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DRUG SYNTHESIS

ANTICONVULSANT EVALUATION OF SOME NEWER BENZIMIDAZOLE DERIVATIVES: DESIGN AND SYNTHESIS

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Abstract: A series of new 2-[(1-substituted phenylethylidine) hydrazine]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamides **(4a-n)** were designed and synthesized to have the pharmacophoric elements essential for anticonvulsant activity. The key step in the synthesis of the title compounds involves the reaction of 2-mercaptobenzimidazole with hydrazine hydrate, substituted acetophenones and phenylisothiocyanate to get the compounds in good yields. All the newly synthesized compounds were screened by two most adopted models, maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ). Interestingly, compounds **4e, 4f, 4g, 4h** and **4j** exhibited potent anticonvulsant results and in the neurotoxicity screening, most of the compounds were devoid of toxicity at the dose of 60 and 100 mg/kg.

Keywords: 2-mercaptobenzimidazole, maximal electroshock seizure test, subcutaneous pentylenetetrazole, anticonvulsant activity, neurotoxicity

Epilepsy, a ubiquitous disease characterized by recurrent seizures inflicts more than 60 million people worldwide according to epidemiology studies. Nearly 95% of clinically available antiepileptic drugs were approved before 1985 and they could provide satisfactory seizures control for 60–70% of patients.

These drugs, however, also cause notable adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia and even life threatening conditions.

Studies indicate that a significant group of patients (20–30%) are resistant to the currently used therapeutic drugs i.e., agents with more effective anticonvulsant activity and exhibiting lower toxicity. Since several of the currently available AEDs have been associated with several side effects and fail to control seizures in about 30% of epileptic patients, there is a substantial need for development of new, more effective and less toxic AEDs.

While searching for compounds with potential antiepileptic action, we directed our attention to the benzimidazole and its derivatives, which possess different biological activities such as: anti-inflammatory (1), diuretic (2), antimicrobial (3), antibacterial (4), antiviral (5), antitumor (6), antiprotozoal (7), antiulcer (8), protein kinase CK2 (9), antioxidants (10), antiasthmatic (11), antidiabetic, cysticidal (12), 5-HT_3 receptor antagonist (13), analgesic (14), hypotensive (15), antimycobacterial (16, 17), anthelmintic (18), histamine H_4 receptor antagonist (19) and anticonvulsant activity (20, 21) .

Jain et al. (22) have reported the QSAR studies of benzimidazole derivatives and suggested that the binding affinity of the drug is strongly dependent upon the thermodynamic properties. Correlation between these properties and anticonvulsant activity was used to synthesize compounds possessing potent anticonvulsant activity.

In our study benzimidazole derivatives having pharmacophore in their structures, were synthesized and evaluated for anticonvulsant activity.

EXPERIMENTAL

Chemistry

All of the chemicals used in synthesis were obtained from s.d. Fine Chem., Spectrochem Pvt. Ltd., and Qualigens. The purity of synthesized com-

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pounds was tested by thin layer chromatography on silica gel G coated plates and solvent system used was benzene : acetone (7:3 v/v). Melting points were determined in open glass capillaries using Hicon melting point apparatus (Hicon, India) and are uncorrected. The infrared spectra of the compounds were recorded in KBr discs on Shimadzu 8416 FT-IR (λ_{max} in cm^{-1}). The proton magnetic resonance (^1H NMR) spectra were recorded on Bruker DRX 300 in DMSO-d_6 at 300 MHz using TMS as an internal standard and mass spectrum on Jeol SX 102/DA-6000 mass spectrometer using methanol as solvent. Iodine chamber and UV lamp were used for visualization of TLC spots. Elemental analysis was performed on CHN analyzer, Carlo Erba 1108. All the chemicals used were of L.R. grade and used without purification.

Synthesis of the title compounds (4a-n)

General procedure for synthesis of 1-(1*H*-benzo[*d*]imidazole-2-yl) hydrazine (2)

To the warm hydrazine hydrate solution (0.02 mol) of 2-mercapto-1*H*-benzo[*d*]imidazole (0.01mol), ethanol (10 mL) was added and then aqueous solution of sodium hydroxide (10%) added and the reaction mixture was refluxed for 6 h. The solid obtained was filtered, dried in vacuum dessicator and recrystallized from absolute ethanol to yield compound **2**, (68.34%), m.p. 298–300°C (23).

General procedure for synthesis of 1-(1*H*-benzo[*d*]imidazole-2-yl)-2-[(1-substituted-phenylethylidene] hydrazones (3a-n)

Equimolar quantity of 1-(1*H*-benzo[*d*]imidazole-2-yl)hydrazine (**2**) (0.12 mmol) and substituted acetophenones (0.12 mmol) in ethanol (10 mL) was refluxed for 6.5 h. After refluxing, the reaction mixture was poured onto crushed ice. The solid obtained was filtered, dried and recrystallized from ethanol to give compounds **3a**–**n** (24).

2-[(*E*)2-(1-substituted phenylethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4a-n)

Equimolar quantity of 1-(1*H*-benzo[*d*]imidazole-2-yl)-2-[(1-substituted phenyl ethylidene] hydrazones (**3a-n**) (0.01 mol) was refluxed with phenylisothiocynate (0.01 mol) in the presence of triethylamine (0.1 mL) and ethanol (10 mL) for 7 h at 80°C. The reaction mixture was poured onto crushed ice and the solid obtained was filtered, dried and recrystallized from ethanol to give compounds **4a**–**n**.

2-[(*E*)2-(1-phenylethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4a)

UV λ_{max} (methanol): 278 nm; IR (KBr, cm^{-1}): 3387 (NH), 3044 (CH), 1482 (C=N), 1328 (C–N), 1076 (C=S); ^1H NMR (DMSO-d_6, δ, ppm): 2.79 (s, 3H, CH_3), 4.09 (s, 1H, N-H), 7.48–6.80 (m, 14H, Ar-H), 10.55 (s, 1H, Ar-N-H); EIMS: 386 (26) $[M+1]^+$, 385 (100) $[M]^+$, 309 (64), 233 (42), 177 (34), 118 (24), 105 (34), 90 (44), 63 (12). Analysis: calcd. for $C_{22}H_{19}N_5S$: C; 68.55, H; 4.97, N; 18.17, S; 8.32%; found: C; 68.45, H; 4.80, N; 18.10; S; 8.30%.

2-[(*E*)2-(1-(4-aminophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4b)

UV λ_{max} (methanol): 314 nm; IR (KBr, cm^{-1}): 3455 (NH), 3011 (CH), 1495 (C=N), 1334 (C–N), 1122 (C=S); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.31 (s, 3H, CH_3), 3.63 (s, 2H, Ar-NH), 4.33 (s, 1H, NH), 7.28–7.74 (m, 13H, Ar-H), 7.56 (s, 1H, Ar-NH); EIMS: 401 (32) $[M+1]^+$, 400 (100) $[M]^+$, 309 (62), 233 (34), 177 (22), 118 (36), 105 (48), 90 (66), 63 (42). Analysis: calcd. for $C_{22}H_{20}N_6S$: C; 65.98, H; 5.03, N; 20.98, S; 8.01%; found: C; 65.85, H; 5.0, N; 20.90, S; 8.0%.

2-[(*E*)2-(1-(4-hydroxyphenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4c)

UV λ_{max} (methanol): 220 nm; IR (KBr, cm^{-1}): 3432 (OH), 3327 (NH), 3022 (CH), 1456 (C=N), 1173 (C=S); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.75 (s, 3H, CH_3), 4.47 (s, 1H, N-H), 7.33–7.99 (m, 4H, Ar-H), 7.24–7.64 (m, 9H, Ar-H), 7.67 (s, 1H, Ar-NH), 10.11 (s, 1H, Ar-OH); EIMS: 402 (32) $[M+1]^+$, 401 (100) $[M]^+$, 384.12 (52), 252.01 (18), 176.02 (50), 117.04 (29). Analysis: calcd. for $C_{22}H_{19}ON_5S$: C; 65.81, H; 4.77, N; 17.44, S; 7.99%; found: C; 65.80, H; 4.7, N; 17.30, S; 7.95%.

2-[(*E*)2-(1-(3-hydroxyphenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4d)

UV λ_{max} (methanol): 352 nm; IR (KBr, cm^{-1}): 3473 (OH), 3302 (NH), 3039 (CH), 1480 (C=N), 1173 (C=S); ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.79 (s, 3H, CH_3), 4.49 (s, 1H, NH), 7.10–7.99 (m, 13H, Ar-H), 7.79 (s, 1H, Ar-NH), 10.11 (s, 1H, Ar-OH); EIMS: 402 (32) $[M+1]^+$, 401 (100) $[M]^+$, 384.12 (52) , 252.01 (18), 176.02 (50), 117.04 (29). Analysis: calcd. for $C_{22}H_{19}ON_5S$: C; 65.81, H; 4.77, N; 17.44, S; 7.99%; found: C; 65.80, H; 4.7, N; 17.30, S; 7.95%.

2-[(*E*)2-(1-(4-bromophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4e)

UV λ_{max} (methanol): 305 nm; IR (KBr, cm^{-1}): 3455 (NH), 3044 (CH), 1559 (C=N), 1344 (C–N), 1122 (C=S), 912 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.48 (s, 3H, CH_3), 4.35 (s, 1H, NH), 7.58 (s, 1H, Ar-NH), 6.75 7–55 (m, 9H, Ar-H), 7.65–7.67 (m, 4H, Ar-H); EIMS: 465.12 (8) $[M+1]^+$, 464.38 (100) $[M]^+$, 384.12 (28), 308.09 (32), 232.06 (18), 175.02 (9), 117.04 (34). Analysis: calcd. for $C_{22}H_{18}BrN_5S$: C; 56.90, H; 3.91, N; 17.21, S; 6.90%; found: C; 56.89, H; 3.90, N; 17.20, S; 6.89%.

2-[(*E*)2-(1-(4-flourophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4f)

UV λ_{max} (methanol): 306 nm; IR (KBr, cm^{-1}): 3405 (NH), 3044 (CH), 1570 (C=N), 1182 (C=S), 835 (C–F); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.99 (s, 3H, CH_3), 4.44 (s, 1H, NH), 6.55–7.41 (m, 9H, Ar-H), 7.43 (s, 1H, NH), 9.16–9.19 (m, 4H, Ar-H); EIMS: 404.48 (12) $[M+1]^+$, 403 (100) $[M]^+$, 384.12 (28), 231.05 (40), 175.02 (28), 117.64 (15). Analysis: calcd. for $C_{22}H_{18}FN_5S$: C; 65.49, H; 4.50, N; 17.36, S; 7.95%; found: C; 65.50, H; 4.51, N; 17.35, S; 7.90%.

2-[(*E*)2-(1-(2-chlorophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4g)

UV λ_{max} (methanol): 378 nm; IR (KBr, cm^{-1}): 3495 (NH), 3144 (CH), 1563 (C=N), 1134 (C=S), 810 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.13 (s, 3H, CH_3), 4.44 (s, 1H, NH), 6.55–6.99 (m, 9H, Ar-H), 7.13 (s, 1H, N-H), 7.19–7.36 (m, 4H, Ar-H); EIMS: 421.23 (32) $[M+2]^+$, 420.12 (28) $[M+1]^+$, 419.93 (100) $[M]^+$, 384.12 (42), 308.12 (26), 232.06 (44), 175.02 (24), 117.04 (48), 117.64 (15). Analysis: calcd. for $C_{22}H_{18}ClN_5S$: C; 62.92, H; 4.32, N; 16.68, S; 7.65, Cl; 8.44%; found: C; 62.90, H; 4.30, N; 16.65, S; 7.63, Cl; 8.40%.

2-[(*E*)2-(1-(4-nitrophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4h)

UV λ_{max} (methanol): 278 nm; IR (KBr, cm^{-1}): 3454 (NH), 3021 (CH), 1582 (C=N), 1331 (NO_2), 1288 (C–N), 1112 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.13 (s, 3H, CH_3), 4.95 (s, 1H, NH), 7.20–7.66 (m, 9H, Ar-H), 7.68–8.10 (m, 4H, Ar-H); EIMS: 431.32 (12) $[M+1]^+$, 430.48 (100) $[M]^+$, 385.12 (18), 233.05 (40), 175.02 (49), 117.04 (12). Analysis: calcd. for $C_{22}H_{18}N_6O_2S$: C; 61.38, H; 4.21, N; 17.52, S; 7.45%; found: C; 61.36, H; 4.20, N; 17.50, S; 7.42%.

2-[(*E*)2-(1-(3-bromophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4i)

UV λ_{max} (methanol): 278 nm; IR (KBr, cm^{-1}): 3347 (NH), 3060.8 (CH), 1461 (C=N), 1155 (C=S), 890 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.48 (s, 3H, CH_3), 4.49 (s, 1H, NH), 6.99–7.89 (m, 13H, Ar- H), 7.29 (s, 1H, Ar-NH), EIMS: 465.12 (8) $[M+1]^+$, 464.38 (100) $[M]^+$, 384.12 (28), 308.09 (32), 232.06 (18), 175.02 (9), 117.04 (34). Analysis: calcd. for $C_{22}H_{18}BrN_5S$: C; 56.90, H; 3.91, N; 15.08, S; 6.90%; found: C; 56.91, H; 3.90, N; 15.06, S; 6.89%.

2-[(*E*)2-(1-(4-chlorophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4j)

UV λ_{max} (methanol): 364 nm; IR (KBr, cm^{-1}): 3308 (NH), 3105 (CH), 1561 (C=N), 1147 (C=S), 740 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.29 (s, 3H, CH_3), 4.54 (s, 1H, NH), 7.26–7.71 (m, 9H, Ar-H), 7.74–8.09 (m, 4H, Ar-H,); EIMS: 421.53 (29) $[M+2]^+$, 420.12 (28) $[M+1]^+$, 419.93 (100) $[M]^+$, 384.12 (42), 308.12 (26), 232.06 (44), 175.02 (24), 117.04 (48), 117.64 (15). Analysis: calcd. for $C_{22}H_{18}ClN_5S$: C; 62.92, H; 4.32, N; 16.68, S; 7.64, Cl; 8.44%; found: C; 62.90, H; 4.30, N; 16.65, S; 7.60, Cl; 8.40%.

2-[(*E*)2-(1-(3-nitrophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4k)

UV λ_{max} (methanol): 344 nm.; IR (KBr, cm^{-1}): 3333 (NH), 3061 (CH), 1574 (C=N), 1504 (C=C str.), 1285 (C–N), 1133 (C=S), 1331 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.31 (s, 3H, CH_3), 4.88 (s, 1H, NH), 7.20–7.66 (m, 9H, Ar-H), 7.68–8.10 (m, 4 H, Ar-H), EIMS: 431.32 (12) $[M+1]^+$, 430.48 (100) $[M]^+$, 385.12 (18), 233.05 (40), 175.02 (49), 117.04 (12). Analysis: calcd. for $C_{22}H_{18}N_6O_2S$: C; 61.38, H; 4.21, N; 19.52, S; 7.45%; found: C; 61.36, H; 4.20, N; 19.50, S; 7.43%.

2-[(*E*)2-(1-(3-aminophenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4l)

UV λ_{max} (methanol): 276 nm; IR (KBr, cm^{-1}): 3409 (NH), 3039 (CH), 1582 (C=N), 1158 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.99 (s, 3H, CH_3), 3.33 (s, 2H, Ar–NH), 4.89 (s, 1H, NH), 6.83–7.98 (m, 13H,

Ar-H), 7.29 (s, 1H, Ar-NH), EIMS: 401 (32) $[M+1]^+$, 400 (100) $[M]^+$, 309 (62), 233 (34), 177 (22), 118 (36), 105 (48), 90 (66), 63 (42). Analysis: calcd. for $C_{22}H_{20}N_6S$: C; 65.98, H; 5.03, N; 20.98, S; 8.01%; found: C; 65.95, H; 5.0, N; 20.95, S; 8.0%.

2-[(*E*)2-(1-(4-methoxyphenyl)ethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamide (4m)

UV λ_{max} (methanol): 278 nm; IR (KBr, cm^{-1}): 3329 (NH), 3025 (CH), 1586 (C=N), 1055 (C=S), 1108 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.20 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.33 (s, 1H, NH), 7.21–7.23 (m, 9H, Ar-H), 7.69 (s, 1H, NH); EIMS: 416.21 (18) $[M+1]^+$, 415.51 (100) $[M]^+$, 384.12 (23), 252.06 (30), 176.02 (11), 117.04 (22). Analysis: calcd. for $C_{23}H_{21}N_5OS$: C; 66.45, H; 5.09, N; 16.85, S; 7.72%; found: C; 66.40, H; 5.0, N; 16.80, S; 7.70%.

2-[(*E*)2-(1-(2,5-dihydroxyphenyl)ethylidine) hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*] imidazole-1-carbothioamide (4n)

UV λ_{max} (methanol): 240 nm; IR (KBr, cm^{-1}): 3463 (OH), 3351 (NH), 3015 (CH), 1490 (C=N), 1095 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.33 (s, 3H, CH_3), 4.85 (s, 1H, NH), 7.22–7.59 (m, 12H, Ar-H), 7.76 (s, 1H, NH), 10.65 (s, 2H, Ar–OH); EIMS: 418.32 (18) $[M+1]^+$, 417.48 (100) $[M]^+$, 384.12 (18), 308.09 (32), 173.08 (56), 116.03 (24). Analysis: calcd. for $C_{22}H_{19}N_5O_2S$: C; 63.29, H; 4.59, N; 16.78, S; 7.68%; found: C; 63.25, H; 4.55, N; 16.75, S; 7.65%.

Pharmacology

The investigations were conducted on albino rats of either sex (150–200 g). Animals were kept under standard conditions at an ambient temperature of 25 ± 2°C and allowed free access to food and water except at the time they were brought out of the cage. All the experimental protocols were carried out with the permission from Institutional Animal Ethics committee (IAEC), form no. 14/10, registration no. is 882-ac/05/CPCSEA Animals were obtained from Animal House Facility, Rajiv Academy for Pharmacy, Mathura, U.P.

Maximal electroshock seizure test (MES)

Each compound was administered through an oral route at dose level of 30, 60 and 100 mg/kg body weight. The maximal electroshocks seizures were elicited in rats by delivering 60 Hz, 150 mA electrical stimuli for 0.2 s *via* ear clip electrodes. The MES-convulsions are divided into five phases such as (a) tonic flexion (b) tonic extensor (c) clonic convulsion (d) stupor (e) recovery or death. The time (s) spent by the animal in each phase of the convulsions was noted. A compound is known to possess anticonvulsant property if it reduces or abolishes the hind limb tonic extensor phase of MES-convulsions (25, 26).

scPTZ-induced seizures test

The scPTZ test utilizes a dose of pentylenetetrazole (70 mg/kg in rats). This produces clonic seizures lasting for a period of at least 5 s. The time needed for the development of clonic seizure activity involving limbs and duration of seizure was carefully noted. Seizure free period of 1 h was considered as protection. The number of animals protected in each group was recorded and percentage of protection was calculated (27, 28).

Neurotoxicity screening (NT)

The minimal motor impairment was measured in rats by the rotorod test. The albino rats (100–250 g) were trained to stay on an accelerating rotorod (diameter 3.2 cm) that rotated at 10 rpm. Only those rats were taken for the test, which can stay on the revolving rod for at least one minute. Trained animals were injected *i.p.* with the test compounds at doses of 100 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at least one minute (29, 30).

Lipophilicity determination

The calculated log P (Clog P) values were generated by using the software ACD Labs 8.0 version. It was found that almost all the compounds have the Clog P values near 2.0, which is considered to be the optimum lipophilicity for the congeners that act on central nervous system (31). This may be the reason behind the rapid onset and long duration of action.

Statistical analysis

All experimental results are given as the means ± SEM. An analysis of variance, ANOVA, followed by Dunnett's multiple comparision test for *post hoc* comparison was used to verify significance compared against the control group (0.1% carboxymethylcellulose, CMC) and standard drug (phenytoin sodium 30 mg/kg); p values less than 0.001 were considered as highly significant.

RESULTS AND DISCUSSION

The synthesis of 2-[(1-substituted phenylethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imida-

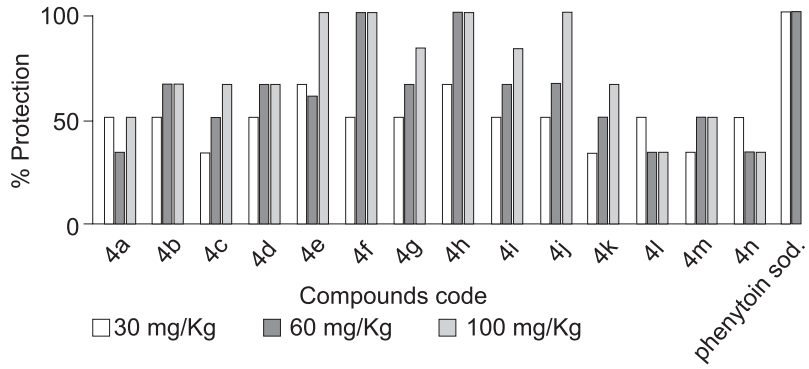


Figure 1. Graphical representation of anticonvulsant activity by MES test

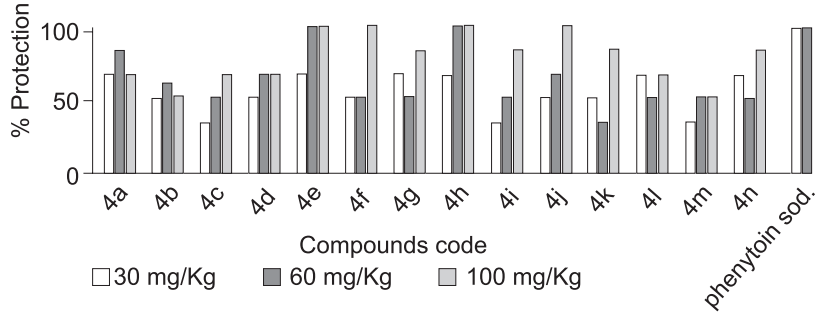
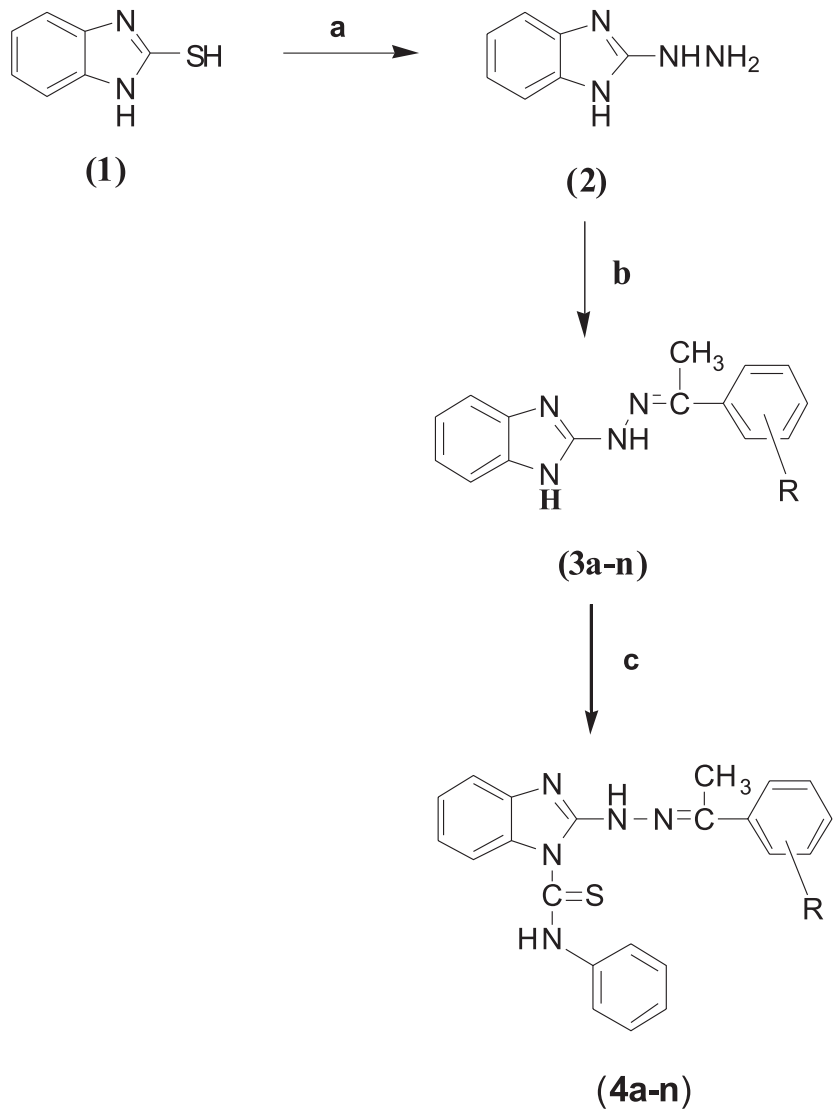


Figure 2. Graphical representation of anticonvulsant activity by scPTZ test

Table 1. Physicochemical parameters of synthesized compounds (**4a-n**).

Compd. No.	Molecular formula	R	Molecular weight	M.p. (°C)	Yield (%)	R_f*	CLog P**
4a	$C_{22}H_{19}N_5S$	H	385.48	294–295	67	0.86	1.91
4b	$C_{22}H_{20}N_6S$	4-NH_2	400.50	298–300	46.49	0.58	2.23
4c	$C_{22}H_{19}ON_5S$	4-OH	401.40	270–272	45.0	0.68	2.40
4d	$C_{22}H_{19}ON_5S$	3-OH	401.48	274–276	72.44	0.65	2.33
4e	$C_{22}H_{18}BrN_5S$	4-Br	464.38	282–284	71.34	0.69	1.97
4f	$C_{22}H_{18}FN_5S$	4-F	403.48	230–232	45.46	0.80	2.14
4g	$C_{22}H_{18}ClN_5S$	2-Cl	419.93	202–204	47.25	0.79	2.26
4h	$C_{22}H_{18}N_6O_2S$	4-NO_2	430.44	234–236	68.61	0.65	1.86
4i	$C_{22}H_{18}BrN_5S$	3-Br	464.38	278–280	68.34	0.61	1.95
4j	$C_{22}H_{18}ClN_5S$	4-Cl	419.93	250–252	80.0	0.66	2.20
4k	$C_{22}H_{18}N_6O_2S$	3-NO_2	430.48	230–232	47.0	0.67	2.44
4l	$C_{22}H_{20}N_6S$	3-NH_2	400.50	296–298	52.0	0.69	2.46
4m	$C_{23}H_{21}N_5OS$	4-OCH_3	415.51	240-242	55.0	0.63	1.92
4n	$C_{22}H_{19}N_5O_2S$	2,5 di-OH	417.48	242–244	65.34	0.72	2.21

*R_f in benzene : acetone (7:3, v/v), **CLog P was calculated using software ACD Labs 8.0 version.



Reactions and conditions: a) Hydrazine hydrate, ethanol and reflux, **b**) substituted acetophenones, glacial acetic acid, ethanol and reflux, **c**) phenylisothiocyanate, triethylamine and reflux.

Scheme 1. Synthetic route to the synthesized compounds (**4a-n**)

zole-1-carbothioamides (**4a-n**) was accomplished as presented in Scheme 1. It involves the reaction of 2-mercaptobenzimidazole with hydrazine hydrate giving 1-(1*H*-benzo[*d*]imidazol-2-yl) hydrazine (2), which was refluxed with substituted acetophenones for 7.5 h to afford 1-(1*H*-benzo[d]imidazol-2-yl)-2-[(1-substituted phenylethylidene] hydrazones (**3a-n**), which on reaction with phenylisothiocyanate in the presence of triethylamine in ethanol afforded final compounds in good yields. Their physicochemical parameters are given in Table 1.

The structures of all synthesized compounds were confirmed by means of FTIR, ^{1}H NMR (300 MHz), MS (ESI) spectral analyses and elemental analysis. The spectra of newly synthesized compounds showed the presence of characteristic absorption bands in the region 3200–3500, 3100–3000, 1500–1600, 1300–1400 and 1200–1050 cm^{-1}. This confirms the presence of NH stretching, Ar-H stretching, C=N stretching, C-N stretching and C=S stretching vibrations of substituted benzimidazole nucleus, respectively.

Table 2. Anticonvulsant activity of synthesized compounds **(4a-n)** using MES model.

Compd. no.	Doses (mg/kg)	Flexion (Mean ± SEM)	Extensor (Mean ± SEM)	Clonus (Mean ± SEM)	Stupor (Mean ± SEM)	Recovery/ Death	Neurotoxicity screen
4a	30	2.5 ± 0.31**	10.8 ± 0.21*	41.9 ± 0.23*	203.5 ± 1.4**	Recovery	x
	60	2.6 ± 0.34**	Absent	23.6 ± 0.7**	126.2 ± 1.6**	Recovery	x
	100	2.5 ± 0.3**	Absent	Absent	203.5 ± 1.4**	Recovery	–
4b	30	2.8 ± 0.36**	13.9 ± 0.23*	36.37 ± 0.24**	206.5 ± 1.5**	Recovery	–
	60	Absent	Absent	Absent	132.5 ± 1.4*	Recovery	–
	100	2.8 ± 0.36**	10.9 ± 0.23	36.37 ± 0.24**	238.5 ± 1.4**	Recovery	–
4c	30	2.2 ± 0.33	14.5 ± 0.24*	34.4 ± 0.25	214.8 ± 1.51*	Recovery	x
	60	2.6 ± 0.34**	26.7 ± 0.7**	23.6 ± 0.7**	126.2 ± 1.6**	Recovery	x
	100	2.0 ± 0.31*	14.6 ± 0.34**	34.4 ± 0.25*	211.8 ± 1.2*	Recovery	–
4d	30	2.6 ± 0.34**	20.9 ± 0.25*	Absent	211.8 ± 1.2**	Recovery	–
	60	2.8 ± 0.8**	23.6 ± 0.7**	18.1 ± 0.3*	141.1 ± 1.2**	Recovery	–
	100	2.6 ± 0.34**	Absent	Absent	211.8 ± 1.2**	Recovery	–
4e	30	2.1 ± 0.29**	Absent	Absent	208.6 ± 1.6*	Recovery	–
	60	Absent	Absent	16.2 ± 0.4**	126.2 ± 1.6**	Recovery	–
	100	Absent	Absent	Absent	208.6 ± 1.6*	Recovery	–
4f	30	Absent	Absent	Absent	234.8 ± 1.2**	Recovery	–
	60	Absent	Absent	Absent	204.2 ± 1.6*	Recovery	–
	100	Absent	Absent	Absent	234.8 ± 1.2**	Recovery	–
4g	30	Absent	Absent	46.32 ± 0.4*	216.7 ± 1.2*	Recovery	–
	60	2.6 ± 0.34**	Absent	23.6 ± 0.7**	126.2 ± 1.6**	Recovery	–
	100	2.3 ± 0.42**	23.6 ± 0.7**	Absent	211.8 ± 1.2*	Recovery	–
4h	30	Absent	Absent	48.34 ± 0.4**	224 ± 1.4**	Recovery	–
	60	2.4 ± 0.3**	Absent	Absent	187.3 ± 1.6*	Recovery	–
	100	Absent	Absent	32.3 ± 0.4**	244 ± 1.4**	Recovery	–
4i	30	Absent	23.6 ± 0.7**	49.63 ± 0.4**	281.8 ± 1.2**	Recovery	x
	60	2.6 ± 0.34**	Absent	23.6 ± 0.7**	126.2 ± 1.6**	Recovery	–
	100	Absent	Absent	Absent	211.8 ± 1.2**	Recovery	–
4j	30	Absent	Absent	Absent	255.3 ± 1.9**	Recovery	–
	60	Absent	Absent	Absent	239 ± 1.6**	Recovery	–
	100	Absent	Absent	Absent	211.8 ± 1.2**	Recovery	–
4k	30	3.2 ± 0.40**	Absent	42.43 ± 0.41**	271.9 ± 1.2**	Recovery	x
	60	2.6 ± 0.34**	23.6 ± 0.7**	23.6 ± 0.7**	126.2 ± 1.6**	Recovery	x
	100	2.2 ± 0.03**	Absent	Absent	211.8 ± 1.2**	Recovery	x
4l	30	4.3 ± 0.43*	17.8 ± 0.23	56.63 ± 0.53**	219.0 ± 1.2**	Recovery	x
	60	Absent	Absent	Absent	278.2 ± 1.7**	Recovery	–
	100	2.2 ± 0.03*	17.8 ± 0.43**	56.63 ± 0.53**	211.8 ± 1.2**	Recovery	–
4m	30	Absent	28.8 ± 0.43**	59.35 ± 0.49*	287.2 ± 1.6*	Recovery	–
	60	Absent	Absent	Absent	278.2 ± 1.7**	Recovery	–
	100	Absent	Absent	Absent	278.2 ± 1.7**	Recovery	–
4n	30	2.0 ± 0.31*	Absent	65.87 ± 0.57**	291.5 ± 1.2**	Recovery	x
	60	2.6 ± 0.34**	23.6 ± 0.7**	23.6 ± 0.7**	126.2 ± 1.6**	Recovery	x
	100	2.6 ± 0.34**	23.6 ± 0.7**	23.6 ± 0.7**	126.2 ± 1.6**	Recovery	–
Control	0.1% CMC	2.7 ± 0.23	13.37 ± 0.23	78.5 ± 0.61	235.2 ± 1.4	–	x
Phenytoin sodium		Absent	Absent	Absent	111.6 ± 1.1	Recovery	–

N = 6 in each group, ***p < 0.001 highly significant, **p < 0.01 significant and *p < 0.05 moderately significant compared against the control group (0.1%) carboxymethylcellulose (CMC) and standard drug (phenytoin sodium 30 mg/kg). – indicates an absence of activity at maximum dose administered (100 mg/kg), x denotes not tested.

Table 3. Anticonvulsant activity of synthesized compounds **(4a-n)** using scPTZ model.

Compd. no.	Doses (mg/kg)	Latency to clonic convulsion (s) (Mean ± SEM)	Duration of seizure (s) (Mean ± SEM)	Recovery/ Death	Neurotoxicty screen
4a	30	212.34 ± 34.12**	48.45 ±3.98**	Recovery	x
	60	242.41 ± 22.43*	68.38 ± 11.13**	Recovery	x
	100	231.83 ± 8.81	84.73 ± 3.32*	Recovery	x
4b	30	223.34 ± 34.12**	79.14 ± 2.87**	Recovery	–
	60	243.41 ± 23.67*	89.34 ± 12.03**	Recovery	–
	100	272.06 ± 29.53**	94.83 ± 1.84**	Recovery	x
4c	30	204.51 ± 16.34*	69.74 ± 11.03**	Recovery	x
	60	265.35 ± 25.7**	94.35 ± 2.87**	Recovery	–
	100	233.13 ± 7.43*	108.66 ± 4.74**	Recovery	–
4d	30	223.34 ± 34.12**	79.14 ± 2.87**	Recovery	–
	60	265.35 ± 25.7**	94.35 ± 2.87**	Recovery	–
	100	231.83 ± 8.0	38.66 ± 2.74**	Recovery	–
4e	30	223.34 ± 34.12**	69.14 ± 2.47**	Recovery	–
	60	268.42 ± 23.67*	Absent	Recovery	–
	100	263.5 3± 8.81*	Absent	Recovery	–
4f	30	223.34 ± 34.12**	Absent	Recovery	–
	60	252.66 ± 24.71*	Absent	Recovery	–
	100	336.56 ± 17.37**	Absent	Recovery	–
4g	30	213.47 ± 17.23**	Absent	Recovery	–
	60	341.45 ± 16.34*	Absent	Recovery	–
	100	234.43 ± 17.81	Absent	Recovery	–
4h	30	223.34 ± 34.12**	Absent	Recovery	–
	60	332.41 ± 32.4**	Absent	Recovery	–
	100	244.53 ± 17.55*	77.54 ± 2.11**	Recovery	–
4i	30	389.14 ± 29.78**	79.14 ± 2.87**	Recovery	–
	60	243.41 ± 23.67*	Absent	Recovery	–
	100	325.10 ± 36.34**	Absent	Recovery	–
4j	30	223.34 ± 34.12**	Absent	Recovery	x
	60	289.21 ± 29.2**	Absent	Recovery	–
	100	329.12 ± 36.45*	Absent	Recovery	–
4k	30	249.76 ± 23.43	77.91 ± 3.34**	Recovery	x
	60	243.83 ± 23.59*	59.65 ± 5.19 **	Recovery	–
	100	253.40 ± 30.54**	Absent	Recovery	–
4l	30	293.34 ± 30.76**	38.45 ± 2.65**	Recovery	x
	60	245.65 ± 21.60*	Absent	Recovery	x
	100	247.30 ± 24.57**	63.16 ± 6.19*	Recovery	–
4m	30	223.34 ± 34.12**	38.45 ± 2.65**	Recovery	–
	60	243.83 ± 23.59*	59.65 ± 5.19 **	Recovery	–
	100	259.63 ± 8.95*	58.66 ± 2.74**	Recovery	–
4n	30	345.47 ± 17.78**	74.25 ± 3.02*	Recovery	x
	60	248.67 ± 21.65*	Absent	Recovery	–
	100	275.03 ± 51.58**	74.12 ± 3.34**	Recovery	–
Control	1 % CMC	148.47 ± 4.23	296.74 ± 31.12	-	x
Phenytoin sodium		Absent	Absent	Recovery	–

N = 6 in each group, ***p < 0.001 highly significant, **p < 0.01 significant and *p < 0.05 moderately significant compared against the control group (0.1%) carboxymethylcellulose (CMC) and standard drug (phenytoin sodium 30 mg/kg). The (-) indicates an absence of activity at maximum dose administered 100 mg/kg, (x) denotes not tested.

^{1}H NMR spectra of synthesized compounds showed the characteristic peaks in the region 0.80–1.50 ppm for CH_3 protons, 3.50–3.90 ppm for O-CH_3 protons and 6.86–7.95 ppm for aromatic protons. These studies provided information about the presence of various functional groups and protons in the compounds, to help in confirmation of their structures. Fragmentations of compounds were studied by mass spectroscopy.

The anticonvulsant screening of the final compounds (**4a-n**) was initially screened in the rats using MES and scPTZ models. Minimal motor impairment in the form of neurotoxicity was measured by rotorod test. The anticonvulsant activity data for the compounds are reported in Tables 2 and 3. At the dose level of 30 mg/kg, all the compounds showed average to good protection. The graphical data are presented as % protection at the dose level of 30, 60 and 100 mg/kg and the results are shown in Figures 1 and 2. Phenytoin was used as a standard at the dose of 30 mg/kg.

Anticonvulsant screening of the synthesized compounds showed protection in MES screen, which showed the good ability of these compounds to stop the spread of seizures. Among the compounds studied, **4a**, **4g**, **4i** and **4m** exhibited their ability to diminish tonic-extensor seizures at the lowest doses of 30 and 60 mg/kg, whereas compounds **4e, 4f, 4h** and **4j** continued to protect from seizures at 100 mg/kg dose, which indicated the promising nature of the compounds showing remarkably reduction of extensor phase time.

In the scPTZ screening, compounds that were found to be active included **4f**, **4g**, **4j** and **4m**, whereas those which showed significant anticonvulsant activity and being active at lower dose of 30 and 60 mg/kg were **4e**, **4h** and **4i**.

In the neurotoxicity screening, compounds **4b**, **4d**, **4e**, **4f**, **4g**, **4h**, **4i** and **4m** were devoid of minimal motor impairment. Compounds **4a, 4c, 4k** and **4n** showed some sign of neurotoxicity but were less toxic than the standard drug, phenytoin.

CONCLUSIONS

In conclusion, a simple and convenient method has been developed to synthesize novel 2-[(*E*)2-(1-phenylethylidine)hydrazinyl]-*N*-phenyl-1*H*-benzo[*d*]imidazole-1-carbothioamides (**4a-n**). Further, the study highlights the importance of the structural features responsible for the anticonvulsant activity. These compounds, being active in both the screens, proved to have broad spectrum of action in dealing with the convulsions. These new data might be beneficial in the future development of benzimidazole derivatives as novel anticonvulsants.

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