



## Synthesis of some derived thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole ring systems from Isonicotinic acid hydrazide: A novel class of potential anticonvulsant agents

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### Abstract

A series of Isonicotinic acid hydrazide (INH) incorporated derivatives of thiazolidin-4-one (**2a-h**, **3a-h**), azetidin-2-one (**4a-h**) and 1,3,4-oxadiazole (**5a-h**) has been synthesized using an appropriate synthetic route and characterized by elemental analysis and spectral data. The anticonvulsant activity of all the synthesized compounds was evaluated against maximal electroshock induced seizures (MES) and subcutaneous pentylenetetrazole (scPTZ) induced seizure models in mice. The neurotoxicity was assessed using the rota-rod method. All the test compounds were administered at doses of 30, 100 and 300 mg/kg body weight and the anticonvulsant activity was noted at 0.5 and 4h time intervals after the drug administration. All the compounds were active in MES and a majority of compounds were active in scPTZ test. All compounds were less neurotoxic than the standard drug phenytoin.

**Keywords:** Thiazolidin-4-one, azetidin-2-one, 1,3,4-oxadiazole, maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ).

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### Introduction

Epilepsy is not a disease, but a syndrome of different cerebral disorders of central nervous system (CNS), and it is characterized by paroxysmal, excessive and hyper synchronous discharges of large numbers of neurons [1]. Despite the increasing understanding of the pathogenesis of seizures and epilepsy, the cellular basis of human epilepsy remains a mystery

[2]. Studies have reported that in India the prevalence rate of epilepsy varies from 1710 to 9780 cases per million population [1]. Epilepsies are common and frequently devastating and affect around 1-2% of the world population. The convulsions of approximately 25% of epileptics are inadequately controlled by the standard drug therapy. The number of drugs useful for the treatment of epilepsy is remarkably small. Fewer than 20 drugs are currently marketed in the United States and of these only five or six are widely used[3]. It has been estimated adequate control of seizures could not be obtained in up to 20% of the patients with epilepsy using first generation of antiepileptic drugs (phenobarbital, phenytoin, carbamazepine, sodium valproate and diazepam)[4]. The limitations with conventional available antiepileptic drugs (AEDs) highlighted the need for developing newer agents for epilepsies [1].

The search for antiepileptic compounds with a more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry[3]. All currently approved antiepileptic drugs have dose-related toxicity and idiosyncratic side effects. In response to the premise that major medical breakthroughs in non-pharmacologic therapies for the treatment of epilepsy in the near future seem remote, the search for new antiepileptic drugs with lower toxicities and fewer side effects continues. During recent years, a large number of new AEDs have been marketed worldwide, but the proportion of patients failing to respond to drug treatment has not been changed in a significant extent [2].

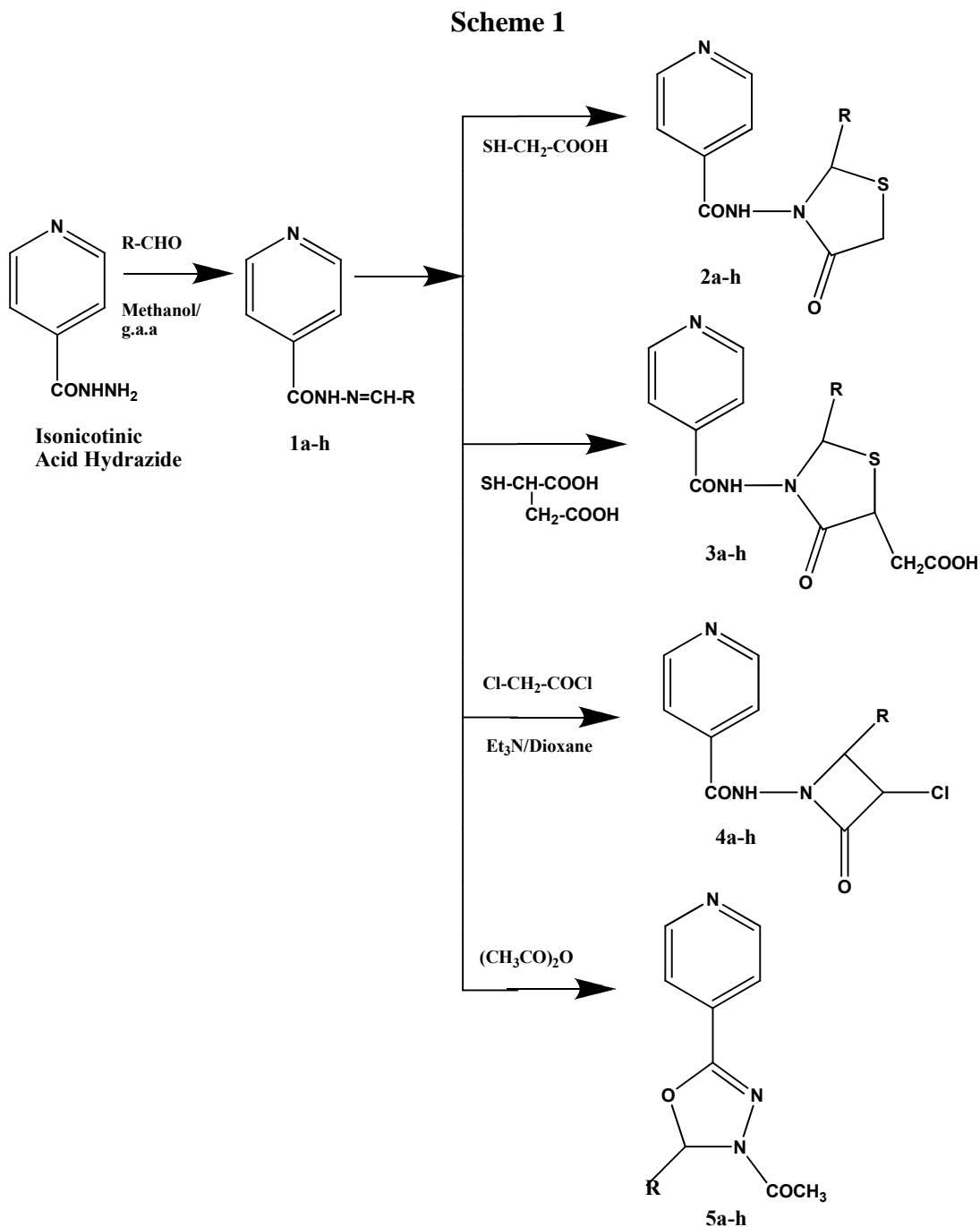
From the study of structures of clinically established drugs, it can be concluded that the anticonvulsant properties have been displayed by various hydrazones (= N-NH-), amides (-CONH) and carbamides (-NHCO-NH-) [1]. The prime need was to search for a molecule that could complement all the above structures in one and benzylidene isonicotinohydrazide was expected to be considered one of them. In the present investigation we have synthesized substituted derivatives of thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole, a versatile hydrophobic molecule possessing preliminary anticonvulsant properties [3], condensed with the isonicotinic acid hydrazide with the hope to potentiate the biological activities with lesser or limited amount of toxicities [Scheme 1].

## **Materials and Methods**

### ***Experimental***

#### ***Animals***

Male albino mice (Swiss, 18-25 gm) were used as experimental animals. The test compounds were suspended in polyethylene glycol (PEG). The animals were maintained on an adequate diet and allowed free access to food and water except during the short time they were removed from cages for testing. The animals were maintained at room temperature (25-30°C). All the experimental protocols were carried out with the permission from Institutional Animal Ethics Committee (IAEC), form no.520. Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi-110062, India. Registration number and date of registration of Animal House Facility (173/CPCSEA, 28, JAN-2000).



**1,2,3,4,5** ( a: R = *o*-C<sub>6</sub>H<sub>5</sub>Cl; b: R = *p*-C<sub>6</sub>H<sub>5</sub>Cl; c: R = *o*-C<sub>6</sub>H<sub>5</sub>OH; d: R = *m*-C<sub>6</sub>H<sub>5</sub>OH; e: R = *p*-C<sub>6</sub>H<sub>5</sub>OCH<sub>3</sub>; f: R = *p*-C<sub>6</sub>H<sub>5</sub>F; g: R = *o*-C<sub>6</sub>H<sub>5</sub>NO<sub>2</sub>; h: R = *p*-C<sub>6</sub>H<sub>5</sub>N(CH<sub>3</sub>)<sub>2</sub> )

### Chemistry

All the solvents were of AR grade and were obtained from Merck, CDH and S.D.Fine chemicals. Melting points were determined in open capillary tubes and are uncorrected. All the compounds were subjected to elemental analysis (CHN) and the measured values agreed within  $\pm 0.4\%$  with

the calculated ones. Thin layer chromatography was performed on silica gel G (Merck). The spots were developed in iodine chamber and visualized with an ultra-violet lamp. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS 135) WIN-IR spectrophotometer.  $^1\text{H-NMR}$  spectra were recorded on a Bruker model DPX 300 FT-NMR spectrometer in  $\text{CDCl}_3$  using tetramethylsilane ( $\text{Me}_4\text{Si}$ ) TMS as an internal standard. The chemical shifts are reported in  $\delta$  ppm scale.

*General procedure for the synthesis of (E)-N'-(substitutedbenzylidene) isonicotinohydrazide (1a-h)*

To an equimolar methanolic solution of isonicotinic acid hydrazide (0.1mol) and substituted benzaldehyde (0.1mol), a few drops of glacial acetic acid were added. The mixture was then refluxed on water bath for 5-6 h. It was then allowed to cool and poured into crushed ice. Recrystallisation of the dried compounds from methanol yielded compounds **1a-h**.

*(E)-N'-(2-chlorobenzylidene) isonicotinohydrazide (1a)*

Yield: 90%; m.p.180°C; IR( $\nu, \text{cm}^{-1}$ ):3300(N-H)str.,1680(C=O)str.,1600(-N=CH-Ar)str.,830 (C-Cl) str.;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 7.72, 8.63 (m,4H,Py), 7.12- 7.15 (m,4H, Ar-H,  $J=9\text{Hz}$ ), 6.1(s,1H,NH),7.9(s,1H,N-CH),MS m/z: 259[ $\text{M}^+$ ], 261[ $\text{M}^++1$ ]; Anal Calcd for:  $\text{C}_{13}\text{H}_{10}\text{N}_3\text{OCl}$ : C,60.10; H,3.79; N,16.12; Found:C,60.12; H,3.88; N,16.18.

*General procedure for the synthesis of N-(2-(substituted phenyl)-4-oxothiazolidin-3-yl) isonicotinamide (2a-h)*

A mixture of **1** (0.01mol) and thioglycolic acid (0.01mol) was heated on an oil-bath at 120-25 °C for 12h.The reaction mixture was cooled and treated with10%sodium bicarbonate solution. The product was isolated and recrystallised from methanol-dioxane (4:1) to give compounds **2a-h**.

*N-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl) isonicotinamide (2a)*

Yield: 85%; m.p.198 °C; IR( $\nu, \text{cm}^{-1}$ ): 3300 (NH) str., 1700(C=O thiazolidinone) str., 1670 (C=O) str.,700 (C-S-C)str., 830 (C-Cl) str., 1619(C=N) str.,1572 (C=C)str. $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) $\delta$  ppm:7.72,8.63(m,4H,Py),5.96(s,1H,-S-CH-Ar),7.12-7.15 (m,4H, Ar- H, $J=9\text{Hz}$ ),9.8(s,1H,CONH-),7.2 (s,1H,N-CH-),3.5(s,2H,CH<sub>2</sub>-C),MS m/z: 333[ $\text{M}^+$ ], 353 [  $\text{M}^++1$  ],334[ $\text{M}^++2$ ]; Anal Calcd for:  $\text{C}_{15}\text{H}_{12}\text{ClN}_3\text{O}_2\text{S}$ : C,53.92; H,3.59; N,12.54; Found:C,53.97; H,3.62; N,12.59.

*General procedure for the synthesis of 2-(2-(2-substitutedphenyl)-3-(isonicotinamido)-4-oxothiazolidin-5-yl) acetic acid (3a-h)*

A mixture of **1** (0.01mol) and thiomalic acid (0.01mol) was heated on an oil-bath at 120-125 °C for 12h. The reaction mixture was cooled and treated with10%sodium bicarbonate solution. The product was isolated and recrystallised from methanol-dioxane (4:1) to give compounds **3a-h**.

*2-(2-(2-chlorophenyl)-3-(isonicotinamido)-4-oxothiazolidin-5-yl) acetic acid (3a)*

Yield: 80%; m.p.185 °C; IR( $\nu, \text{cm}^{-1}$ ): 3200 (NH) str., 1700 (C=O thiazolidinone) str., 1666(C=O) str.,700(C-S-C)str., 830 (C-Cl) str., 1610(C=N)str., 1572 (C=C)str. $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 7.73, 8.63 (m,4H,Py),5.95(s,1H,-S-CH-Ar), 6.17- 6.15 (m, 4H,Ar-H, $J=6\text{Hz}$ ),9.8(s,1H,CONH-),7.2 (s,1H,N-CH-),10.0(s,1H,COOH), MS m/z: 391[ $\text{M}^+$ ], 393

[M<sup>+</sup>+1]; Anal Calcd for: C<sub>17</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>4</sub>S: C,52.09; H,3.57; N,10.69; Found: C,52.11; H,3.60; N,10.72.

**General procedure for the synthesis of N-(3-chloro-2-(2-substitutedphenyl)-4-oxaazetidin-1-yl)isonicotinamide (4a-h)**

A solution of **1** (0.01mol) in dioxane (20mL) was added to a well stirred mixture of chloroacetylchloride (0.012mol) and triethylamine (Et<sub>3</sub>N) (0.012mol) in dioxane (10mL) at 0-5 °C. The reaction mixture was then stirred for 8h, kept for 2days at room temperature and then treated with cold water. The solid thus obtained was filtered, washed with water and recrystallised from methanol to yield **4a-h**.

**N-(3-chloro-2-(2-chlorophenyl)-4-oxaazetidin-1-yl) isonicotinamide (4a)**

Yield:75%; m.p. >300 °C; IR(v,cm<sup>-1</sup>): 3250 (NH) str., 1745 (C=O β lactam ring) str.,1670(C=O) str.,742(C-Cl) str., 1600(C=N)str., 1560 (C=C)str.<sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ ppm: 7.73, 8.61 (m,4H,Py),6.61- 6.63 (m,4H,Ar-H,J=6Hz), 9.4(s,1H,CONH-), 7.7 (s,1H,N-CH-), MS m/z: 335 [M<sup>+</sup>], 337 [M<sup>+</sup>+1]; Anal Calcd for: C<sub>17</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>4</sub>S: C,53.56; H,3.26; N,12.46; Found: C,53.59; H,3.30; N,12.50.

**General procedure for the synthesis of 1-(2-(2-substitutedphenyl)-5-(pyridine-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5a-h)**

A mixture of **1**(0.003mol) and acetic anhydride (10mL) was heated under reflux for 4h. After the reaction mixture attained room temperature, excess acetic anhydride was decomposed by water and the mixture was stirred for further 30 min. The separated product was filtered, washed with water, dried and recrystallised in appropriate solvent systems to give the products **5a-h**.

**1-(2-(2-chlorophenyl)-5-(pyridine-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5a)**

Yield:65%; m.p.182 °C; IR(v,cm<sup>-1</sup>): 1660(acetyl C=O) str. 1614(C=N)str.,1560 (C=C)str.,830 (C-Cl) str.,1500(C-O-C)str.;<sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ ppm: 7.72, 8.64 (m,4H,Py), 7.12- 7.14 (m,4H, Ar-H, J=6Hz),7.19(s,1H,CH-oxadiazole);MS m/z:301[M<sup>+</sup>],303[M<sup>+</sup>+1]; Anal Calcd for: C<sub>15</sub>H<sub>12</sub>ClN<sub>3</sub>O<sub>2</sub>: C,59.68; H,3.98; N,13.90; Found:C,59.71; H,4.01; N,13.93.

Similarly other compounds (**1b-h**, **2b-h**, **3b-h**, **4b-h** & **5b-h**) of the series were synthesized and their structures are confirmed by their spectral and elemental analysis.

**Anticonvulsant screening:**

**Electroshock-induced seizures (MES test)**

Albino mice (20-25g) were used in this test. Animals were divided in groups of six and were stimulated through corneal electrodes to 50mA current at a pulse of 60 Hz alternating current for 2s.The mice were previously administered *i.p.* with the test drug solution in polyethylene glycol at three dose levels (30,100 and 300 mg/kg), the anticonvulsant activity was assessed after 30 min. and 4h intervals of administration. The abolition of hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity [5].

***Subcutaneous pentylenetetrazole seizure threshold test***

scPTZ was conducted by administering PTZ dissolved 0.9% sodium chloride solution in the posterior midline of the animals. A minimal time of 30 min subsequent to sc administration of PTZ was used for seizure detection. Protection was referred to as the failure to observe an episode of clonic spasms of at least 5s duration during this time period [3].

***Neurotoxic effects******Rota-rod test***

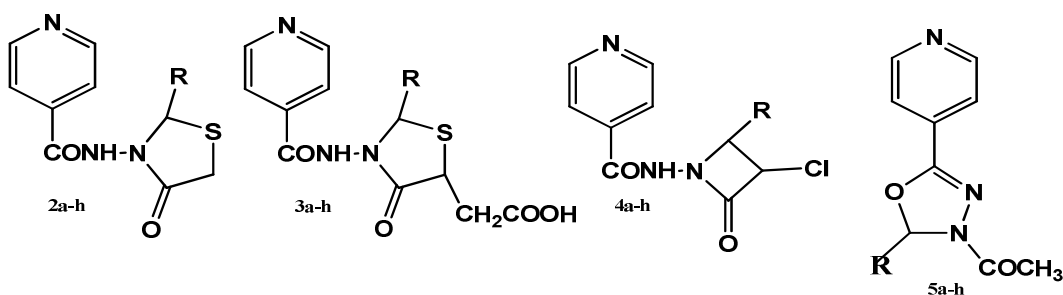
Minimal motor impairment was measured in mice by the rotorod test [6]. The mice were trained to stay on an accelerating rota-rod that rotates at 10 revolutions/min. The rod diameter was 30.2 cm. Trained animals were given i.p. injection of the test compounds 30, 100, 300 mg/kg. Neurotoxicity was indicated by the inability of the animals to maintain equilibrium on the rod for at least 1 min. in each of the trials.

**Results and Discussion**

The pharmacological evaluation of the compounds (**2a-h, 3a-h, 4a-h & 5a-h**) were initially carried out according to the protocols of antiepileptic drug development program (ADD), Epilepsy Branch, INH. The methods employed have been previously described [5]. The compounds were initially screened in the mouse MES test. Minimal motor impairment was measured by rotorod test. Data is represented in (Table 1). All the isonicotinic acid hydrazide incorporated derivatives of thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole ring systems were active in MES test at a dose of 300 mg/kg indicative of their ability to protect the seizure spread. At a dose of 30 mg/kg, compounds that showed protection in half or more tested mice were (**2a, 2c, 2f, 2g, 3a-c, 4a-c, 5a, 5c, 5f & 5g**) after 0.5h time interval. These compounds also showed protection after 4h but at a higher dose of 100 mg/kg. The compounds (**2b, 2d, 3d, 3f, 3g, 4d, 4e, 4g, 5b, 5d & 5e**) showed protection at a dose of 100 mg/kg after 0.5h. These compounds also showed protection after 4h but at a higher dose of 300 mg/kg. Compounds (**2e, 2h, 3e, 3h, 4h & 5h**) showed protection in MES test at 300 mg/kg both after 0.5h and 4h duration.

In the scPTZ screen compounds (**2a, 2c, 2f, 2g, 3a, 4c, 4f, 5a, 5c & 5g**) had shown activity at 30 mg/kg dose level after 0.5h time interval and 100 mg/kg dose level after 4h time interval but compounds (**2d, 3b, 3c, 4a, 4b, 4d & 5f**) had shown activity at the dose level of 100 mg/kg after 0.5h time interval. These compounds also showed protection after 4h but at a higher dose of 300 mg/kg. Rest of the compounds (**2b, 2h, 3e, 3f, 3h, 4h, 5b, 5e & 5h**) had shown activity at the dose level of 300 mg/kg at both time intervals except compounds (**2e, 3d, 3g, 4e, 4g & 5d**) which had inactivity.

In neurotoxicity screening, the compounds (**2a, 2d, 3d, 3e, 3g, 4a, 4e, 4g, 5a & 5g**) were neurotoxic at a maximal dose of 300 mg/kg after 0.5h. Compounds (**2e, 2g, 3c, 4b, 4d, 5d & 5e**) showed neurotoxicity at a dose of 100 mg/kg after 4h. All the compounds showed neurotoxicity at a higher dose of 300 mg/kg after 0.5h time interval. However all the compounds were less neurotoxic than phenytoin.

**Table1. Anticonvulsant profile and rota-rod toxicity of the examined compounds (2a-h, 3a-h, 4a-h, 5a-h) in mice**

Compound	Intraperitoneal injection in mice <sup>a</sup>					
	MES screen		scPTZ		Neurotoxicity screen	
	0.5h	4h	0.5h	4h	0.5h	4h
<b>2a</b>	30	100	30	100	300	-
<b>2b</b>	100	300	300	300	300	300
<b>2c</b>	30	100	30	100	300	300
<b>2d</b>	100	300	100	300	300	-
<b>2e</b>	300	300	-	-	300	100
<b>2f</b>	30	100	30	100	300	300
<b>2g</b>	30	100	30	100	300	100
<b>2h</b>	300	300	300	300	300	300
<b>3a</b>	30	100	30	100	300	300
<b>3b</b>	30	100	100	300	300	300
<b>3c</b>	30	300	100	300	300	100
<b>3d</b>	100	300	-	-	300	-
<b>3e</b>	300	300	300	300	300	-
<b>3f</b>	100	300	300	300	300	300
<b>3g</b>	100	300	-	-	300	-
<b>3h</b>	300	300	300	300	300	300
<b>4a</b>	30	100	100	300	300	-
<b>4b</b>	30	100	100	300	300	100
<b>4c</b>	30	100	30	100	300	300
<b>4d</b>	100	300	100	300	300	100
<b>4e</b>	100	300	-	-	300	-
<b>4f</b>	30	100	30	100	300	300
<b>4g</b>	100	300	-	-	300	-
<b>4h</b>	300	300	300	300	300	300
<b>5a</b>	30	100	30	100	300	-
<b>5b</b>	100	300	300	300	300	300
<b>5c</b>	30	100	30	100	300	300
<b>5d</b>	100	300	-	-	300	100
<b>5e</b>	100	300	300	300	300	100
<b>5f</b>	30	100	100	300	300	300

<b>5g</b>	30	100	30	100	300	-
<b>5h</b>	300	300	300	300	300	300
<b>Phenytoin<sup>b</sup></b>	30	30	-	-	100	100
<b>Carbamazepine<sup>b</sup></b>	30	100	100	100	100	300

<sup>a</sup>Doses of 30,100,300 mg/kg were administered. The figure in the table indicates the minimum dose whereby bioactivity was demonstrated in half or more of the animals (n=6).The animals were examined 0.5h and 4h after administration. The (-) indicates an absence of activity at maximum dose administered (300mg/kg).

<sup>b</sup>Data from references [7,8].

## Conclusion

In general, substitution of *o*-Cl, *o*-OH, *o*-NO<sub>2</sub> and *p*-F at a distal phenyl ring showed potent activity against MES and scPTZ test. The presence of *p*-Cl, *m*-OH and *p*-OCH<sub>3</sub> at distal phenyl ring showed moderate activity against MES and scPTZ test. Substitution with N(CH<sub>3</sub>)<sub>2</sub> showed protection against MES test at a higher dose of 300mg/kg. In conclusion, the majority of the compounds of isonicotinic acid hydrazide incorporated derivatives of thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole were active in MES and scPTZ test and all the compounds were less neurotoxic than phenytoin.

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