A New Class of Anticonvulsants Possessing 6 Hz Psychomotor Seizure Test Activity: 2-(1*H*-Benzotriazol-1-yl)-*N*'-[Substituted] Acetohydrazides

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Abstract: A series of 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted]acetohydrazides was designed and synthesized keeping in view the structural requirement of pharmacophore and evaluated for anticonvulsant activity and neurotoxicity. The new compounds were characterized using FT-IR, ^{1}H NMR, mass spectral data and elemental analysis. The anticonvulsant activity of the titled compounds was assessed using the 6 Hz psychomotor seizure test. The neurotoxicity was assessed using the rotarod method. The most active compound of the series was *N*'-[4-(1,3-Benzodioxol-5-yloxy)benzylidene]-2-(1*H*-benzotriazol-1-yl)acetohydrazide **(BTA 9)**, which showed good activity with 75 % protection (3/4, 0.5 h) at a dose of 100 mg/kg in mice. None of the compounds exhibited neurotoxicity. A computational study was carried out for the calculation of pharmacophore pattern and prediction of pharmacokinetic properties. Titled compounds have also exhibited good binding properties with epilepsy molecular targets such as glutamate, GABA (A) delta, GABA (A) alpha-1 receptors and Na/H exchanger, in Lamarckian genetic algorithm based flexible docking studies.

Keywords: Anticonvulsant, Benzotriazole, Computational study, Docking study, 6 Hz Psychomotor Seizure test, Neurotoxicity.

INTRODUCTION

Epilepsy affects 1% of world's population according to the epidemiological studies. Current clinically available drugs produce satisfactory seizure control in 60–70% of patients [1]. Several new anticonvulsants like oxacarbazepine, vigabatrin, lamotrigine, gabapentin, topiramate, felbamate, rufinamide and levetiracetam have been put in clinical practice. Despite familiarity with established antiepileptic drugs and the introduction of these new agents in the past decade, upto one third of epilepsy patients remain resistant to optimum drug treatment [2]. These facts triggered the search for newer more effective and less toxic anticonvulsants.

Benzotriazole derivatives constitute an important class of heterocyclic compounds and present a wide range of bioactivities. Among the most important are: anticonvulsant [3-5], CNS depressant [6], antimicrobial [7, 8], anticancer [9], analgesic and anti-inflammatory activity [10]. Several derivatives of benzotriazole are reported as agonists of peroxisome proliferator activated receptors [11]. Synthesis and biological activity of 1*H*-benzotriazole analogs as inhibitors of the NTpase / helicase and some related Flavivirade have been extensively investigated [12].

In the twenties, 1*H*-benzotriazole moiety containing compounds such as benzotriazole and benzofuran-based heterocycles **1** & **2** [3], 1-(2-Amino phenyl)-2-[5-(2-benzotriazol-2-yl-ethyl)-tetrazol-2-yl]-ethanone **3** and 1-(4-Amino phenyl)-2-[5-(2-benzotriazol-2-yl-ethyl)-tetrazol-2-yl]-ethanone **4** [4] were reported as potential anticonvulsants. In fact, these evidences suggest that the 1*H*-benzotriazole moiety, possesses a pharmacophoric character for anticonvulsant activity. In addition, the 4-(2-phenoxyphenyl)semicarbazones **5** (Fig. **1**) were reported as potential anticonvulsants [13].

Continuing our studies on benzfused derivatives that are attractive candidates as anticonvulsant agents [14], we designed a new series of functionalized 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted]acetohydrazides compounds **BTA 1-10,** exploring 1*H*-benzotriazole, as starting material. The rational design of these new derivatives **BTA 1-10**, was planned by molecular hybridization of substituted 1*H*-benzotriazole **7**, and 4-(aryloxy) phenyl semicarbazones **6** (Fig. **2**).

Based on the literature review, we are the first to report the synthesis and anticonvulsant activities of 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted]acetohydrazides. Their chemical structures were characterized using IR, ^{1}H-NMR, MS and elemental analysis techniques. All the synthesized titled compounds comprised of the essential pharmacophoric elements (Fig. **3**) that are necessary for good anticonvulsant activity as suggested by Unverferth *et al.* [15]. In addition, their anticonvulsant activity was evaluated by using 6 Hz psychomotor seizure test in mice. The rotorod assay was performed in mice to evaluate the neurotoxicity of the compounds. Computational study was also carried out to highlight the pharmacophore distance mapping, Log *P* calculation and prediction of pharmacokinetic parameters. In this study, we have used AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking of compounds with six established epilepsy molecular targets and evaluated the affinity and hydrogen bonding.

MATERIALS AND METHODS

Chemistry

All the chemicals and solvents, purchased from Merck (India), Spectrochem (India), Himedia (India) and S. d. Fine

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1875-6638/12 $58.00+.00

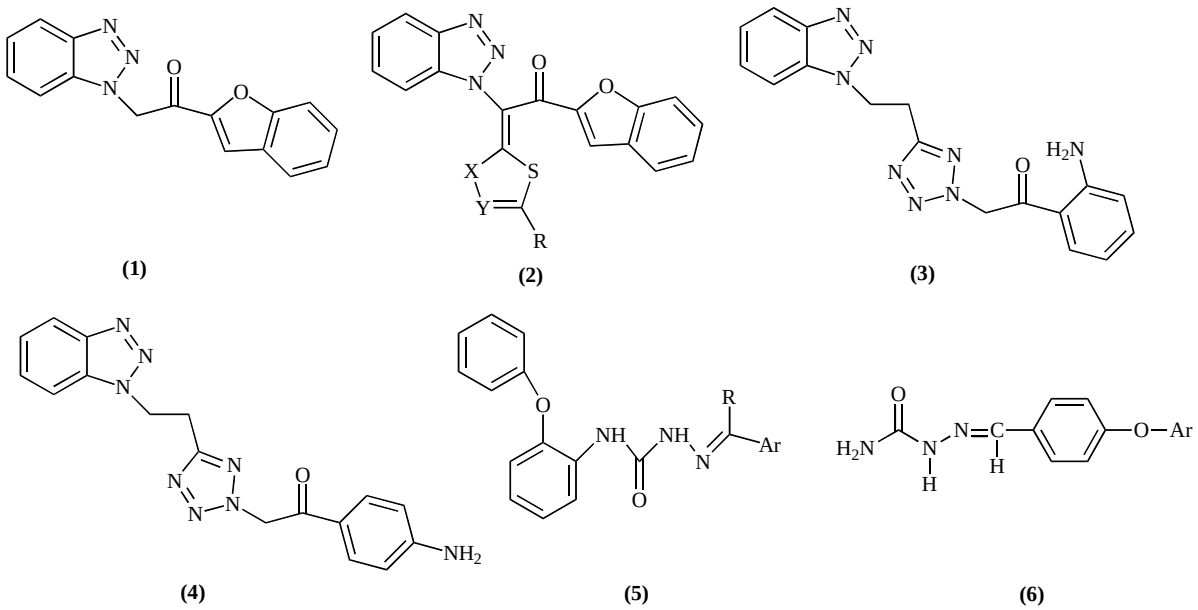


Fig. (1). Chemical structure of benzotriazole and benzofuran-based heterocycles **1** & **2**
1-(2-Amino phenyl)-2-[5-(2-benzotriazol-2-yl-ethyl)-tetrazol-2-yl]-ethanone **3**
1-(4-Amino phenyl)-2-[5-(2-benzotriazol-2-yl-ethyl)-tetrazol-2-yl]-ethanone **4**
4-(2-phenoxyphenyl) semicarbazones **5,** and 4-(aryloxy)phenyl semicarbazones **6**

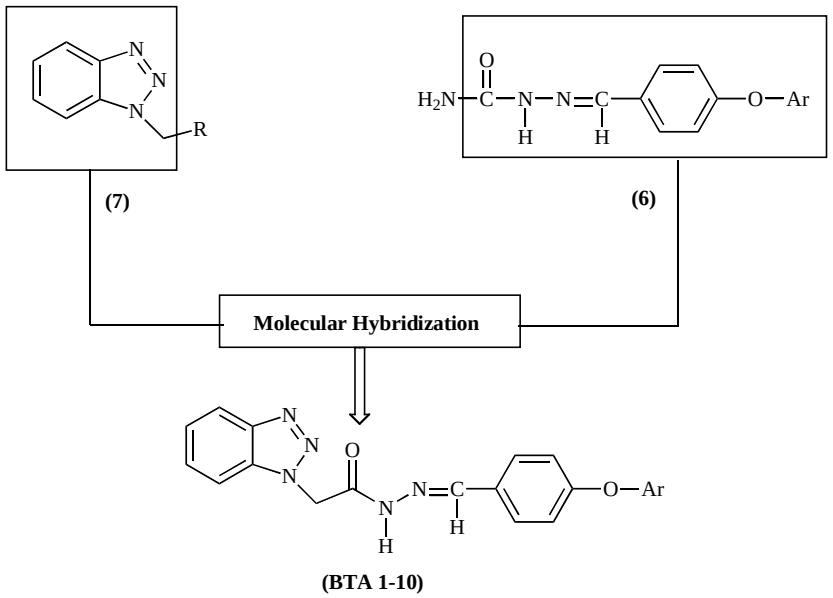


Fig. (2). Rational concept to new 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted] acetohydrazides **BTA 1-10**.

were used without further purification. The compounds were synthesised according to Scheme **1**. The progress of reaction was monitored by thin layer chromatography, performed on a silica gel 60 F_{254} coated aluminium sheet. The melting points were determined by using Thomas- Hoover melting point apparatus and were uncorrected. The FT-IR spectra were recorded on Perkin- Elmer Spectrum BX-II Spectrophotometer. The ^{1}H-NMR spectra were recorded on Bruker 300 MHz High Resolution NMR spectrometer using TMS as an internal standard. Chemical shifts were reported in ppm (δ) and signals were described as singlet (s), doublet (d), triplet (t) and multiplet (m). All exchangeable protons were confirmed by the addition of D_2O. The mass spectra were recorded on a Waters Micromass ZQ 2000 mass spectrometer. Elemental analysis (C, H, N) was undertaken with Perkin Elmer Model 240C analyzer.

Synthesis of Ethyl 1H-benzotriazol-1-ylacetate (2): A mixture of 1*H*-Benzotriazole (**1**) (0.1 mole), chloroethyl acetate (0.1 mole) and potassium carbonate (3 g) was stirred in dry acetone for 6 h. The solvent was removed under reduced pressure and the solid mass obtained was extracted with ether. The ether was removed under reduced pressure to get needle shaped white crystals of compound **(2).** Yield: 86%;

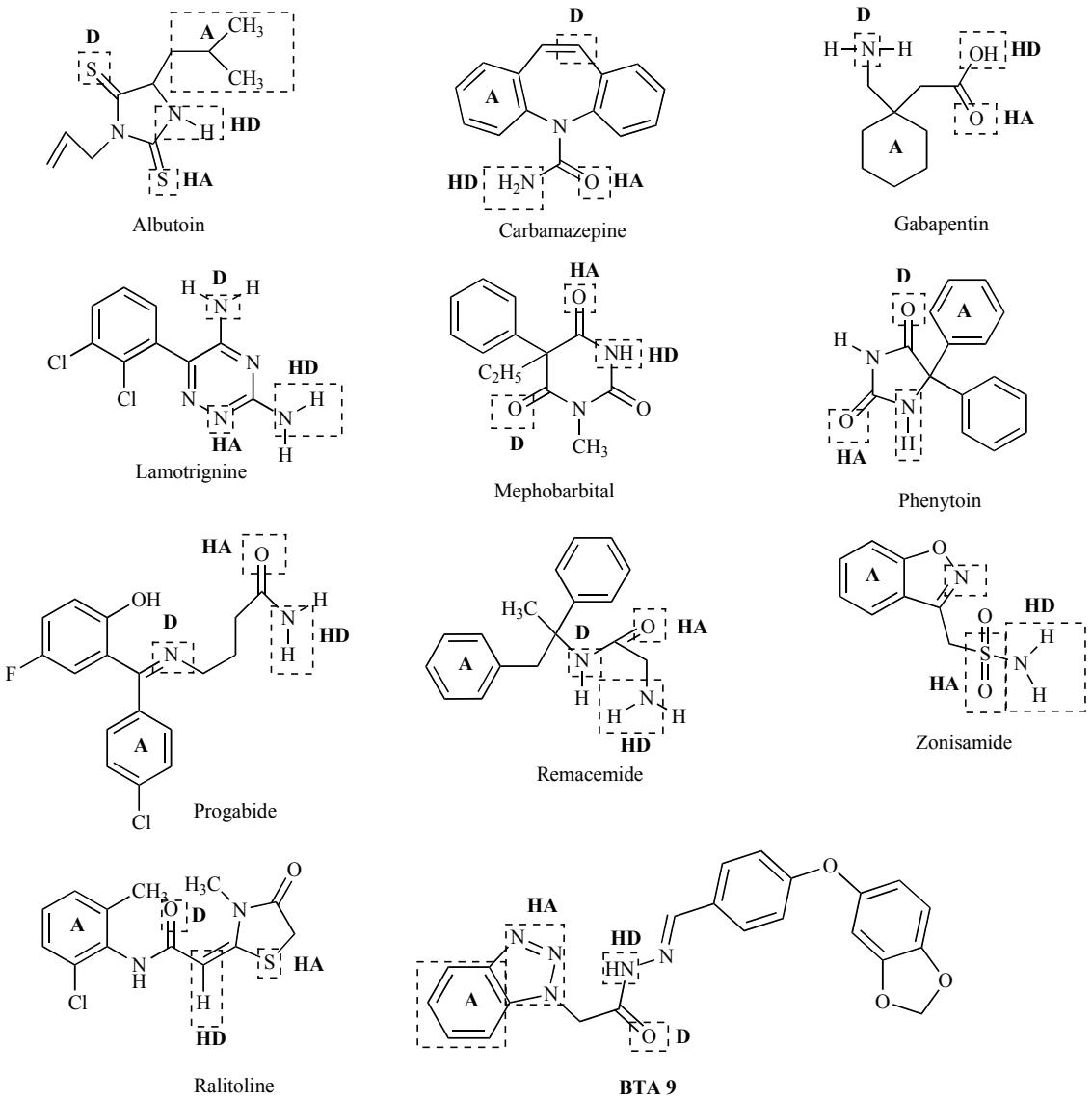


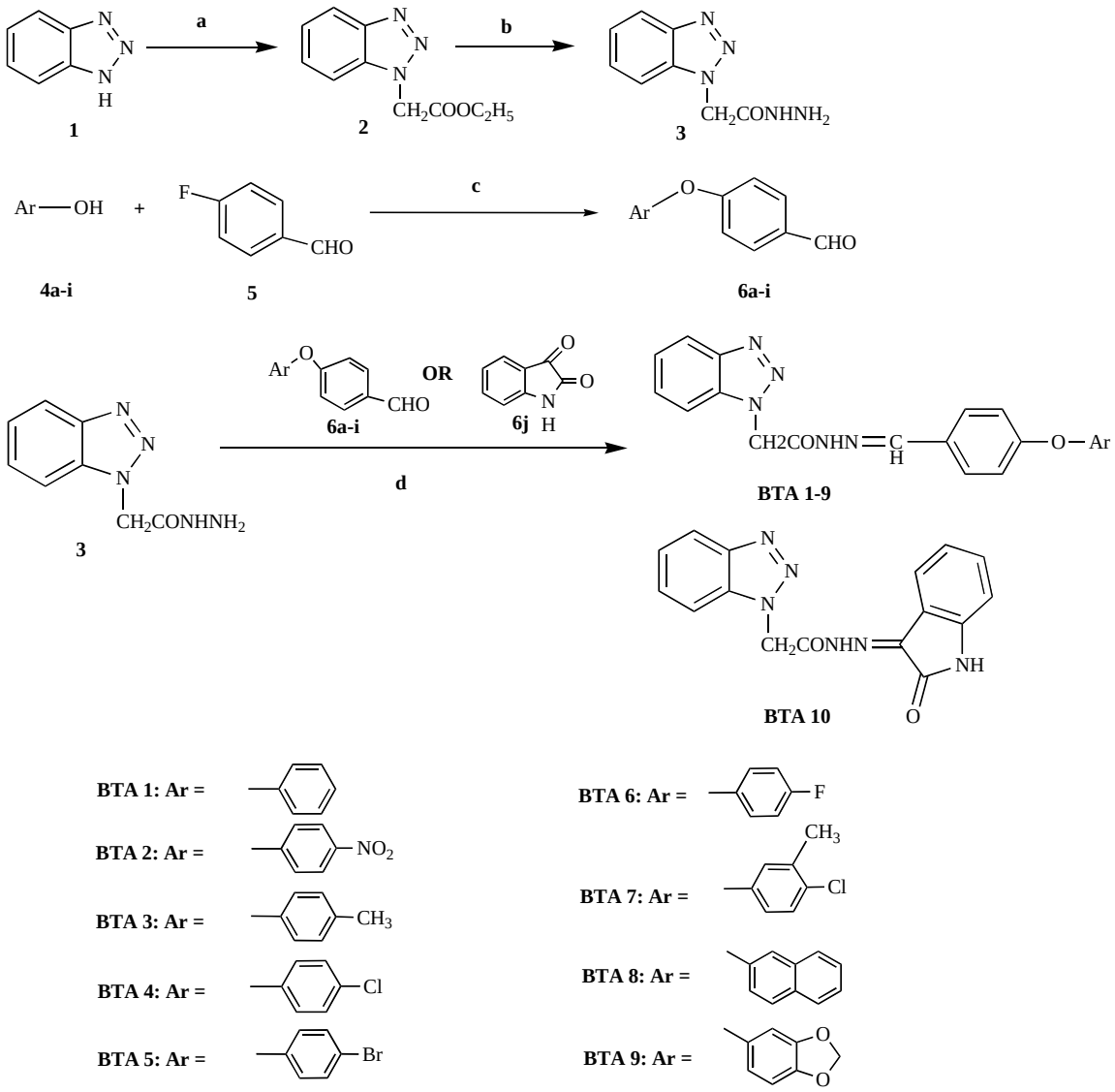
Fig. (3). Pharmacophoric pattern of well- known anticonvulsants and *N*'-[4-(1,3- Benzodioxol-5-yloxy)benzylidene]-2-(1*H*-benzotriazol-1-yl)acetohydrazide **BTA 9**.

m.p.: 40°C; IR (KBr, cm^{-1}) υ: 1742 (C=O), 1464, 1237 (N-CH_2); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.30 (t, 3H, CH_3), 4.27 (q, 2H, $COOCH_2$), 5.81 (s, 2H, N-CH_2), 6.80-7.94 (a set of signals, 4H, Ar-H); MS (m/z, %): 206.16 (M^+ + 1, 76.9); Anal. Calcd. for $C_{10}H_{11}N_3O_2$ (205.21): C, 58.53; H, 5.40; N, 20.48. Found: C, 58.49; H, 5.41; N, 20.45.

Synthesis of 2-(1H-Benzotriazol-1-yl)acetohydrazide (3): Compound (2) (0.1 mole) and hydrazine hydrate (0.3 mole) in ethanol (50 mL) were stirred for 1h and then refluxed for 2h. The excess of solvent was removed under reduced pressure and recrystallized from chloroform-hexane (3:1) to yield white crystals of compound (3). Yield: 81%; m.p.: 178°C; IR (KBr, cm^{-1}) υ: 3337 (-$NHNH_2$), 1672 (C=O), 1461, 1232 (N-CH_2); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 9.88 (s, 1H, NH), 5.81 (s, 2H, N-CH_2), 4.68 (s, 2H,-NH_2), 6.83-7.91 (a set of signals, 4H, Ar-H); MS (m/z, %): 192.13 (M^+ + 1, 71.1); Anal. Calcd. for $C_8H_9N_5O$ (191.19): C, 50.26; H, 4.74; N, 36.63. Found: C, 50.21; H, 4.71; N, 36.65.

Synthesis of 4-substituted benzaldehyde (6a-i): A mixture of substituted phenol (4a-i) (37.4 mmol), 4-fluorobenzaldehyde (5) (37.4 mmol) and potassium carbonate (38.8 mmol) in *N,N*-dimethylformamide (30 mL) was refluxed for 16-18 h under nitrogen. After cooling, the product was extracted from the reaction mixture and purified by chromatography. (6f) Yield: 70%; m.p.: 41°C; IR (KBr, cm^{-1}) υ: 1665 (C=O), 1240 (-O-); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 9.87 (s, 1H, -CHO), 6.23-7.87 (a set of signals, 8H, Ar-H); MS (m/z, %): 216.17 (M^+ + 1, 67.2); Anal. Calcd. for $C_{13}H_9FO_2$ (216.21): C, 72.22; H, 4.20; O, 14.80. Found: C, 72.19; H, 4.16; O, 14.77.

Synthesis of 2-(1H-Benzotriazol-1-yl)-N'-[substituted] acetohydrazide (BTA 1-10): Equimolar quantities (0.01 mol) of 4-substituted benzaldehydes (6a-i)/ isatin (6j) and 2-(1*H*-Benzotriazol-1-yl)acetohydrazide (3) were dissolved in warm ethanol containing 0.5 ml of glacial acetic acid. The reaction mixture was refluxed for 4-6 h and set aside. The resultant solid was washed with ethanol and recrystallized



Scheme 1. Synthesis of 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted] acetohydrazides **(BTA 1-10).** Reagents and conditions: a) $ClCH_2COOC_2H_5$, K_2CO_3, Acetone, Stirring, 6h; b) $NH_2NH_2.H_2O$, Ethanol, Reflux, 2h; c) K_2CO_3, DMF, Reflux, 16-18h; d) Ethanol, GAA, Reflux, 4-6h.

from 90% ethanol. The physical, elemental analysis and spectral data of the titled compounds (BTA 1-10) are given below.

2-(1H-Benzotriazol-1-yl)-N'-(4-phenoxybenzylidene) acetohydrazide (BTA 1): Yield: 73%; m.p.: 146°C; IR (KBr, cm^{-1}) υ: 3217 (-NH-), 1684 (C=O), 1604 (-N=CH-), 1478, 1260 (-N-CH_2-), 1229 (-O-); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.894 (s, 2H, N-CH_2), 6.420-8.07 (a set of signals, 13H, Ar-H), 7.713 (s, 1H, -CH=N-), 9.586 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 372.16 (M^+ + 1, 89.9); Anal. Calcd. for $C_{21}H_{17}N_5O_2$ (371.39): C, 67.91; H, 4.61; N, 18.86. Found: C, 67.90; H, 4.61; N, 18.85.

2-(1H-Benzotriazol-1-yl)-N'-[4-(4-nitrophenoxy)benzylidene]acetohydrazide (BTA 2): Yield: 74%; m.p.: 138°C; IR (KBr, cm^{-1}) υ: 3218 (-NH-), 1686 (C=O), 1601 (-N=CH-), 1525 (N=O), 1478, 1261 (-N-CH_2-), 1227 (-O-); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.890 (s, 2H, N-CH_2), 6.421-8.04 (a set of signals, 12H, Ar-H), 7.711 (s, 1H, -CH=N-), 9.582 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 417.14 (M^+ + 1, 72.5); Anal. Calcd. for $C_{21}H_{16}N_6O_4$ (416.39): C, 60.57; H, 3.87; N, 20.18. Found: C, 60.56; H, 3.84; N, 20.19.

2-(1H-Benzotriazol-1-yl)-N'-[4-(4-methylphenoxy) benzylidene]acetohydrazide (BTA 3): Yield: 75%; m.p.: 155°C; IR (KBr, cm^{-1}) υ: 3216 (-NH-), 1681 (C=O), 1605 (-N=CH-), 1481, 1261 (-N-CH_2-), 1231 (-O-); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.353 (s, 3H, -CH_3), 5.892 (s, 2H, N-CH_2), 6.422-8.068 (a set of signals, 12H, Ar-H), 7.710 (s, 1H, -CH=N-), 9.580 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 386.17 (M^+ + 1, 68.9); Anal. Calcd. for $C_{22}H_{19}N_5O_2$ (385.42): C, 68.56; H, 4.97; N, 18.17. Found: C, 68.52; H, 4.95; N, 18.16.

2-(1H-Benzotriazol-1-yl)-N'-[4-(4-chlorophenoxy)benzylidene]acetohydrazide (BTA 4): Yield: 76%; m.p.: 188°C; IR (KBr, cm^{-1}) υ: 3215 (-NH-), 1681 (C=O), 1603 (-N=CH-), 1479, 1263 (-N-CH_2-), 1231 (-O-); ^{1}H NMR

($CDCl_3$, 300 MHz) δ in ppm: 5.893 (s, 2H, N-CH_2), 6.421-8.067 (a set of signals, 12H, Ar-H), 7.711 (s, 1H, -CH=N-), 9.582 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 406.12 (M^+ + 1 for ^{35}Cl, 100.00), 408.10 (M^+ + 1 for ^{37}Cl, 33.8); Anal. Calcd. for $C_{21}H_{16}ClN_5O_2$ (405.84): C, 62.15; H, 3.97; N, 17.26. Found: C, 62.13; H, 3.92; N, 17.23.

2-(1H-Benzotriazol-1-yl)-N'-[4-(4-bromophenoxy)benzylidene]acetohydrazide (BTA 5): Yield: 78%; m.p.: 215°C; IR (KBr, cm^{-1}) υ: 3219 (-NH-), 1689 (C=O), 1607 (-N=CH-), 1475, 1260 (-N-CH_2-), 1232 (-O-); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.890 (s, 2H, N-CH_2), 6.423-8.07 (a set of signals, 12H, Ar-H), 7.715 (s, 1H, -CH=N-), 9.581 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 452.10 (M^+ + 1 for ^{81}Br, 100.00), 450.07 (M^+ + 1 for ^{79}Br, 98.3); Anal. Calcd. for $C_{21}H_{16}BrN_5O_2$ (450.29): C, 56.01; H, 3.58; N, 15.55. Found: C, 56.0; H, 3.56; N, 15.53.

2-(1H-Benzotriazol-1-yl)-N'-[4-(4-fluorophenoxy)benzylidene]acetohydrazide (BTA 6): Yield: 74%; m.p.: 204°C; IR (KBr, cm^{-1}) υ: 3216 (-NH-), 1687 (C=O), 1602 (-N=CH-), 1477, 1260 (-N-CH_2-), 1233 (-O-); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.891 (s, 2H, N-CH_2), 6.425-8.076 (a set of signals, 12H, Ar-H), 7.710 (s, 1H, -CH=N-), 9.582 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 390.16 (M^+ + 1, 85.3); Anal. Calcd. for $C_{21}H_{16}FN_5O_2$ (389.38): C, 64.78; H, 4.14; N, 17.99. Found: C, 64.73; H, 4.12; N, 17.98.

2-(1H-Benzotriazol-1-yl)-N'-[4-(4-chloro-3-methylphenoxy)benzylidene]acetohydrazide (BTA 7): Yield: 72%; m.p.: 195°C; IR (KBr, cm^{-1}) υ: 3217 (-NH-), 1687 (C=O), 1602 (-N=CH-), 1477, 1261 (-N-CH_2-), 1231 (-O-); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.351 (s, 3H, -CH_3), 5.892 (s, 2H, N-CH_2), 6.421-8.072 (a set of signals, 11H, Ar-H), 7.7130(s, 1H, -CH=N-), 9.581 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 420.12 (M^+ + 1 for ^{35}Cl, 100.00), 422.08 (M^+ + 1 for ^{37}Cl, 34.7); Anal. Calcd. for $C_{22}H_{18}ClN_5O_2$ (419.86): C, 62.93; H, 4.32; N, 16.68. Found: C, 62.90; H, 4.28; N, 16.64.

2-(1H-Benzotriazol-1-yl)-N'-[4-(naphthalen-2-yloxy) benzylidene]acetohydrazide (BTA 8): Yield: 77%; m.p.: 172°C; IR (KBr, cm^{-1}) υ: 3216 (-NH-), 1681 (C=O), 1601 (-N=CH-), 1478, 1260 (-N-CH_2-), 1233 (-O-), 836, 821 (β-naphthyl); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.895 (s, 2H, N-CH_2), 6.412-8.05 (a set of signals, 15H, Ar-H), 7.709 (s, 1H, -CH=N-), 9.585 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 422.17 (M^+ + 1, 63.5); Anal. Calcd. for $C_{25}H_{19}N_5O_2$ (421.45): C, 71.25; H, 4.54; N, 16.62. Found: C, 71.21; H, 4.52; N, 16.59.

N'-[4-(1,3-Benzodioxol-5-yloxy)benzylidene]-2-(1H-benzotriazol-1-yl)acetohydrazide (BTA 9): Yield: 71%; m.p.: 165°C; IR (KBr, cm^{-1}) υ: 3215 (-NH-), 1687 (C=O), 1602 (-N=CH-), 1480,1262 (-N-CH_2-), 1228(-O-); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.892 (s, 2H, N-CH_2), 5.989 (s, 2H, O-CH_2), 6.521-8.09 (a set of signals, 11H, Ar-H), 7.711 (s, 1H, -CH=N-), 9.588 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 416.15 (M^+ + 1, 65.3); Anal. Calcd. for $C_{22}H_{17}N_5O_4$ (415.40): C, 63.61; H, 4.12; N, 16.86. Found: C, 63.60; H, 4.10; N, 16.84.

2-(1H-Benzotriazol-1-yl)-N'-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]acetohydrazide (BTA 10): Yield: 79%; m.p.: 260°C; IR (KBr, cm^{-1}) υ: 3263 (-NH- of isatin), 3215 (-NH-), 1687 (C=O), 1601 (-N=C-), 1478, 1260 (-N-CH_2-); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.89 (s, 2H, N-CH_2), 6.72-7.81 (a set of signals, 8H, Ar-H), 9.58 (s, 1H, -NH-, D_2O exchangeable), 12.83 (s, 1H, -NH of isatin, D_2O exchangeable); MS (m/z, %): 321.12 (M^+ + 1, 81.7); Anal. Calcd. for $C_{16}H_{12}N_6O_2$ (320.31): C, 60.00; H, 3.78; N, 26.24. Found: C, 59.9; H, 3.77; N, 26.22.

Pharmacology

The evaluation of anticonvulsant activity and neurotoxicity was carried out by the Epilepsy Branch, National Institute of Neurological Disorder and Stroke, National Institute of Health, Bethesda, USA following the reported procedures. Male albino mice (CF-1 strain, 18-25g) and male albino rats (Sprague-Dawley, 100-150g) were used as experimental animals. The synthesized derivatives were suspended in 0.5% methyl cellulose and the test compound is usually manipulated with a motar pestle to help in the preparation of suspension. The titled compounds **BTA 1-10** were subjected to anticonvulsant screening by 6 Hz psychomotor seizure test to identify the anticonvulsant activity of the compounds at five different time points, i.e., 0.25 h, 0.5 h, 1.0 h, 2.0 h and 4.0 h after i.p. administration in mice at a dose of 100 mg/kg. Neurotoxicity was observed by minimal motor impairment which was measured by the rotorod (neurotoxicity) test.

6 Hz Psychomotor Seizure Test

6 Hz psychomotor seizure or minimal clonic seizure test was used to assess a compound's efficacy against electrically induced seizures but used a lower frequency (6 Hz) and longer duration of stimulation (3s). Test compounds were pre-administered to mice via i.p. injection. At varying times, individual mice (four mice per time point) were challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% animals (32 mA for 3 s). The untreated mice would display seizure characterized by a minimal clonic phase followed by stereotyped, automatistic behaviors, described originally as being similar to the aura of human patients with partial seizure. Animals not displaying this behavior are considered to be protected [16, 17].

Neurotoxicity- Minimal Motor Impairment (MMI)

Minimal motor impairment was measured by the rotarod (neurotoxicity) test. When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for a long period of time. The compound was considered toxic if the treated animal fell off this rotating rod 3 times during 1 minute period. Mice were placed on the elevated accelerating rod (rotarod, 3 cm in diameter) beginning at 5 rpm/min for four trials per day for three consecutive days. Each trial lasted a maximum of 10 min, during which time the rotating rod underwent a linear acceleration from 4 to 40 rpm over the first 5 min of the trial and then remained at maximum speed for the remaining 5 min. Animals were scored for their latency (in seconds) to fall (height = 20 cm) for each trial. Animals rested a minimum of 10 min between trials to avoid fatigue.

Computational Studies

Distance Mapping

The pharmacophore pattern studies in which distance between the various groups postulated as essential for anticonvulsant activity were done on the 3D optimized structures using ACD/3D viewer version 12.01 and Argus Lab 4.0 Mark A. Thompson Planaria Software LLC. In conformational analysis of the ten clinically effective, well known and structurally different anticonvulsant drugs such as albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide, zonisamide**;** a molecular model was suggested on the basis of molecular dynamics distance estimations [18].

Calculation of Physicochemical Properties

A computational study of titled compounds was performed for the prediction of ADME properties. Polar surface area (TPSA) [19], miLog P, number of rotable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five [20] were calculated using Molinspiration online property calculation toolkit [21]. Absorption (%ABS) was calculated by: %ABS = 109-(0.345 × TPSA) [22].

Log *P* Determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature [23]. 10 mL of octanol and 10 mL phosphate buffer were taken in a glass stoppered graduated tube and 5 mg of accurately weighed compound was added. The mixture was then shaken with the help of a mechanical shaker for 24 h at room temperature and then transferred to a separating funnel and allowed to dynamically equilibrate for 6 h. The aqueous and octanol phases were separated and filtered through membrane filter and drug content in aqueous phase was analyzed by UV spectroscopy. Theoretical miLog P for synthesized compounds was then compared with the experimental Log P data.

Docking Studies

Compound **BTA 9** was selected as a ligand for docking studies with six established epilepsy receptors namely GABA(A) alpha-1, GABA(A) delta, glutamate, Na/H exchanger, Na channel and T-type calcium channel (Fig. **5**). These receptors are the most important molecular targets in the design and discovery of successful antiepileptic drugs [24]. In the present study, AutoDock 4.0 with its Lamarckian genetic algorithm (LGA) was used for automated flexible ligand docking of **BTA 9** with above mentioned receptors and affinity (Kcal/mol) and H-bond properties were evaluated.

The grid maps were calculated using AutoGrid. In all dockings, a grid map with 60 × 60 × 60 points, a grid spacing of 0.375 A ° (roughly a quarter of the length of a carbon–carbon single bond) were used, and the mps were centered on the ligand binding site. For all dockings, 100 independent run with, an initial population of random individuals with a population size of 150 individuals, a maximum number of 2.5×10^6 energy evaluations, maximum number of generations of 27,000, an elitism value of 1 and a number of active torsion of 9 were used. AutoDock 4.0 was used to generate both grid and docking parameter files (i.e., .gpf and .dpf files) and docking affinity (Kcal/mol) and count of probable H-bonds were determined.

RESULTS AND DISCUSSION

Synthesis and Characterization of BTA 1-10

The key intermediate 2-(1*H*-Benzotriazol-1-yl)acetohydrazide **3** was synthesized by reacting 1*H*-Benzotriazole **1** with chloroethyl acetate and potassium carbonate in dry acetone to yield ethyl 1*H*-benzotriazol-1-ylacetate **2,** which was refluxed with hydrazine hydrate in ethanol to afford the **3.** A mixture of substituted phenol **4a-i**, 4-fluorobenzaldehyde **5** and potassium carbonate in *N*,*N*-dimethylformamide was refluxed to afford 4-substituted benzaldehydes **6a-i.** The IR spectra of compound **3** showed peaks at 3337, 1672 and 1461, 1232 cm^{-1} indicating the presence of NH, C=O, N-CH_2 group respectively. Its ^{1}H NMR spectra showed a singlet at δ 9.88 ppm due to NH group; a singlet at δ 5.81 due to N-CH_2; a singlet at δ 4.68 for NH_2 and a multiplet at δ 6.83-7.91 ppm for aromatic (4H) protons. The absence of a triplet at δ 1.30 for CH_3 and a quartet at δ 4.27 for $COOCH_2$ of **2** confirms the conversion of **2** to **3**. Data from the elemental analyses were found to be in conformity with the assigned structure. Further the $[M^+ +1]$ peak recorded in the mass spectra is also in agreement with the molecular weight of the compound. The formation of 4-substituted benzaldehydes **6a-i** from 4-substituted phenols **4a-i** was confirmed by its IR and ^{1}H-NMR spectral studies. The IR spectrum of 4-(4-fluorophenoxy) benzaldehydes **6f** showed bands at 1665, 3035 and 1240 cm^{-1} indicating the presence of C=O *str*, C-H *str* and diaryl ether linkage (-O-). The absence of broad band of phenolic -OH at 2900-3000 cm^{-1} confirms the conversion of 4-fluoro phenol **4f** to **6f**. In its ^{1}H-NMR spectrum a singlet at δ 9.87 ppm indicates the presence of -CHO group, where as the absence of a singlet around δ 9.288 ppm for phenolic –OH confirms the conversion of **4f** to **6f**.

The IR spectrum of the title compound **BTA 9** showed C=O *str* at 1687 cm^{-1}, N-H *str* at 3215 cm^{-1}, CH=N *str* at 1602 cm^{-1}, N-CH_2 *str* at 1480, 1262 cm^{-1} and diaryl ether linkage (-O-) at 1228 cm^{-1}. Its ^{1}H-NMR spectrum showed a singlet of imine (CH=N) proton at δ 7.711 ppm; a singlet at δ 5.892 ppm for N-CH_2; a singlet at δ 5.989 ppm for O-CH_2; a multiplet at δ 6.521-8.09 for aromatic (11H) protons and –NH proton resonated as a broad singlet at δ 9.588 ppm that was D_2O exchangeable. The presence of CH=N *str* at 1602 cm^{-1} in IR spectrum and a singlet for imine (CH=N) proton at δ 7.711 ppm confirm the formation of **BTA 9**. The mass spectrum of the compound showed $[M^+ +1]$ peak at 416.15 corresponding to their molecular formula and are in conformity with the assigned structure. Elemental (C, H, N) analyses satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Pharmacology

The newly synthesized 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted] acetohydrazides **BTA 1-10** were subjected to anticonvulsant screening by 6 Hz psychomotor seizure or

Table 1. 6 Hz psychomotor seizure test activity and neurotoxicity of BTA 1-10

Compound	Test	Dose (mg/kg)	Time (h) to Peak Effect (N/F)*				
			0.25	0.5	1.0	2.0	4.0
BTA 1	6 Hz	100	0/4	1/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 2	6 Hz	100	0/4	0/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 3	6 Hz	100	0/4	1/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 4	6 Hz	100	1/4	1/4	2/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 5	6 Hz	100	0/4	2/4	1/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 6	6 Hz	100	0/4	0/4	1/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 7	6 Hz	100	1/4	1/4	0/4	0/4	¼
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 8	6 Hz	100	0/4	2/4	1/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 9	6 Hz	100	0/4	3/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BTA 10	6 Hz	100	0/4	1/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4

*N/F= number of animals active or toxic over the number tested.

minimal clonic seizure test to identify their anticonvulsant activity at five different time points, i.e., 0.25 h, 0.5 h, 1.0 h, 2.0 h and 4.0 h after i.p. administration in mice at a dose of 100 mg/kg. The results of screening at five different points are summarized in Table **1**. As observed from the results of various tested 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted] acetohydrazides, compound **BTA 9** was the most active one in this series with 75 % protection (3/4, 0.5 h) at a dose of 100 mg/kg. At a dose of 100 mg/kg, compounds **BTA 4, BTA 5** and **BTA 8** showed 50% protection (2/4) at a time point of 1.0 h, 0.5 h and 0.5 h respectively. Other compounds showed mild to moderate activity. These active compounds contain 1,3-benzodioxol-5-yl, 4-chlorophenyl, 4-bromophenyl and naphthalen-2-yl substitution attached to basic molecular structure. None of the compounds showed neurotoxicity in the highest administered dose.

Computational Studies

Distance Mapping

The present work involves the correlation of the structural requirement of well known and structurally different anticonvulsant compounds with the titled compounds. The two- dimensional (2D) modeling on anticonvulsants has identified that at least one aryl unit, one or two electron donor atoms, and/or an NH group in a special spatial arrangement are recommended for anticonvulsant activity. In the present study, the 10 well-known and structurally different compounds with anticonvulsant activity- albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide and zonisamide (Fig. **3**) with different mechanism of action, were selected so as to propose a generalized pharmacophore model. The pharmacophore group's distance estimation was done by molecular mechanics calculation with the force fields based on both CHARMm force fields and MM3 parametrization. In the present work, energy minimization was performed on above mentioned ten well known anticonvulsants and titled compounds using Argus Lab 4.0. Distance between the various structural components essential for activity was determine by ACD/3D viewer version 12.01. The crucial structural components that were included in the four-point pharmacophore model (Fig. **4**) were the aryl ring center or the lipophilic group (A), an electron donor atom (D), a hydrogen bond acceptor (HA), and a hydrogen bond donor (HD). An aver-

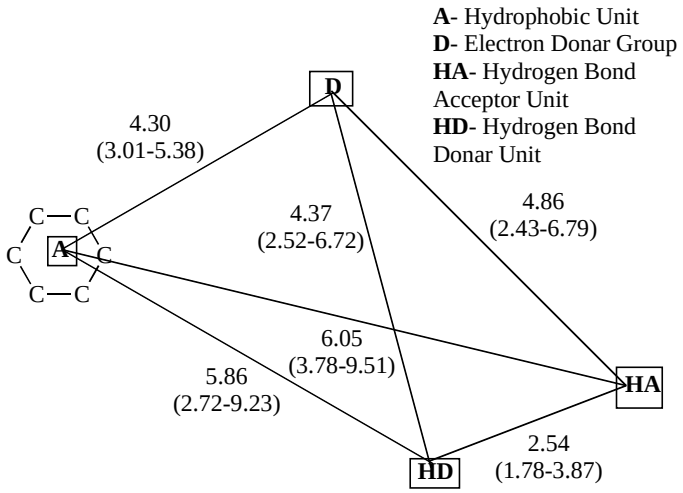


Fig. (4). Four-point 3D pharmacophore model for anticonvulsants derived by using MM3 and CHARMm parametrization (Argus Lab 4.0 and ACD/3D viewer).

Table 2. Distance ranges between the essential structural elements A, D and HA – HD[a]

Compounds	A-HA	A-HD	A-D	HA-HD	HD-D	HA-D
Albutoin	5.37	2.72	4.51	2.72	4.03	5.40
Carbamazepine	4.28	4.28	4.25	2.33	5.75	5.67
Gabapentin	4.26	4.93	3.83	2.23	3.57	4.50
Lamotrigine	5.30	7.42	4.54	2.42	4.94	4.25
Mephobarbital	3.78	5.50	4.81	2.34	4.63	5.23
Phenytoin	6.20	4.01	4.35	2.63	3.88	5.17
Progabide	9.51	9.23	3.79	2.41	6.72	6.79
Ralitoline	8.30	5.55	4.56	2.75	2.52	4.85
Remacemide	7.51	8.75	5.38	3.87	3.96	2.43
Zonisamide	6.02	6.22	3.01	1.78	3.71	4.31
Mean±standard deviation	6.05 ± 1.87	5.86 ± 2.09	4.30 ± 0.64	2.54 ± 0.54	4.37 ± 1.19	4.86 ± 1.13
BTA 9	3.29	6.07	6.56	3.14	2.37	4.66

[a]Distances calculated for 3D optimized structures using MM3 and CHARMm parameterization (Argus Lab 4.0 and ACD/3D viewer).

age distance range for every point was obtained and compared to the titled compounds. Now it may be interesting to examine whether the titled compounds reflect the conditions of the derived pharmacophore model. Our analyses of the distance relationship showed that titled compounds did fulfill the essential demands of the pharmacophore when compared to the average distance requirement (Table **2**).

Prediction of ADME Properties

A computational study for the prediction of ADME properties of titled compounds was performed. Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestines and blood-brain barrier crossing [17]. The percentage of absorption (%ABS) was calculated using TPSA. From all these parameters, it can be observed that all titled compounds exhibited a great %ABS ranging from 65.1 to 80.9% (Table **3**). Furthermore, except **BTA 7** and **BTA 8**, none of the compounds violated Lipinski's parameters, making them potentially promising agents for epilepsy therapy.

Log *P* Determination

Titled compounds showed dependence of biological activity on lipophilic character in a congeneric series. In par-

Table 3. Pharmacokinetic parameters important for good oral bioavailability of compounds BTA 1-10[a]

Compound	% ABS	TPSA (A^2)	n-ROTB	MW	Molecular Volume	n-OHNH Donors	n-OH Acceptors	Lipinski's Violation
Rule	--	--	--	<500	--	<5	<10	≤1
BTA 1	80.9	81.41	6	371.4	328.34	1	7	0
BTA 2	65.1	127.23	7	416.39	351.674	1	10	0
BTA 3	80.9	81.41	6	385.43	344.901	1	7	0
BTA 4	80.9	81.41	6	405.85	341.875	1	7	0
BTA 5	80.9	81.41	6	450.29	346.225	1	7	0
BTA 6	80.9	81.41	6	389.39	333.271	1	7	0
BTA 7	80.9	81.41	6	419.87	358.436	1	7	1
BTA 8	80.9	81.41	6	421.46	372.331	1	7	1
BTA 9	74.5	99.88	6	415.41	352.269	1	9	0
BTA 10	72.7	105.04	3	320.312	268.49	2	8	0

[a] %ABS, percentage of absorption; TPSA, topological polar surface area; n-ROTB, number of rotatable bonds; MW, molecular weight; MV, molecular volume; n-OHNH, number of hydrogen bond donors; n-ON, number of hydrogen bond acceptors.

Table 4. Log *P* value for compounds BTA 1-10

Compounds	Experimental Log *P*	Theoretical Log *P* (miLog *P*[a])
Rule	--	≤5
BTA 1	3.892	4.184
BTA 2	3.813	4.143
BTA 3	4.356	4.633
BTA 4	4.567	4.862
BTA 5	4.644	4.993
BTA 6	4.144	4.348
BTA 7	4.923	5.239
BTA 8	4.961	5.367
BTA 9	3.870	4.074
BTA 10	1.630	1.665

[a]miLog *P*, logarithm of compound partition coefficient between n-octanol and water calculated as per Molinspiration Online Property Toolkit.

ticular, for drugs acting on central nervous system to be potent, they have to cross blood brain barrier (BBB), thus potency has been correlated with optimum lipophilicity (Log *P*) near 2. In this study, we attempted to correlate the anticonvulsant activity of congeners with their calculated Log P value. The experimental Log *P* values were determined using the octanol- phosphate buffer method. The data are presented in Table **4**. As observed some of the experimental values were in good agreement with the theoretical values. All the titled compounds showed lipophilic character.

Docking Studies

In this study, we have used AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking of compound **BTA 9** with six established epilepsy molecular targets namely GABA (A) alpha-1 receptor, GABA (A) delta receptor, glutamate receptor, Na/ H exchanger, Na channel receptor, T-type calcium channel receptor and evaluated their affinity (Kcal/mol) and hydrogen bonding. The crystallographic structure of receptors is shown in Fig. (**5**). **BTA 9** has exhibited good binding properties with GABA (A) delta receptor (Affinity value -6.3 Kcal/mol and 4 H-bonds), GABA (A) alpha-1 receptor (Affinity value -5.5 Kcal/mol and 1 H-bond), glutamate receptor (Affinity value -5.8 Kcal/mol and 1 H-bond) and Na/H exchanger (Affinity value -5.7 Kcal/mol and 1 H-bond). The docking study results are shown in Table **5** and Fig. (**6**). Docking study results shows that the **BTA 9** exhibited good binding properties with

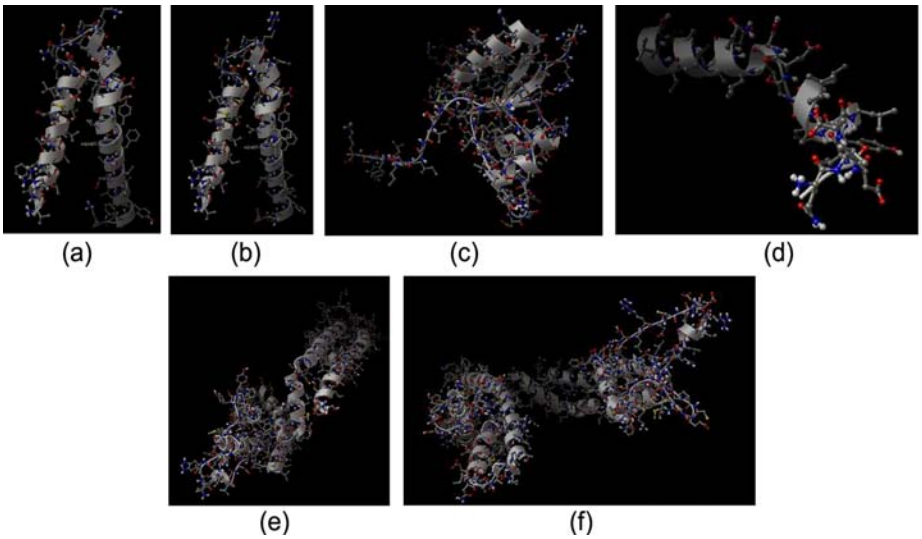


Fig. (5). Crystallographic structure of (**a**) GABA(A) alpha-1 receptor, (**b**) GABA(A) delta receptor, (**c**) Glutamate receptor, (**d**) Na/H exchanger, (**e**) Na channel receptor, (**f**) T-type calcium channel receptor.

Table 5. Docking study results of compound BTA 9[a]

Ligand	Receptor	Affinity (Kcal/mol)	H-bonds	H- BindingLigand			H- Binding Receptor			
				Element	Atom ID	Type	Residue	Element	Atom ID	Type
BTA 9	GABA(A) alpha-1	-5.5	01	N	8	Acceptor	Ser299	O	185	Both
	GABA(A) delta	-6.3	04	N	12	Donor	Tyr318	O	400	Both
				N	06	Acceptor	Tyr318	O	400	Both
				N	07	Acceptor	Tyr318	O	400	Both
				N	08	Acceptor	Tyr318	O	400	Both
	Glutamate	-5.8	01	N	12	Donor	Leu469	O	250	Acceptor
	Na/H exchanger	-5.7	01	N	06	Acceptor	Phe253	N	96	Donor
	Na Channel	-0.0	00	--	--	--	--	--	--	--
	T-type Calcium	-0.0	00	--	--	--	--	--	--	--

[a]Affinity and H-bonds calculations were determined by docking studies using Autodoc 4.0 software.

GABA (A) delta, GABA (A) alpha-1, glutamate receptor and Na/H exchanger.

CONCLUSION

A series of 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted] acetohydrazide was designed, synthesized, and their anticonvulsant activity was evaluated after intraperitoneal administration in 6 Hz psychomotor seizure test. A computational study was also carried out, including the calculation of pharmacophore pattern, prediction of pharmacokinetic properties and docking studies. The compound **BTA 9** displayed significant protection and emerged as a lead in this series. Further, compounds **BTA 4, BTA 5** and **BTA 8** came out as a potential candidate for further investigation. Furthermore, except **BTA 7** and **8**, none of the compounds violated Lipinski's parameters, making them potentially promising agent for epilepsy therapy. Docking study results show that the compounds exhibited good binding properties with GABA (A) delta, GABA (A) alpha-1, glutamate receptor and Na/H exchanger. The docking study data strongly support the assumption that these receptors may be involved in observed anticonvulsant activity of 2-(1*H*-Benzotriazol-1-yl)-*N*'-[substituted]acetohydrazides. However, further studies need to be carried out to ascertain the precise mechanism of action of anticonvulsant activity of these molecules.

DISCLOSURE

Some part of information included in this chapter has been previously published MEDICINAL CHEMISTRY RE-

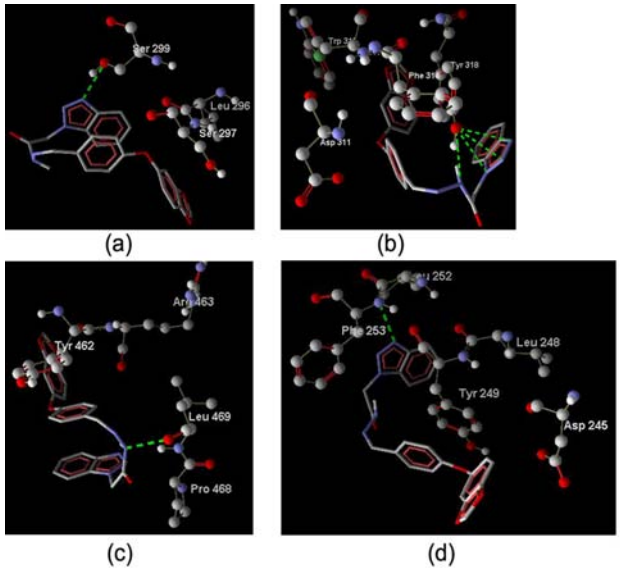


Fig. (6). Docking images of **BTA 9** with (**a**) GABA(A) alpha-1 receptor, (**b**) GABA(A) delta receptor, (**c**) Glutamate receptor, (**d**) Na/H exchanger.

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