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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new benzimidazole and phenylhydrazinecarbothiomide hybrids and their anticonvulsant activity

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Abstract A series of new benzimidazole derivatives (**4a–p**) were synthesized and evaluated for anticonvulsant activity in albino mice against two most adopted models, i.e. maximal electroshock seizure (MES)- and subcutaneous pentylenetetrazole (scPTZ)-induced seizures. Synthesized compounds were also screened for possible neurotoxicity using rotarod test. Among the synthesized compounds, **4p** showed the most promising activity in MES and scPTZ screens, which was further subjected for oral activity in rats. At a dose of 30 mg/kg, it showed tremendous activity in the scPTZ screen. The acute toxicity study (LD_{50}) of compounds showed that only two tested compounds **4f** and **4m** did not produce any mortality at any of the dose level. Molecular properties and pharmacokinetic parameters of the titled compounds were also determined using Lipinski's rule of five. The promising results encourage future investigation on the rational modification of this nucleus for development of better compounds.

Keywords Anticonvulsant · Glutamate · Benzimidazole · In silico · In vivo · Molecular docking

Introduction

Epilepsy is simply defined as the tendency to developed seizures. Epilepsy is characterized clinically by periodic development of seizures which are often distressing, and educe fear and misunderstanding. The distribution of epilepsy is uniform globally with no racial, geographical or social limits (Sander, 2003). This has led to social consequences and has added effect to the disease burden. With the advancement in neurological science, a number of antiepileptic drugs (AEDs), pharmacotherapies and neurosurgical techniques have been developed and new ones are under development.

$GABA_A$ receptors, voltage gated K^+ and Na^+ channels, T-type Ca^{2+} channels and glutamate receptors are primary targets for which antiepileptic drugs have been developed (Brauner-Osborne *et al.*, 2000). The identification of gamma-amino butyric acid (GABA) as an important inhibitory neurotransmitter and glutamate (Glu) as most important excitatory neurotransmitter in mammalian brain led to the identification of agents which can be targeted to inhibit seizures through enhancing the activity of GABA. Glutamate performs through the activation of either ionotropic or metabotropic receptors (Dingledine *et al.*, 1999). It was proposed that pharmacological intervention with *i*GluRs antagonists may have a potential therapeutic benefit in case of epilepsy (Lees, 2000; Auberson, 2001). Especially, the non-competitive NMDA antagonists acting at

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the glutamate coagonist glycine binding site on the NMDA receptor complex have been identified as possible therapeutic agents for the epilepsy (Danysz and Parsons, 1998; Kulagowski, 1996; Kulagowski and Leeson, 1995; Donati and Micheli, 2000; Leeson and Iversen, 1994). Antagonists of these glutamate receptors help against epilepsy. Convulsion can be prevented by antagonism of ionotropic glutamate receptors, which are the main grounds of seizing (Sarro *et al.*, 2005).The BCATc is expressed in neuronal tissue of CNSand catalyses the transfer of an amino group from branched chain amino acids to α-ketoglutarate led to synthesis of glutamate in CNS (Lain-Yen *et al.*, 2006). The inhibition of BCATc can reduce the release of glutamate synthesis during excitation in neuronal tissues which is likely to be useful for the treatment variety of neurodegenerative disorder such as epilepsy.

Within the vast range of heterocycles, benzimidazoles are found to be trendy scaffold used for finding of drugs in the pharmaceutical and medicinal chemistry field. The exclusive structural features of benzimidazole and a vast range of biological activities of its derivatives made it privileged structure in drug discovery. Benzimidazole is a heterocyclic aromatic system, found in some natural and many synthetic compounds. Benzimidazoles and its derivatives are found to be biologically active against many diseases which have made it a crucial anchor for development of promising therapeutic agents (Kruse *et al.*, 1989; Jun *et al.*, 2005; Castro *et al.*, 2011; Anelia *et al.*, 2006; Kaur *et al.*, 2014; Iemura *et al.*, 1986; Cole *et al.*, 1974; Kubo *et al.*, 1993; Blaszczak-Swiqtkiewicz and Mikiciuk-Olasik, 2015; Porcari *et al.*, 1998). Recently, benzimidazole scaffold has emerged as a pharmacophore of choice for designing anticonvulsant agents active on different clinically approved targets. The structural modification of existing leads (arylsemicarbazone) to aryl thiosemicarbazide by biosteric replacement of oxygen with sulphur and saturation of imine, led to development of newer aryl hydrazinecarbothioamide as promising anticonvulsant agents (Dimmock *et al.*, 1996). In designing a drug molecule, computational biology and bioinformatics display a most important role and have the potential of speeding up the drug discovery process. Molecular docking of the drug molecule with the receptor gives important information about drug–receptor interactions and is commonly used to find out the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity. On the basis of above facts and in continuation of our research on antiepileptics, we report here the synthesis, molecular docking and anticonvulsant activity of a new series of benzimidazole derivatives.

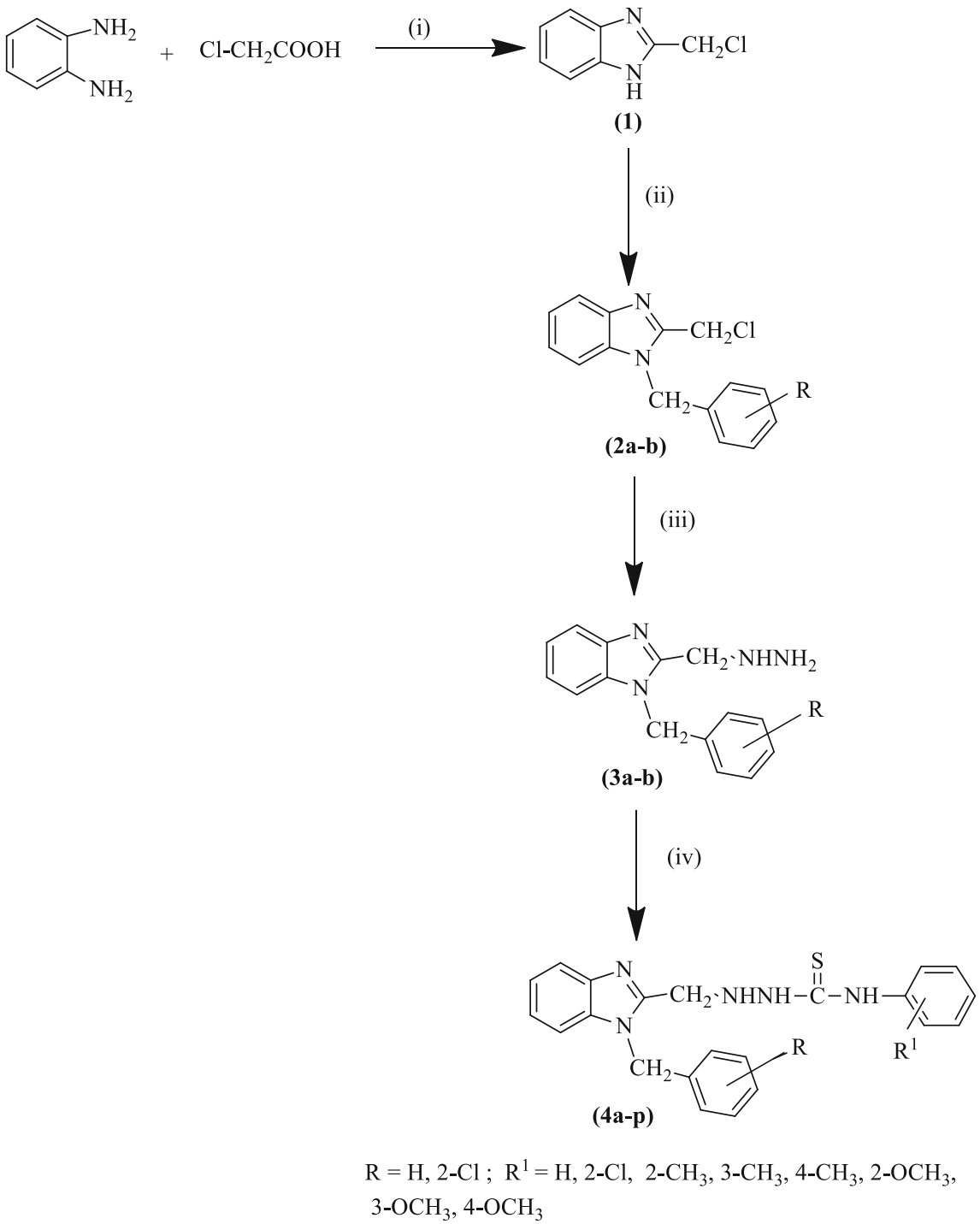
Materials and methods

Chemistry

All chemicals used in the synthesis were procured from E. Merck and S D Fine-Chem Limited. Thin-layer chromatography (TLC) was performed with silica gel 60 F254 TLC aluminium sheet (Merck) using toluene:ethyl acetate:formic acid (5:4:1) and benzene:acetone (9:1) as eluents. Spots were visualized under UV light (254 nm) and in iodine chamber. Ashless Whatmann No. 1 filter paper was used for vacuum filtration. Melting points were determined by using open capillary tubes in a Hicon melting point apparatus (Hicon, India) and are uncorrected. The purity of the compounds was confirmed through elemental analysis. The elemental analyses (C, H, N and S) of all compounds were performed on the CHNS Elimentar (Analysen systime, GmbH) Germany Vario EL III, and results were within ±0.4 % of the theoretical values. Fourier transform infrared (FT-IR) spectra were recorded in KBr pellets on a Shimadzu FT-IR spectrometer. ^{1}HNMR and ^{13}CNMR spectra in DMSO-d_6/$CDCl_3$ solutions were, respectively, recorded at 400 and 100 MHz with Bruker 400 ultrashield TM NMR spectrometer using TMS [$(CH_3)_4Si$] as internal standard. Splitting patterns are nominated as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; m, multiplet. The NH protons were D_2O exchanged for their spectral characterization. The mass spectra were recorded using Waters micromass ZQ 2000 spectrophotometer (Jamia Hamdard, New Delhi, India).

2-[(1-(2-Substitutedbenzyl)-1H-benzo*[d]*imidazol-2-yl)methyl]-*N*-substitutedphenylhydrazinecarbothioamides (**4a**–**p**) were synthesized and presented in Scheme 1.

(Chloromethyl)-1H-benzo*[d]*imidazole (**1**) was prepared from heating a mixture of *o*-phenylene diamine and 2-chloroacetic acid in a dry conical flask. Compounds (**2a**, **b**) were synthesized by stirring a mixture of compound (**1**), anhydrous potassium carbonate in acetone and substitutedbenzyl chloride. On refluxing, compounds (**2a**, **b**) with hydrazine hydrate in the presence of ethanol, 1-[(1-substitutedbenzyl-1H-benzo*[d]*imidazol-2-yl) methyl]hydrazines (**3a**, **b**) were prepared. Finally titled compounds 2-[(1-(2-substitutedbenzyl)-1H-benzo*[d]*imidazol-2-yl)methyl]-*N*-substitutedphenylhydrazinecarbothioamides (**4a**–**p**) were prepared on refluxing different substitutedphenylisothiocyanates with compounds **3a**, **b**. The physicochemical parameters of all the final compounds (**4a**–**p**) are presented in Table 1.



Scheme 1 Reagents and conditions: (*i*) Heated for 1.5 h, cooled and basified with 10 % NaOH sol^{n}, (*ii*) Anhy. K_2CO_3, acetone, substitutedbenzyl chloride, stirred for 8 h, (*iii*) $NH_2NH_2{\cdot}H_2O$, C_2H_5OH, refluxed for 20–22 h, (*iv*) ArNCS, absolute ethanol, refluxed for 5–6 h

General procedure for the synthesis of titled compounds 4a–p

2-(Chloromethyl)-1H-benzo*[d]*imidazole (**1**): A mixture of o-phenylenediamine (0.01 mol) and 2-chloroacetic acid (0.01 mol) was taken in a dry conical flask, and mouth of the flask was plugged with cotton. The mixture was heated on a boiling water bath for 1.5 h. Flask was cooled down under tap water, and the product was basified with 10 % NaOH solution, filtered and washed with cold water, and compound **1** obtained was recrystallized with hot water.

Table 1 Physicochemical parameters of compounds (**4a**–**p**)

Compd.	R	R'	Mol. Formula[a]	Log*P*[b] found (calculated.)	[c]R_f (R_m)[d]	[e]Elemental analysis (%)		
						C	H	N
4a	H	H	$C_{22}H_{21}N_5S$	4.21 (3.99)	0.79 (−0.57)	68.19	5.46	18.07
4b	H	2-Cl	$C_{22}H_{20}ClN_5S$	4.57 (4.70)	0.82 (−0.65)	62.62	4.78	16.60
4c	H	2-CH_3	$C_{23}H_{23}N_5S$	4.20 (4.48)	0.80 (−0.60)	68.80	5.77	17.44
4d	H	3-CH_3	$C_{23}H_{23}N_5S$	4.38 (4.48)	0.90 (−0.95)	68.62	5.98	17.04
4e	H	4-CH_3	$C_{23}H_{23}N_5S$	4.21 (4.48)	0.91 (−1.00)	68.95	5.57	17.20
4f	H	2-OCH_3	$C_{23}H_{23}N_5OS$	4.19 (3.90)	0.94 (−1.19)	66.16	5.55	16.77
4g	H	3-OCH_3	$C_{23}H_{23}N_5OS$	4.14 (3.90)	0.83 (−0.68)	66.56	5.15	16.34
4h	H	4-OCH_3	$C_{23}H_{23}N_5OS$	4.10 (3.90)	0.94 (−1.19)	66.34	5.37	16.67
4i	2-Cl	H	$C_{22}H_{20}ClN_5S$	4.67 (4.70)	0.83 (−0.68)	62.60	4.74	16.57
4j	2-Cl	2-Cl	$C_{22}H_{19}Cl_2N_5S$	4.33 (4.41)	0.94 (−1.19)	57.90	4.20	15.35
4k	2-Cl	2-CH_3	$C_{23}H_{22}ClN_5S$	5.16 (5.20)	0.77 (−0.52)	63.36	5.09	16.06
4l	2-Cl	3-CH_3	$C_{23}H_{22}ClN_5S$	5.41 (5.20)	0.76 (−0.50)	63.72	5.34	16.37
4m	2-Cl	4-CH_3	$C_{23}H_{22}ClN_5S$	5.52 (5.20)	0.94 (−1.19)	63.52	5.28	16.46
4n	2-Cl	2-OCH_3	$C_{23}H_{22}ClN_5OS$	4.25 (4.62)	0.77 (−0.52)	61.12	4.91	15.50
4o	2-Cl	3-OCH_3	$C_{23}H_{22}ClN_5OS$	4.44 (4.62)	0.94 (−1.19)	61.36	4.62	15.79
4p	2-Cl	4-OCH_3	$C_{23}H_{22}ClN_5OS$	4.51 (4.62)	0.78 (−0.54)	61.02	5.09	15.85

[a] Solvent of crystallization-ethanol

[b] Log*P* was determined by octanol:phosphate buffer method; *C*Log*P* was calculated using software ChemDraw Ultra 8.0

[c] Solvent system—toluene:ethyl acetate:formic acid (5:4:1)

[d] A logarithmic function of R_f value was also calculated; $R_m = \log (1 - 1/R_f)$

[e] Elemental analysis for C, H, N were within ±0.4 % of the theoretical value

2-(Chloromethyl)-1-(2-substitutedbenzyl)-1H-benzo*[d]*imidazoles (**2a**, **b**): To a solution of compound **1** (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in acetone (30 mL), substitutedbenzyl chloride (0.01 mol) was added dropwise. The mixture was stirred at room temp for about 8 h. The mixture was then poured into water and extracted with ethyl acetate, dried over sodium sulphate anhydrous and concentrated under vacuum to give the pure compound. Desired compound **2a**, **b** was finally recrystallized with ethanol.

1-[(1-Substitutedbenzyl-1H-benzo*[d]*imidazol-2-yl)methyl] hydrazines (**3a**, **b**): Hydrazine hydrate (10 mL) was placed in a round bottom flask, and compound **2a**, **b** (0.01 mol) was added. Contents were diluted with a sufficient quantity of dry ethanol till clear solution was obtained, and the reaction mixture was refluxed for 20–22 h. After completion of the reaction, ethanol was distilled off till a small volume was left. On cooling, crystals of compounds (**3a**, **b**) were formed and were filtered and recrystallized with ethanol.

2-[(1-(2-Substitutedbenzyl)-1H-benzo*[d]*imidazol-2-yl) methyl]-*N*-substitutedphenylhydrazinecarbothioamides (**4a**–**p**): A mixture of compound (**3a**, **b**, 0.01 mol) and substitutedphenylisothiocyanates (0.01 mol) in 20 mL of absolute ethanol was refluxed for 5–6 h. After completion of the reaction the reaction, mixture was concentrated and kept overnight at room temperature. The needle-shaped crystals thus obtained were purified by repeated washing with petro leum ether (see Supplementary material).

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-phenylhydrazinecarbothioamide (**4a**)*

Yield: 64 %; m.p. 180 °C; IR (KBr) υ cm^{-1}: 3576 ($NH_{str.}$), 3373 ($NH_{str.}$), 3200 ($NH_{str.}$-thioamide), 3089 (Ar–$CH_{str.}$), 2957 ($CH_{str.}$), 1602 (C=N, cyclic), 1528 (C=$S_{str.}$); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.66 (s, 2H, CH_2), 4.05 (s, 2H, CH_2–Ar), 6.59–7.37 (m, 10H, Ar–H), 7.01–7.67 (m, 4H, Bz–H), 8.46 (s, 1H, NH), 8.67 (s, 1H, NH), 10.50 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 42.24, 45.63, 109.41, 117.32, 120.24, 122.62, 123.53, 124.64, 126.42, 127.12, 127.35, 127.63, 128.23, 129.52, 130.54, 130.87, 134.23, 135.12, 138.54, 148.46, 153.26, 161.24; MS: *m/z* 386 (M-1).

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-(2-chlorophenyl)hydrazinecarbothioamide (**4b**)*

Yield: 52 %, m.p. 190 °C. IR (KBr) υ cm^{-1}: 3525 ($NH_{str.}$), 3345 ($NH_{str.}$), 3265 ($NH_{str.}$-thioamide), 3008 (Ar–$CH_{str.}$), 2985 ($CH_{str.}$), 1608 (C=N, cyclic), 1537 (C=$S_{str.}$), 805 (C–Cl); ^{1}H NMR δ (ppm) ($CDCl_3$): 3.58 (s, 2H, CH_2), 4.25 (s,

2H, CH_2–Ar), 6.64–7.86 (m,9H, Ar–H), 7.04–7.36 (m, 4H, Bz–H), 8.24 (s, 1H, NH, D_2O exchangeable), 8.95 (s, 1H, NH, D_2O exchangeable), 10.24 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 43.44, 45.53, 110.28, 118.22, 119.82, 122.82, 123.63, 125.25, 126.19, 127.28, 127.39, 127.84, 128.81, 129.28, 130.10, 130.92, 133.43, 135.27, 137.34, 148.28, 152.16, 163.82.

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl) methyl]-N-o-tolylhydrazinecarbothioamide (**4c**)*

Yield: 51 %; m.p. 175 °C; IR (KBr) υ cm^{-1}: 3371 ($NH_{str.}$), 3194 ($NH_{str.}$), 3091 ($NH_{str.}$-thioamide), 2990 (Ar–$CH_{str.}$), 2943 ($CH_{str.}$), 1685 (C=N, cyclic), 1540 (C=$S_{str.}$); ^1H NMR ($CDCl_3$) δ (ppm): 2.48 (s, 3H, CH_3) 3.89 (s, 2H, CH_2), 4.09 (s, 2H, CH_2–Ar), 7.07–7.97 (m, 9H, Ar–H), 7.22–7.91 (m, 4H, Bz–H), 8.00 (s, 1H, NH, D_2O exchangeable), 9.25 (s, 1H, NH, D_2O exchangeable), 10.01 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 18.24, 41.27, 43.48, 110.17, 118.29, 119.83, 121.29, 124.10, 125.53, 126.82, 127.16, 127.23, 127.72, 128.73, 129.18, 130.28, 130.96, 133.14, 134.47, 136.17, 149.27, 151.92, 164.92; MS: *m/z* 403 (M+2).

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-m-tolylhydrazinecarbothioamide (**4d**)*

Yield: 44 %; m.p. 210 °C; IR (KBr) υ cm^{-1}: 3345 ($NH_{str.}$), 3173 ($NH_{str.}$), 3065 ($NH_{str.}$-thioamide), 2985 (Ar–$CH_{str.}$), 2932 ($CH_{str.}$), 1647 (C=N, cyclic), 1564 (C=$S_{str.}$); ^1H NMR ($CDCl_3$) δ (ppm): 2.45(s, 3H, CH_3) 3.58 (s, 2H, CH_2), 4.05 (s, 2H, CH_2–Ar), 7.04–7.85 (m, 9H, Ar–H), 7.12–7.99 (m, 4H, Bz–H), 8.05 (s, 1H, NH, D_2O exchangeable), 9.29 (s, 1H, NH, D_2O exchangeable), 10.17 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 17.26, 42.47, 44.29, 112.21, 117.13, 120.15, 121.72, 124.27, 125.17, 126.73, 127.33, 127.74, 127.85, 128.83, 129.92, 130.12, 130.83, 133.26, 134.18, 136.92, 147.22, 153.12, 162.61; MS: *m/z* 401 (M+1).

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-p-tolylhydrazinecarbothioamide (**4e**)*

Yield: 65 %; m.p. 215 °C; IR (KBr) υ cm^{-1}: 3321 ($NH_{str.}$), 3173 ($NH_{str.}$), 3054 ($NH_{str.}$-thioamide), 2974 (Ar–$CH_{str.}$), 2965 ($CH_{str.}$), 1627 (C=N, cyclic), 1538 (C=$S_{str.}$); ^1H NMR ($CDCl_3$) δ (ppm): 2.44(s, 3H, CH_3), 3.48 (s, 2H, CH_2), 4.04 (s, 2H, CH_2–Ar), 7.06–7.84 (m, 9H, Ar–H), 7.14–7.47 (m, 4H, Bz–H), 8.45 (s, 1H, NH, D_2O exchangeable), 9.04 (s, 1H, NH, D_2O exchangeable), 10.14 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 18.25, 40.23, 43.18, 113.41, 118.92, 120.62, 121.16, 124.68, 125.15, 126.92, 127.17, 127.82, 127.44, 128.21, 129.34, 130.28, 131.83, 132.23, 134.35, 138.32, 147.45, 152.39, 160.91; MS: *m/z* 402 (M+1).

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-(2-methoxyphenyl)hydrazinecarbothioamide (**4f**)*

Yield: 63 %; m.p. 170 °C; IR (KBr) υ cm^{-1}: 3424 ($NH_{str.}$), 3299 ($NH_{str.}$), 3117 ($NH_{str.}$-thioamide), 2961 (Ar–$CH_{str.}$), 2901 ($CH_{str.}$), 1601 (C=N, cyclic), 1536 (C=$S_{str.}$), 1169 (OCH_3); ^1H NMR (DMSO-d_6) δ(ppm): 3.17 (s, 2H, CH_2), 3.40 (s, 3H, OCH_3) 4.15 (s, 2H, CH_2–Ar), 6.58–8.15 (m, 9H, Ar–H), 7.22–8.76 (m, 4H, Bz–H), 8.82 (s, 1H, NH, D_2O exchangeable), 9.19 (s, 1H, NH, D_2O exchangeable), 11.11 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.24, 42.84, 55.25, 114.26, 119.28, 120.27, 121.38, 124.84, 125.28, 126.94, 127.21, 127.23, 127.49, 128.48, 129.28, 130.47, 131.23, 133.40, 134.35, 138.24, 147.36, 150.58, 163.41; MS: *m/z* 419 (M+2).

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-(3-methoxyphenyl)hydrazinecarbothioamide (**4g**)*

Yield: 61 %; m.p. 151 °C; IR (KBr) υ cm^{-1}: 3458 ($NH_{str.}$), 3273 ($NH_{str.}$), 3123 ($NH_{str.}$-thioamide), 2984 (Ar–$CH_{str.}$), 2928 ($CH_{str.}$), 1648 (C=N, cyclic), 1521 (C=$S_{str.}$), 1114 (OCH_3); ^1H NMR ($CDCl_3$) δ (ppm): 3.25 (s, 2H, CH_2), 3.63 (s, 3H, OCH_3), 4.27 (s, 2H, CH_2–Ar), 6.24–8.56 (m,9H, Ar–H), 7.02–8.21 (m, 4H, Bz–H), 8.15 (s, 1H, NH, D_2O exchangeable), 9.01 (s, 1H, NH, D_2O exchangeable), 11.23 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 41.25, 43.95, 57.28, 111.37, 119.83, 120.73, 121.49, 122.39, 125.25, 126.29, 127.35, 127.57, 127.21, 128.24, 129.35, 130.64, 131.24, 133.87, 137.25, 141.23, 148.26, 150.35, 165.45.

*2-[(1-Benzyl-1H-benzo[d]imidazol-2-yl)methyl]-N-(4-methoxyphenyl)hydrazinecarbothioamide (**4h**)*

Yield: 59 %; m.p. 200 °C; IR (KBr) υ cm^{-1}: 3445 ($NH_{str.}$), 3273 ($NH_{str.}$), 3154 ($NH_{str.}$-thioamide), 2921 (Ar–$CH_{str.}$), 2958 ($CH_{str.}$), 1625 (C=N, cyclic), 1556 (C=$S_{str.}$), 1147 (OCH_3); ^1H NMR ($CDCl_3$) δ (ppm): 3.26 (s, 2H, CH_2), 3.58 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2–Ar), 6.14–8.56 (m,9H, Ar–H), 7.05–8.12 (m, 4H, Bz–H), 8.17 (s, 1H, NH, D_2O exchangeable), 9.06 (s, 1H, NH, D_2O exchangeable), 11.31 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 40.34, 45.24, 53.26, 114.26, 118.26, 120.23, 121.24, 123.34, 125.36, 126.45, 127.56, 127.68, 127.48, 128.23, 129.42, 129.62, 132.54, 133.35, 139.24, 140.24, 146.36, 149.85, 164.43.

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-phenylhydrazinecarbothioamide (**4i**)*

Yield: 67 %; mp 173 °C. IR (KBr) υ cm^{-1}: 3576 ($NH_{str.}$), 3471 ($NH_{str.}$), 3223 ($NH_{str.}$-thioamide), 2960 (Ar–$CH_{str.}$), 2905 ($CH_{str.}$), 1625 (C=N, cyclic), 1595 (C=$S_{str.}$), 745 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.52 (s, 2H, CH_2), 3.31 (s, 2H, CH_2–Ar), 7.14–8.20 (m, 9H, Ar–H), 7.20–8.23 (m, 4H, Bz–H), 8.76 (s, 1H, NH, D_2O exchangeable), 10.42 (s, 1H, NH, D_2O exchangeable), 11.52 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 38.23, 42.65, 106.43, 119.39, 120.47, 122.68, 123.35, 125.24, 126.24, 127.35, 127.68, 127.58, 128.37, 129.28, 130.68, 130.76, 133.12, 137.46, 138.35, 147.17, 156.58, 161.97; MS: *m*/*z* 423 (M+2).

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-(2-chlorophenyl)hydrazinecarbothioamide (**4j**)*

Yield: 57 %; m.p. 144 °C; IR (KBr) υ cm^{-1}: 3545 ($NH_{str.}$), 3473 ($NH_{str.}$), 3365 ($NH_{str.}$-thioamide), 3023 (Ar–$CH_{str.}$), 2941 ($CH_{str.}$), 1632 (C=N, cyclic), 1575 (C=$S_{str.}$), 712 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.12 (s, 2H, CH_2), 3.62 (s, 2H, CH_2–Ar), 7.11–8.25 (m,8H, Ar–H), 7.24–8.56 (m, 4H, Bz–H), 8.25 (s, 1H, NH, D_2O exchangeable), 10.23 (s, 1H, NH, D_2O exchangeable), 11.74 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.43, 40.65, 108.44, 119.35, 121.42, 122.46, 124.89, 125.84, 126.45, 127.74, 127.84, 127.57, 128.36, 129.32, 130.14, 132.46, 135.58, 137.34, 139.43, 144.58, 154.23, 165.27.

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-o-tolylhydrazinecarbothioamide (**4k**)*

Yield: 64 %; m.p. 300 °C; IR (KBr) υ cm^{-1}: 3506 ($NH_{str.}$), 3484 ($NH_{str.}$), 3463 ($NH_{str.}$-thioamide), 3063 (Ar–$CH_{str.}$), 2950 ($CH_{str.}$), 1610 (C=N, cyclic), 1409 (C=$S_{str.}$), 801 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.50 (s, 3H, CH_3), 3.37 (s, 2H, CH_2), 5.24 (s, 2H, CH_2–Ar), 6.47–7.96 (m,8H, Ar–H), 7.02–7.97 (m, 4H, Bz–H), 9.85 (s, 1H, NH, D_2O exchangeable), 10.39 (s, 1H, NH, D_2O exchangeable), 10.67 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.36, 39.35, 47.74, 118.87, 119.37, 120.34, 121.72, 124.87, 125.53, 126.57, 127.45, 127.93, 127.95, 128.35, 129.49, 130.86, 131.09, 132.46, 133.67, 136.42, 144.67, 153.37, 158.94.

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-m-tolylhydrazinecarbothioamide (**4l**)*

Yield: 56 %; m.p. 300 °C; IR (KBr) υ cm^{-1}: 3545 ($NH_{str.}$), 3444 ($NH_{str.}$), 3412 ($NH_{str.}$-thioamide), 3085 (Ar–$CH_{str.}$), 2953 ($CH_{str.}$), 1641 (C=N, cyclic), 1485 (C=$S_{str.}$), 805 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.55 (s, 3H, CH_3), 3.42 (s, 2H, CH_2), 5.66 (s, 2H, CH_2–Ar), 6.63–7.95 (m,8H, Ar–H), 7.69–7.14 (m, 4H, Bz–H), 9.12 (s, 1H, NH, D_2O exchangeable), 10.74 (s, 1H, NH, D_2O exchangeable), 10.34 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.35, 40.95, 44.57, 117.96, 119.85, 120.74, 121.73, 124.42, 125.58, 126.37, 127.15, 127.27, 127.34, 128.43, 129.79, 130.23, 131.42, 132.47, 135.35, 138.32, 143.35, 155.27, 158.23.

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-p-tolylhydrazinecarbothioamide (**4m**)*

Yield: 53 %; m.p. 180 °C; IR (KBr) υ cm^{-1}: 3565 ($NH_{str.}$), 3484 ($NH_{str.}$), 3442 ($NH_{str.}$-thioamide), 3432 (Ar–$CH_{str.}$), 2985 ($CH_{str.}$), 1612 (C=N, cyclic), 1442 (C=$S_{str.}$), 812 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.54 (s, 3H, CH_3), 3.45 (s, 2H, CH_2), 5.32 (s, 2H, CH_2–Ar), 6.52–7.85 (m, 8H, Ar–H), 7.56–7.46 (m, 4H, Bz–H), 9.85 (s, 1H, NH, D_2O exchangeable), 10.32 (s, 1H, NH, D_2O exchangeable), 10.47 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 26.67, 41.24, 46.43, 118.57, 119.34, 120.34, 121.48, 124.97, 125.97, 126.45, 127.24, 127.74, 127.64, 128.23, 129.96, 130.35, 131.75, 132.95, 135.86, 136.98, 144.78, 154.26, 157.22.

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-(2-methoxyphenyl)hydrazinecarbothioamide (**4n**)*

Yield: 57 %; m.p. 185 °C; IR (KBr) υ cm^{-1}: 3497 ($NH_{str.}$), 3484 ($NH_{str.}$), 3474 ($NH_{str.}$-thioamide), 3074 (Ar–$CH_{str.}$), 2826 ($CH_{str.}$), 1670 (C=N, cyclic), 1488 (C=$S_{str.}$), 1105 (OCH_3), 733 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.31 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.85 (s, 2H, CH_2–Ar), 6.97–7.89 (m, 8H, Ar–H), 7.00–7.96 (m, 4H, Bz–H), 7.98 (s, 1H, NH, D_2O exchangeable), 9.08 (s, 1H, NH, D_2O exchangeable), 10.72 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 37.24, 44.45, 59.37, 109.43, 118.32, 118.35, 120.42, 123.54, 125.53, 126.56, 127.17, 127.32, 127.35, 128.47, 129.09, 130.85, 132.56, 133.75, 137.64, 140.43, 147.32, 149.32, 165.35.

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-(3-methoxyphenyl)hydrazinecarbothioamide (**4o**)*

Yield: 54 %; m.p. 300 °C; IR (KBr) υ cm^{-1}: 3466 ($NH_{str.}$), 3454 ($NH_{str.}$), 3412 ($NH_{str.}$-thioamide), 3021 (Ar–$CH_{str.}$), 2854 ($CH_{str.}$), 1654 (C=N, cyclic), 1484 (C=$S_{str.}$), 1131 (OCH_3), 745 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.32 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.56 (s, 2H, CH_2–Ar), 6.54–7.86 (m,8H, Ar–H), 7.05–7.32 (m, 4H, Bz–H), 7.85 (s, 1H, NH, D_2O exchangeable), 9.12 (s, 1H, NH, D_2O exchangeable), 10.41 (s, 1H, NH–Ar, D_2O exchangeable).

^{13}C NMR (DMSO-d_6) δ (ppm): 39.34, 46.75, 55.76, 105.35, 117.54, 118.38, 121.27, 124.25, 125.43, 126.54, 127.35, 127.65, 127.76, 128.23, 129.67, 131.23, 132.55, 133.46, 136.24, 139.44, 145.46, 147.57, 161.38.

*2-[(1-(2-Chlorobenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-(4-methoxyphenyl)hydrazinecarbothioamide (**4p**)*

Yield: 57 %; m.p. 190 °C; IR (KBr) υ cm^{-1}: 3423 ($NH_{str.}$), 3412 ($NH_{str.}$), 3358 ($NH_{str.}$-thioamide), 3054 (Ar–$CH_{str.}$), 2896 ($CH_{str.}$), 1612 (C=N, cyclic), 1431 (C=$S_{str.}$), 1154 (OCH_3), 736 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.12 (s, 2H, CH_2), 3.14 (s, 2H, CH_2–Ar), δ 3.50 (s, 3H, OCH_3), 6.65–7.84 (m,8H, Ar–H), 7.01–7.32 (m, 4H, Bz–H), 7.45 (s, 1H, NH, D_2O exchangeable), 9.22 (s, 1H, NH, D_2O exchangeable), 10.51 (s, 1H, NH–Ar, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ (ppm): 37.36, 44.56, 56.47, 103.38, 117.56, 118.43, 120.37, 121.23, 124.45, 125.46, 127.23, 127.37, 127.87, 128.16, 129.48, 131.48, 132.47, 133.65, 136.67, 138.36, 144.26, 146.32, 167.84.

Pharmacology

The pharmacological testing of all the final compounds has been performed by National Institute of Neurological Disorders and Stroke (NINDS), USA, under Anticonvulsant Screening Program (ASP), following the protocol adopted by the Antiepileptic Drug Development (ADD) programme. The screening for anticonvulsant activity was carried out by maximal electroshock seizure (MES) test and subcutaneous pentylenetetrazole (scPTZ)-induced seizure test, whereas neurotoxicity evaluation was done by the rotarod method. The tested compounds (**4a**–**p**) were injected intraperitoneally (i.p) at a dose of 30, 100 and 300 mg/kg for both methods. Phenytoin and carbamazepine were used as standard drugs for comparison.

Maximal electroshock seizure (MES) test

MES test is a model for generalized tonic–clonic seizures and identifies those compounds which prevent seizure spread. Animals were randomly divided into different treatment groups. For induction of seizures in mice, a 60 Hz alternating current of 50 mA intensity was used. The current was applied for 0.2 s using ear clip electrodes, coated with an electrolyte solution containing an anaesthetic agent. Abolition of the hind limb and tonic maximal extension was regarded as end point protection against MES-induced seizures (Krall *et al.*, 1978). The observations were taken at two different time intervals (0.5 h in one group and 4.0 h in other group, respectively).

Subcutaneous pentylenetetrazole (scPTZ)-induced seizure test

This model primarily identifies those compounds that raises seizure threshold. The subcutaneous dose of PTZ (85 mg/kg) at which 97 % of the animals showed clonic seizure induction was calculated using dose response method. Animals were divided into different groups, and test drugs were administered. The test compounds were administered intraperitoneally at three different doses, i.e. 30, 100, 300 mg/kg. At the anticipated time of testing, PTZ was administered subcutaneously as 0.5 % solution in the posterior midline of mice. Animals were observed after 30 min and 4.0 h. The absence of clonic spasm in half or more animals was regarded as endpoint protection against scPTZ-induced clonic seizures (Swinyard *et al.*, 1989).

Rotarod test

Mice were divided into different experimental groups and trained to stay on a knurled rotarod of diameter 3.2 cm rotating at 10 rpm. Trained animals were injected with the test compounds. Motor impairment was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials (Dunham and Miya, 1957).

Quantification studies

For the determination of effective dose ED_{50}, that is, the dose of the drug required to elicit the biological response in 50 % of animals and TD_{50} values, that is, median toxic dose, groups of six mice were given a range of i.p. doses of the test drug until at least three points were established in the range of 10–90 % seizure protection or minimal observed neurotoxicity. From the plot of these data, the respective ED_{50} and TD_{50} values, 95 % confidence intervals, slope of the regression line and the standard error of the slope were calculated by means of the computer program (Litchfield and Wilcoxon, 1949).

Computational studies

MM-GBSA binding free energy estimation

To calculate ligand binding energies and ligand strain energies, binding energy estimation can be used for a set of ligand and a single receptor human cytosolic branched chain amino transferase (hBCATc) using the Prime molecular mechanics-generalized Born surface area (MM-GBSA) method, Maestro 10.1. LigPrep and protein preparation wizard was used to prepare the ligands and the receptors.

Molecular docking

To predict binding modes of ligands to receptor and good anticonvulsant activity on the basis of structures, molecular docking studies of all the compounds were carried out by taking X-ray crystal structure data to establish the interaction of the synthetic compounds with hBCATc enzyme (PDB code: 2COI) using Glide extra precision (XP) Maestro 10.1 Schrodinger, running on Linux 64 operating system (Schrödinger, 2015a, b). It involves few steps, i.e. selection and preparation of appropriate protein, grid generation, ligand preparation followed by docking and its analysis. The docking score, binding free energy and hydrogen bonds and pi–pi interaction formed with the surrounding amino acids are used to envisage their binding affinities and proper alignment of these compounds at the active site of the hBCATc enzyme.

Results and discussion

Chemistry

All the final compounds were characterized by different spectral analytical techniques such as IR spectroscopy, ^{1}HNMR, ^{13}C NMR and mass spectrometry, and the data confirmed the structures of synthesized compounds. The structures and purity of the final compounds were confirmed on the basis of spectral and elemental analyses, and the data were within ±0.4 % of the theoretical values. The FT-IR bands at the 3576–3173, 3474–3054, 3432–2921, 1685–1602 and 1576–1409 cm^{-1} confirmed the presence of $NH_{str.}$, $NH_{str.}$-thioamide, Ar–$CH_{str.}$, C=N, cyclic and C=$S_{str.}$ functionalities, respectively. The ^{1}H NMR spectrum showed multiplet at δ 7.01–8.76 confirmed benzimidazole aromatic protons and singlet at δ 3.12–3.89, δ 8.00–10.74 and δ 10.01–11.74 confirms methylene, thiourea NH and NH-aromatic protons, respectively. ^{13}C NMR of different carbon atoms of the compounds were found to be in the range of δ 17.26–167.84. The mass spectrometry of the prototype compound **4a** was performed, and it showed a base peak at the *m/z* value 386 that represents the M-1 peak corresponding to the molecular weight of the compound.

Pharmacology

Synthesized titled compounds **4a**–**p** of Scheme 1 were screened for protection against MES and scPTZ seizures. Some of these compounds showed promising results in Phase 1 screening in mice (Table 2). The compounds **4e**, **4f**, **4j**, **4l**,

Table 2 Anticonvulsant screening project (ASP)

Compd.	MES screening						scPTZ screening						Minimal motor impairment screening					
	30 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg	
	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F	0.5 h N/F	4.0 h N/F
4a	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
4b	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
4c	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
4d	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
4e	0/1	0/1	0/3	**1/3**	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	**0/4**	**0/2**	**0/8**	**0/4**	**0/4**	**0/2**
4f	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	**1/5**	0/1	0/1	**0/4**	**0/2**	**0/8**	**0/4**	**0/4**	**0/2**
4g	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/4	0/2	0/8	0/4	0/4	0/2
4h	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
4i	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
4j	0/1	0/1	0/3	0/3	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/0	**0/4**	**0/2**	**0/8**	**0/4**	**0/4**	1/2
4k	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
4l	0/1	0/1	0/3	0/3	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	**0/4**	**0/2**	**0/8**	**0/4**	**0/4**	**0/2**
4m	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**0/4**	**0/2**	**0/8**	**0/4**	**0/4**	**0/2**
4n	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
4o	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
4p	0/1	0/1	0/3	**2/3**	0/1	0/0	0/1	0/1	0/1	**1/1**	**4/5**	0/0	**0/4**	**0/2**	**0/8**	3/4	1/4	2/2

Bold values indicate that molecules were active at at least one of the tested dose and did not show any toxicity

Phase I anticonvulsant screening of synthesized compounds (**4a**–**p**)

'×' indicated not tested

MES maximal electroshock seizure, *scPTZ* subcutaneous pentylenetetrazole, route of administration: ip

4m and **4p** were most potent anticonvulsants. In MES test, compounds **4e** and **4p** were active at dose of 100 mg/kg (1/3 and 2/3 animals protected) 4.0 h after administration; however, compound **4p** showed toxicity at 100 and 300 mg/kg dose levels. Interestingly compound **4e** was also active at dose of 300 mg/kg after 4.0 h of administration. Further, compounds **4e**, **4j** and **4l** were active 4.0 h after administration of 300 mg/kg dose. In this case also, toxicity was observed with **4j**, 4.0 h after 300 mg/kg dose administration.

In scPTZ screening, compounds **4f** and **4p** were active at doses of 100 mg/kg (1/5 and 1/1 animals protected) 4.0 h after administration; interestingly, compound **4f** did not show any toxicity at any dose. Dramatically compound **4p** was found to be most potent at the dose of 100 mg/kg (1/1 animal protected) after 4.0 h and at the dose of 300 mg/kg (4/5 animals protected) after 0.5 h administration; however, it showed some toxicity at higher doses of 100 and 300 mg/kg.

The compound **4p** that displayed a marked MES activity in mice at the dose of 100 mg/kg i.p (2/3 animals protected) was evaluated for oral activity in rats, Test 2 results (Table 3). Compound **4p** did not show any activity in the MES screen at 30 mg/kg (p.o. dose) but tremendously showed far-fetched activity in the scPTZ screen at 30 mg/kg (p.o. dose) (1/4, 1/4 and 2/4 animals protected) 1.0, 2.0 and 4.0 h after administration. It is interesting that compound **4p** showed lack of any toxicity at 30 mg/kg p.o. dose) in any time interval.

Compound **4m** was evaluated for 6 Hz assay in mice, Test 7 results (Table 4). Compound **4m** showed protection in the minimal clonic seizure (6 Hz) test at 100 mg/kg (i.p dose) (1/4 animal protected) 0.5 h after administration. Interestingly it lacks any toxicity at given dose in any time intervals.

The acute toxicity study (LD_{50}) was performed on the albino mice in order to assess any mortality or toxic effects shown by the synthesized compounds at increased doses, and the data are presented in Table 5. It was found that the tested compounds **4f** and **4m** did not produce any mortality or gross effect on the central nervous system at any of the dose levels, i.e. 5, 25, 125, 250 and 400 mg/kg. However, the animals have exhibited some of the toxic signs such as chewing, licking, salivation followed by brief period of sedation. But at the doses of 125, 250 and 400 mg/kg, compound **4p** showed mortality (1/6, 3/6 and 3/6, respectively). There was not any toxicity seen in the vehicle alone as evident in the control group of animals.

These results suggest that thioamide as hydrogen bonding domain (HBD), nitrogen of benzimidazole as electron donor (D), substitution of 2/4-methoxy or methyl as electron donating agent in hydrophobic domain (A) and unsubstitution or 2-chloro substitution in distal hydrophobic domain (R) made the compound potent in the MES screen at 100 and 300 mg/kg doses, scPTZ screen at 100 and 300 mg/kg (i.p dose) in mice and 30 mg/kg (p.o. dose)

Table 3 Anticonvulsant screening project (ASP)

Compd.	Test	Time (h)				
		0.25	0.5	1.0	2.0	4.0
4p	MES[a]	0/4	0/4	0/4	0/4	0/4
	scPTZ[b]	0/4	0/4	**1/4**	**1/4**	**2/4**
	TOX[c]	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**

Bold values indicate active compounds in one row and compounds without toxicity in other row

Test 2 results in rats

Dose = 30 mg/kg

[a] Maximal electroshock test (number of animal protected/number of animals tested)

[b] Subcutaneous pentylenetetrazole test (number of animal protected/number of animals tested)

[c] Rotarod toxicity (number of animals exhibiting toxicity/number of animals tested) endpoint: unable to maintain balance on rotarod

Table 4 Anticonvulsant screening project (ASP)

Compd.	Test	Time (h)				
		0.25	0.5	1.0	2.0	4.0
4m	6 Hz[a]	0/4	**1/4**	0/4	0/4	0/4
	TOX[b]	**0/4**	**0/4**	**0/4**	**0/4**	**0/4**

Bold values indicate active compounds in one row and compounds without toxicity in other row

Test 7 results in mice; 6 Hz assay

Dose = 100 mg/kg

[a] Qualitative 6 Hz assay (number of animal protected/number of animals tested)

[b] Rotarod toxicity (number of animals exhibiting toxicity/number of animals tested) endpoint: unable to maintain balance on rotarod

Table 5 Acute toxicity study data (LD_{50}) of active compounds

Compd.	Number of animals dead/Total number of animals tested				
	Dose (mg/kg), i.p.				
	5	25	125	250	400
Control	0/6	0/6	0/6	0/6	0/6
4f	0/6	0/6	0/6	0/6	0/6
4m	0/6	0/6	0/6	0/6	0/6
4p	0/6	0/6	1/6	3/6	3/6
PHY	0/6	0/6	0/6	3/6	4/6
Carbamazepine	0/6	0/6	0/6	3/6	3/6
Phenobarbital	0/6	0/6	0/6	3/6	3/6

Vehicle used: polyethylene glycol (0.4 mL, i.p.)

Table 6 Docking score and binding free energy of all the compounds at the active sites of hBCATc (PDB ID: 2COI) GBN-A-420

Comp.	Docking score	Binding free energy (kcal/mol)
4a	−5.679	−41.313
4o	−4.652	−64.205
4p	−6.286	−60.438
4n	−6.172	−76.141
4m	−4.102	−66.226
4k	−4.468	−69.816
4l	−5.082	−72.537
4j	−4.732	−63.909
4i	−5.094	−70.725
4h	−5.732	−57.073
4g	−4.631	−49.009
4f	−4.219	−69.571
4e	−4.180	−63.286
4d	−4.685	−56.504
4c	−5.038	−71.011
4b	−5.879	−64.231
Co-crystal ligand	−4.367	−38.147

in rats as well as minimal clonic seizure (6 Hz) test at 100 mg/kg (i.p dose) in mice.

Computational studies

MM-GBSA binding free energy estimation

All the waters were deleted from the designed complexes prior to dG binding free energy calculation using solvation model VSGB by Prime MM-GBSA, Maestro 10.1. All the compounds are well fitted in the active sites of human cytosolic branched chain amino transferase (hBCATc). The binding poses for each compound were analysed by examining their free energy scores. The more energetically favourable conformation was selected as the best pose. The dG binding energy of compounds was found in the range of −41.313 to −76.141 kcal/mol for enzyme hBCATc active sites (Table 6). All the compounds displayed a higher binding free energy (dG bind) for hBCATc which indicates that compounds may have higher selectivity towards hBCATc enzyme except **4a**. However, there are considerable differences in free energy binding for the compounds towards

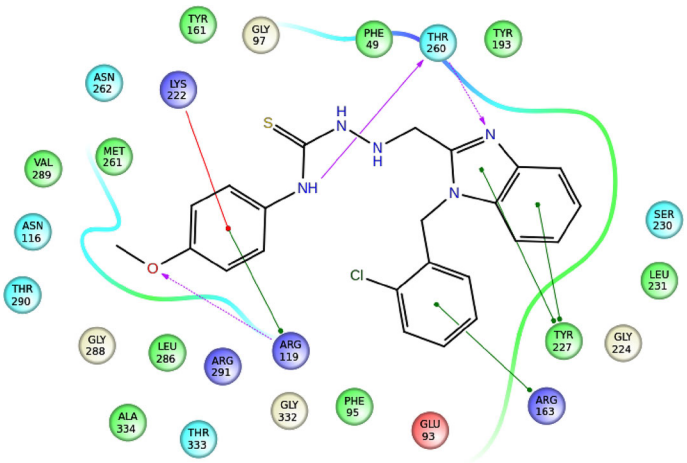


Fig. 1 2D ligand interaction representation of compound **4p** showing hydrogen bond interaction with THR 260 (*purple colour arrow line*) and ARG 119 (*purple colour dash arrow line*) and pi–pi stacking (*green line*) with TYR 227, ARG 163 and ARG 119; pi-cation with LYS 222 (*red line*) (Color figure online)

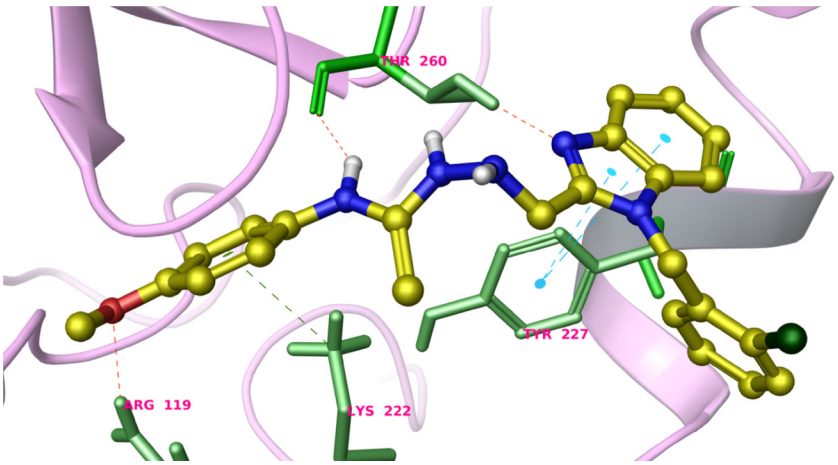


Fig. 2 Docked pose of compound **4p** represented as stick in the binding site of hBCATc showing hydrogen bond interaction with THR 260 and ARG 119; pi–pi stacking with TYR 227, ARG 163 and ARG 119; pi-cation with LYS 222. The *red dash lines*, sky *blue dash lines* and *green dash lines* denote the hydrogen bonds, pi–pi interaction and pi-cation, respectively. The amino acids are represented as *green colour thin line* (Color figure online)

hBCATc. The binding energy of co-crystal ligand was found to be −38.147 kcal/mol and less than the synthesized compounds (**4a**–**p**). This result justify that the synthesized compounds have high affinity for hBCATc than co-crystal.

Molecular docking

The glide scores, binding energy and interaction with amino acids of the titled compounds and co-crystal ligand with the active site of human cytosolic branched chain amino transferase (hBCATc) are summarized in Table 6. The crystal structure of hBCATc was prepared for docking with the protein preparation wizard workflow of Maestro that allows addition of hydrogen atoms which were subsequently minimized with OPLS-2005 force field and optimize the protonation state. The receptor grid was generated by applying a van der Waals radii of non-polar atoms, which decreases penalties for close contacts (scaling

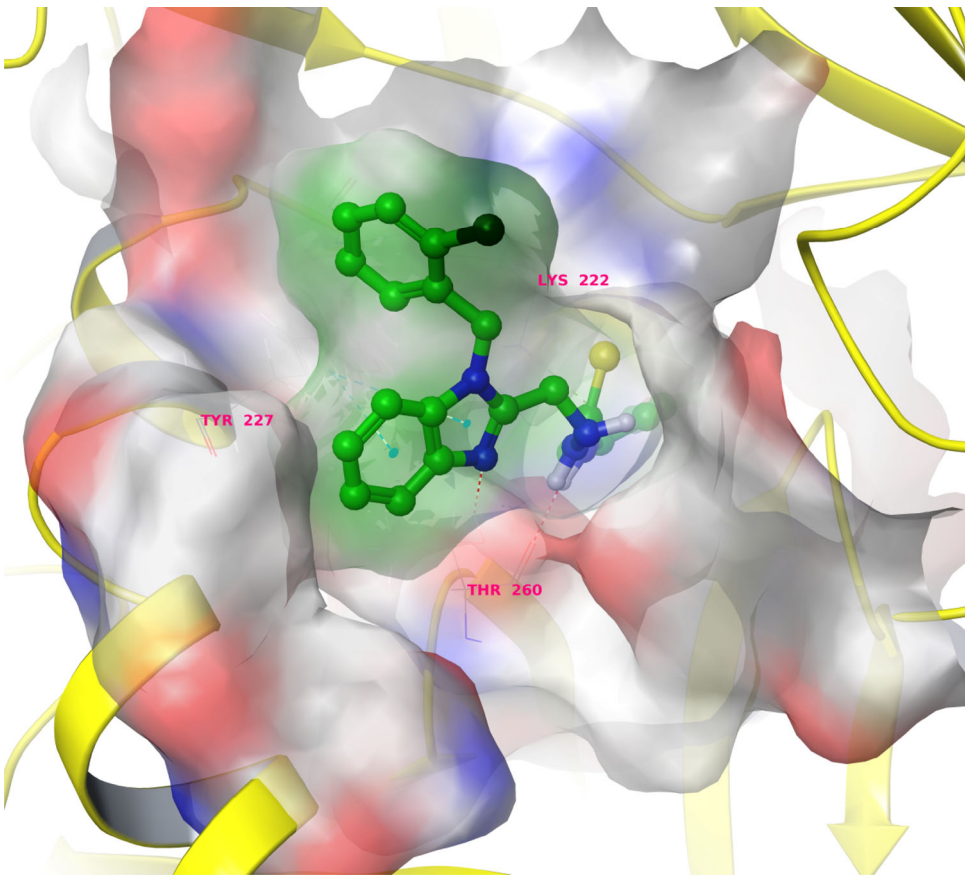


Fig. 3 Receptor surface model of compound **4p** represented as ball and stick in the binding site of hBCATc showing hydrogen bond interactions

factor = 1.00 and partial charge cut off = 0.25). Before docking calculation, all the compounds (**4a**–**p**) were subjected to ligand preparation with the LigPrep tool. Finally the ligand docking were run using receptor grid file and LigPrep out file in the Glide tool of application view.

Among all the titled compounds for hBCATc, compound **4p** was found to be most potent and have high docking score. The compound **4p** also assumes favourable orientation within the hBCATc binding site. The 2D ligand interaction diagram of **4p** is represented in Fig. 1. The nitrogen atom of benzimidazole ring and hydrogen atom of NH of hydrazinecarbothioamide linker of compound **4p** formed strong hydrogen bonds with THR 260 approximately at distances of 2.12 Å (N···HO) and 2.01 Å (H···O), respectively. The **4p** also form additional hydrogen bonding with ARG 119 at a distance 2.57 Å (O···HN). Interestingly **4p** also form a four pi–pi interaction with TYR 227 (phenyl···phenyl. 5.14 Å and imidazole···phenyl, 5.13 Å), ARG 163 (benzyl···C=, 5.27 Å) and ARG 119 (4-methoxy phenyl···C=, 5.48 Å).

The docked pose of compound **4p** is shown in Fig. 2. The benzimidazole scaffold is surrounded by residues TYR 193, LEU 251, SER 230 and PHE 95. The docking studies of **4p** also revealed that the substitution of methoxy group at *para* position of phenyl thiourea scaffold showed the additional hydrogen bonding with ARG 119 and hydrophobic interaction with VAL 289. In the present study, we try to prove that the addition of π–π interaction between the aromatic ring of ligand and amino acid residue of receptor has significantly improve the activity and their docking scores is the rationale for high affinity as compare to the co-crystal ligand which have no such types of interactions. The receptor surface model of compound **4p** is represented in Fig. 3.

Conclusion

In the present study, a series of substitutedbenzimidazoles have been synthesized. Chemical structures of all new compounds were confirmed by spectral and elemental analysis. All the synthesized compounds were evaluated for their anticonvulsant activity. Most of the compounds showed significant activity in MES model. In MES test, compounds 4e and 4p were found to be most active, whereas in scPTZ compound 4p was found to be most potent. The molecular docking study of the compounds was performed for the better understanding of drug–receptor interactions. The anticonvulsant activity of these compounds was fully supported by the in silico molecular docking study.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

References

Anelia TM, Kamelya KA, Dimitar IV, Jordan AT, Pavletta SD, Magdalena SK, Mitka KM (2006) Antihelminthic activity of some newly synthesized 5(6)-(un)substituted-1*H*-benzimidazol-2-ylthioacetylpiperazine derivatives. Eur J Med Chem 41:1412–1420

Auberson YP (2001) Competitive AMPA antagonism: a novel mechanism for antiepileptic drugs. Drugs Fut 26:463–471

Blaszczak-Swiqtkiewicz K, Mikiciuk-Olasik E (2015) Some characteristics of activity of potential chemotherapeutics–benzimidazole derivatives. Adv Med Sci 60:125–132

Brauner-Osborne H, Egebjerg J, Nielsen EO, Adsen U, Krogsgaard-Larsen P (2000) Ligands for glutamate receptors: design and therapeutic prospects. J Med Chem 43:2609–2645

Castro AR, Rivera IL, Rojas LC, Vazquez GN, Rodríguez AN (2011) Synthesis and preliminary evaluation of selected 2-aryl-5 (6)-nitro-1H-benzimidazole derivatives as potential anticancer agents. Arch Pharm Res 34:181–189

Cole ER, Crank G, Salam-Sheikh A (1974) Antioxidant properties of benzimidazoles. J Agric Food Chem 22:918

Danysz W, Parsons CG (1998) Glycine and *N*-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol Rev 50:597–664

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 screen. J Med Chem 39:3984–3997

Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7–61

Donati D, Micheli F (2000) Recent development in glycine antagonists. Exp Opin Ther Patents 10:667–673

Dunham NW, Miya TA (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Sci 46:208–209

Hu L-Y, Boxer PA, Kesten SR, Lei HJ, Wustrow DJ, Moreland DW, Zhang L, Ahn K, Ryder TR, Liu X, Rubin JR, Fahnoe Kelly, Carroll RT, Dutta S, Fahnoe DC, Probert AW, Roof RL, Rafferty MF, Kostlan CR, Scholten JD, Hood M, Ren X-D, Schielke GP, Su T-Z, Taylor CP, Mistry A, McConnell P, Hasemann C, Ohren J (2006) The design and synthesis of human branched-chain amino acid aminotransferase inhibitors for treatment of neurodegenerative diseases. Bioorg Med Chem Lett 16:2337–2340

Iemura R, Kawashima T, Fukuda T, Ito K, Tsukamoto G (1986) Synthesis of 2-(4-substituted-1-piperazinyl)benzimidazoles as H1-antihistaminic agents. J Med Chem 29:1178–1183

Jun C, Jiangtao X, Xianjin L (2005) Synthesis and antiviral activity against Coxsackie virus B3 of some novel benzimidazole derivatives. Bioorg Med Chem Lett 15:267–269

Kaur G, Kaur M, Silakari O (2014) Benzimidazoles: an ideal privileged drug scaffold for the design of multitargeted anti-inflammatory ligands. Mini Rev Med Chem 14:747–767

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

Kruse LI, Ladd DL, Harrsch PB, McCabe FL, Mong SM, Faucette L, Johnson R (1989) Synthesis, tubulin binding, antineoplastic evaluation, and structure-activity relationship of oncodazole analogs. J Med Chem 32:409–417

Kubo K, Kohara Y, Yoshimura Y, Inada Y, Shibouta Y, Furukawa Y, Kato T, Nishikawa K, Naka T (1993) Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of potential prodrugs of benzimidazole-7-carboxylic acids. J Med Chem 36:2343–2349

Kulagowski JJ (1996) Glycine-site NMDA receptor antagonists: an update. Exp Opin Ther Patents 6:1069–1079

Kulagowski JJ, Leeson PD (1995) Glycine-site NMDA receptor antagonists. Exp Opin Ther Patents 5:1061–1075

Lees GJ (2000) Pharmacology of AMPA/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders. Drugs 59:33–78

Leeson PD, Iversen LL (1994) The glycine site on the NMDA receptor: structure-activity relationships and therapeutic potential. J Med Chem 37:4053–4067

Litchfield JT, Wilcoxon FA (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99–113

Porcari AR, Devivar RV, Kucera LS, Drach JC, Townsend LB (1998) Design, synthesis, and antiviral evaluations of 1-(substituted benzyl)-2-substituted-5,6-dichlorobenzimidazoles as nonnucleoside analogues of 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole. J Med Chem 41:1252–1262

Sander JW (2003) The epidemiology of epilepsy revisited. Curr Opin Neurol 16:165–170

Sarro GD, Gitto R, Russo E, Ibbadu GF, Barreca ML, Luca LD, Chimirri A (2005) AMPA receptor antagonists as potential anticonvulsant drugs. Curr Top Med Chem 5:31–42

Schrödinger Release 2015-1 (2015a) Maestro, version 9.8, Schrödinger, LLC, New York, NY

Schrödinger Release 2015-1 (2015b) Schrödinger Suite 2014-2 Protein preparation wizard; Epik version 2.8, Schrödinger, LLC, New York, NY, 2014; Impact version 6.3, Schrödinger, LLC, New York, NY, 2015; Prime version 3.5, Schrödinger, LLC, New York, NY, (2015)

Swinyard EA, Woodhead JH, White HS, Franklin MR (1989) Antiepileptic drugs, 3rd edn. Raven-Press, New York, pp 85–102