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RESEARCH ARTICLE

SYNTHESIS, ANTICONVULSANT ACTIVITY OF SOME NOVEL BENZIMIDAZOLE ACETOHYDRAZIDES

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ABSTRACT:

A series of some novel benzimidazole acetohydrazide derivatives was synthesized and characterized by TLC, MP, elemental analysis, IR, Mass and ^{1}H NMR spectroscopy. These compounds were screened for anticonvulsant activities by Maximum Electroshock seizure model in male wistar rats and compared with the standard drug phenytoin.

Keywords: Benzimidazole, hydrazide, Anticonvulsant, MES

INTRODUCTION:

Benzimidazole is very useful heterocyclic compound/intermediate for the development of molecules of pharmaceutical or biological interest.[1] Derivatives of benzimidazole possess a wide range of pharmacological activities such as Anti-neoplastic, analgesic, Anthelmintics, Anti-ulcer, anti-inflammatory, Antifungal, Anti-histaminic, Antiviral and Anti-diabetic.[2-7]

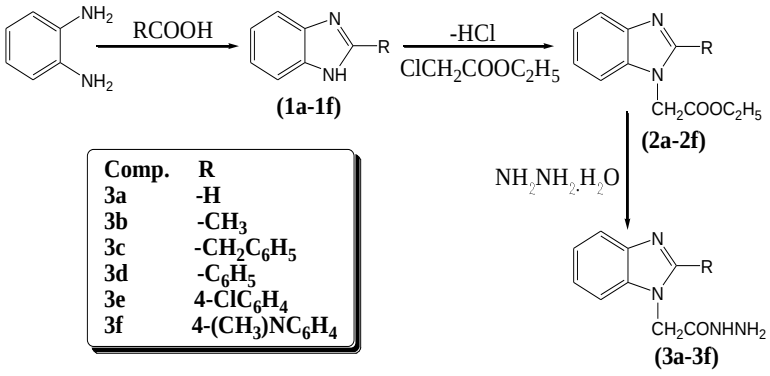
Numerous publication have shown that benzimidazole derivatives also have anticonvulsant activity.[8-12]

An attempt has been made in our study to synthesize Benzimidazole acetohydrazide derivatives to get as potent anticounvulsant compounds. The newly synthesized Benzimidazole acetohydrazide derivatives were evaluated for anticonvulsant activity by the MES method using phenytoin as standard.

MATERIAL AND METHODS:

*1H-benzimidazole (**1a**).* A mixture of *o*-phenylenediamine (27 g, 0.25 mol) and formic acid (16 ml, 85 %) was refluxed on a water bath for 2 h. The reaction mixture was cooled and its pH was adjusted to 8 by addition of sodium hydroxide (10 %) solution. The crude product so obtained was filtered, thoroughly washed with ice cold water and dried. The dried product was boiled with decolourising charcoal (2 g) in water (400 ml) for 15 min and filtered while hot. The solution thus obtained was allowed to cool at 10 °C for 30 min, filtered and dried. The completion of reaction was monitored by running TLC.

Yield: 88 %, R_f value: 0.46, M.p. 170-172 ºC. IR (KBr in cm^{-1}): 3115 (N-H), 3048 (Ar. C-H), 1362 (C=N). Anal. calcd. for $C_7H_6N_2$ (118.14): C, 71.17; H, 5.12; N, 23.71. Found: C, 71.22; H, 5.16; N, 23.31 %. LCMS m/z: 118.1 (M+).



Comp.	R
3a	-H
3b	$-CH_3$
3c	$-CH_2C_6H_5$
3d	$-C_6H_5$
3e	$4\text{-}ClC_6H_4$
3f	$4\text{-}(CH_3)NC_6H_4$

Scheme 1 Reaction Protocol for the synthesis of **3a-3f**

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Similar procedure was used to prepare **1b-1f** by using corresponding aliphatic and aromatic carboxylic acid.

*2-Methyl-1H-benzimidazole (**1b**):* Yield: 76 %, R_f value: 0.39, M.p. 174-176 ºC. IR (KBr in cm^{-1}): 3194 (N-H), 3066, 2998(Ar. C-H), 1386(C=N). Anal. calcd. for $C_8H_8N_2$ (132.16): C, 72.70; H, 6.10; N, 21.20 %. Found: C, 72.57; H, 6.04; N, 21.12 %. LCMS m/z: 132.1 (M+).

*2-Benzyl-1H-benzimidazole (**1c**)*: Yield: 79 %, R_f value: 0.67, M.p. 192-194 ºC. IR (KBr in cm^{-1}): 3023 (N-H), 2964(Ar. C-H), 2614(-CH_2), 1418(C=N). Anal. calcd. for $C_{14}H_{12}N_2$ (208.26: C, 80.74; H, 5.81; N, 13.45 %. Found: C, 80.63; H, 5.92; N, 13.61 %. LCMS m/z: 208.1 (M+).

*2- Phenyl-1H-benzimidazole (**1d**)*: Yield: 81 %, R_f value: 0.57, M.p. 290-292 ºC. IR (KBr in cm^{-1}): 3183 (N-H), 2943(Ar. C-H), 1427 (C=N). Anal. calcd. for $C_{13}H_{10}N_2$ (194.23): C, 80.39; H, 5.19; N, 14.42 %. Found: C, 80.51; H, 5.26; N, 14.23 %. LCMS m/z: 195.1 (M+).

*2-(4-Chlorophenyl)-1H-benzimidazole (**1e**)*: Yield: 87 %, R_f value: 0.77, M.p. 234-236 ºC. IR (KBr in cm^{-1}): 3188 (N-H), 2971(Ar. C-H), 1412 (C=N), 816 (C-Cl). Anal. calcd. for $C_{13}H_9ClN_2$ (228.68): C, 68.28; H, 3.97; N, 12.25 %. Found: C, 68.39; H, 4.03; N, 12.36 %. LCMS m/z: 228.3.

*4-Dimethyl aminophenyl-1H-benzimidazole (**1f**)*: Yield: 83 %, R_f value: 0.66, M.p. 176-178 ºC. IR (KBr in cm^{-1}): 3192 (N-H), 2987 (Ar. C-H), 1428 (C=N), 1293 (C-N). Anal. calcd. for $C_{15}H_{17}N_3$ (239.32): C, 75.28; H, 7.16; N, 17.56 %. Found: C, 75.34; H, 7.23; N, 17.62 %. LCMS m/z: 239.6 (M+).

*Ethyl-2-(1H-benzimidazol-1-yl)ethanoate (**2a**)*: A solution of Benzimidazole **1a** (3.54 g, 0.03 mol) in acetone was refluxed with chloroethylacetate (3.69 g, 3.20 ml, 0.03 mol) and potassium carbonate (8.28 g, 0.06 mol) for 4 h. The reaction mixture was filtered while hot and evaporated to dryness. The completion of reaction was monitored by running TLC.

Yield: 74 %, R_f value: 0.49, M.p.160-164 ºC. IR (KBr in cm^{-1}): 2978 (Ar H str), 1689 (C=O),1396 (tert. N), 1226 (C-O). Anal. calcd. for $C_{11}H_{12}N_2O_2$ (204.23): CC, 64.69; H, 5.92; N, 13.72 %. Found: C, 64.82; H, 5.76; N, 13.63 %. LCMS m/z: 205.1 (M+).

Similar procedure was used to prepare **2b-2f** by using corresponding 2-subtituted benzimidazoles (**1b-1f**).

*Ethyl-2-(2-methyl-1H-benzimidazol-1-yl)ethanoate (**2b**)*: Yield: 79 %, R_f value: 0.68, M.p. 80-82 ºC. IR (KBr in cm^{-1}): 2998 (Ar H str), 1692 (C=O),1387 (tert. N), 1248 (C-O). Anal. calcd. for $C_{17}H_{19}N_5O$ (309.37): C, 66.00; H, 6.19; N, 22.64 %. Found: C, 65.91; H, 6.24; N, 22.55 %. LCMS m/z: 309.2 (M+).

*Ethyl-2-(2-benzyl-1H-benzimidazol-1-yl)ethanoate (**2c**)*: Yield: 83 %, R_f value: 0.49, M.p. 92-96 ºC. IR (KBr in cm^{-1}): 2974 (Ar H str), 1678 (C=O), 1392 (tert. N), 1253 (C-O). Anal. calcd. for $C_{18}H_{18}N_2O_2$ (294.35): C, 73.45; H, 6.16; N, 9.52 %. Found: C, 73.61; H, 6.19; N, 9.67 %. LCMS m/z: 294.14 (M+).

*Ethyl-2-(2-phenyl-1H-benzimidazol-1-yl)ethanoate (**2d**):* Yield: 81 %, R_f value: 0.69, M.p. 74-78 ºC. IR (KBr in cm^{-1}): 2963 (Ar H str), 1689 (C=O), 1374 (tert. N), 1265 (C-O). Anal. calcd. for $C_{17}H_{16}N_2O_2$ (280.32): C, 72.84; H, 5.75; N, 9.99 %. Found: C, 72.79; H, 5.81; N, 9.79 %. LCMS m/z: 280.1 (M+).

*Ethyl-2-(2-(4-chloro)phenyl-1H-benzimidazol-1-yl)ethanoate (**2e**)*: Yield: 67 %, R_f value: 0.53, M.p. 88-90 ºC. IR (KBr in cm^{-1}): 2943 (Ar H str), 1678 (C=O), 1381 (tert. N), 1272 (C-O), 8-24 (C-Cl). Anal. calcd. for $C_{17}H_{15}ClN_2O_2$ (314.77) C, 64.87; H, 4.80; N, 8.90 %. Found: C, 64.92; H, 4.89; N, 8.78 %. LCMS m/z: 314.1 (M+).

*Ethyl 2-(2-(4-dimethyl amino)phenyl-1H-benzimidazol-1-yl)ethanoate (**2f**)*: Yield: 88 %, R_f value: 0.76, M.p. 102-106 ºC. IR (KBr in cm^{-1}): ^{1}H-NMR (DMSO δ ppm): . Anal. calcd. for $C_{19}H_{21}N_3O_2$ (323.39): C, 70.57; H, 6.55; N, 12.99 %. Found: C, 70.72; H, 6.61; N, 12.93 %. LCMS m/z: 323.2 (M+).

*2-(1H-Benzimidazol-1-yl) acetohydrazide (**3a**)*: A mixture of Ethyl-2-(1H-benzimidazol-1-yl) ethanoate **2a** (2.04 g, 0.01 mol) and hydrazine hydrate (0.75 g, 0.73 ml, 0.015 mol) in ethanol (25 ml) was refluxed on a water bath for 3 h. The reaction mixture was cooled, made acidic to get precipitate. The separated solid was filtered and purified by recrystallisation from ethanol. The completion of reaction was monitored by running TLC. Yield: 73 %, R_f value: 0.65, M.p. 180-182 ºC. IR (KBr in cm^{-1}): 3362 (NH), 2972 (Ar C-H str), 1689 (C=O), 1391 (tert. N). ^{1}H-NMR (DMSO δ ppm): 7.92 (s, 1H, Ar-CH), 7.23-7.73 (m, 4H, Ar-CH), 6.34 (s, 1H, NH), 5.29 (s, 2H, CH_2), 3.39-3.44 (s, 2H, NH_2). Anal. calcd. for $C_9H_{10}N_4O$ (190.2): C, 56.83; H, 5.30; N, 29.46 %. Found: C, 56.63; H, 5.23; N, 29.29 %. LCMS m/z: 190.1 (M+).

Compounds (**2b-2f)** were used to prepare **3b-3f** by using similar procedure.

*2-(2-Methyl-1H-benzimidazol-1-yl)acetohydrazide (**3b**)*: Yield: 79 %, R_f value: 0.29, M.p. 240-242 ºC. IR (KBr in cm^{-1}): 3455(NH), 3138(NH), 3051, 2905(Ar CH str), 1635(C=O), 1510(C=N), 1323(tert. N), 1290(C-N). ^{1}H-NMR (DMSO δ ppm): 7.14-7.59 (m, 4H, Ar-CH), 6.28 (s, 1H, NH), 5.23 (s, 2H, CH_2), 3.29-3.31 (s, 2H, NH_2), 2.58 (s, 3H, CH_3). Anal. calcd. For $C_{10}H_{12}N_4O$ (204.23): C, 58.81; H, 5.92; N, 27.43; O, 7.83 %. Found: C, 58.81; H, 5.87; N, 27.53; O, 7.69 %. LCMS m/z: 204.6 (M+).

*2-(2-Benzyl-1H-benzimidazol-1-yl)acetohydrazide (**3c**)*: Yield: 82 %, R_f value: 0.36, M.p. 184-186 ºC. IR (KBr in cm^{-1}): 3342 (-NH), 2894 (Ar CH str), 1652 (C=O), 1509 (C=N), 1328 (tert. N), 1310 (C-N). ^{1}H-NMR (DMSO δ ppm): 7.09-7.64 (m, 9H, Ar-CH), 6.47 (s, 1H, NH), 5.16 (s, 2H, CH_2), 3.94 (s, 2H, CH_2), 3.29-3.31 (s, 2H, NH_2). Anal. calcd. for $C_{16}H_{16}N_4O$ (280.32): C, 68.55; H, 5.75; N, 19.99 %. Found: C, 68.73; H, 5.82; N, 19.86 %. LCMS m/z: 280.1 (M+).

*2-(2-Phenyl-1H-benzimidazol-1-yl)acetohydrazide (**3d**)*: Yield: 79 %, R_f value: 0.36, M.p. 294-298 ºC. IR (KBr in cm^{-1}): 3358 (NH), 3051, 2961 (Ar H str), 1696 (C=O), 1380 (tert. N). ^{1}H-NMR (DMSO δ ppm): 7.11-7.86 (m, 9H, Ar-CH), 6.29 (s, 1H, NH), 5.27 (s, 2H, CH_2), 3.08 (s, 2H, NH_2). Anal. calcd. for $C_{15}H_{14}N_4O$ (266.3): C, 67.65; H, 5.30; N, 21.04 %. Found: C, 67.75; H, 5.49; N, 21.14 %. LCMS m/z: 266.1 (M+).

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*2-(2-(4-Chloro) phenyl-1H-benzimidazol-1-yl)acetohydrazide (**3e**)*: Yield: 74 %, R_f value: 0.29, M.p. 252-256 ºC. IR (KBr in cm^{-1}): 3369 (NH), 2914 (Ar H str), 1699 (C=O), 1375 (tert. N), 733 (C-Cl). ^{1}H-NMR (DMSO δ ppm): 7.03-7.68 (m, 8H, Ar-CH), 6.34 (s, 1H, NH), 5.19 (s, 2H, CH_2), 3.13 (s, 2H, NH_2). Anal. calcd. for $C_{15}H_{13}ClN_4O$ (300.74): C, 59.91; H, 4.36; Cl, 11.79; N, 18.63 %. Found: C, 59.82; H, 4.24; N, 18.48 %. LCMS m/z: 300.1(M+).

*2-(2-(4-Dimethyl amino) phenyl-1H-benzimidazol-1-yl)acetohydrazide (**3f**)*: Yield: 85 %, R_f value: 0.73, M.p. 204-206 ºC. IR (KBr in cm^{-1}): 3380 (NH), 2924 (Ar H str), 1681 (C=O), 1384 (tert. N). ^{1}H-NMR (DMSO δ ppm): 7.01-7.61 (m, 8H, Ar-CH), 6.42 (s, 1H, NH), 5.28 (s, 2H, CH_2), 3.18 (s, 2H, NH_2), 2.89 (s, 6H, $N(CH_3)_2$). Anal. calcd. for $C_{17}H_{19}N_5O$ (309.37): C, 66.00; H, 6.19; N, 22.64 %. Found: C, 66.11; H, 6.12; N, 22.52 %. LCMS m/z: 309.2 (M+).

Pharmacology

Animals: Male wistar rats procured from BN College of Pharmacy, Udaipur (150-200 g) were used in the present study. The animals were housed in colony cages, conditions of constant temperature (22±2 °C), a 12 h light/dark schedule, and allowed free access to standard diet and tap water except during the experiment. The animals were allowed to habituate to the laboratory environment for 24 h before the experiments were initiated.

All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The protocol of the study was approved by Institutional Animal Ethical Committee.

Anticonvulsant activity

Maximal Electroshock Seizure Model (MES): Maximal electroshock seizure model was used in the present study to evaluate the anticonvulsant activity of the compounds on male wistar rats. Seizures were induced in rats by delivering electroshock of 150 mA for 0.2 sec by means of an electro-convulsiometer through a pair of ear clip electrodes. The test compounds (100 mg/kg) were administered by oral route in the form of solution (The compounds were dissolved in 1% sodium carboxymethyl cellulose), 30 min before the maximal electroshock seizure (MES) test.[13] Phenytoin (100 mg/kg) was used as a standard drug. The reduction in time or abolition of tonic extensor phase of MES- convulsions was noted.[14] The data are calculated & expressed as mean extensor phase duration in sec. followed by % protection and % potency in comparison with the standard as shown in Table 1 using the following formula:

% Protection = (MEPDnc - MEPDsample/ MEPD) X100

Where MEPDnc is the mean extensor phase duration of normal control in sec. and MEPD is the mean extensor phase duration of sample or standard in sec.

% Potency = (MEPDnc - MEPD/ MEPDnc -MEPDstd) X 100

Where MEPDstd is the mean extensor phase duration of standard control in sec.

Statistical analysis

The results are expressed as the mean±SEM per group and the data were analyzed by one-way analysis of Variance (ANOVA) followed by Dunnett's test as post hoc test. *p* value <0.05 was considered statistically significant.

Table 1 Anticonvulsant activity of title compounds

Compound Nos.	Dose (mg/kg)	Extensor phase duration(Sec.)	Protection (%)	Potency (%)
Control	-	18.4±0.927	-	-
Phenytoin	100	7.6±0.509**	58.69	100
3a	100	14.8±0.860**	19.56	33.33
3b	100	13.0±0.447**	29.35	50.00
3c	100	12.6±0.678**	31.52	53.70
3d	100	17.6±0.812ns	4.35	7.41
3e	100	15.6±1.116ns	15.22	25.92
3f	100	15.2±0.374*	17.39	29.63

Data analyzed by one way ANOVA followed by Dunnett's test, (n = 6), **P* < 0.05, ***P* < 0.01 significant from control; ns, not significant.

RESULTS & DISCUSSION:

All title compounds were synthesized as per Scheme 1 and their structure was confirmed by IR, ^{1}H NMR, LCMS, and elemental analysis. The anticonvulsant activity was determined by MES method on wistar rats using phenytoin as standard drug (Table 1). Compounds **3a**, **3b**, **3c** and **3f** showed significant protection against maximum electroshock seizures. Compounds **3b** and **3c** exhibited more potent activity than that of other compounds. Compound **3c** was found to be most potent among all the compounds.

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