepine	50	0.5	100	20	0
4b	100	0.5	0	0	0

Table 3
Effect of compound **4b** on Isoniazid-induced convulsant in mice

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO		0.5	100	100	100
Carbamazepine	50	0.5	10	10	0
4b	100	0.5	0	0	0

*3.1.1. 7-Nitro-2H-1,4-benzoxazin-3(4H)-one (**2**)*

Compound **1** (10 g, 0.065 mol), sodium bicarbonate (21.8 g, 0.26 mol) and TEBA (14.8 g, 0.065 mol) were placed into a round-bottomed flask containing 200 ml of acetonitrile, and the mixture was put in an ice–salt bath to keep the temperature 0–5 °C, then, 2-chloroacetyl chloride (9.5 g, 0.084 mol) was added with moderate speed. 2-Chloroacetyl chloride (9.5 g, 0.084 mol) was added with moderate speed and reacted for 40 min. Replaced the ice–salt bath with oil-bath, heated to reflux for 2 h, then remove the solvent under reduced pressure. The resultant product was purified by recrystallization with ethanol–water (1:1). M.p. 219–222 °C, yield = 87%. ^{1}H NMR (DMSO, 300 MHz) δ 4.48 (s, 2H, $-COCH_2$), 7.69 (d, $J = 7.3$ Hz, 1H, C_8–H), 7.75 (d, $J = 9.0$ Hz, 1H, C_5–H), 8.30 (d, $J = 9.0$ Hz, 1H, C_6–H), 9.85 (s, 1H, CO–NH).

*3.1.2. 7-Amino-2H-1,4-benzoxazin-3(4H)-one (**3**)*

Compound **2** (8.84 g, 0.046 mol) and iron powder (7.7 g, 0.138 mol) were placed into a three-necked round-bottomed flask containing 200 ml ethanol (95%). The mixture was stirred and heated with a blade stirrer and oil-bath until the temperature attains 80–85 °C. Then 4 ml of hydrochloric acid (1.2 M) was added and the mixture was stirred at 80–85 °C for 4 h. The mixture was filtered immediately with a hot filter under reduced pressure. Adjusting the filtrate's pH value to 7–8 using saturated $NaHCO_3$ resulted in precipitation of solid **3** (5.6 g). M.p. 212–215 °C, yield = 75%. ^{1}H NMR (DMSO, 300 MHz) δ 4.41 (s, 2H, $-COCH_2$), 4.88 (s, 2H, $-NH_2$), 6.17–6.57 (m, 3H, Ar–H), 10.27 (s, 1H, –CONH).

*3.2. General procedure for the preparation of 7-benzylamino-2H-1,4-benzoxazin-3(4H)-ones (**4a–4n**)*

Compound **3** (2 g, 0.012 mol) was placed into a round-bottomed flask containing 120 ml methanol. Benzaldehydes (0.015 mol) diluted by methanol was dropwise added into the mixture. After stirring for 1–4 h, $NaBH_4$ (1.76 g, 0.038 mol) was divided into multiple portions and added one portion at a time. When $NaBH_4$ addition was over (about 2–4 h), the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (30 ml), washed with water (30 ml × 3) and dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product, which was purified by silica gel column chromatography with ethyl acetate–petroleum ether (1:1 or 2:1) to a solid.

Table 4
Effect of compound **4b** on Strychnine-induced convulsant in mice

Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO		0.5	100	100	100
Carbamazepine	50	0.5	40	30	0
4b	100	0.5	40	30	10

*3.2.1. 7-(Benzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4a**)*

M.p. 138–140 °C; yield 94%. ^{1}H NMR (DMSO, 300 MHz) δ 4.18 (s, 2H, Ar–CH_2), 4.40 (s, 2H, $-COCH_2$), 6.10–7.37 (m, 8H, Ar–H), 10.26 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3408 (NH), 1703 (C=O). MS *m/z* 255 (M + 1). Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02. Found: C, 71.07; H, 5.36; N, 10.78.

*3.2.2. 7-(4-Fluorobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4b**)*

M.p. 170–172 °C; yield 92%. ^{1}H NMR (DMSO, 300 MHz) δ 4.23 (s, 2H, Ar–CH_2), 4.44 (s, 2H, $-COCH_2$), 6.24–7.37 (m, 7H, Ar–H), 10.26 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3421 (NH), 1689 (C=O). MS *m/z* 273 (M + 1). Anal. Calcd for $C_{15}H_{13}FN_2O_2$: C, 66.17; H, 4.81; N, 10.29. Found: C, 66.31; H, 5.12; N, 10.37.

*3.2.3. 7-(3-Fluorobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4c**)*

M.p. 134–136 °C; yield 51%. ^{1}H NMR (DMSO, 300 MHz) δ 4.27 (s, 2H, Ar–CH_2), 4.45 (s, 2H, $-COCH_2$), 6.23–7.31 (m, 7H, Ar–H), 10.25 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3421 (NH), 1689 (C=O). MS *m/z* 273 (M + 1). Anal. Calcd for $C_{15}H_{13}FN_2O_2$: C, 66.17; H, 4.81; N, 10.29. Found: C, 66.40; H, 4.59; N, 10.39.

*3.2.4. 7-(2-Fluorobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4d**)*

M.p. 136–138 °C; yield 38%. ^{1}H NMR (DMSO, 300 MHz) δ 4.16–4.24 (m, 2H, Ar–CH_2), 4.40 (s, 2H, $-COCH_2$), 6.0–7.3 (m, 7H, Ar–H), 10.27 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3421 (NH), 1689 (C=O). MS *m/z* 273 (M + 1). Anal. Calcd for $C_{15}H_{13}FN_2O_2$: C, 66.17; H, 4.81; N, 10.29. Found: C, 66.41; H, 4.92; N, 10.34.

*3.2.5. 7-(4-Chlorobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4e**)*

M.p. 138–140 °C; yield 44%. ^{1}H NMR (DMSO, 300 MHz) δ 4.19 (s, 2H, Ar–CH_2), 4.40 (s, 2H, $-COCH_2$), 6.14–7.93 (m, 7H, Ar–H), 10.27 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3415 (NH), 1678 (C=O). MS *m/z* 290 (M + 1). Anal. Calcd for $C_{15}H_{13}ClN_2O_2$: C, 62.40; H, 4.54; N, 9.70. Found: C, 62.46; H, 4.21; N, 9.86.

*3.2.6. 7-(3-Chlorobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4f**)*

M.p. 172–174 °C; yield 39%. ^{1}H NMR (DMSO, 300 MHz) δ 4.20 (s, 2H, Ar–CH_2), 4.40 (s, 2H, $-COCH_2$), 6.16–7.37 (m, 7H, Ar–H), 10.28 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3415

(NH), 1678 (C=O). MS 290 *m/z* (M + 1). Anal. Calcd for $C_{15}H_{13}ClN_2O_2$: C, 62.40; H, 4.54; N, 9.70. Found: C, 62.06; H, 4.56; N, 9.86.

*3.2.7. 7-(2-Chlorobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4g**)*

M.p. 160–163 °C; yield 33%. ^{1}H NMR (DMSO, 300 MHz) δ 4.19 (s, 2H, Ar–CH_2), 4.41 (s, 2H, –$COCH_2$), 6.14–7.56 (m, 7H, Ar–H), 10.28 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3415 (NH), 1678 (C=O). MS *m/z* 290 (M + 1). Anal. Calcd for $C_{15}H_{13}ClN_2O_2$: C, 62.40; H, 4.54; N, 9.70. Found: C, 62.38; H, 4.36; N, 9.52.

*3.2.8. 7-(2,4-Dichlorobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4h**)*

M.p. 165–169 °C; yield 55%. ^{1}H NMR (DMSO, 300 MHz) δ 4.23 (s, 2H, Ar–CH_2), 4.41 (s, 2H, –$COCH_2$), 6.12–7.78 (m, 6H, Ar–H), 10.30 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3415 (NH), 1678 (C=O). MS *m/z* 323 (M + 1); Anal. Calcd for $C_{15}H_{112}Cl_2N_2O_2$: C, 55.75; H, 3.74; N, 8.67. Found: C, 55.49; H, 3.46; N, 8.52.

*3.2.9. 7-(4-Bromobenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4i**)*

M.p. 143–146 °C; yield 56%. ^{1}H NMR (DMSO, 300 MHz) δ 4.18 (s, 2H, Ar–CH_2), 4.40 (s, 2H, –$COCH_2$), 6.14–7.50 (m, 7H, Ar–H), 10.27 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3415 (NH), 1678 (C=O). MS *m/z* 334 (M + 1). Anal. Calcd for $C_{15}H_{13}BrN_2O_2$: C, 54.07; H, 3.93; N, 8.41. Found: C, 54.26; H, 4.19; N, 8.31.

*3.2.10. 7-(4-Methylbenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4j**)*

M.p. 170–173 °C; yield 55%. ^{1}H NMR (DMSO, 300 MHz) δ 2.25 (s, 3H, –CH_3), 4.14 (s, 2H, Ar–CH_2), 4.39 (s, 2H, –$COCH_2$), 6.05–7.21 (m, 7H, Ar–H), 10.26 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3404 (NH), 1703 (C=O). MS *m/z* 269 (M + 1). Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.76; H, 5.86; N, 10.65.

*3.2.11. 7-(4-Hydroxybenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4k**)*

M.p. 192–196 °C; yield 54%. ^{1}H NMR (DMSO, 300 MHz) δ 4.05 (s, 2H, Ar–CH_2), 4.40 (s, 2H, –$COCH_2$), 6.14–7.13 (m, 7H, Ar–H), 9.22 (s, 1H, Ar–OH), 10.26 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3415 (NH), 1668 (C=O). MS *m/z* 271 (M + 1). Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.93; H, 5.32; N, 10.06.

*3.2.12. 7-(4-Methoxybenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4l**)*

M.p. 179–181 °C; yield 58%. ^{1}H NMR (DMSO, 300 MHz) δ 3.71 (s, 3H, –OCH_3), 4.11 (s, 2H, Ar–CH_2), 4.40 (s, 2H, –$COCH_2$), 6.01–7.25 (m, 7H, Ar–H), 10.26 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3371 (NH), 1685 (C=O). MS *m/z* 285 (M + 1). Anal. Calcd for $C_{16}H_{16}N_2O_3$: C, 67.59; H, 5.67; N, 9.85. Found: C, 67.71; H, 5.42; N, 10.12.

*3.2.13. 7-(4-Ethoxybenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4m**)*

M.p. 138–141 °C; yield 50%. ^{1}H NMR (DMSO, 300 MHz) δ 1.32 (t, 3H, $J = 7.85$ Hz, –CH_2CH_3), 3.97 (2H, $J = 7.0$, –OCH_2), 4.09 (s, 2H, Ar–CH_2), 4.40 (s, 2H, –$COCH_2$), 5.97–7.24 (m, 7H, Ar–H), 10.26 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3371 (NH), 1685 (C=O). MS *m/z* 299 (M + 1). Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.38; H, 5.95; N, 9.66.

*3.2.14. 7-(3,4-Methylenedioxybenzylamino)-2H-1,4-benzoxazin-3(4H)-one (**4n**)*

M.p. 170–173 °C; yield 60%. ^{1}H NMR (DMSO, 300 MHz) δ 4.09 (s, 2H, Ar–CH_2), 4.40 (s, 2H, –$COCH_2$), 5.95 (s, 2H, –OCH_2O), 6.04–6.91 (m, 7H, Ar–H), 10.26 (s, 1H, –CONH). IR (KBr) cm^{-1}: 3404 (NH), 1701 (C=O). MS *m/z* 299 (M + 1). Anal. Calcd for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.39; H, 4.79; N, 9.69.

*3.2.15. 7-Benzoylamino-2H-1,4-benzoxazin-3(4H)-one (**5**)*

The benzoyl chloride (1.1 g, 0.0078 mol) was added to a round-bottomed flask containing toluene (30 ml) which dissolved compound **3** (1 g,0.006 mol) and sodium bicarbonate (1 g, 0.012 mol). The stirred reaction mixture was refluxed for 6 h. After removing the solvent under reduced pressure, the residue was dissolved in dichloromethane (30 ml), washed with water (30 ml × 3) and dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product, which was purified by silica gel column chromatography with ethyl acetate–petroleum ether (1:1) to a white solid. M.p. 217–219 °C; yield = 11%. ^{1}H NMR (DMSO, 300 MHz) δ 4.56 (s, 2H, –$COCH_2$), 6.83–7.93 (m, 8H, Ar–H), 10.25 (s, 1H, –CONH), 10.67 (s, 1H, ArCONH). IR (KBr) cm^{-1}: 3361 (NH), 1656 (C=O). MS *m/z* 269 (M + 1). Anal. Calcd for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44; O, 17.89.

*3.2.16. 7-(4-Fluorobenzylamino)-4-methyl-2H-1,4-benzoxazin-3(4H)-one (**6**)*

Compound **4b** (0.5 g, 0.0018 mol) and dimethyl sulfate (0.91 g, 0.0072 mol) were placed into a round-bottomed flask containing 30 ml of toluene, potassium hydroxide powder was added (0.4 g, 0.0072 mol) while the mixture was stirred. The mixture was stirred at 35–40 °C for 16 min. Then the solvent was removed under reduced pressure on a rotary evaporator below 40 °C. The resulting solid residue was partitioned between water and dichloromethane, and the aqueous layer was extracted with dichloromethane (30 ml × 3). The combined layers of dichloromethane were dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product, which was purified by silica gel column chromatography with ethyl acetate–petroleum ether (2:3) to a light yellow liquid; yield 70%. ^{1}H NMR (DMSO, 300 MHz) δ 3.32 (s, 3H, –$CONCH_3$), 4.28 (s, 2H, Ar–CH_2), 4.57 (s, 2H, –$COCH_2$), 6.35–7.36 (m, 7H, Ar–H). IR (KBr) cm^{-1}: 3439 (NH), 1691.57 (C=O). MS *m/z* 287 (M + 1). Anal. Calcd for $C_{16}H_{15}FN_2O_2$: C, 67.12; H, 5.28; N, 9.78. Found: C, 66.86; H, 9.64; N, 10.01.

3.2.17. 7-((4-Fluorobenzyl)(methyl)amino)-4-methyl-2H-1,4-benzoxazin-3(4H)-one (7)

Compound **4b** (0.6 g, 0.0022 mol) and dimethyl sulfate (1.1 g, 0.0088 mol) were placed into a round-bottomed flask containing 30 ml of toluene, potassium hydroxide powder was added (0.49 g, 0.0088 mol) while the mixture was stirred. The mixture was stirred at 110–115 °C for 40 min, and then removing the solvent under reduced pressure on a rotary evaporator. The resulting solid residue was partitioned between water and dichloromethane, and the aqueous layer was extracted with dichloromethane (30 ml × 3). The combined layers of dichloromethane were dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product, which was purified by silica gel column chromatography with ethyl acetate–petroleum ether (2:3) to a light yellow liquid; yield 76.2%. ^{1}H NMR (DMSO, 300 MHz) δ 3.02 (s, 3H, $-CONCH_3$), 3.32 (s, 3H, $-CH_2NCH_3$), 4.51 (s, 2H, Ar$-CH_2$), 4.57 (s, 2H, $-COCH_2$), 6.40–7.36 (m, 7H, Ar–H). IR (KBr) cm^{-1}: 1687 (C=O). MS *m/z* 301 (M + 1). Anal. Calcd for $C_{17}H_{17}FN_2O_2$: C, 67.99; H, 5.71; N, 9.33. Found: C, 67.89; H, 5.89; N, 9.28.

3.3. Pharmacology

All compounds were tested for anticonvulsant activity with C57B/6 mice in the 18–25 g weight range purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. The tested compounds were dissolved in DMSO. All compounds were tested by the MES test; otherwise, compound **4b** was tested by sc-PTZ test, isoniazid test and strychnine test.

3.4. Anticonvulsant effects in the maximal electroshock seizure (MES) test [10,11]

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. Abolition of the hind-leg tonic-extensor component of the seizure indicated protection against the spread of MES-induced seizures.

3.4.1. sc-PTZ-induced seizures test [10,12]

At 30 min after the administration of the compounds, 85 mg/kg pentylenetetrazole dissolved in saline was administered s.c. The animals were placed in individual cages and observed for 30 min. The number of clonic and tonic seizures as well as the number of deaths was noted.

3.4.2. Isoniazid-induced seizures test [13,14]

Animals were injected i.p. with Isoniazid 250 mg/kg 30 min after the administration of **4b**, and the number of clonic seizures, tonic seizures and lethality was recorded. Data of the control group were compared to data of the group treated with **4b**. The positive control group received Carbamazepine, 50 mg/kg.

3.4.3. Strychnine-induced seizures test [12]

At 30 min after the administration of the compounds, the animals received a s.c. injection of a solution of strychnine chlorhydrate in saline (1.2 mg/kg). The mice were placed in individual cages and observed for 30 min. The time of onset of the seizure, the number of clonic seizures, tonic seizures, and the lethality were recorded.

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