Synthesis and anticonvulsant activity of 1-substituted benzyl-*N*-substituted-1, 2, 3-triazole-4-formamides*

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Abstract Substituted benzyl azids were synthesized through the reaction of substituted benzyl chloride and sodium azid, which subsequently underwent cyclization with ethyl propiolate and amidation to give thirteen 1-substituted benzyl-*N*-substituted-1, 2, 3-triazole-4-formamide derivatives (3a-3m). The structure of the synthesized compounds was confirmed by IR, ^{1}H-NMR, MS and elemental analysis. Their anticonvulsant activity against maximal electrolshock (MES) induced seizure was tested and the result showed that all these compounds possess anticonvulsant activity in different degrees. Among those, the compounds containing chloro atoms on the phenyl ring were less potent in anticonvulsant activity, while introducing one or two fluorin atoms on benzyl system increased its activity. Furthermore, their activity decreased when there was substituent on the nitrogen atom of carboxamide, and the larger the substituent, the lower the activity.

Keywords: **1-substituted benzyl-*N*-substituted-1, 2, 3-triazole-4-formamide, anticonvulsant, synthesis.**

Epilepsy, a ubiquitous disease, has more than sixty million people sufferring from seizure according to epidemiological studies[1]. Nearly 95% of clinically available drugs in the treatment of epilepsy were approved before 1985, and 60%—70% of patients were benefited with satisfactory seizure control. However, the healing would accompany notable adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia[2—4]. Some of epilepsy patients could be worsen eventually into the even life threatening state[5]. Research to find more effective and safer antiepileptic drugs is, therefore, imperative and challenging in medicinal chemistry.

The derivatives of triazole possess a wide variety of activities such as antitumor,[5] anti-inflammation,[6] antimicorbial,[7,8] antifungi,[9] antithrombotic,[10] antiplatelet,[11] and antiviral[12] activities. According to the report of Meier[13], 1-benzyl-1, 2, 3-triazole-4-formamides possess potent anticonvulsant activity. To search for better compounds and elucidate structure versus activity relationships, we introduced different substituent groups on the phenyl ring and nitrogen atom of formyl amide, thereby, synthesized thirteen 1-substituted benzyl-*N*-substituted-1, 2, 3-triazole-4-formamides (Scheme 1). Their structures were characterized by IR, ^{1}H-NMR, MS, and elemental analysis. The anticonvulsant activities of the new compounds were evaluated by the maximal eletroshock (MES) test.

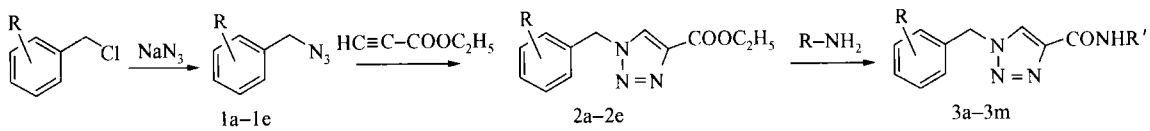

Fig. 1. Synthesis of compounds 3a-3m.

1 Experimental

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded on a FT-IR1730 spectrometer as KBr disc (cm^{-1}). The proton nuclear magnetic resonance spectra were recorded on a BRUKER AV-300 spectrometer using

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the δ scale with reference to tetramethylsilane for DMSO-d_6 solution. Mass spectra of the compounds were recorded under positive atmospheric pressure chemical ionization ($APCI^+$) with an API2000 mass spectrometer. Elemental analyses were performed on a Perkin-Elmer 204Q elemental analyzer for C, H and N, and all analytical data were within ±0.4% of the theoretical values. All the solvents were of analytic reagent or chemical purity grade.

1.1 Synthesis of thirteen 1-substituted benzyl-*N*-substituted-1,2,3-triazole-4-formamides

Synthesis of compounds 3a-3m is shown in Scheme 1.

Preparation of compounds 1a-1e: To a solution of NaN_3 1.01 g (0.016 mol) in 10 mL anhydrous DMF was added substituted benzyl chloride (0.016 mol). The mixture was stirred for 24 h at room temperature. To this was added water 40 mL, extracted with ethyl ether, and dried over anhydrous magnesium sulfate. After removing solvent under reduced pressure, oily product 1a-1e was obtained with yield of 75%—95% and used without purification.

Preparation of 2a-2e: To a 100 mL round-bottomed flask was added compounds 1a-1e (12 mmol), ethyl propiolate 1.2 mL (12 mmol) and ethanol 15 mL. The mixture was refluxed for 5 h. Removal of solvents gave crude product, that was recrystallized in petroleum ether: methanol (15∶2), afforded compound 2a-2e. The data of yield, melting point, elemental analysis, IR, MS and ^{1}H-NMR are listed in Tables 1 and 2.

Table 1. Yield, melting point and elemental analysis data of compounds 2a-2e and 3a-3m

Compd.	R	R′	Yield(%)	m.p.(℃)	Elemental analysis (%)[a]		
					C	H	N
2a	-H	—	56	89—91	62.12(62.33)	5.81(5.67)	17.89(18.17)
2b	4-Cl	—	66	126—128	54.02 (54.25)	4.69 (4.55)	15.71 (15.82)
2c	4-F	—	73	90—93	58.08 (57.83)	4.90 (4.85)	16.66 (16.86)
2d	2,4-Cl_2	—	73	99—100	47.93 (48.02)	3.88 (3.69)	14.16 (14.00)
2e	2,6-F_2	—	62	112—114	53.77 (53.93)	4.37 (4.15)	15.98 (15.72)
3a	-H	-H	69	237—239	59.48 (59.40)	4.92 (4.98)	27.86 (27.71)
3b	-H	-n-C_3H_7	97	169—170	63.80 (63.91)	6.78 (6.60)	22.77 (22.93)
3c	4-Cl	-H	77	237—241	50.89 (50.75)	4.09 (3.83)	23.49 (23.67)
3d	4-Cl	-*n*-C_3H_7	91	178—181	55.89 (56.02)	5.34 (5.42)	20.21 (20.10)
3e	4-F	-H	71	248—250	54.61 (54.54)	4.32 (4.12)	25.12 (25.44)
3f	4-F	-CH_3	68	192—194	56.22 (56.41)	4.91 (4.73)	23.87 (23.92)
3g	4-F	-n-C_3H_7	69	181—184	59.66 (59.53)	5.69 (5.76)	21.23 (21.36)
3h	2,4-Cl_2	-H	97	250—251	44.59 (44.30)	3.12 (2.97)	20.51 (20.67)
3i	2,4-Cl_2	-CH_3	90	190—192	46.03 (46.34)	3.40 (3.54)	19.76 (19.65)
3j	2,4-Cl_2	-n-C_3H_7	97	169—172	49.98 (49.86)	4.37 (4.51)	18.01 (17.89)
3k	2,6-F_2	-H	92	236—237	50.19 (50.42)	3.51 (3.39)	23.69 (23.52)
3l	2,6-F_2	-CH_3	68	199—201	52.31 (52.38)	4.13 (4.00)	22.05 (22.21)
3m	2,6-F_2	-n-C_3H_7	86	179—181	55.61 (55.71)	5.24 (5.03)	20.18 (19.99)

a) Measured values (calculated values)

Table 2. IR, MS and ^{1}H-NMR data of compounds 2a-2e and 3a-3m

Compd.	IR $\tilde{\nu}$ (cm^{-1})	MS (m/z) $[M+H]^+$	^{1}H-NMR (δ)
2a	1717 ($\nu_{C=O}$)	232	1.40 (t, J=7.2 Hz, 3H, CH_3), 4.41 (q, J=7.2 Hz, 2H, CH_2), 5.56 (s, 2H, $PhCH_2$), 7.65—7.28 (m, 5H, ArH), 8.31 (s, 1H, =CH)
2b	1726 ($\nu_{C=O}$)	266	1.28 (t, J=6.9 Hz, 3H, CH_3), 4.29 (q, J=6.9 Hz, 2H, CH_2), 5.66 (s, 2H, $PhCH_2$), 7.45—7.35 (m, 4H, ArH), 8.86 (s, 1H, =CH)
2c	1718 ($\nu_{C=O}$)	250	1.28 (t, J=7.05 Hz, 3H, CH_3), 4.29 (q, J=7.05 Hz, 2H, CH_2), 5.65 (s, 2H, $PhCH_2$), 7.18—7.46 (m, 4H, ArH), 8.88 (s, 1H, =CH)

To be continued

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Continued

Compd.	IR $\tilde{v}$ (cm^{-1})	MS (m/z) $[M+H]^+$	^{1}H-NMR (δ)
2d	1720 ($v_{C=O}$)	301	1.29 (t, J = 6.9 Hz, 3H, CH_3), 4.30 (q, J = 6.9 Hz, 2H, CH_2), 5.75 (s, 2H, $PhCH_2$), 7.69—7.31 (m, 3H, ArH), 8.82 (s, 1H, =CH)
2e	1729 ($v_{C=O}$)	268	1.30 (t, J = 6.9 Hz, 3H, CH_3), 4.29 (q, J = 6.9 Hz, 2H, CH_2), 5.70 (s, 2H, $PhCH_2$), 7.45—7.03 (m, 3H, ArH), 8.59 (s, 1H, =CH)
3a	3413, 3184 (v_{N-H}), 1636 ($v_{C=O}$)	203	5.65 (s, 2H, $PhCH_2$), 7.37—7.41 (m, 5H, ArH), 8.35 (s, 1H, =CH)
3b	3430, 3320 (v_{N-H}), 1649 ($v_{C=O}$)	245	0.84 (t, J = 7.2 Hz, 3H, CH_3), 1.50 (m, J = 7.2 Hz, 2H, CH_2), 3.19 (m, 2H, $NHCH_2$), 5.64 (s, 2H, $PhCH_2$), 7.35—7.40 (m, 5H, ArH), 8.47 (s, 1H, NH), 8.59 (s, 1H, =CH)
3c	3405, 3170 (v_{N-H}), 1652 ($v_{C=O}$)	237	5.65 (s, 1H, $PhCH_2$), 7.33—7.42 (m, 4H, ArH), 8.38 (s, 1H, =CH)
3d	3316 (v_{N-H}), 1646 ($v_{C=O}$)	279	0.84 (t, J = 7.2 Hz, 3H, CH_3), 1.50 (m, J = 7.2 Hz, 2H, CH_2), 3.19 (m, 2H, $NHCH_2$), 5.64 (s, 2H, $PhCH_2$), 7.35—7.46 (m, 4H, ArH), 8.47 (m, 1H, NH), 8.61 (s, 1H, =CH)
3e	3413, 3184 (v_{N-H}), 1637 ($v_{C=O}$)	221	5.64 (s, 2H, $PhCH_2$), 7.18—7.45 (m, 4H, ArH), 7.49 (s, 1H, NH), 7.88 (s, 1H, NH), 8.61 (s, 1H, =CH)
3f	3413, 3335 (v_{N-H}), 1648 ($v_{C=O}$)	235	2.75 (m, 3H, CH_3), 5.64 (s, 2H, $PhCH_2$), 7.19—7.45 (m, 4H, ArH), 8.48 (m, 1H, NH), 8.63 (s, 1H, =CH)
3g	3432, 3320 (v_{N-H}), 1647 ($v_{C=O}$)	263	0.85 (t, J = 7.2 Hz, 3H, CH_3), 1.50 (m, J = 7.2 Hz, 2H, CH_2), 3.18 (m, 2H, $NHCH_2$), 5.63 (s, 2H, $PhCH_2$), 7.19—7.45 (m, 4H, ArH), 8.51 (m, 1H, NH), 8.61 (s, 1H, =CH)
3h	3397, 3173 (v_{N-H}), 1650 ($v_{C=O}$)	272	5.78 (s, 2H, $PhCH_2$), 7.35—7.58 (m, 3H, ArH), 8.38 (s, 1H, =CH)
3i	3342 (v_{N-H}), 1650 ($v_{C=O}$)	286	2.76 (m, 3H, CH_3), 5.99 (s, 2H, $PhCH_2$), 7.29—7.71 (m, 3H, ArH), 8.49 (m 1H, NH), 8.58 (s, 1H, =CH)
3j	3346 (v_{N-H}), 1650 ($v_{C=O}$)	314	0.85 (t, J = 7.5 Hz, 3H, CH_3), 1.50 (m, J = 7.2 Hz, 2H, CH_2), 3.19 (m, 2H, $NHCH_2$), 5.74 (s, 2H, $PhCH_2$), 7.29—7.69 (m, 3H, ArH), 8.48 (m, 1H, NH), 8.55 (s, 1H, =CH)
3k	3412, 3190 (v_{N-H}), 1633 ($v_{C=O}$)	239	5.68 (s, 2H, $PhCH_2$), 7.03—7.50 (m, 3H, ArH), 8.38 (s, 1H, =CH)
3l	3364 (v_{N-H}), 1651 ($v_{C=O}$)	253	2.75 (m, 3H, CH_3), 5.72 (s, 2H, $PhCH_2$), 7.15—7.56 (m, 3H, ArH), 8.42 (m, 1H, NH), 8.54 (s, 1H, =CH)
3m	3338 (v_{N-H}), 1647 ($v_{C=O}$)	281	0.97 (t, J = 7.5 Hz, 3H, CH_3), 1.63 (m, J = 7.2 Hz, 2H, CH_2), 3.34 (m, 2H, $NHCH_2$), 5.76 (s, 2H, $PhCH_2$), 7.05—7.54 (m, 3H, ArH), 8.34 (s, 1H, =CH)

Preparation of 3a-3m: Compounds 2a-2e (0.37 mmol) were placed in an appropriate saturated solution of ammonia and methylamine in methanol and *n*-propylamine, respectively. The mixture was stirred at room temperature. A solid product was obtained after filtration, which was then purified by recrystallization in methanol. The data of yield, melting point, elemental analysis, IR, MS and ^{1}H-NMR are also shown in Tables 1 and 2.

1.2 Antiepileptic activity test

Antiepileptic activity test was performed according to the method reported previously[14]. Kunming mice (18—25 g) of either sex were used in this test.

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MES were induced by the application of 50 mA current for 0.2 s via ear electors. Those with entasia of hind legs were used in the later test which was performed at least 24 h later. Compounds were suspended in 30% polyethylene glycol 400 and administered intraperitoneally to animals. MES were induced 30 min after drug treatment by application of 50 mA current for 0.2 s via ear electors. The protection was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. The dose given to the mice was according to the reported dose previously[15]. Groups of six mice were administered i.p. at doses from 25 mg/kg to 300 mg/kg with 25 mg/kg as intervals, and those exhibited anticonvulsant activity at 25 mg/kg were given doses of 15 mg/kg, 10 mg/kg and 5 mg/kg afterwards. The dose that made all the six mice exhibit anticonvulsant activity was considered as the effective dose of the compound. The result is illustrated in Table 3 along with the comparison with phenobarbital.

Table 3. Antiepileptic activity of compounds (3a-3m) in mice

Compd.	MES (mg/kg)	Compd.	MES (mg/kg)
3a	50	3h	200
3b	200	3i	225
3c	200	3j	300
3d	200	3k	10
3e	15	3l	25
3f	15	3m	50
3g	100	Phenobarbital	25

MES: *Maximal electroshock-induced seizure*

2 Result and discussion

2.1 Chemosynthesis

Triazoles can be obtained through the cycloaddition reaction of azides with acetylenes or the base catalyzed condensation reaction of azide with methylenes. The latter reaction is usually used in the synthesis of compounds possessing carbonyl group on the forth position and hydroxyl or amino group on the fifth position[16]. Products in the former route are varied with the difference of acetylenes. However, cycloaddition of azides to ethyl propiolate provided a sole product of 5-substituted derivative. And, therefore, the former route was preferred in our synthesis of 5-substituted-1,2,3-triazole.

Cyclization of substituted benzyl azide with ethyl propiolate is 1,3-dipolar cycloaddition reaction which is similar to Diels-Alder reaction. Crude products were obtained by refluxing the mixture of reactants in ethanol and were refined through recrystallization in petroleum ether and methanol. In order to augment the consistency of reactants and shorten the reaction time, it was better to use ethanol as little as possible.

Carboxamido ester, which is also called aminate of ester, was accomplished by stirring at room temperature. Crude products were obtained after filtering the precipitates that could be recrystallized in methanol to give 3a-3m compounds. Introduction of halogen into the phenyl ring leads to an increase in lipophilicity of compounds, which would in turn allow a facile penetration of them into the biological membrane. To enhance the affinity of compound to the acceptor, we introduced formamide group on the forth position of heterocycle. Based on this assumption, a series of halogen substituted aryl compounds were designed to achieve anticonvulsant activity. And to evaluate the effect of substituents on the nitrogen atom of formyl amide toward the anticonvulsant activity, alkyl substituted derivatives on the amino group were designed and synthesized.

2.2 Antiepileptic activity of compounds

The test of antiepileptic activity revealed that all the thirteen synthesized compounds showed anticonvulsant activities that differ in degree. Among them, compounds 3e, 3f and 3k exhibited the most potent activities with effective doses of 15 mg/kg, 15 mg/kg and 10 mg/kg, respectively, which were less than that of Phenobarbital. The anticonvulsant activity of compound 3l was comparable to Phenobarbital while the rest were less effective.

By the study on the structure versus activity relationship, it was observed that introduction of chloro atom to the phenyl ring results in a decrease in activity, and the more the chloro atoms were introduced, the lower the activity could be obtained (3a 50 mg/kg, 3c 200 mg/kg). This might be ascribed to the presence of chlorinated phenyl ring which weakens the binding between the compound and the receptor. However, introduction of fluoro atoms enhanced its activity significantly (3e 15 mg/kg), while the compound with two fluoro atoms substituted on the phenyl ring was the most potent (3k 10 mg/kg). This might be due to the electronic and steric effects due to halogen atoms substituted. Besides, fluoro atom also could increase the lipophilic property of compounds and make them easy to enter the biological

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membrane.

On the contrary, their anticonvulsant activity decreased when there were substituent groups on the nitrogen atom of formyl amide, and the larger the substituent, the lower the activity (3a 50 mg/kg, 3b 200 mg/kg), probably because the introduction of substituents on the nitrogen atom would make the bond between formyl amide and receptor difficult, which in turn led to the decrease of activity.

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