Synthesis and anticonvulsant evaluation of 2-(substituted benzylidene/ethylidene)-N-(substituted phenyl)hydrazinecarboxamide analogues

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Abstract In the present investigation, we described herein the molecular properties prediction by Molinspiration (2008) and synthesized a series of 17 2-(substituted benzylidene/ethylidene)-*N*-(substituted phenyl)hydrazine-carboxamide analogues. All the title compounds (**4a–q**) followed the Lipinski "Rule of Five." The synthesized compounds were characterised by elemental analyses and spectral data followed by anticonvulsant activity according to the Antiepileptic Drug Development Programme Protocol. 2-(4-Hydroxybenzylidene)-*N*-(2-chlorophenyl)hydrazinecarboxamide (**4j**) was found to be the most active compound of the series showing protection at 4.0 h at a dose of 100 mg/kg against maximal electroshock seizure test and 50 % (2/4, 0.25, 1–2 h) and 100 % (4/4, 0.5 h) protection in 6 Hz psychomotor seizure test without showing any neurotoxicity. *N*-(2-chlorophenyl)hydrazine carboxamide (**3b**) showed 100 % (4/4, 0.25–2 h) and 66.6 % (2/3, 4 h) protection in 6 Hz psychomotor seizure test.

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Introduction

Epilepsy is a common neurological disorder, affecting 1–2 % of the world's population (White, 2003). In spite of new developments in the antiepileptic drug (AED) research, roughly 28–30 % patients still remain poorly treated with the available AEDs (Meador, 2003). Also most of the AEDs have dose-related toxicity and idiosyncratic side effects (Dimmock *et al.*, 1995a; Duncan, 2002). Thus, the search for the new and effective AEDs continues to be an active area of investigation in medicinal chemistry. CPP 115 and vigabatrin were found to be effective in 6 Hz psychomotor seizure test (Antiepileptic Drug and Device Trials XI Conference, 2011).

Semicarbazones have been reported as potent anticonvulsant agents possibly act by inhibiting voltage sensitive Na^+ channel (Dimmock *et al.*, 1995b). The pharmacophore model for anticonvulsant activity includes hydrophobic domain, a hydrogen binding site and an electron donor moiety (Dimmock *et al.*, 2000; Pandeya and Raja, 2002). Aryl semicarbazones, a structurally dissimilar to existing AEDs, can be assumed as such novel compounds that would not have the side effects commonly seen with many of the currently available medications (Azam *et al.*, 2010). Their activity is mainly attributed to the presence of aryl binding site with aryl/alkyl hydrophobic group, hydrogen bonding domain and electron donor group as
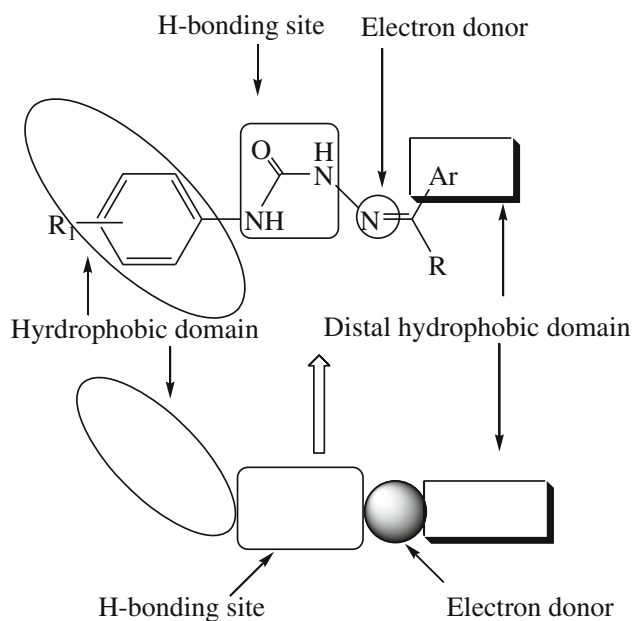
Fig. 1 Pharmacophore model for anticonvulsant activity

reported earlier. Furthermore, it was proposed that introduction of the one more aryl ring in the structure brings about the enhanced van der Waals bonding at the receptor site that leads to increased potency (Pandeya and Raja, 2002). The proposed element for anticonvulsant activity is shown in Fig. 1 for in vivo interaction with macromolecules. In the current work, we studied the molecular properties prediction of 2-(substituted benzylidene/ethylidene)-*N*-(substituted phenyl)hydrazinecarboxamide analogues by Molinspiration and synthesized 17 compounds (**4a–q**) followed by anticonvulsant evaluation. Earlier we have reported the anticonvulsant and antimicrobial activities of semicarbazone analogues (Amir *et al.*, 2010; Ahsan *et al.*, 2011).

Materials and methods

Chemistry

The entire chemicals were supplied by E. Merck (Germany), and S.D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. The completion of reactions was monitored by TLC (silica gel G) using mobile phase chloroform–methanol (9:1), and the spots were located under iodine vapours or UV light. IR spectra were obtained on a Schimadzu 8201 PC, FT-IR spectrometer, Japan (KBr pellets). ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer, Germany using TMS as internal standard in DMSO-d_6. Mass spectra were recorded on a Bruker Esquire LCMS, Germany using ESI and elemental analyses were performed on Perkin-Elmer 2400 Elemental Analyzer, USA.

Synthesis of substituted phenyl urea (2a–b)

Aromatic aniline (0.1 mol) was dissolved in 20 ml of glacial acetic acid and 10 ml of hot water and sodium cyanate (6.5 g, 0.1 mol) in 80 ml of hot water was added with stirring. It was allowed to stand for 30 min, then cooled in ice bath and filtered with suction, dried, and recrystallized from boiling water (Pandeya and Raja, 2002; Singhal and Paul, 2011).

Synthesis of substituted phenyl semicarbazide (3a–b)

Equimolar quantities (0.05 mol) of substituted phenyl urea (**2a–b**) and hydrazine hydrate (AR 99–100 %) (2.5 ml, 0.05 mol) in ethanol were refluxed for 48 h with stirring. The two-third volume of alcohol was distilled by vacuum distillation and then poured into the crushed ice. The resultant precipitate was filtered, washed with water, and dried. The solid mass was recrystallized from 50 ml of absolute ethanol (Pandeya and Raja, 2002; Singhal and Paul, 2011).

Synthesis of substituted phenyl semicarbazone (4a–q)

To a solution of substituted phenyl semicarbazide (**3a–b**) (0.005 mol) in 1 ml conc. HCl and 25 ml water, a solution of sodium acetate (0.41 g, 0.005 mol) in 2 ml water was added. About 25 ml ethanol was added to clear turbidity. This solution mixture was added to an equimolar quantity of the appropriate aldehyde or ketone in alcohol. The solution was stirred for 15 min immediately precipitation occurred (if immediate precipitation was not occurred, reaction mixture was allowed to stand for 1–2 h) and the solids were filtered, dried, and recrystallized from hot ethanol (Amir *et al.*, 2010).

*2-(2-Chlorobenzylidene)-N-(4-methylphenyl)hydrazinecarboxamide (**4a**)* IR (KBr) cm^{-1}: 3375 (NH), 1675 (C=O), 1532 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.16 (3H, s, CH$_3$), 7.16–7.91 (8H, m, aromatic), 7.93 (1H, s, CH=N), 9.17 (1H, s, ArNH, D$_2$O exchangeable), 11.02 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 287 (M^{+1}), 288 (M+H)$^{+}$; Cal/Ana: [C (62.61) 62.59 H (4.90) 4.92 N (14.60) 14.63].

*2-(3-Methoxy-4-hydroxybenzylidene)-N-(4-methylphenyl)hydrazinecarboxamide (**4b**)* IR (KBr) cm^{-1}: 3378 (NH), 1685 (C=O), 1542 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.36 (3H, s, CH$_3$), 3.79 (3H, s, OCH$_3$), 7.19–7.89 (7H, m, aromatic), 8.09 (1H, s, CH=N), 9.12 (1H, s, ArNH, D$_2$O exchangeable), 10.09 (1H, s, CONH, D$_2$O exchangeable), 11.57 (1H, s, OH). EI-MS (*m/z*): 299 (M^{+1}), 300 (M+H)$^{+}$; Cal/Ana: [C (64.24) 64.20 H (5.70) 5.72 N (14.06) 14.04].

2-(4-Hydroxybenzylidene)-N-(4-methylphenyl)hydrazine-carboxamide (4c) IR (KBr) cm^{-1}: 3372 (NH), 1682 (C=O), 1540 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.24 (3H, s, CH$_3$), 7.17–7.92 (8H, m, aromatic), 8.19 (1H, s, CH=N), 9.16 (1H, s, ArNH, D$_2$O exchangeable), 10.29 (1H, s, CONH, D$_2$O exchangeable), 11.02 (1H, s, OH). EI-MS (*m/z*): 269 (M^{+1}), 270 (M+H)$^+$; Cal/Ana: [C (66.92) 66.90 H (5.63) 5.61 N (15.59) 15.60].

2-[(2-Hydroxyphenyl)ethylidene)]-N-(4-methylphenyl) hydrazinecarboxamide (4d) IR (KBr) cm^{-1}: 3378 (NH), 1672 (C=O), 1534 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.28 (3H, s, CH$_3$), 7.19–7.96 (8H, m, aromatic), 9.24 (1H, s, ArNH, D$_2$O exchangeable), 10.24 (1H, s, CONH, D$_2$O exchangeable), 10.92 (1H, s, OH). EI-MS (*m/z*): 283 (M^{+1}), 284 (M+H)$^+$; Cal/Ana: [C (67.86) 67.83 H (6.04) 6.05 N (14.81) 14.83].

2-[(2,4-Dihydroxyphenyl)ethylidene)]-N-(4-methylphenyl)-hydrazinecarboxamide (4e) IR (KBr) cm^{-1}: 3376 (NH), 1682 (C=O), 1540 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.18 (3H, s, CH$_3$), 7.22–7.98 (7H, m, aromatic), 9.02 (1H, s, ArNH, D$_2$O exchangeable), 10.27 (1H, s, CONH, D$_2$O exchangeable), 11.02 (1H, s, OH). EI-MS (*m/z*): 299 (M^{+1}), 300 (M+H)$^+$; Cal/Ana: [C (64.18) 64.20 H (5.70) 5.72 N (14.05) 14.04].

2-Cyclohexylidene-N-(4-methylphenyl)hydrazinecarboxa-mide (4f) IR (KBr) cm^{-1}: 3373 (NH), 1672 (C=O), 1533 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.16 (3H, s, CH$_3$), 2.22–2.54 (10H, m, cyclohexyl), 7.16–7.74 (4H, m, aromatic), 9.17 (1H, s, ArNH, D$_2$O exchangeable), 10.67 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 245 (M^{+1}); Cal/Ana: [C (68.54) 68.55 H (7.81) 7.83 N (17.13) 17.14].

2-N-(4-methylphenyl)-2-(4-oxopentan-2-ylidene)hydrazin-ecarboxamide (4g) IR (KBr) cm^{-1}: 3376 (NH), 1678 (C=O), 1537 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 1.12 (3H, s, CH$_3$), 2.36 (3H, s, CH$_3$), 2.52 (2H, s, CH$_2$), 7.06–7.51 (4H, m, aromatic), 8.64 (1H, s, ArNH, D$_2$O exchangeable), 9.67 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 247 (M^{+1}); Cal/Ana: [C (63.13) 63.14 H (6.91) 6.93 N (16.97) 16.99].

2-(4-Chlorobenzylidene)-N-(2-chlorophenyl)hydrazine-carboxamide (4h) IR (KBr) cm^{-1}: 3379 (NH), 1685 (C=O), 1543 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 7.19–7.74 (8H, m, aromatic), 8.96 (1H, s, CH=N), 9.07 (1H, s, ArNH, D$_2$O exchangeable), 10.87 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 308 (M^{+1}), 309 (M+H)$^+$; Cal/Ana: [C (54.57) 54.55 H (3.60) 3.63 N (13.64) 13.65].

2-(2,4-Dichlorobenzylidene)-N-(2-chlorophenyl)hydrazine-carboxamide (4i) IR (KBr) cm^{-1}: 3377 (NH), 1670 (C=O), 1531 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 7.22–7.98 (7H, m, aromatic), 8.83 (1H, s, CH=N), 9.02 (1H, s, ArNH, D$_2$O exchangeable), 10.72 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 342 (M^{+1}), 343 (M+H)$^+$; Cal/Ana: [C (49.08) 49.09 H (2.94) 2.93 N (12.26) 12.27].

2-(4-Hydroxybenzylidene)-N-(2-chlorophenyl)hydrazine-carboxamide (4j) IR (KBr) cm^{-1}: 3375 (NH), 1675 (C=O), 1532 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 7.02–7.64 (8H, m, aromatic), 8.10 (1H, s, CH=N), 8.27 (1H, s, ArNH, D$_2$O exchangeable), 10.02 (1H, s, CONH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d_6) ppm: 116.21, 122.23, 126.12, 130.42, 129.13, 130.11, 134.02, 143.01, 159.09 (C=O), 160.12. EI-MS (*m/z*): 389 (M^{+1}), 390 (M+H)$^+$; Cal/Ana: [C (58.04) 58.05 H (4.17) 4.16 N (14.50) 14.51].

2-(3,4-Dimethoxybenzylidene)-N-(2-chlorophenyl)hydra-zinecarboxamide (4k) IR (KBr) cm^{-1}: 3372 (NH), 1678 (C=O), 1538 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 3.81 (3H, s, OCH$_3$), 3.83 (3H, s, OCH$_3$), 7.39–7.98 (7H, m, aromatic), 8.98 (1H, s, CH=N), 9.17 (1H, s, ArNH, D$_2$O exchangeable), 11.67 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 333 (M^{+1}), 334 (M+H)$^+$; Cal/Ana: [C (57.58) 59.33 H (4.83) 4.66 N (12.59) 13.85].

2-(3-Hydroxybenzylidene)-N-(2-chlorophenyl)hydrazine-carboxamide (4l) IR (KBr) cm^{-1}: 3377 (NH), 1674 (C=O), 1542 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 7.21–7.98 (8H, m, aromatic), 8.62 (1H, s, CH=N), 9.18 (1H, s, ArNH, D$_2$O exchangeable), 10.97 (1H, s, CONH, D$_2$O exchangeable), 11.04 (1H, s, OH). EI-MS (*m/z*): 289 (M^{+1}), 290 (M+H)$^+$; Cal/Ana: [C (58.05) 58.04 H (4.18) 4.17 N (14.49) 14.50].

2-[1-(4-Methoxyphenyl)ethylidene]-N-(2-chlorophenyl) hydrazinecarboxamide (4m) IR (KBr) cm^{-1}: 3377 (NH), 1680 (C=O), 1531 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 3.82 (3H, s, OCH$_3$), 7.29–7.88 (8H, m, aromatic), 9.17 (1H, s, ArNH, D$_2$O exchangeable), 10.37 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 317 (M^{+1}), 318 (M+H)$^+$; Cal/Ana: [C (60.47) 60.48 H (5.08) 5.09 N (13.22) 13.23].

2-[1-(4-Methylphenyl)ethylidene]-N-(2-chlorophenyl) hydrazinecarboxamide (4n) IR (KBr) cm^{-1}: 3370 (NH), 1678 (C=O), 1539 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.18 (3H, s, OCH$_3$), 7.19–7.79 (8H, m, aromatic), 9.26 (1H, s, ArNH, D$_2$O exchangeable), 10.57 (1H, s, CONH, D$_2$O exchangeable). EI-MS (*m/z*): 301 (M^{+1}), 302 (M+H)$^+$; Cal/Ana: [C (63.67) 63.68 H (5.33) 5.34 N (13.93) 13.92].

2-[(4-Chlorophenyl)ethylidene)]-N-(2-chlorophenyl)hydra-zinecarboxamide (4o) IR: 3373 (NH), 1671 (C=O), 1531 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 7.42–8.09 (8H, m, aromatic), 9.02 (1H, s, ArNH, D_2O exchangeable), 10.52 (1H, s, CONH, D_2O exchangeable). EI-MS (*m/z*): 322 (M^{+1}), 323 (M+H)$^+$; Cal/Ana: [C (55.92) 55.93 H (4.07) 4.06 N (13.04) 13.05].

2-(Furan-2-ylmethylidene)-N-(2-chlorophenyl)hydrazine-carboxamide (4p) IR (KBr) cm^{-1}: 3375 (NH), 1674 (C=O), 1531 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 7.02–7.58 (7H, m, aromatic), 8.38 (1H, s, CH=N), 9.17 (1H, s, ArNH, D_2O exchangeable), 10.12 (1H, s, CONH, D_2O exchangeable). EI-MS (*m/z*): 263 (M^{+1}), 264 (M+H)$^+$; Cal/Ana: [C (54.66) 54.65 H (3.38) 3.39 N (15.94) 15.93].

2-Cyclopentylidene-N-(2-chlorophenyl)hydrazinecarbox-amide (4q) IR (KBr) cm^{-1}: 3378 (NH), 1682 (C=O), 1543 (C=N). ^{1}H NMR (DMSO-d_6) ppm: 2.31–2.63 (8H, m, cyclopentyl), 7.16–7.54 (4H, m, aromatic), 9.02 (1H, s, ArNH, D_2O exchangeable), 10.17 (1H, s, CONH, D_2O exchangeable). EI-MS (*m/z*): 251 (M^{+1}), 252 (M+H)$^+$; Cal/Ana: [C (57.22) 57.26 H (5.62) 5.61 N (16.67) 16.69].

Pharmacology

Maximal electroshock (MES)

The anticonvulsant evaluations were undertaken by the National Institute of Health, using their reported procedures. For all tests based on MES convulsions, 60 Hz of alternating current (50 mA in mice and 150 mA in rat) was delivered for 0.2 s by corneal electrodes which have been primed with an electrolyte solution containing anaesthetic agent (0.5 % tetracaine HCl). The mice were tested at various (0.5 and 4 h) intervals following doses of 30, 100, and 300 mg/kg of test compound given by i.p. injection of a volume of 0.01 ml/g. An animal is considered "protected" from MES-induced seizures upon abolition of the hindlimb tonic extensor component of the seizure.

Subcutaneous metrazole-induced seizure (scMET)

Subcutaneous injection of the convulsant metrazole produces clonic seizures in laboratory animals. The scMET test detects the ability of a test compound to raise the seizure threshold of an animal, and thus, protect it from exhibiting a clonic seizure. Animals were pre-treated with various doses of the test compound (in a similar manner to the MES test, although a dose of 50 mg/kg (p.o.) is the standard for scMET). An animal is considered "protected"

from scMET-induced seizures upon the absence of episode of clonic spasms, approximately 3–5 s, of the fore and/or hindlimbs, jaws, or vibrissae.

Hz psychomotor seizure test

Some clinically useful AEDs are ineffective in the standard MES and scMET tests but still have anticonvulsant activities in vivo. In order to identify potential AEDs with this profile, compounds were tested in the minimal clonic seizure (6 Hz or "psychomotor") test. The title compounds were tested in the minimal clonic seizure test at dose of 100 mg/kg to four mice. Like the MES test, the minimal clonic seizure (6 Hz) test is used to assess a compound's efficacy against electrically induced seizures but uses a lower frequency (6 Hz) and longer duration of stimulation (3 s).

Neurotoxicity or minimal motor impairment

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotate at 10 rpm. The rod diameter was 3.2 cm trained animals were given i.p. injection of the test compound in doses of 30, 100, and 300 mg/kg, neurotoxicity was indicated by the inability of the mice to maintain equilibrium on the rod for at least 1 min in each of the three trails.

In vitro hippocampal slice culture neuroprotection assay (NP): primary screen experiment

The "Primary Screen Experiment" is a qualitative assessment of the ability of a compound to prevent excitotoxic cell death. Organotypic hippocampal slice cultures are treated with N-methyl-D-aspartate (NMDA) or kainic acid (KA) to induce neuronal cell death. Propidium iodide, a membrane-impermeant compound, is included in all wells of the culture plate. Dying cells have compromised cell membranes, thus, propidium iodide may diffuse into the cell, intercalate with DNA and fluoresce. Thus, the intensity of the propidium iodide fluorescence is proportional to the amount of cell death in the individual slices. Hippocampal slice cultures are treated with the excitotoxin alone, or where indicated above, with the excitotoxin and either one or two investigational compounds at the concentrations indicated. If NP occurs as a consequence of the added compound, slice cultures will have a visibly reduced fluorescent intensity when compared to the slice cultures that have been treated with the excitotoxin alone.

Results and discussion

Molecular properties prediction

Molecular properties, mainly hydrophobicity, molecular size, flexibility and the presence of various pharmacophoric features influence the behaviour of molecules in the living organism, including bioavailability. Thus, in order to achieve good oral drugs, we have subjected a series of 2-(substituted benzylidene/ethylidene)-*N*-(substituted phenyl)-hydrazinecarboxamide analogues (**4a–q**) for prediction of lipophilicity and Lipinski "Rule of Five" and other properties for filtering compounds for synthesis and anticonvulsant evaluation. For good membrane permeability, log *P* value should be ≤5. All the title compounds (**4a–q**) have miLog *P* value 1.997–5.327. It is revealed that for passing oral bioavailability criteria, number of rotatable bond should be ≤10 (Veber *et al.*, 2002). The compounds in this series (**4a–q**) in general possess sufficient number of rotatable bonds (1–5) and therefore, exhibit good conformational flexibility. Molecular polar surface area (TPSA) is a very useful parameter for the prediction of drug transport properties. TPSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. TPSA and volume is inversely proportional to percentage of absorption (%ABS). TPSA was used to calculate the %ABS

according to the equation: %ABS = 109 ± 0.345 × TPSA (Wang *et al.*, 1997). From all these parameters, it can be observed that all the title compounds (**4a–q**) exhibited a great %ABS ranging from 76.59 to 90.55 % and followed the Lipinski "Rule of Five" which states that the molecules with good membrane permeability have log *P* ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors' ≤5 (Lipinski *et al.*, 2001). This rule is widely used as a filter for drug-like properties. The pharmacokinetic parameters were calculated online from Molinspiration Chemoinformatics (http://www.molinspiration.com) and are given in Table 1.

Chemistry

The aryl semicarbazone analogues (**4a–q**) described in this study are shown in Table 2 and the reaction sequence for the synthesis is summarized in Scheme 1. In the initial step, substituted aromatic amines were treated with sodium cyanate in glacial acetic acid at room temperature giving substituted phenyl urea (**2a–b**). In the subsequent step, substituted phenyl urea was treated with hydrazine hydrate in ethanol giving phenyl semicarbazide (**3a–b**). In the final step, treatment of phenyl semicarbazide with appropriate aldehyde/ketone in the presence in sodium acetate in ethanol furnished the title compounds (**4a–q**). The yields of

Table 1 Pharmacokinetic parameters important for good oral bioavailability of 2-(substituted benzylidene/ethylidene)-*N*-(substituted phenyl)-hydrazinecarboxamide analogues (**4a–q**)

Compound	% ABS	Volume (A3)	TPSA (A2)	NROTB	*n*-ON acceptor	*n*-OHNH donors	miLog *P*	MW	Lipinski's violations
Rule	–	–	–	–	<10	<5	≤5	<500	≤1
4a	90.55	252.598	53.489	3	4	2	4.492	287	0
4b	80.38	272.625	82.951	4	6	3	3.201	299	0
4c	83.57	247.08	73.717	3	5	3	3.359	269	0
4d	83.57	263.641	73.717	3	5	3	3.716	283	0
4e	76.59	271.658	93.945	3	6	4	3.213	299	0
4f	90.55	240.82	53.489	2	4	2	3.723	245	0
4g	84.66	236.561	70.56	4	5	2	1.997	247	0
4h	90.55	249.572	53.489	3	4	2	4.721	308	0
4i	90.55	263.108	53.489	3	4	2	5.327	342	1
4j	83.57	244.054	73.717	3	5	3	3.564	289	0
4k	84.17	287.128	71.957	5	6	2	3.69	333	0
4l	83.57	244.054	73.717	3	5	3	3.54	289	0
4m	87.36	278.143	62.723	4	5	2	4.013	317	0
4n	90.55	269.159	53.489	3	4	2	4.405	301	0
4o	90.55	266.133	53.489	3	4	2	4.635	322	0
4p	86.01	217.605	66.629	3	5	2	3.3	263	0
4q	90.55	220.993	53.489	2	4	2	3.398	251	0

%ABS percentage of absorption, *TPSA* topological polar surface area, *NROTB* number of rotatable bonds, *MW*, molecular weight, *miLog P* logarithm of compound partition coefficient between *n*-octanol and water, *n-OHNH* number of hydrogen bond donors, *n-ON* number of hydrogen bond acceptors

Table 2 Physical constant of *N*-(substituted phenyl)hydrazinecarboxamide and 2-(substituted benzylidene/ethylidene)-*N*-(substituted phenyl) hydrazinecarboxamide analogues
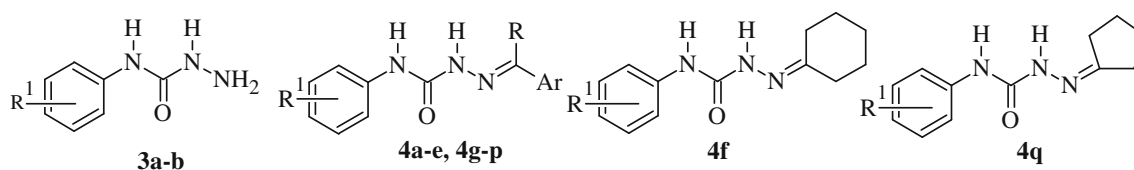
Compound	ADD No.	R^1	R	Ar	Yield (%)	R_f^a	Mp (°C)
3a	433050	Methyl-	–	–	86	0.64	125
3b	439081	2-Chloro	–	–	82	0.58	105
4a	433052	4-Methyl-	H	2-Chlorophenyl-	78	0.65	172
4b	433054	4-Methyl-	H	3-Methoxy-4-hydroxyphenyl-	62	0.72	188
4c	439077	4-Methyl-	H	3-Hydroxyphenyl-	58	0.48	122
4d	433055	4-Methyl-	Methyl-	2-Hydroxyphenyl-	84	0.70	168
4e	433058	4-Methyl-	Methyl-	2,4-Dihydroxyphenyl-	72	0.81	220
4f	433059	4-Methyl-	–	–	66	0.59	178
4g	433060	4-Methyl-	Methyl	2-Propanonyl-	76	0.79	70
4h	433061	2-Chloro-	H	4-Chlorophenyl-	72	0.60	182
4i	433062	2-Chloro-	H	2,4-Dichlorophenyl-	69	0.73	184
4j	433063	2-Chloro-	H	4-Hydroxyphenyl-	67	0.68	202
4k	433064	2-Chloro-	H	3,4-Dimethoxyphenyl-	92	0.78	172
4l	433065	2-Chloro-	H	3-Hydroxyphenyl-	82	0.77	186
4m	433067	2-Chloro-	Methyl-	4-Methoxyphenyl-	84	0.75	72
4n	433068	2-Chloro-	Methyl-	4-Methylphenyl-	88	0.61	169
4o	439085	2-Chloro-	Methyl-	4-Chlorophenyl-	62	0.66	92
4p	433070	2-Chloro-	H	2-Furfuryl-	64	0.63	78
4q	433069	2-Chloro-	–	–	62	0.56	128

ADD No. Antiepileptic Drug Development Number (National Institute of Neurodegenerative Disorders and Stroke, National Institute of Health, USA)

[a] Mobile phase: chloroform: methanol (9:1)

the titled compounds were ranging from 62 to 92 % after recrystallization with absolute ethanol. The completion of reactions was monitored TLC using mobile phase chloroform: methanol (9:1) and purity was checked by elemental analyses. Both the analytical and spectral data (IR, ^{1}H NMR) of all the synthesized compounds were in full accordance with the proposed structures. In general, the IR spectra of the compounds afforded imine C=N stretching at 1,528–1,545 cm^{-1}, and carbamoyl group N–H stretching at 3,366–3,390 cm^{-1} and C=O stretching at 1,670–1,686 cm^{-1} bands. In the Nuclear Magnetic Resonance spectra (^{1}H NMR), the signals of the respective protons of the synthesized titled compounds were verified on the basis of their chemical shift and multiplicities in DMSO-d_6. The spectra showed singlet at δ 2.02–2.96 ppm corresponding to CH$_3$; a singlet at δ 3.80–3.85 ppm corresponding to OCH$_3$ group; multiplet at δ 7.06–7.98 ppm corresponding to aromatic protons; a singlet at δ 7.93–9.08 ppm

corresponding to imine H; a singlet at δ 9.02–9.27 ppm corresponding to aromatic NH (D$_2$O exchangeable); broad singlet at δ 9.97–11.87 ppm corresponding to CONH (D$_2$O exchangeable) and a singlet at 10.92–11.67 ppm corresponding to aromatic OH. The elemental analysis results were within ±0.4 % of the theoretical values.

Anticonvulsant activity

All the compounds were evaluated for their anticonvulsant activity according to the Antiepileptic Drug Development (ADD) Programme Protocol reported elsewhere (Swingyard *et al.*, 1989; White *et al.*, 1995a, b; Dunham and Miya, 1957; Barton *et al.*, 2001; Toman *et al.*, 1952). Initially, the compounds were administered i.p. at doses of 30, 100 and 300 mg/kg in mice and activity was established using the MES-induced seizure and scMET model to identify their anticonvulsant activity at two intervals (0.5 and 4.0 h).

Scheme 1 Synthetic protocol: (*a*) reagents: NaCNO/AcOH, (*b*) NH$_2$NH$_2$·H$_2$O/EtOH and (*c*) aldehyde/ketone/EtOH
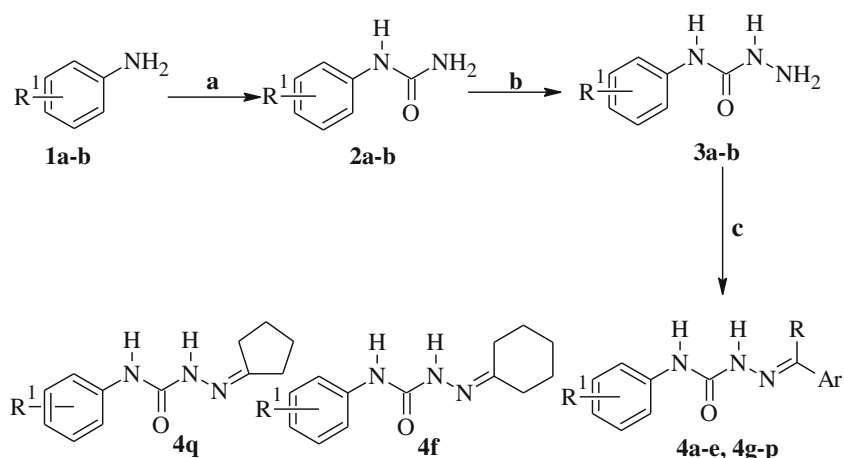

Table 3 Anticonvulsant evaluation of *N*-(substituted phenyl)hydrazinecarboxamide and 2-(substituted benzylidene/ethylidene)-*N*-(substituted phenyl)hydrazinecarboxamide analogues

Compound	Intraperitoneal injection in mice[a]				Intraperitoneal injection in mice (100 mg/kg)									
	MES screen		Toxicity screen		Time to peak effect (N/F)[b]					Toxicity				
	0.5 h	4.0 h	0.5 h	4.0 h	0.25 h	0.5 h	1.0 h	2.0 h	4.0 h	0.25 h	0.5 h	1.0 h	2.0 h	4.0 h
3a	300	–	–	300[d]	4/4	2/4	2/4	1/4[c]	0/3[d]	0/4	0/4	0/4	1/4	2/4
3b	–	–	–	–	4/4	4/4	4/4	4/4	2/3	1/4	1/4	1/4	0/4	1/4[e]
4a	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4b	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4c	–	–	–	–	2/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
4d	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4e	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4f	–	300	–	–	1/4	4/4	2/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
4g	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4h	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4i	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4j	–	100	–	–	2/4	4/4	2/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4
4k	–	100	–	–	1/4	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
4l	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4m	300	300	–	300	×	×	×	×	×	×	×	×	×	×
4n	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4o	–	100	–	–	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
4p	–	–	–	–	×	×	×	×	×	×	×	×	×	×
4q	–	–	–	–	×	×	×	×	×	×	×	×	×	×
Phenytoin[f]	30	30	100	100	×	×	×	×	×	×	×	×	×	×
Carbamazepine[f]	30	100	100	300	×	×	×	×	×	×	×	×	×	×

A dash (–) indicate lack of activity

A cross (×) indicates activity not determined

[a] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4 h after administration

[b] N/F = number of animals active or toxic over the number tested at a dose of 100 mg/kg

[c] Clonic seizure in 1/4 animal

[d] Death in 2/3 animal

[e] Death of one mice followed by clonic seizure

[f] Activity reported (Pandeya and Raja, 2002)

Neurotoxicity or toxicity was observed by minimal motor impairment which was measured in mice by rotorod test. Seven compounds were screened in 6 Hz psychomotor seizure test to identify their anticonvulsant activity at five different time points viz. 0.25, 0.5, 1.0, 2.0 and 4.0 h after i.p. administration in mice. Two compounds were also evaluated in the MES test and toxicity after oral administration to rat at 30 mg/kg dose and in vitro hippocampal slice culture NP assay (Noraberg *et al.*, 2005).

All the compounds were evaluated for their anticonvulsant activity against MES, scMET model and toxicity at two intervals (0.5 and 4.0 h), and the results are given in Table 3. None of the compounds showed any protection in scMET screen while 6 compounds showed significant protection in MES screening. In 6 Hz psychomotor seizure test four among the seven compounds showed significant protection against MES-induced seizure. *N*-(4-methylphenyl)hydrazinecarboxamide **3a**, showed protection in MES screening at 0.5 h at 300 mg/kg dose but associated with toxicity followed by death of the mice. 2-Cyclohexylidene-*N*-(4-methylphenyl)hydrazinecarboxamide **4f**, 2-(4-hydroxybenzylidene)-*N*-(2-chlorophenyl)hydrazinecarboxamide **4j** and 2-(3,4-dimethoxybenzylidene)-*N*-(2-chlorophenyl)hydrazinecarboxamide **4k** showed protection against MES-induced seizure at 4.0 h at 300, 100 and 300 mg/kg dose, respectively, devoid of any toxicity. 2-[1-(4-Methoxyphenyl)ethylidene]-*N*-(2-chlorophenyl)hydrazinecarboxamide **4m** showed protection against MES-induced seizure at 0.5 and 4.0 h at 300 mg/kg dose and associated with neurotoxicity at 4.0 h (300 mg/kg). The compounds, **4j** and **4k** did not show any protection against MES-induced seizure test in rat at 30 mg/kg dose after oral administration. Also the compounds **4j** and **4k** in pairs did not show NP against KA or NMDA-induced cytotoxicity in vitro hippocampal slice culture NP assay. The compounds **3a**, **3b**, **4f**, and **4j** showed significant protection in 6 Hz psychomotor seizure test. The compound **3a** showed 100 % (4/4, 0.25 h), 50 % (2/4, 0.5–1.0 h) and 25 % (1/4, 2.0 h) protection; compound **3b** showed 100 % (4/4, 0.25–2.0 h) and 66.6 % (2/3, 4.0 h) protection; compound **4f** showed 25 % (1/4, 0.25 and 4.0 h), 100 % (4/4, 0.5 h) and 50 % (2/4, 1.0 h) protection; compound **4j** showed 50 % (2/4, 0.25 and 1–2 h) and 100 % (4/4, 0.5 h) protection. The compound **4k** showed 25 % (1/4, 0.25, 2.0 and 4.0 h), while compound **4o** showed 50 % (2/4, 1.0 h) in 6 Hz psychomotor seizure test. We have observed that **4j** was the most active compounds of the series. In the title compounds (**4a–p**), aryl semicarbazone with electronegative group substitution such as 2-chloro, 4-chloro, 2,4-dichloro and electron releasing group such as 2-hydroxy and 3-hydroxy in the distal hydrophobic aryl domain showed decreased activity in MES screening while electron releasing group such as

4-hydroxy, 4-methoxy and 3,4-dimethoxy group showed increased activity in MES screening. 4-Hydroxy group present on distal hydrophobic aryl domain showed maximum activity in 6 Hz psychomotor seizure test without any neurotoxicity or toxicity. Also semicarbazone analogue with cyclohexyl group (**4f**) showed significantly more activity than cyclopentyl group (**4q**).

Conclusion

All the compounds were synthesized in good yields. Aryl semicarbazone with electronegative group substitution such as 2-chloro, 4-chloro, 2,4-dichloro and electron releasing group such as 2-hydroxy and 3-hydroxy in the distal hydrophobic aryl domain showed decreased activity in MES screening while electron releasing group such as 4-hydroxy, 4-methoxy and 3,4-dimethoxy group showed increased activity in MES screening. 4-Hydroxy group present on distal hydrophobic aryl domain showed maximum activity in 6 Hz psychomotor seizure test without any neurotoxicity or toxicity. Also semicarbazone analogue with cyclohexyl group showed significantly more activity than cyclopentyl group.

Conflict of interest The authors reported no conflict of interest.

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