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Anticonvulsant and Neurotoxicity Evaluation of Some Novel 2-(1*H*-Benzotriazol-1-yl)-N'-[substituted] acetohydrazide

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A series of novel 2-(1*H*-benzotriazol-1-yl)-N'-[substituted] acetohydrazide were synthesized and screened for anticonvulsant activity in maximal electroshock induced seizure (MES) and subcutaneous metrazol (scMET) induced seizure models in mice. The neurotoxicity was assessed using the Rotorod method. The compound N'-[4-(1,3-benzodioxol-5-yloxy)benzylidene]-2-(1*H*-benzotriazol-1-yl)aceto-hydrazide (**BTA 9**) emerged as the most promising one with anti-MES activity in mice i.p. All the compounds exhibited no neurotoxicity in Rotorod method.

Key Words: Benzotriazole, Anticonvulsant, 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 anticonvulsant 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 presenting a wide range of bioactivities. Among the most important are: anti-convulsant[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 analogues as inhibitors of the NT pase/ helicase and some related flavivirade has been extensively investigated[12]. In the present study, we have designed and synthesized a series of benzotriazol-1-yl-acetic acid (substituted)-hydrazide derivatives and investigated their anticonvulsant activity by MES and scMET models. The neurotoxicity evaluation was done by Rotorod method.

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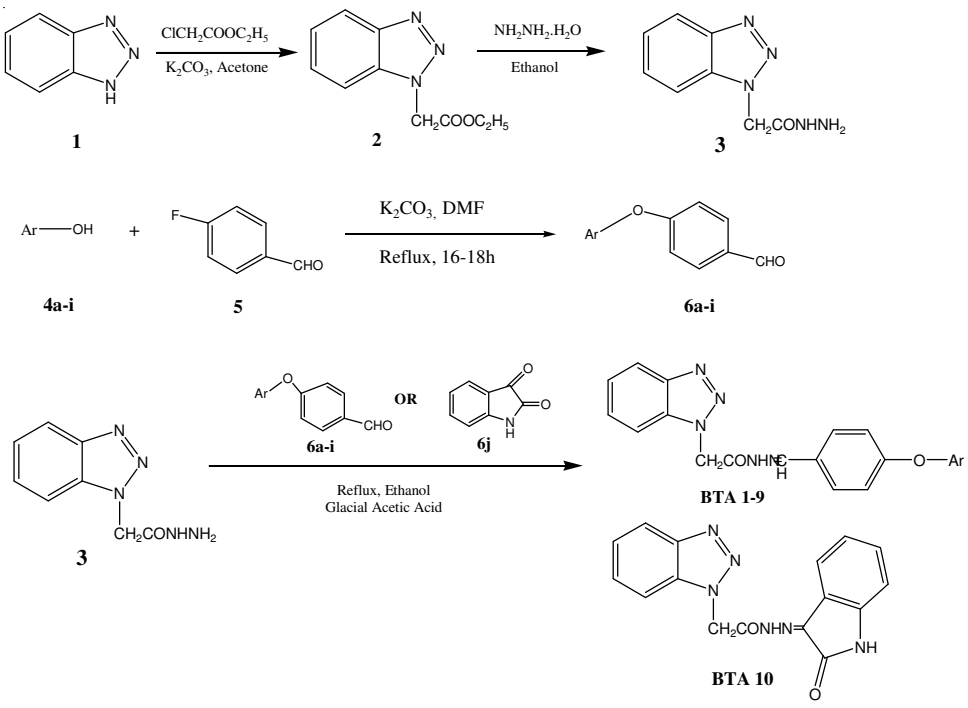
EXPERIMENTAL

All the chemicals and solvents, purchased from Merck (India), Spectrochem (India), Himedia (India) and S. D. Fine were used without further purification. 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 are 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 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.

The reaction sequence leading to the formation of the titled compounds, *viz.* 2-(1*H*-benzotriazol-1-yl)-N'-[substituted] acetohydrazide (**BTA 1-10**) is shown in **Scheme-I**.



Scheme-I: Synthesis of 2-(1*H*-benzotriazol-1-yl)-N'-[substituted] acetohydrazide (**BTA 1-10**)

A mixture of 1*H*-benzotriazole (**1**), chloroethyl acetate and potassium carbonate was stirred in dry acetone to obtain ethyl-1*H*-benzotriazol-1-ylacetate (**2**). The ethyl-1*H*-benzotriazol-1-ylacetate (**2**) was converted to 2-(1*H*-benzotriazol-1-yl)-acetohydrazide (**3**) by reacting with hydrazine hydrate. Various 4-substituted benzaldehydes (**6a-i**) were prepared by refluxing various substituted phenol (**4a-i**) with 4-fluoro benzaldehyde (**5**) in presence of potassium carbonate. The 2-(1*H*-benzotriazol-1-yl)acetohydrazide (**3**) was condensed with various 4-substituted benzaldehyde (**6a-i**)/ isatin (**6j**) to yield the titled compounds (**BTA 1-10**).

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

Synthesis of 2-(1*H*-benzotriazol-1-yl)acetohydrazide (3): Compound (**2**) (0.1 mol) and hydrazine hydrate (0.3 mol) in ethanol (50 mL) was stirred for 1 h and then refluxed for 2 h. The excess of solvent was removed under reduced pressure and recrystallized from chloroform-hexane (3:1) to yield white crystals of compound (**3**).

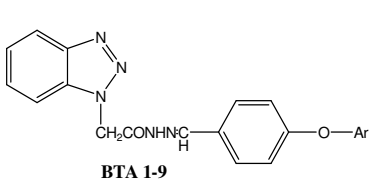
Synthesis of 4-substituted benzaldehyde (6a-i): A mixture of substituted phenol (**4a-i**) (37.4 mmol), 4-fluorobenzaldehyde (**5**) (37.4 mmol) and potassium carbonate (38.8 mmol) in N,N-dimethylformamide (30 mL) was refluxed for 16-18 h under nitrogen. After cooling, the product was extracted from the reaction mixture and purified by chromatography.

Synthesis of 2-(1*H*-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 from 90 % ethanol. The physical data and elemental analysis data of the titled compounds (**BTA 1-10**) are presented in Table-1. The spectral data of titled compounds (**BTA 1-10**) are given below.

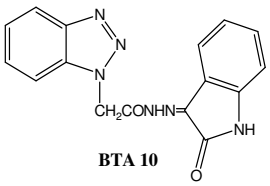
2-(1*H*-Benzotriazol-1-yl)-N'-(4-phenoxybenzylidene)acetohydrazide (BTA 1): IR (KBr, ν_{max}, cm^{-1}): 3217 (-NH-), 1684 (C=O), 1604 (-N=CH-), 1478, 1260 (-N-CH_2-), 1229 (-O-); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.894 (s, 2H, N-CH_2), 6.420-8.07 (a set of signals, 13H, Ar-H), 7.713 (s, 1H, -CH=N-), 9.586 (s, 1H, -NH-, D_2O exchangeable); MS (m/z, %): 372.16 (M^+ + 1, 100).

2-(1H-Benzotriazol-1-yl)-N'-[4-(4-nitrophenoxy)benzylidene]acetohydrazide (BTA 2): IR (KBr, ν_{max}, cm^{-1}): 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, 100).

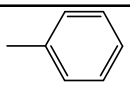
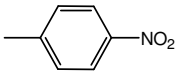
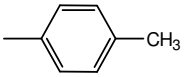
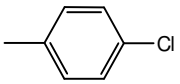
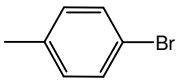
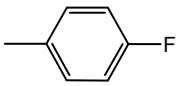
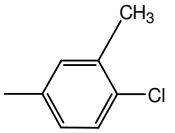
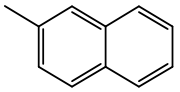
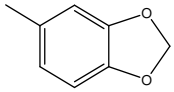
TABLE-1
PHYSICAL AND ELEMENTAL ANALYSIS DATA OF 2-(1*H*-BENZOTRIAZOL-1-YL)-N'-[SUBSTITUTED] ACETOHYDRAZIDE (**BTA 1-10**)



BTA 1-9



BTA 10

Compd.	Ar	Yield, % (m.p., ºC)	m.f. (m.w.)	Elemental analysis (%): Found (calcd.)		
				C	H	N
BTA 1		73 (146)	$C_{21}H_{17}N_5O_2$ (371.39)	67.90 (67.91)	4.61 (4.61)	18.85 (18.86)
BTA 2		74 (138)	$C_{21}H_{16}N_6O_4$ (416.39)	60.56 (60.57)	3.84 (3.87)	20.19 (20.18)
BTA 3		75 (155)	$C_{22}H_{19}N_5O_2$ (385.42)	68.52 (68.56)	4.95 (4.97)	18.16 (18.17)
BTA 4		76 (188)	$C_{21}H_{16}ClN_5O_2$ (405.84)	62.13 (62.15)	3.92 (3.97)	17.23 (17.26)
BTA 5		78 (215)	$C_{21}H_{16}BrN_5O_2$ (450.29)	56.0 (56.01)	3.56 (3.58)	15.53 (15.55)
BTA 6		74 (204)	$C_{21}H_{16}FN_5O_2$ (389.38)	64.73 (64.78)	4.12 (4.14)	17.98 (17.99)
BTA 7		72 (195)	$C_{22}H_{18}ClN_5O_2$ (419.86)	62.90 (62.93)	4.28 (4.32)	16.64 (16.68)
BTA 8		77 (172)	$C_{25}H_{19}N_5O_2$ (421.45)	71.21 (71.25)	4.52 (4.54)	16.59 (16.62)
BTA 9		71 (165)	$C_{22}H_{17}N_5O_4$ (415.40)	63.60 (63.61)	4.10 (4.12)	16.84 (16.86)
BTA 10	–	79 (260)	$C_{16}H_{12}N_6O_2$ (320.31)	59.9 (60.00)	3.77 (3.78)	26.22 (26.24)

2-(1*H*-Benzotriazol-1-yl)-N'-[4-(4-methylphenoxy)benzylidene]acetohydrazide (BTA 3): IR (KBr, ν_{max}, cm^{-1}): 3216 (-NH-), 1681 (C=O), 1605 (-N=CH-), 1481, 1261 (-N-CH_2-), 1231 (-O-); ^1H 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, 100).

2-(1*H*-Benzotriazol-1-yl)-N'-[4-(4-chlorophenoxy)benzylidene]acetohydrazide (BTA 4): IR (KBr, ν_{max}, cm^{-1}): 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).

2-(1*H*-Benzotriazol-1-yl)-N'-[4-(4-bromophenoxy)benzylidene]acetohydrazide (BTA 5): IR (KBr, ν_{max}, cm^{-1}): 3219 (-NH-), 1689 (C=O), 1607 (-N=CH-), 1475, 1260 (-N-CH_2-), 1232 (-O-); ^{1}H 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).

2-(1*H*-Benzotriazol-1-yl)-N'-[4-(4-fluorophenoxy)benzylidene]acetohydrazide (BTA 6): IR (KBr, ν_{max}, cm^{-1}): 3216 (-NH-), 1687 (C=O), 1602 (-N=CH-), 1477, 1260 (-N-CH_2-), 1233 (-O-); ^{1}H 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, 100).

2-(1*H*-Benzotriazol-1-yl)-N'-[4-(4-chloro-3-methylphenoxy)benzylidene]-acetohydrazide (BTA 7): IR (KBr, ν_{max}, cm^{-1}): 3217 (-NH-), 1687 (C=O), 1602 (-N=CH-), 1477, 1261 (-N-CH_2-), 1231 (-O-); ^{1}H 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).

2-(1*H*-Benzotriazol-1-yl)-N'-[4-(naphthalen-2-yloxy)benzylidene]-acetohydrazide (BTA 8): IR (KBr, ν_{max}, cm^{-1}): 3216 (-NH-), 1681 (C=O), 1601 (-N=CH-), 1478, 1260 (-N-CH_2-), 1233 (-O-), 836, 821 (β-naphthyl); ^{1}H 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, 100).

N'-[4-(1,3-Benzodioxol-5-yloxy)benzylidene]-2-(1*H*-benzotriazol-1-yl)acetohydrazide (BTA 9): IR (KBr, ν_{max}, cm^{-1}): 3215 (-NH-), 1687 (C=O), 1602 (-N=CH-), 1480,1262 (-N-CH_2-), 1228(-O-); ^{1}H 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, 100).

2-(1*H*-Benzotriazol-1-yl)-N'-[(3Z)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]acetohydrazide (BTA 10): IR (KBr, ν_{max}, cm^{-1}): 3263 (-NH- of isatin), 3215 (-NH-), 1687 (C=O), 1601 (-N=C-), 1478, 1260 (-N-CH_2-); ^{1}H 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, 100).

Anticonvulsant and neurotoxicity evaluation: Male albino mice (CF-1 strain, 18-25 g) were used as experimental animal. The animals were housed in metabolic cages and allowed free access to food and water. The synthesized derivatives were suspended in 0.5 % methyl cellulose/ water mixture or in polyethylene glycol (PEG 200). The test compound is usually manipulated with a motar pestle to help preparation of suspension. In the preliminary screening each compound was administered as an i.p. injection at three dose levels (30, 100 and 300 mg/kg) and anticonvulsant and neurotoxic effects were assessed at 0.5 and 4.0 h intervals after administration. Anticonvulsant efficacy was measured by MES and scMET tests and the data are presented in Table-2. The preliminary animal research study was carried out according to the protocols approved by the Institutional animal ethical committee, S.D. College of Pharmacy and Vocational Studies, Muzaffarnagar (Reg No. 876/AC/05/CPCSEA) and advanced anticonvulsant screening were carried out at Epilepsy Branch, National Institute of Neurological Disorder and Stroke, National Institute of Health, Bethesda, USA.

TABLE-2
ANTICONVULSANT AND NEUROTOXIC ACTIVITY OF 2-(1*H*-BENZOTRIAZOL-1-YL)-N'-[SUBSTITUTED] ACETOHYDRAZIDE (**BTA 1-10**)

Compd.	Intra-peritoneal injection in mice[a]					
	MES screen (h)		scMET screen (h)		Neurotoxicity screen (h)	
	0.5	4.0	0.5	4.0	0.5	4.0
BTA 1	–	–	–	–	–	–
BTA 2	–	–	–	–	–	–
BTA 3	–	–	–	–	–	–
BTA 4	–	–	–	–	–	–
BTA 5	–	–	–	–	–	–
BTA 6	–	–	–	–	–	–
BTA 7	–	–	–	–	–	–
BTA 8	–	–	–	–	–	–
BTA 9	–	300[b]	–	–	–	–
BTA 10		–				
Phenytoin	30	30	–	–	100	100
Sodium valproate	–	–	300	–	–	–

[a]Dose of 30, 100 and 300 mg/kg were administered. The figure in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more mice. The dash (–) indicates an absence of activity at maximum dose administered (300 mg/kg).
[b]In the MES screen compound **BTA 9** showed protection (1/1, 4h) at a dose of 300 mg/kg.

RESULTS AND DISCUSSION

In the present study 2-(1*H*-benzotriazol-1-yl)-N'-[substituted] acetohydrazide have been synthesized by refluxing 2-(1*H*-benzotriazol-1-yl)acetohydrazide with various 4-substituted benzaldehyde/isatin in the presence of catalytic amount of glacial acetic acid. All compounds gave satisfactory elemental analysis (Table-1). IR, ^{1}H NMR and mass spectra were consistent with the assigned structures.

All titled compounds were evaluated for anticonvulsant activity in maximal electroshock induced seizure (MES) and subcutaneous metrazol (scMET) induced seizure models in mice using doses of 30, 100, 300 mg/kg. The observation was carried out at two different time intervals (0.5 and 4.0 h). The pharmacological test results were shown in Table-2. Compound N'-[4-(1,3-benzodioxol-5-yloxy)benzylidene]-2-(1*H*-benzotriazol-1-yl) acetohydrazide (**BTA 9**) showed 100 % protection (1/1, 4.0 h) at a dose of 300 mg/kg in MES test, indicating the compounds ability to prevent seizure spread. All the titled compound exhibited no neurotoxicity at the highest administered dose (300 mg/kg) in Rotorod method.

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