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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization and pharmacological evaluation of (*E*)-*N*′-(substituted-benzylidene)isonicotinohydrazide derivatives as potent anticonvulsant agents

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Abstract A series of (*E*)-*N*′-(substituted-benzylidene)isonicotinohydrazide derivatives were synthesized by coupling it with different substituted aldehydes, acetophenone, and benzophenones in presence of absolute ethanol along with catalytic amount of glacial acetic acid. All the synthesized compound were confirmed and characterized by using various spectral technique like IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy studies. Anticonvulsant evaluations of all the synthesized compounds were done using various seizures models like maximal electroshock-induced seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) at a dose of 30, 100, and 300 mg/kg body weight and anticonvulsant activity was noted at 0.5 h and 4 h time intervals after the drug administration. Compound **1a** (*E*)-*N*′-2-benzylidene isonicotinohydrazide, **1g** (*E*)-*N*′-2-ethoxybenzylidene isonicotinohydrazide, **1k** (*E*)-*N*′-3-flourobenzylidene isonicotinohydrazide and **3a** (*E*)-*N*′-diphenylmethylene isonicotinohydrazide showed protection in MES model, which indicates that these compounds have the ability to prevent the spread of seizure at 300 mg/kg dose and showed protection at 0.5 h duration. Compound **3a** was also found to be active in scPTZ screen at a dose of 300 mg/kg. In neurotoxicity screen, all the synthesized compounds were found non-toxic except compounds **1n**, **2a**, and **3b**. Further compounds **1a**, **1g**, **1k**, and **3a** were also evaluated in the minimal clonic seizure model and exhibited potent anticonvulsant activity with lower neurotoxicity. Among all synthesized derivatives, analogue **3a** was found to exhibit protection in MES and scPTZ seizure models. This study proved that isonicotinoyl hydrazides synthesized by condensing isoniazid with various aldehydes and ketones displayed moderate to potent anticonvulsant activity.

Keywords Isoniazid · (*E*)-*N*′-(substituted-benzylidene)isonicotinohydrazide · Anticonvulsant · Pentylenetetrazole · Neurotoxicity

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Introduction

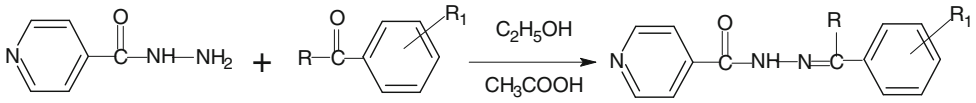
In the modern world, the drugs affecting central nervous system plays significant role. Centrally acting drugs are of special interest to mankind. So, over the last century, large numbers of compounds with specific effects on brain and behavior have been discovered (Aggarwal *et al.*, 2009).

Epilepsy is a central nervous system disorder characterize by paroxysmal and excessive discharges of large number of neurons (Kaushik *et al.*, 2010). There is urgent need for improved drugs for the treatment because the presence of large number of patients who are resistant to the available antiepileptic drugs. The long established AEDs control seizures in 50% of patients developing partial seizures and in 60–70% of those developing generalized seizures (Kulandasamy *et al.*, 2009a, b; Duncan, 2002). During past decade numerous new drugs were approved but despite the advances in the new drug treatment of epilepsy, approved antiepileptic drugs have dose-related toxicity and idiosyncratic side effects (Ghogare *et al.*, 2010). Mechanistic approaches are increasingly being facilitated by the wave of research in the epileptics (Eadie *et al.*, 1989). Some of the currently available active drugs have not been directly linked with any specific receptor within the brain. It is too difficult to identify common pharmacophore responsible for prevention or arrest of seizure activity mainly because of the chemical diversity of organic compounds and their multiple mechanism of action in controlling the seizures. Hence, the search for antiepileptic compounds with more selective activity and minimum toxicity continues to be an area of investigation in medicinal chemistry (Kulig *et al.*, 2011). So, the anticonvulsant drug discovery has given a high priority. Further anticonvulsant properties displayed by various established drugs containing hydrazones group open a new path for the treatment of epilepsy. The Semicarbazones have been considered as a new class of anticonvulsants with oral activity (Angelusiu *et al.*, 2010). Semicarbazone have documented consistent advances in the design of novel anticonvulsant agents, through the work of Dimmock and his collogues (Dimmock *et al.*, 1999). A number of (aryl-oxy) aryl semicarbazones possessed greater protection in the maximal electroshock seizure MES screen (Dimmock *et al.*, 1996). Semicarbazones displaying activity in the MES screen interact at a specific binding site referred as the hydrogen bonding area and aryl binding site, respectively (Dimmock *et al.*, 1996). In the initial studies on aryl semicarbazones, it was found to possess potent anticonvulsant activity and the importance of the terminal amino group of semicarbazone was implicated in hydrogen bonding (Pandeya *et al.*, 1998, Pandeya *et al.*, 2002). The aryl acid hydrazones contains large hydrophobic groups similar to semicarbazones perform the structural requirements of maximal electroshock (MES) screen (Dimmock *et al.*, 1995). Semicarbazones act at a specific binding site called as hydrogen bonding area and aryl binding site (Yogeeswari *et al.*, 2004, 2005). The gamma amino butyric acid an inhibitory neurotransmitter gets degradated by pyridoxal phosphate dependent enzyme known as gamma amino butyrate amino transferase (Jones and Woodbury 1982). Also, it is well documented that attenuation of GABAergic neurotransmission is involved in the pathophysiology of several CNS disorders in humans, namely anxiety, pain, and epilepsy. It is one of the important targets in the design and discovery of successful antiepileptic drugs (Gajcy *et al.*; 2010, Loscher *et al.*, 1985; Curtis *et al.*, 1978). Until now, aryl and heteroaryl semicarbazones and thiosemicarbazones are very well-established anticonvulsants. By considering the anticonvulsant potential displayed by these class of compounds, we tried to develop aryl acid hydrazide–hydrazones as new congeners to join this, as this group mimics the cyclic ureide group of semicarbazone along with the other fundamental requirement. In terms of interaction at the binding site, as suggested by Dimmock *et al.* (1996; Puthucode *et al.*, 1998) the pharmacophoric elements were thought to be lipophilic aryl ring and hydrogen bonding acid hydrazide moiety. The conformational analysis of previously well established drugs showed that for anticonvulsant activity, the model must be comprised of two aromatic rings (Yogeeswari *et al.*, 2005) as proximal aryl ring and distal ring to increase the van der Waal's bonding at the binding site and to increase the potency as reported in a favoured orientation and a third region, usually the number of hydrogen bonding forming functional groups. Also the specific placement of these hydrogen bonding groups in this region appeared to be less important than the proper orientation and correct conformation (Wong *et al.*, 1986). The presence of electron rich group at ortho and para position of the aryl ring has shown increased potency in the MES screen. The presence of halogens particularly, showed low ED_{50} values in the rat oral MES screen. It was further associated with higher protection index values (Yogeeswari *et al.*, 2005). Halogen substituted semicarbazone was reported as the prototype molecule from the class of semicarbazones (Dimmock and Baker 1994). Keeping all this in background, we tried to develop and explore this particular class of compounds as potential anticonvulsant agents with minimal number of side effects. In this work, we highlight the synthesis, characterization, anticonvulsant, and neurotoxicity profile of aryl acid hydrazones.

Chemistry

The synthesis of target compounds were carried outline in Scheme 1. Compounds **1a–1n**, **2a**, and **3a–3b** is readily prepared in good yields and purity. Equimolar quantity of substituted benzaldehydes, acetophenone, and benzophenones with equimolar quantity of isoniazid in ethanolic medium with catalytic amount of glacial acetic acid was refluxed for 3–9 h to form acid hydrazones. The completion of reaction was confirmed by thin layer chromatography (TLC). The reaction mixture was then poured in ice

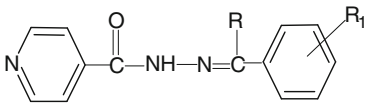
Scheme 1 Synthetic pathway for the formation of the title compounds



Isoniazid | Substituted benzaldehydes, acetophenone and benzophenones | Substituted aryl acid hydrazones (**1a-1n, 2a, 3a-3b**)

cold water and the precipitate obtained was filtered and dried in oven at low temperature. The products were recrystallised from absolute ethanol. The percentage yield was calculated. Physical Data of Substituted aryl acid hydrazones is shown in Table 1. The purity of the compounds was checked by TLC, elemental analyses, and characterized by spectral data. In general, IR spectra of most compounds **1a**–**1n**, **2a**, and **3a**–**3b** showed absorption band at around 3365–3199, 3071–3029, 1691–1659, 1662–1625, 1569–1542 cm^{-1} regions, conforming the presence of NH, CH, C=N, C=O, C=C, respectively. The ^{1}H NMR spectra, the signals of the respective prepared most derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of most compounds showed the characteristic NH proton δ 12.63–11.52 ppm, 1H proton of –N=C–H at δ 8.92–8.30 ppm, 4H proton of pyridine were at around δ 8.92–7.50 ppm, and characteristic protons of benzylidene at δ 8.79–6.75 ppm.

Table 1 Physical Data of Substituted aryl acid hydrazones



Compounds	R	R_1	Yield (%)	MP (°C)	R_f^a
1a	H	H	82	205–207	0.45
1b	H	2-Cl	75	218–220	0.27
1c	H	4-Br	72	215–217	0.38
1d	H	3-NO_2	68	222–224	0.35
1e	H	2,4-NO_2	72	220–222	0.42
1f	H	4- OCH_3	58	147–149	0.55
1 g	H	2-OC_2H_5	64	205–207	0.47
1 h	H	4-OC_3H_7	58	157–159	0.45
1i	H	4-OH, 3-OCH_3	72	212–214	0.39
1j	H	3,4-$(OCH_3)_2$	65	188–190	0.48
1 k	H	3-F	62	210–212	0.53
1 l	H	2-OH	68	203–205	0.55
1 m	H	4-OH	71	187–189	0.52
1n	H	4-$N(CH_3)_2$	69	207–209	0.43
2a	CH_3	H	74	169–171	0.47
3a	C_6H_5	–	68	113–115	0.38
3b	C_6H_5	4-Br	71	158–160	0.44

[a] R_f calculated in chloroform:ethyl acetate (1:1)

^{13}C-NMR spectra of most compounds have characteristic C=O signals appeared at around δ 164.52–162.92 ppm, pyridine δ 149.89–121.11 ppm, –N=C–H δ 143.72–142.69 ppm, benzylidene δ 168.17–113.64 ppm.

Experimental

Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart SMP10 melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel plates kiesel gel 0.25 mm, 60 GF_{254}, precoated sheets obtained from Merck, Darmstadt (Germany) were used for TLC and the spots were visualized by iodine vapors/ultraviolet light as visualizing agent. The IR spectra (υ, cm^{-1}) were obtained with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. ^{1}H-NMR spectra (δ, ppm) were recorded in DMSO-d_6 solutions on a Varian-Mercury 300 MHz spectrometer using tetramethylsilane as the internal reference. ^{13}C NMR spectra were recorded on in DMSO-d_6 solutions on a Bruker Avance II 400 spectrometer at 400 MHz using tetramethylsilane as the internal reference. Mass spectra were recorded on a shimadzu GCMS-QP 1000 EX. Elemental analyses were performed on an ECS 4010 Elemental Combustion System. The necessary chemicals were purchased from Sigma Aldrich companies.

General procedure for the synthesis of aryl acid hydrazones

The synthesis of the 17 isonicotinohydrazide derivatives **1a**–**1n**, **2a** and **3a**–**3b** were synthesized, among them **1e**, **1g**, **1h**, **1i**, **1l**, **1m**, **1n**, **3a**, and **3b** being unpublished, was performed with good yields from commercially available materials. The compounds **1a**–**1d**, **1f**, **1j**, **1k**, and **2a** have been reported with their anti-tubercular activity elsewhere (Lourenco *et al.*, 2007, 2008). The corresponding substituted benzaldehydes otherwise acetophenone or else benzophenones (0.01 mol) was reacted with Isoniazid (1.37 g, 0.01 mol) in ethanolic medium with catalytic amount of glacial acetic acid was refluxed for 3–9 h to form acid hydrazones. The completion of reaction was confirmed by thin layer chromatography. The reaction

mixture was then poured in ice cold water and the precipitate obtained was filtered and dried the solid residue in a desiccator, was recrystallised from absolute ethanol to yield compounds. The percentage yield was calculated.

*(E)-N′-(2,4-dinitrobenzylidene)isonicotinohydrazide (**1e**)*

IR (υ, cm^{-1}): 3199, 3052, 1685, 1629, 1556, 1529, 1342, 1163. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 12.63 (s, 1H, –NH–N=), 8.92 (s, 1H, –N=C–H), 8.79 (s, 1H, benzylidene), 8.79 (d, 2H, pyridine, $J = 4.2$ Hz), 8.61 (d, 2H, pyridine, $J = 3.7$ Hz), 7.85 (d, 2H, benzylidene, $J = 8.2$ Hz); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 162.92, 153.17, 149.79, 142.84, 139.66, 131.76, 129.72, 127.34, 121.18, 117.66. MS (ESI) $m/z = 316$ (M + 1): Anal. Calcd. for $C_{13}H_9N_5O_5$ (315.24): C 49.53, H 2.88, N 22.22. Found: C 49.47, H 2.91, N 22.25.

*(E)-N′-(2-ethoxybenzylidene)isonicotinohydrazide (**1g**)*

IR (υ, cm^{-1}): 3249, 3045, 1659, 1635, 1550, 1467, 1372, 1253, 1023. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 12.07 (s, 1H, –NH–N=), 8.82 (s, 1H, –N=C–H), 8.75 (d, 2H, pyridine, $J = 4.4$ Hz), 7.89 (d, 2H, pyridine, $J = 3.7$ Hz), 7.84 (d, 2H, benzylidene, $J = 7.5$ Hz), 7.38 (d, 2H, benzylidene, $J = 7.2$ Hz), 4.08 (m, 2H, CH_2) 1.33 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.54, 158.74, 149.59, 143.52, 139.79, 132.12, 128.79, 122.72, 120.18, 117.94, 114.72, 64.92, 13.89. MS (ESI) $m/z = 270$ (M + 1): Anal. Calcd. for $C_{15}H_{15}N_3O_2$ (269.32): C 66.90, H 5.61, N 15.60. Found: C 66.86, H 5.59, N 15.66.

*(E)-N′-(4-propoxybenzylidene)isonicotinohydrazide (**1h**)*

IR (υ, cm^{-1}): 3259, 3029, 1665, 1642, 1546, 1464, 1371, 1251, 1023. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.92 (s, 1H, –NH–N=), 8.77 (d, 2H, pyridine, $J = 4.5$ Hz), 8.39 (s, 1H, –N=C–H), 7.81 (d, 2H, pyridine, $J = 4.1$ Hz), 7.68 (d, 2H, benzylidene, $J = 8.2$ Hz), 7.01 (d, 2H, benzylidene, $J = 7.5$ Hz), 3.97 (t, 2H,CH_2), 1.75 (m, 2H, CH_2) 0.98 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.49, 159.79, 149.82, 143.23, 139.79, 129.57, 125.55, 121.87, 114.18, 72.29, 22.36, 11.88. MS (ESI) $m/z = 298$ (M + 1): Anal. calcd. for $C_{16}H_{17}N_3O_2$ (283.33): C 67.83, H 6.05, N 14.83. Found: C 67.85, H 6.07, N 14.79.

*(E)-N′-(4-hydroxy-3-methoxybenzylidene) isonicotinohydrazide (**1i**)*

IR (υ, cm^{-1}): 3336, 3256, 3029, 1661, 1639, 1542, 1292, 1251, 1029. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.86 (s, 1H, –NH–N=), 8.76 (d, 2H, pyridine, $J = 4.3$), 8.34 (s, 1H, –N=C–H), 7.80 (d, 2H, pyridine, $J = 3.9$), 7.32 (s, 1H, benzylidene), 7.09 (d, 1H, benzylidene, $J = 8.2$), 5.08 (s, 1H, OH, D_2O exchangeable), 3.82 (s, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.42, 151.94, 149.53, 147.65, 142.83, 140.38, 128.12, 123.74, 122.91, 116.85, 113.64, 55.89. MS (ESI) $m/z = 272$ (M + 1): Anal. Calcd. for $C_{14}H_{13}N_3O_3$ (271.27): C 61.99, H 4.83, N 15.49. Found: C 61.97, H 4.76, N 15.58.

*(E)-N′-(3,4-dimethoxybenzylidene)isonicotinohydrazide (**1j**)*

IR (υ, cm^{-1}): 3232, 3047, 1675, 1645, 1560, 1249, 1026. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.82 (s, 1H, –NH–N=), 8.56 (d, 2H, pyridine, $J = 4.8$ Hz), 8.43 (s, 1H, –N=C–H), 7.80 (d, 2H, pyridine, $J = 4.3$), 7.35 (d, 2H, benzylidene, $J = 8.4$), 7.11 (s, 1H, benzylidene), 3.82 (s, 6H, $2CH_3$); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.59, 152.98, 149.71, 149.12, 143.72, 139.82, 126.89, 122.82, 122.11, 114.28, 56.72. MS (ESI) $m/z = 286$ (M + 1): Anal. Calcd. for $C_{15}H_{15}N_3O_3$ (285.32): C 63.15, H 5.30, N 14.73. Found: C 63.14, H 5.16, N 14.88.

*(E)-N′-(2-hydroxybenzylidene)isonicotinohydrazide (**1l**)*

IR (υ, cm^{-1}): 3345, 3284, 3071, 1677, 1648, 1553, 1283. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.47 (s, 1H, –NH–N=), 8.92 (d, 2H, pyridine, $J = 4.8$), 8.79 (s, 1H, –N=C–H), 7.85 (d, 2H, pyridine, $J = 4.2$), 7.54 (d, 2H, benzylidene, $J = 8.3$), 7.38 (d, 2H, benzylidene, $J = 7.7$), 4.84 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.75, 149.77, 143.18, 139.78, 130.57, 124.89, 121.85, 116.45. MS (ESI) $m/z = 242$ (M + 1): Anal. Calcd. for $C_{13}H_{11}N_3O_2$ (283.33): C 64.72, H 4.60, N 17.42 Found: C 64.65, H 4.55, N 17.54.

*(E)-N′-(4-hydroxybenzylidene)isonicotinohydrazide (**1m**)*

IR (υ, cm^{-1}): 3368, 3238, 3059, 1671, 1638, 1556, 1289. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.42 (s, 1H, –NH–N=), 8.89 (d, 2H, pyridine, $J = 4.7$), 8.74 (s, 1H, –N = C–H), 7.89 (d, 2H, pyridine, $J = 4.2$), 7.64 (d, 2H, benzylidene, $J = 7.8$), 7.35 (d, 2H, benzylidene, $J = 7.2$), 5.08 (s, 1H, OH, D_2O exchangeable); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.72, 159.89, 143.52, 139.76, 130.44, 125.57, 121.94, 116.37. MS (ESI) m/z = 242 (M + 1): Anal. Calcd. for $C_{13}H_{11}N_3O_2$ (241.25): C 64.72, H 4.60, N 17.42 Found: C 64.69, H 4.62, N 17.43.

*(E)-N′-(4-dimethylaminobenzylidene)isonicotinohydrazide (**1n**)*

IR (υ, cm^{-1}): 3245, 3064, 1675, 1637, 1555, 1168. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.76 (s, 1H,

–NH–N=), 8.75 (d, 2H, pyridine, $J = 4.7$), 8.30 (s, 1H, –N=C–H), 7.79 (d, 2H, pyridine, $J = 4.2$), 7.55 (d, 2H, benzylidene, $J = 8.7$), 6.75 (d, 2H, benzylidene, $J = 8.2$), 2.98 (s, 6H, CH_3); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.18, 152.16, 149.77, 143.27, 139.86, 130.55, 121.79, 114.88, 41.56. MS (ESI) $m/z = 269$ (M + 1): Anal. Calcd. for $C_{15}H_{16}N_4O$ (268.31): C 67.15, H 6.01, N 20.83 Found: C 67.18, H 5.95, N 20.31.

*(E)-N′-(diphenylmethylene)isonicotinohydrazide (**3a**)*

IR (υ, cm^{-1}): 3244, 3062, 1691, 1662, 1569. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.52 (s, 1H, –NH–N=), 8.67 (d, 2H, pyridine, $J = 4.6$), 7.84 (d, 2H, pyridine, $J = 4.1$) 7.58 (m, 10 ArH, benzylidene); ^{13}C-NMR (400 MHz, DMSO-d_6) $\delta = 163.37$, 156.18, 149.64, 139.79, 133.12, 131.28, 127.15, 122.84. MS (ESI) $m/z = 302$ (M + 1): Anal. Calcd. for $C_{19}H_{15}N_3O$ (301.34): C 75.73, H 5.02, N 13.94 Found: C 75.82, H 5.05, N 13.82.

*(E)-N′-4-(bromophenylphenylmethylene) isonicotinohydrazide (**3b**)*

IR (υ, cm^{-1}): 3256, 3054, 1689, 1662, 1557, 649. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.65 (s, 1H, –NH–N=), 8.68 (d, 2H, pyridine, $J = 4.8$), 7.72 (d, 2H, pyridine, $J = 4.2$), 7.50 (m, 4H, benzylidene), 7.37 (m, 5H, phenyl); ^{13}C-NMR (400 MHz, DMSO-d_6, δ ppm): 163.33, 154.46, 149.77, 138.74, 131.81, 131.45, 131.15, 127.91, 125.43, 121.68. MS (ESI) $m/z = 381$ (M + 1): Anal. Calcd. for $C_{19}H_{14}BrN_3O$ (268.31): C 60.02, H 3.71, N 11.05 Found: C 60.05, H 3.69, N 11.04.

Pharmacology

Maximal electroshock seizures (MES), subcutaneous pentylene tetrazole-induced seizures (scPTZ), minimal clonic seizure (6 Hz), and rotarod test were carried out by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological Disorders and Stroke (NINDS) program following previously described testing procedures (Krall *et al.*, 1978; Porter *et al.*, 1984; Barton *et al.*, 2001). Compounds were dissolved in polyethylene glycol 400 and evaluated for anticonvulsant activity with both sexes of C57B/6 mice in the 18–22 g weight range and Sprague-Dawley rats in the 120–125 g weight range in both qualitative and quantitative screening methods. In quantitative screening, groups of eight mice were given a range of intraperitoneal doses (per oral for rats) of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal-observed neurotoxicity.

Maximal electroshock seizure

Seizures were elicited in mice with (50 mA) or in rats (150 mA) 60 Hz alternating current. 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 tonic maximal extension component of the seizure. At 0.5 h and 4.0 h after administration of the test compounds, activities were evaluated in the MES test.

Subcutaneous pentylene tetrazole-induced seizures

Seizures were produced in mice and rats with subcutaneous administration of pentylene tetrazole (85 mg/kg for mice and 70 mg/kg for rats). The test compounds were administered intraperitoneally in mice and orally in rats followed by subcutaneous injection of pentylene tetrazole. Protection against the spread of scPTZ-induced seizures was defined as the absence of clonic spasm. At 0.5 h and 4.0 after administration of the compounds, activities were evaluated in the scPTZ test.

Rotarod test

At 30 min after the administration of test compounds, the animals were tested on a 1-in. diameter knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in each of three trials.

Minimal clonic seizure test

The minimal clonic seizure test is used to assess a compound's efficacy against electrically induced seizures but uses low frequency (6 Hz) and longer duration of stimulation (3 s). Test compounds are pre-administered to mice via i.p. injection. At varying times, individual mice are challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% of animals (32 mA for 3 s). Protection was defined as the absence of minimal clonic phase with stereotyped, automatistic behaviors. This behavior is described as being similar to the aura of human patients with partial seizures.

Result and discussions

All the synthesized hydrazones were subjected to anticonvulsant screening by maximal electroshock seizures test

(MES) and subcutaneous pentylenetetrazole test (scPTZ) using doses of 30, 100, and 300 mg/kg and observations were carried out at two different time intervals (0.5 and 4.0 h). Among all the synthesized derivatives the compounds **1a**, **1g**, **1k**, **1n**, **2a**, **3a–3b** was found to be active and the rest of the compounds were inactive as shown in Table 2. The result of the MES results revealed that compound **1a**, **1g**, **1k** and **3a** exhibited anti MES activity at a dose of 300 mg/kg at 0.5 h duration. The compound **1a** and **3a** have phenyl group to hydrazone has shown very good anticonvulsant activity. As it was reported by (Kulandasamy *et al.*, 2009a, b) the presence of phenyl group is necessary for a compound to exhibit potent anticonvulsant activity. However, none of the compound was found to be active at 4 h thus, indicate that these compounds had the ability to prevent the spread of seizure and having a rapid onset and short duration of action.

All the test compounds were found to be inactive except **3a** in the scPTZ test, a test used to identify compounds that elevate seizure threshold. The compound (**3a**) displayed anticonvulsant activity at a dose of 300 mg/kg at 0.5 h of time period indicating that the compound is short acting.

As seen from the results of neurotoxicity screening, compounds **1n** and **2a** exhibited toxicity at a dose of 300 mg/kg where as compound (**3b**) showed at a dose of 100 mg/kg at 4 h but did not exhibit any toxicity at 5 h interval. It is interesting to note that the remaining compounds did not exhibit any neurotoxicity at a maximum administered dose level of 300 mg/kg. These results clearly indicated that toxicity is not attributed due to the basic moiety but it is due to the presence of other substituents attached to it. In addition to these tests, compound displaying activity in MES screen, were selected for evaluation of their anticonvulsant activity in minimal clonic seizure model (6 Hz) where smaller amount of electric current was given for longer duration of time as shown in Table 3. In this screen, compounds **1a**, **1g**, **1k**, and **3a** displayed strong anticonvulsant activity with lower neurotoxicity.

From the results it has been noticed that the introduction of phenyl group to hydrazone (**1a**) has caused moderate activity while the substitution by 2-hydroxyphenyl (**1l**) group has resulted in loss of activity on the other hand replacement of hydroxy group with ethoxy group (**1g**) in position 2 of phenyl ring imparts activity to the compounds as reported by (Ghogare *et al.*, 2010) even the presence of electron donating group impart a very good anticonvulsant activity. Thus the presence of electron donating group at *o*-position of phenyl ring (**1g**) contributes toward anticonvulsant activity as the analogue if having electronegative group (**1b**) at 2-position of phenyl ring resulted in the loss of activity. Further, the compounds having substituent at the 4-position (**1c**, **1f**, **1h**, **1m**, and **3b**) were found to be inactive. Even the disubstituted compounds **1e** and **1i** does not exhibit any protection. It is intresting to note that the incorporation of 4-fluoro phenyl group to hydrazone (**1k**) impart anticonvulsant activity to the compound activity probably due to the bioisosteric resemblance of flourine with hydrogen atom. As earlier reported by (Sinha *et al.*, 2010) six nicotinic acid hydrazone derivatives D_2, D_4, D_8, D_9, D_{10} and D_{17} were found to be exhibit significant anticonvulsant activity, all the derivatives contain electron withdrawing group. Thus from the results it has been noticed the nature and position of the substituent has marked effect on anticonvulsant activity. Analogue (**3a**), diphenyl hydrazone derivative in which the hydrogen atom of NH group was replaced by a phenyl group exhibit protection in both MES, scPTZ induced seizures model at a dose of 300 mg/kg and devoid of any neurotoxicity. Thus, from the results, it has been noticed the nature and position

Table 2 Anticonvulsant evaluation of compounds **1a**, **1g**, **1k**, **1n**, **2a**, **3a–3b**

Comp. no.	Intraperitoneal injection in mice[a]					
	MES screen		scPTZ screen		Neurotoxicity	
	0.5	4.0	0.5	4.0	0.5	4.0
1a	300	–	–	–	–	–
1g	300	–	–	–	–	–
1k	300	–	–	–	–	–
1n	–	–	–	–	–	300
2a	–	–	–	–	300	300
3a	300	–	300	–	–	–
3b	–	–	–	–	–	100
Phenytoin	30	30	–	–	100	100

The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. – indicates absence of activity at maximum dose administered (300 mg/kg) a doses of 30, 100, and 300 mg/kg were administered

Compounds **1a**, **1g**, **1k**, **1n**, **2a**, **3a–3b** was found to be active and the rest of the compounds were inactive as shown in Table 2

Table 3 Anticonvulsant activity of compounds **1a**, **1g**, **1k**, and **3a** at 6 Hz

Comp. no.	Dose (mg/kg)	MES screen				
		0.25 h	0.5 h	1.0 h	2.0 h	4.0 h
1a	100	3/4	2/4	1/4	0/4	0/4
1g	100	3/4	3/4	0/4	0/4	0/4
1k	100	2/4	1/4	0/4	0/4	0/4
3a	100	3/4	3/4	2/4	0/4	0/4

Dose of 100 mg/kg was administered. The data indicate the number of mice of four that were protected

of the substituent has marked effect on anticonvulsant activity of the synthesized compounds.

Conclusion

This study reports the synthesis of (*E*)-*N*'-(mono-substituted-benzylidene)isonicotinohydrazide derivative and all were characterized by spectral and elemental analysis. All the compounds were subjected to antiepileptic screening by standard methods. The compounds **1a**, **1g**, **1k**, and **3a** were found to exhibit protection in MES model, were also found to exhibit potent anticonvulsant activity in 6 Hz model at a dose of 100 mg/kg and displayed lesser neurotoxicity. Among the tested model the 6 Hz results were promising. Thus, hydrazone derivatives of isoniazid displayed moderate to good anticonvulsant activity. Here the activity is attributed to the presence of favorable structural environment such as aryl binding site with a hydrophobic group, hydrogen bonding domain –NHCO– group, electron donar, electron withdrawing group and another hydrophobic aryl ring in these hydrazone. Rather increase in the hydrophobicity in the synthesized molecules brings about same degree of activity in the series. So, hydrazone derivative **3a** found to be the most promising analogue displaying protection in all tested seizures models with least contribution toward the neurotoxicity and emerged as lead in these series. Further, the **1a**, **1g**, and **1k** come out as potential candidates for further investigation. Finally, it can be readily conclude that the substitution pattern in the phenyl ring influences the activity as well as toxicity of the different substituted hydrazones.

References

Aggarwal N, Mishra P, Nagori BP, Aggarwal R, Jain J (2009) Anticonvulsant and neurotoxicity evaluation of Some N4 phenyl substituted pyridyl semicarbazones. Cent Nerv Syst Agents Med Chem 9:295–299

Angelusiu MA, Barbuceanu SF, Draghici C, Almajan GL (2010) New Cu (II), Co (II), Ni (II) complexes with aroyl-hydrazone based ligand. Synthesis, spectroscopic characterization and in vitro antibacterial evaluation. Eur J Med Chem 45:2055–2062. doi:10.1016/j.ejmech.2010.01.033

Barton ME, Klein BD, Wolf HH, White HS (2001) Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. Epilepsy Res 47:217–227

Curtis DR, Larsen KP, Kruger SJ, Kofoed H (1978) GABA Neurotransmitters: pharmacochemical biochemical and pharmacological aspects. Munksgaard, Copenhagen, pp 17–27

Dimmock JR, Baker GB (1994) Anticonvulsant activities of 4-bromobenzaldehyde semicarbazone. Epilepsia 35:648–655. doi:10.1111/j.1528-1157.1994.tb02486

Dimmock JR, Pandeya SN, Quail JW, Pugazhenthi U, Allen TM, Kao GY, Balzarini J, Declercq E (1995) Evaluation of the semicarbazones, thiosemicarbazones and bis-carbohydrazones of some aryl alicyclic ketones for anticonvulsant and other biological properties. Eur J Med Chem 30:303–314. doi:10.1016/0223-5234(96)88238-9

Dimmock JR, Puthucode RN, Smith JM, Hetherington M, Quail JW, Pugazhenthi U, Lechler T, Stables JP (1996) Aryloxy aryl semicarbazones and related compounds: a novel class of anticonvulsant agents possessing high activity in the maximal electroshock dcreen. J Med Chem 39:3984–3997. doi:10.1021/jm9603025

Dimmock JR, Sample HA, John S, Beazely MA, Abrams GD (1999) Isolation and identification of the major urinary metabolite of 4-(4-fluorophenoxy) benzaldehyde semicarbazone after oral dosing to rats. Die Pharmazie 54:260–262

Duncan JS (2002) The promise of new antiepileptic drugs. Br J Clin Pharmacol 53:123–131. doi:10.1046/j.0306-5251.2001.01540

Eadie MJ, Tyrer JH, Kutt H (1989) Anticonvulsant therapy: pharmacological basis and practice. Churchill Livingstone, Edinburgh

Gajcy K, Kala JP, Fra BZ, Wojtasek C, Librowski T, Lochyn S (2010) Stereochemistry of terpene derivatives. Part 7: Novel rigidified amino acids from (+)-3-carene designed as chiral GABA analogues. Tetrahedron Asymmetr 21:2015–2020. doi:10.1016/j.tetasy.2010.06.026

Ghogare JG, Bhandari SV, Bothara KG, Madgulkar AR, Parashar GA, Sonawane BG, Inamdar PR (2010) Design, synthesis and pharmacological screening of potential anticonvulsant agents using hybrid approach. Eur J Med Chem 45:857–863. doi:10.1016/j.ejmech.2009.09.014

Jones GL, Woodbury DM (1982) Anticonvulsant structure–activity relationships: historical development and probable causes of failure. Drug Dev Res 2:333–355

Kaushik D, Khan SA, Chawla G, Kumar S (2010) N'-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]2/4-substituted hydrazides: synthesis and anticonvulsant activity. Eur J Med Chem 45:3943–3949. doi:10.1016/j.ejmech.2010.05.049

Krall RJ, Penry JK, White BG, Kupferberg HJ, Swinyard EA (1978) Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia 19(4):409–428

Kulandasamy R, Adhikari AV, Stables JP (2009a) Synthesis and anticonvulsant activity of some bishydrazone derived from 3,4-dipropyloxythiophene. Eur J Med Chem 44:3672–3679. doi:10.1016/j.ejmech.2009.02.009

Kulandasamy R, Adhikari AV, Stables JP (2009b) A New class of anticonvulsant possessing 6 Hz activity: 3,4-Dialkyloxythiophene bishydrazones. Eur J Med Chem 44:4376–4384. doi:10.1016/j.ejmech.2009.05.026

Kulig K, Wieckowski K, Wieckowski A, Gajda J, Pochwat B, Hofner GC, Wanner KT, Malawska B (2011) Synthesis and biological evaluation of new derivatives of 2-substituted 4-hydroxybutanamides as GABA uptake inhibitors. Eur J Med Chem 46:183–190. doi:10.1016/j.ejmech.2010.11.001

Loscher W, Bartholini G, Bossi L, Lloyd KG, Morselli PL (1985) Epilepsy and GABA receptor agonists: basic and therapeutic research. L.E.R.S. monograph series. Raven Press, New York, pp 109–119

Lourenco MCS, Souza MVND, Pinheiro AC, Ferreira MDL, Goncalves RSB, Nogueira TCM, Peralta MA (2007) Evaluation of anti-tubercular activity of nicotinic and isoniazid analogue. Arkivoc 15:181–191

Lourenco MCDS, Ferreira MDL, Souza MVND, Peralta MA, Vasconcelos TRA, Henriques MDGM (2008) Synthesis and anti-mycobacterial activity of (*E*)-*N*'-(substituted-benzylidene)-isonicotinohydrazide derivatives. Eur J Med Chem 43:1344–1347. doi:10.1016/j.ejmech.2007.08.003

Pandeya SN, Mishra V, Singh PN, Rupainwar DC (1998) Anticonvulsant activity of thioureido derivatives of acetophenone semicarbazone. Pharmacol Res 37:17–22

Pandeya SN, Raja AS, Stables JP (2002) Synthesis of isatin semicarbazones as potential anticonvulsants. Role of hydrogen bonding. J Pharm Pharm Sci 5:266–271

Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferburg HJ, Scoville B (1984) Antiepileptic drug development program. Cleve Clin Q 51:293–305

Puthucode RN, Pugazhenthi U, Quail JW, Stables JP, Dimmock JR (1998) Anticonvulsant activity of various aryl, arylidene and aryloxyaryl semicarbazones. Eur J Med Chem 33:595–607. doi:10.1016/S0223-5234(98)80018-4

Sinha R, Sara UVS, Khosa RL, Stables J, Jainendra J (2010) Nicotinic acid hydrazone: a novel anticonvulsant pharmacophore. Med Chem Res. doi:10.1007/s0004–010-9396–0

Wong MG, Dejina JA, Andrews PR (1986) Conformational analysis of clinically active anticonvulsant drugs. J Med Chem 29:562–572

Yogeeswari P, Sriram D, Pandeya SN, Stables JP (2004) 4-Sulphamoyl phenyl semicarbazones with anticonvulsant activity. IL Farmaco 59:609–613. doi:10.1016/j.farmac.2004.01.009

Yogeeswari P, Sriram D, Veena V, Kavya R, Rakhra K, Mehta S, Raghuvendran JV, Thirumurugan R, Stables JP (2005) Synthesis of aryl semicarbazones as potential anticonvulsant agents. Biomed Pharmacother 59:51–55. doi:10.1016/j.biopha.2004.04.013