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Synthesis and Evaluation on Anticonvulsant Activities of Pyrazolyl Semicarbazones

XIAN-QING DENG*, MING-XIA SONG and HAI-HONG YU

Medical College, Jinggangshan University, No 28, Xueyuan Road, Ji'an 343009, Jiangxi, P.R. China

*Corresponding author: Tel/Fax: +86 796 8117893; E-mail: dengxianqing1121@126.com

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In this paper, a series of 2-[(5-phenoxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-methylene]hydrazine carboxamides were synthesized and evaluated for their anticonvulsant activities using the maximal electroshock method. Their neurotoxicities were determined applying the rotarod test. Interestingly, all compounds showed anticonvulsant activity with long duration of protection effects in the maximal electroshock test. Among which, compound **5m** was found to have promising anticonvulsant activity, which gave an ED_{50} of 42.4 mg/kg and a protective index value of 3.7, possessing better anticonvulsant activity and higher safety than marketed drugs valproate, but weaker than phenobarbital. Furthermore, the antagonistic activity against seizures induced by pentylenetetrazole of the compound **5m** was also established, which suggested that compound **5m** may exert anticonvulsant activity through γ-aminobutyric acid (GABA)-mediated mechanisms.

Keywords: Anticonvulsant, Pyrazole, Semicarbazone, Maximal electroshock.

INTRODUCTION

Epilepsy, one of the most frequent neurological afflictions in men characterized by recurrent unprovoked seizures, inflicts more than 60 million people worldwide[1,2]. The conventional antiepileptic drugs (AEDs) like phenytoin, carbamazepine, valproic acid and barbiturates, though widely prescribed, exhibited unfavorable side effects such as drowsiness, ataxia and hepatotoxicity[3-6]. Recently, several new antiepileptic drugs such as lamotrigine, oxcarbazepine, felbamate and rufinamide have been approved with an improved efficacy and lower toxicity. However, it is roughly estimated that up to 28-30 % of patients with epilepsy have inadequate control of seizures with the currently available antiepileptic drugs. Therefore, it is essential to search for newer chemical entities with better efficacy and lower toxicity for the treatment of epilepsy.

Semicarbazide has been recognized earlier as a promising pharmacophore and a number of semicarbazide derivatives have been synthesized and tested for their anticonvulsant activity[7-11]. Different aryl and heteroaryl moieties have been clubbed to the semicarbazide pharmacophore as hydrophobic domain which led to the increase in the activity significantly. Recently, several semicarbazones have emerged as structurally novel anticonvulsants and were found to act by blocking the voltage-gated Na^+ channels[12-14].

Pyrazoles are important five-membered heterocyclic compounds with double nitrogen, whose derivatives possess important pharmacological activities. Therefore they are useful materials in drug research and they are of interest as potential anticonvulsant agents[15-18].

Based on the above facts, herein we planned to attach the semicarbazide to substituted pyrazoles moiety expecting to obtain some hybrid compounds having a synergistic effect in dealing with epilepsy. A series of 2-[(5-phenoxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene]hydrazine carboxamide derivatives were synthesized and evaluated for anticonvulsant activity. Their structures were confirmed by infrared spectra (IR), nuclear magnetic resonance (NMR) and HRMS (High resolution mass spectra). Their anticonvulsant activities and neurotoxicities were evaluated using the maximal electroshock (MES)-induced seizure model and the rotarod assay in mice, respectively. The anticonvulsant activity of some of the target compounds possessing better activity were quantified and the compound **5m** was tested in subcutaneous pentylenetetrazole (sc-PTZ) induced seizure test.

EXPERIMENTAL

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on IR Prestige-21 (Shimadzu, Japan). ^{1}H NMR spectra were measured on an AV-300 (Bruker, Switzerland) and all chemical shifts were given in ppm relative to tetramethysilane. High resolution mass spectra were measured on an MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Germany). The major chemicals

were purchased from Aldrich Chemical Corporation (St Louis, USA).

Procedures for synthesis of compounds 2, 3 and 4: Compound **2**, **3** and **4** was previously reported[19].

Procedures for preparation of compounds 5a-5m: To a solution of compound **4** (0.01 mol) in 50 % ethanol solution was added an equimolar quantity of hydrazine carboxamide hydrochloride (SEM HCl) and the mixture was refluxed for 0.5-3 h until the completion of the reaction. The solid formed was collected by filtration to give crude product, which was re-crystallized from ethanol to afford the products in 71-87 % yield.

Characterization for the target compounds (5a-5m)

2-[(5-Phenoxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-methylene]hydrazine carboxamide (5a): m.p.: 226-228 ºC, yield: 78 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.43 (s, 3H, CH_3), 5.83 (br.s, 2H, NH_2), 6.95-7.64 (m, 11H, Ar-H, CH=N), 10.01 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3434 3182 3296 (N-H), 1686 (C=O). ESI-HRMS calcd for $C_{18}H_{18}N_5O_2^+$ ([M + H]$^+$): 336.1455; found: 336.1445.

2-[{5-(4-Fluorophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5b): m.p.: 202-204 ºC, yield: 69 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.42 (s, 3H, CH_3), 5.88 (br.s, 2H, NH_2), 7.00-7.62 (m, 10H, Ar-H, CH=N), 10.01 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3486 3244 3267 (N-H), 1689 (C=O). ESI-HRMS calcd for $C_{18}H_{17}FN_5O_2^+$ ([M + H]$^+$): 354.1361; found: 354.1352.

2-[{5-(2-Chlorophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5c): m.p.: 195-197 ºC, yield: 79 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.42 (s, 3H, CH_3), 5.72 (br.s, 1H, NH_2), 6.18 (br.s, 1H, NH_2), 7.09-7.62 (m, 10H, Ar-H, CH=N), 10.03 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3499 3279 3175 (N-H), 1691 (C=O). ESI-HRMS calcd for $C_{18}H_{17}ClN_5O_2^+$ ([M + H]$^+$): 370.1065; found: 370.1060.

2-[{5-(3-Chlorophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5d): m.p. 170-172 ºC, yield: 87 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.42 (s, 3H, CH_3), 5.77 (br.s, 1H, NH_2), 6.20 (br.s, 1H, NH_2), 6.91-7.59 (m, 9H, Ar-H), 67.64 (s, 1H, CH=N), 10.03 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3490 3281 3178 (N-H), 1690 (C=O). ESI-HRMS calcd for $C_{18}H_{17}ClN_5O_2^+$ ([M + H]$^+$): 370.1065; found: 370.1070.

2-[{5-(4-Chlorophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5e): m.p. 214-215 ºC, yield: 85 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.42 (s, 3H, CH_3), 5.89 (br.s, 2H, NH_2), 7.02 (d, 2H, J = 7.7 Hz, Ar-H), 7.30-7.47 (m, 5H, Ar-H), 7.57 (d, 2H, J = 7.7 Hz, Ar-H), 7.63 (s, 1H, CH=N), 10.02 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3490 3292 3188 (N-H), 1690 (C=O). ESI-HRMS calcd for $C_{18}H_{17}ClN_5O_2^+$ ([M + H]$^+$): 370.1065; found: 370.1059.

2-[{5-(2,4-Dichlorophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5f): m.p. 195-196 ºC, yield 73 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.42 (s, 3H, CH_3), 5.75 (br.s, 1H, NH_2), 6.21 (br.s, 1H, NH_2), 6.87-7.75 (m, 9H, Ar-H, CH=N), 10.05 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3504 3389 3276 (N-H), 1686 (C=O). ESI-HRMS calcd for $C_{18}H_{16}Cl_2N_5O_2^+$ ([M + H]$^+$): 404.0676; found: 404.0678.

2-[{5-(2,6-Dichlorophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5g): m.p.: 120-121 ºC, yield: 78 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.34 (s, 3H, CH_3), 6.07 (br.s, 2H, NH_2), 7.07-7.70 (m, 9H, Ar-H, CH=N), 10.03 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3492 3374 3283 (N-H), 1685 (C=O). ESI-HRMS calcd for $C_{18}H_{16}Cl_2N_5O_2^+$ ([M + H]$^+$): 404.0676; found: 404.0675.

2-[{5-(4-Bromophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5h): m.p.: 214-215 ºC, yield: 81 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.42 (s, 3H, CH_3), 6.09 (br.s, 2H, NH_2), 6.95-7.58 (m, 9H, Ar-H), 7.62 (s, 1H, CH=N), 10.03 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3484 3269 3190 (N-H), 1690 (C=O). ESI-HRMS calcd for $C_{18}H_{17}BrN_5O_2^+$ ([M + H]$^+$): 414.0560; found: 414.0559.

2-[{5-(2-Methylphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5i): m.p.: 190-192 ºC, yield: 77 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.33 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 5.92 (br.s, 2H, NH_2), 6.54-7.60 (m, 10H, Ar-H, CH=N), 9.99 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3475 3271 3196 (N-H), 1687 (C=O). ESI-HRMS calcd for $C_{19}H_{20}N_5O_2^+$ ([M + H]$^+$): 350.1612; found: 350.1621.

2-[{5-(4-Methylphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5j): m.p.: 204-206 ºC, yield: 81 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.22 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 5.88 (br.s, 2H, NH_2), 6.84 (d, 2H, J = 8.6 Hz, Ar-H), 7.13 (d, 2H, J = 8.6 Hz, Ar-H), 7.28-7.62 (m, 6H, Ar-H, CH=N), 9.99 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3471 3268 3198 (N-H), 1688 (C=O). ESI-HRMS calcd for $C_{19}H_{20}N_5O_2^+$ ([M + H]$^+$): 350.1612; found: 350.1614.

2-[{5-(2,4-Dimethylphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5k): m.p.: 173-174 ºC, yield: 74 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.18 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 5.94 (br.s, 2H, NH_2), 6.43-7.59 (m, 9H, Ar-H, CH=N), 9.99 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3457 3266 3189 (N-H), 1685 (C=O). ESI-HRMS calcd for $C_{20}H_{22}N_5O_2^+$ ([M + H]$^+$): 364.1768; found: 364.1763.

2-[{5-(2-Methoxyphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5l): m.p.: 202-204 ºC, yield 75 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.40 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 5.95 (br.s, 2H, NH_2), 6.70-7.62 (m, 10H, Ar-H, CH=N), 9.99 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3479 3247 3164 (N-H), 1692 (C=O). ESI-HRMS calcd for $C_{19}H_{20}N_5O_3^+$ ([M + H]$^+$): 366.1561; found: 366.1556.

2-[{5-(4-Methoxyphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (5m): m.p.: 195-196 ºC, yield: 71 %. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 2.42 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 5.94 (br.s, 2H, NH_2), 6.86-7.61 (m, 10H, Ar-H, CH=N), 10.01 (s, 1H, NH). IR (KBr, ν_{max}, cm^{-1}): 3477 3250 3168 (N-H), 1694 (C=O). ESI-HRMS calcd for $C_{19}H_{20}N_5O_3^+$ ([M + H]$^+$): 366.1561; found: 366.1559.

Pharmacology: Male KunMing mice (supplied from the Laboratory of Animal Research, Yanbian University, China) weighting 18-22 g were used for pharmacological study. Animals were allowed free access to food and water except during the experiment and housed at controlled room temperature with 12 h light/dark schedule. All compounds were dissolved in DMSO with the injection volume of 0.05 mL per 20 g, which had no effect on the test system.

Anticonvulsant activity of the synthesized compounds was determined through the evaluation of the ability of the compounds to protect mice against maximal electroshock-induced seizures. The maximal electroshock test was carried out following procedures proposed by the NIH anticonvulsant drug development (ADD) program[20,21]. Seizures were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied *via* clip electrodes for 0.2 s. Protection against the spread of maximal electroshock-induced seizures was defined as the abolition of tonic maximal extension of the hind leg. In preliminarily screening, each compound was administered at the dose levels of 30, 100 and 300 mg/kg for evaluating preliminarily the anticonvulsant activity and neurotoxicity at 0.5 and 4 h interval after intraperitoneal administration (*i.p.*). For determination of the median effective dose (ED_{50}) and the median toxic dose (TD_{50}), the next phase screening was carried out. Groups of 10 mice were given a range of *i.p.* doses of the tested compound until at least three points were established in the range of 10-90 % seizure protection or neurotoxicity. From the plot of this data, the respective ED_{50}, TD_{50} values and 95 % confidence intervals were calculated by probit analysis.

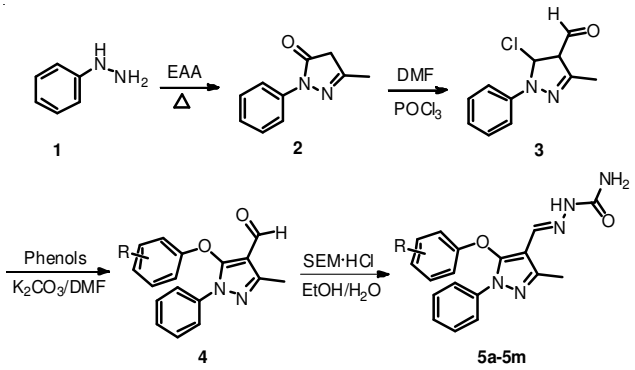
In the pentylenetetrazole (PTZ)-induced seizures, animals were divided into 3 groups (10 mice in one group), which were administrated compound **5m**, carbamazepine and vehicle DMSO, respectively. At 2.5 h after the administration, 85 mg/kg pentylenetetrazole dissolved in saline was administered subcutaneously (*s.c.*) for each group. Then the animals were placed in individual cages and observed for 0.5 h. The numbers of clonic and tonic seizures as well as the number of deaths were noted[20,21].

The neurotoxicity of the compounds was measured in mice by the rotarod test[20,21]. The mice were trained to stay on a rotarod of diameter 3.2 cm which rotates at 10 rpm. Trained animals were given *i.p.* injection of the test compounds. Neurotoxicity was tested at 0.5 h (or 2.5 h for the quantification test) after the administration and indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials.

RESULTS AND DISCUSSION

The target compounds (**5a-5m**) were synthesized according to the route as shown in Fig. 1. Briefly, phenylhydrazine was reacted with ethyl acetoacetate (EAA) to afford 3-methyl-1-phenyl-2-pyrazolin-5-one (**2**). Compound **2** was subjected to a Vilsmeier Haack reaction to provide 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxaldehyde (**3**), which was subsequently reacted with different substituted-phenols to obtain the intermediates **4**. Finally, the target compounds were synthesized by refluxing **4** with hydrazine carboxamide hydrochloride (SEM HCl) in 50 % ethanol. The structures of the target compounds were confirmed by IR, ^{1}H NMR and mass spectral.

A very important step in antiepileptic drug discovery is the choice of an appropriate animal model for the initial screening. At present, there are three models *in vivo* - the maximal electroshock, the pentylenetetrazole and the kindling model - which are routinely used by most antiepileptic drugs discovery



EAA: ethyl acetoacetate; DMF: dimethyl formamide; SEM: hydrazinecarboxamide

Fig. 1. Synthesis route of the target compounds (**5a-5m**)

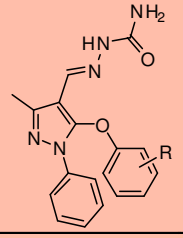
programs. Of these, the maximal electroshock and pentylenetetrazole seizure models represent the two animal seizure models most widely used in the search for new antiepileptic drugs[22,23]. The maximal electroshock test is thought to predict drugs effective against generalized seizures of the tonic-clonic (grand mal) type, whereas the pentylenetetrazole test is used to find drugs effective against the generalized seizures of the petit mal (absence) type. In this study, the maximal electroshock model was used for screening the anticonvulsant activity of target compounds. In the preliminary evaluation of anticonvulsant activity, doses of 30, 100 and 300 mg/kg were used and the results were presented in Table-1.

All the tested compounds showed anti-maximal electroshock activity indicative of their ability to prevent seizure spread. At the dose of 100 mg/kg, all the compounds showed protection against the maximal electroshock model at 0.5 h period and **5b-5m** kept the protection to 4 h, which indicated that these compounds are potent having a rapid onset of action and long duration of action. Compounds showed protection against the maximal electroshock model at 30 mg/kg including **5c**, **5d**, **5i**, **5j**, **5m**. Among them, compounds **5j** and **5m** showed activity at 0.5 h and 4 h periods, while compounds **5c**, **5d** and **5i** showed activity only at 4 h. In the acute neurotoxicity test, compounds **5a**, **5b**, **5e-5h**, **5k**, **5l** did not show neurotoxicity at the dose of 100 mg/kg, while the others showed neurotoxicity in different degree at the same dose. None is found to be neurotoxic at the dose of 30 mg/kg.

From the data in Table-1, it can be seen that each compound has almost equal activity at the end of 0.5 h and 4 h. This fact indicated that the time to peak effect (TPE) of drugs are likely to present in the time range of 0.5 to 4 h. To obtain the TPE of compounds **5a-5m**, we conducted a time-course test for the selected **5m**, in which compound **5m** reached the TPE at 2.5 h after *i.p.* (Fig. 2). Therefore, the 2.5 h time interval was chosen as the assessment time for the test compounds in the next quantitative tests.

On the basis of the results recorded in the preliminary screening, compounds **5c**, **5d**, **5i**, **5j**, **5k** and **5m** were subjected to the next phase of trials concerning quantification of their anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) in mice. Results of the quantitative tests for the selected compounds, together with the corresponding data for the currently marketed antiepileptic drugs, including phenobarbital and valproate, are shown in Table-2.

TABLE-1
PRELIMINARY ANTICONVULSANT ACTIVITY AND NEUROTOXOCITY OF COMPOUNDS **5a-5m** ADMINISTERED INTRAPERITONEALLY (i.p.) TO MICE



Compds.	R	MES [a] (mg/kg)						Toxicity [b] (mg/kg)					
		0.5 h			4 h			0.5 h			4 h		
		30	100	300	30	100	300	30	100	300	30	100	300
5a	H	0/3	1/3	3/3	0/3	0/3	3/3	-[c]	0/3	2/3	-	0/3	3/3
5b	4-F	0/3	2/3	3/3	0/3	2/3	3/3	-	0/3	3/3	-	0/3	3/3
5c	2-Cl	0/3	3/3	3/3	1/3	3/3	3/3	-	0/3	3/3	0/3	1/3	3/3
5d	3-Cl	0/3	3/3	3/3	1/3	3/3	3/3	0/3	2/3	3/3	0/3	3/3	3/3
5e	4-Cl	0/3	2/3	3/3	0/3	2/3	3/3	-	0/3	3/3	-	0/3	3/3
5f	2,4-2Cl	0/3	1/3	3/3	0/3	2/3	3/3	-	0/3	3/3	-	0/3	3/3
5g	2,6-2Cl	0/3	1/3	3/3	0/3	2/3	3/3	-	0/3	3/3	-	0/3	3/3
5h	4-Br	0/3	1/3	3/3	0/3	1/3	3/3	-	0/3	1/3	-	0/3	2/3
5i	2-CH_3	0/3	3/3	3/3	1/3	3/3	3/3	0/3	2/3	3/3	0/3	2/3	3/3
5j	3-CH_3	1/3	3/3	3/3	1/3	3/3	3/3	-	0/3	3/3	0/3	1/3	3/3
5k	2,4-2CH_3	0/3	3/3	3/3	0/3	2/3	3/3	-	0/3	3/3	-	0/3	3/3
5l	2-OCH_3	0/3	1/3	3/3	0/3	1/3	3/3	-	0/3	1/3	-	0/3	2/3
5m	4-OCH_3	1/3	3/3	3/3	1/3	3/3	3/3	-	0/3	3/3	0/3	1/3	3/3

All positive reaction numbers are in bold italic; [a]Maximal electroshock test (number of animals protected/number of animals tested), the number of mice is three; [b]Acute neurotoxicity (number of animals toxic/number of animals tested), the number of mice is three; [c]Not tested

TABLE-2
QUANTITATIVE ANTICONVULSANT EVALUATION IN MICE AFTER INTRAPERITONEAL ADMINISTRATION (*i.p.*)

Compounds	R	ED_{50} [a] (mg/kg) (MES)	TD_{50} [b] (mg/kg) (NT)	PI[c]
5c	2-Cl	52.8 (45.8-60.9) [d]	152.1 (131.8-175.5)	2.9
5d	3-Cl	63.4 (55.0-73.1)	81.8 (71.6-93.5)	1.3
5i	2-CH_3	58.9 (51.5-67.3)	94.7 (82.1-109.3)	1.6
5j	3-CH_3	76.0 (65.9-87.7)	118.3 (102.5-136.5)	1.6
5k	2,4-2CH_3	56.8 (49.2-65.5)	176.0 (152.6-203.0)	3.1
5m	4-OCH_3	42.4 (37.1-48.5)	157.8 (136.8-182.0)	3.7
Phenobarbital	-	21.8 (21.8-25.5)	69.1 (62.8–72.9)	3.2
Valproate	-	272 (247-338)	426 (369-450)	1.6

[a]ED_{50}: Median effective dose; [b]TD_{50}: Median toxic dose; [c]PI = Protective index (TD_{50}/ ED_{50}); [d]The 95 % confidence limits

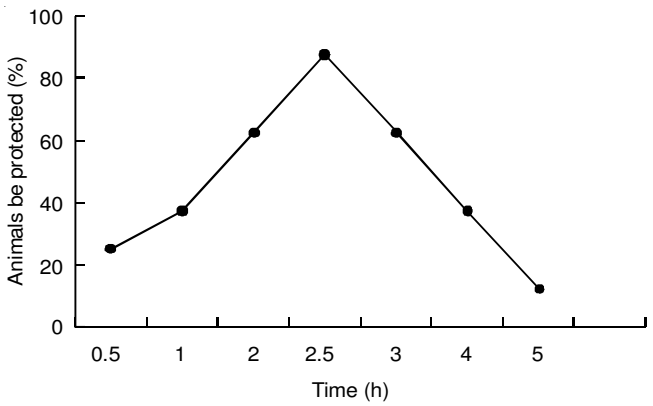


Fig. 2. Time-course of compound **5m** (50 mg/kg) in the maximal electroshock seizure test (*i.p*)

As shown in Table-2, all the test compounds showed weaker anticonvulsant activity than phenobarbital, but better than valproate. Interestingly, 2-[{5-(4-Methoxyphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl}methylene]hydrazine carboxamide (**5m**) possessed nice anti-maximal electroshock activity with an ED_{50} of 42.4 mg/kg and a protective index of 3.7, which was superior to phenobarbital and valproate in the aspect of safety.

For further exploring the anticonvulsant activity of these compounds, pentylenetetrazole-induced seizure model was made to **5m**. As shown in Table-3, 80 % protection against pentylenetetrazole induced tonic seizures and death was observed when pretreated by **5m** (50 mg/kg), which suggested compound **5m** could work against the seizure spread induced by pentylenetetrazole. Pentylenetetrazole has been reported to produce seizures by inhibiting γ-aminobutyric acid neurotransmission[24,25]. γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures, while enhancement of GABAergic neurotransmission is known

TABLE-3
EFFECTS OF COMPOUND 5M ON PENTYLENETETRAZOL-INDUCED SEIZURES IN MICE (i.p.)

Chemical substances	Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
Pentylenetetrazol	DMSO	-	0.5	100	100	100
	Carbamazepine	50	0.5	100	0	10
	5m	50	2.5	80	20	20

to inhibit or attenuate seizures[26]. Based on these, it is speculated that the mechanism of action of the compound **5m** may be involved in the GABAergic neurotransmission.

Conclusion

A series of 2-[(5-phenoxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methylene]-hydrazine carboxamide derivatives (**5a-5m**) were synthesized and studied for their anticonvulsant activity. The results of this study demonstrated that pyrazolyl semicarbazones have potent anticonvulsant activity. Among the compounds synthesized, compound **5m** was found to have promising anticonvulsant activity, which gave an ED_{50} of 42.4 mg/kg and a TD_{50} of 157.8 mg/kg, resulting in a PI value of 3.7. In addition, compound **5m** demonstrated antagonistic activity against seizures induced by pentylenetetrazole, which suggested that compound **5m** may exert anticonvulsant activity through γ-aminobutyric acid-mediated mechanisms. This study provide a new nuclear/structure for further design of new anticonvulsant agents.

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