International Journal of Farmacia

Journal Home page: www.ijfjournal.com

Synthesis and characterization of new benzotriazole derivatives for possible CNS activity

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ABSTRACT

In view of the biological prominence of benzotriazole derivatives, it is planned to synthesize new benzotriazole derivatives. So, some new 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazides (Va-Vg) have been synthesized as depicted in scheme-I. The intermediates and final compounds were purified and their chemical structures have been confirmed by IR, ^{1}H NMR, Mass and by elemental analysis. All the newly synthesized compounds were screened for their CNS activity (Gross behavioral studies and loco motor activity). Among the compounds tested, compound Vb with 4-Chloro substitution on the phenyl ring showed more promising depressant activity among all the test compounds followed by Vg and Ve.

Keywords: Benzotriazole, Anti-inflammatory agents, CNS activity, Gross behavioral studies and loco motor activity.

INTRODUCTION

A heterocyclic is an organic compound with a ring containing one or more carbons and at least one other element, namely O, S and N. About half of the known organic compounds contain at least one heterocyclic component, thus heterocyclic compounds are very widely distributed in nature. Their functions are often of fundamental importance to living systems as they play a vital role in the metabolism of all living cells [1].

Benzotriazole is a benzofusedtriazole moiety. Benzotriazole containing compounds have been found to possess varied applications in organic synthesis in medicines and industry as biologically active systems, as dye stuffs and fluorescent compounds, as corrosion inhibitors, as photostabilizers [2]. They show anticancer, antimicrobial, antifungal, anticonvulsants and antinociceptive activity. The fungicides containing triazoles are known germicides absorbed inside by plants basing on the high efficiency and the qualities of disinfecting the plants, the triazolone, triazolol have become important type of fungicides [3].

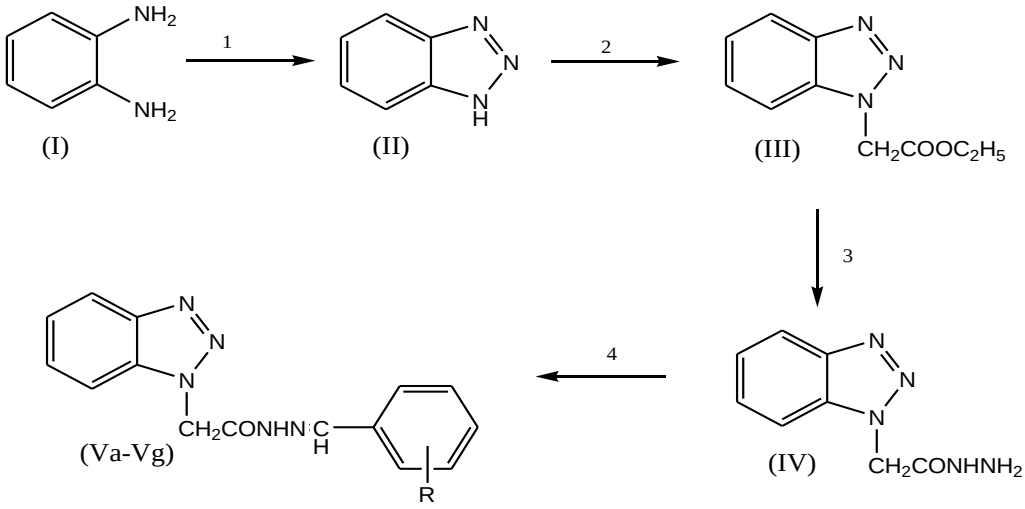
A survey of literature reveals that the benzotriazole nucleus is found to have various

pharmacological activities like anti-inflammatory [4], antimicrobial [5], antifungal [6] and anticancer activities [7]. It is also known from the literature that molecules containing benzotriazole nucleus possess CNS activity and various other pharmacological activities [8]. It has been considered as prime importance to take up such synthesis of new compounds containing benzotriazole nucleus with a view to get more potent compounds and screen them for CNS activity. In view of these valid observations in our present study, we reported the synthesis and screening of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazides.

MATERIALS AND METHODS

The chemicals and solvents used for the experimental work were commercially procured from E. Merck, India, S.D. Fine Chem, India and Qualigens, India. Silica gel G used for analytical chromatography (TLC) was obtained from S.D. Fine Chem, India. Melting points were determined in an open glass capillary using a Kjeldahl flask containing liquid paraffin and are uncorrected. The proton magnetic resonance spectra (1H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO/CDCl3 using TMS as internal standard. Chemical shifts (δ) are expressed in ppm. The infrared spectra of compounds were recorded in KBr on a FTIR- -8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer. Mass spectra were recorded on LC-MS/MS (API-4000 TM), Applied BioSystems, MDS SCIEX (Canada).

Scheme-1



R = H ; 4-Cl ; 3-OCH_3, 4-OH ; 3-OCH_3 ;
2-Cl ; 2-OH ; 4-$N(CH_3)_2$

1) $NaNO_2$, HCl & Glacial acetic acid.

2) Ethylchloroacetate, Anhydrous K_2CO_3 & Dry acetone.

3) $NH_2.NH_2.H_2O$ (99%) & Methanol.

4) Various Aromatic aldehydes, methanol & feew drops of glacial acetic acid.

METHODOLGY

Synthesis of benzotriazole (II)

A mixture of O-phenylenediamene (10.8g, 0.1 mol), glacial acetic acid (11.5 ml, 0.2 mol) and 30 ml of water was taken in a 250 ml beaker. The mixture was heated slightly. The clear solution of sodium nitrite was added. The reaction mixture became warm and within 2–3 minutes reached a temperature of about 85ºC. After cooling the resulting pale brown

compound was filtered and washed thoroughly with ice-cold water [9]. The compound was purified by recrystallization using boiling water, at 99 ºC - 100ºC.

Synthesis of Ethyl 2-(1H-benzo[d][1,2,3]triazol-1-yl)acetate (III)

A mixture of benzotriazole (II, 0.01 mol), anhydrous potassium carbonate (0.02 mol) and ethylchloroacetate (0.01 mol) in dry acetone was stirred on a magnetic stirrer for 20 hrs. The inorganic solids were filtered and solvent was removed on a rotavapour. The residue was poured onto crushed ice. The compound thus separated was washed with cold water and recrystallized from ethanol, m.p.; 72^{o}C, yield; 50%.

Synthesis of 2-(1H-benzo[d][1,2,3]triazol-1-yl) acetohydrazide (IV)

A mixture of Ethyl 1H-benzotriazol-1-yl acetate (III, 0.01 mol) in ethanol (75 ml), and hydrazine hydrate (0.02 mol, 99%) was refluxed for 1.5 hrs. After cooling the resulting solid was filtered, washed thoroughly with cold water, dried and recrystallized from ethanol, m.p.; 170^{o}C - 172^{o}C, yield; 60%. **IR (KBr)(cm^{-1}):** 3373(N-H_2 str.), 3202(N-H str.), 3155 (Ar-H str.), 1687 (C=O str.), 1650-1540 (C=C str.). **H^1 NMR (DMSO-*d6*): δ** 9.6, (s, 1H, CONH), 8.1-7.3 (m, 4H, Ar-H), 5.4 (s, 2H, -CH_2-), 4.4 (s, 2H, -NH_2). **EI-MS: *m/z*=** 191(M^+).

Synthesis of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va - Vg)

A mixture of an appropriate aromatic aldehyde (0.01 mol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl) acetohydrazide (IV, 0.01 mol) in methanol (50 ml) containing 3-4 drops of glacial acetic acid was refuxed on a water bath for about 30 min. and cooled. The crystalline solid which separated out during raction was filtered and recrystallized from suitable solvent(s). The products were characterized by TLC & spectral data. Seven new compounds were prepared by following the above detailed procedure and their physical data is presented in Table-1.

Spectral data of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(phenylmethylidene) acetohydrazide (Va)

m.p.; 232^{o}C - 234^{o}C, yield; 65%.IR (KBr)(cm^{-1}): 3228 (N-H str.), 3142 (Ar-H str.), 1673 (C=O str.), 1623-1558 (C=C str.), 1543 (C=N str.). H^1 NMR (DMSO-*d6*): δ 10.8, (s, 1H, NH acid hydrazide), 8.2-6.9 (m, 10H, Ar-H including benzylidine proton), 6.1 (s, 2H, -CH_2-). EI-MS: *m/z* = 281(M^+).

Spectral data of (E)-N'-(4-chlorobenzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (Vb)

m.p.; 236^{o}C - 238^{o}C, yield; 70%.IR (KBr)(cm^{-1}): 3235 (N-H str.), 3148 (Ar-H str.), 1662 (C=O str.), 1635-1560 (C=C str.), 1540 (C=N str.), 765 (C-Cl). H^1 NMR (DMSO-*d6*): δ 10.2, (s, 1H, NH acid hydrazide), 8.4-7.1 (m, 9H, Ar-H including benzylidine proton), 5.8 (s, 2H, -CH_2-). EI-MS: *m/z* = 313(M^+).

Spectral data of (E)-N'-(4-methoxybenzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (Vc)

m.p.; 220^{o}C - 222^{o}C, yield; 65%.IR (KBr)(cm^{-1}): 3230 (N-H str.), 3140 (Ar-H str.), 1660 (C=O str.), 1639-1568 (C=C str.), 1547 (C=N str.). H^1 NMR (DMSO-*d6*): δ 10.4, (s, 1H, NH acid hydrazide), 8.6-7.4 (m, 9H, Ar-H including benzylidine proton), 5.8 (s, 2H, -CH_2-), 4.4 (s, 3H, OCH_3). EI-MS: *m/z* = 309(M^+).

Spectral data of (E)-N'-(4-hydroxy-3-methoxybenzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (Vd)

m.p.; 210^{o}C - 212^{o}C, yield; 70%.IR (KBr)(cm^{-1}): 3415 (OH str.), 3275 (N-H str.), 3152 (Ar-H str.), 1665 (C=O str.), 1630-1560 (C=C str.), 1541 (C=N str.). H^1 NMR (DMSO-*d6*): δ 10.6, (s, 1H, NH acid hydrazide), 8.5-7.2 (m, 8H, Ar-H including benzylidine proton), 5.6 (s, 2H, -CH_2-), 4.9 (s, 1H, OH), 4.1 (s, 3H, OCH_3). EI-MS: *m/z* = 325(M^+).

Spectral data of (E)-N'-(4-(dimethylamino)benzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (Ve)

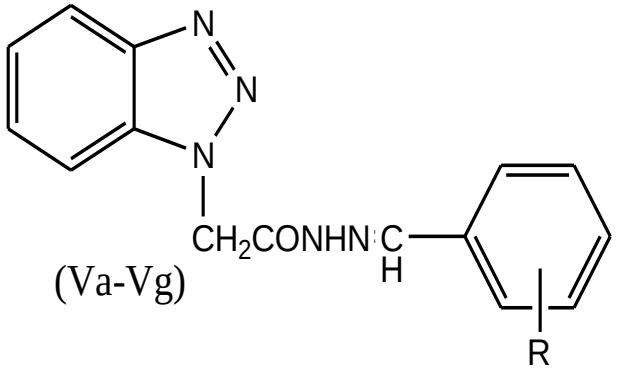
m.p.; 224^{o}C - 226^{o}C, yield; 70%.IR (KBr)(cm^{-1}): 3264 (N-H str.), 3137 (Ar-H str.), 2920 (CH_3 C-H), 1658 (C=O str.), 1628-1569 (C=C str.), 1547 (C=N str.). H^1 NMR (DMSO-*d6*): δ 10.1, (s, 1H, NH acid hydrazide), 8.4-7.1 (m, 9H, Ar-H including benzylidine proton), 5.3 (s, 2H, -CH_2-), 3.8 (s, 6H, $N(CH_3)_2$). EI-MS: *m/z* = 322(M^+).

Spectral data of (E)-N'-(4-(dimethylamino)benzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (Vf)

m.p.; 218^{o}C - 220^{o}C, yield; 60%.IR (KBr)(cm^{-1}): 3428 (OH str.), 3258 (N-H str.), 3124 (Ar-H str.), 1652 (C=O str.), 1621-1563 (C=C str.), 1549 (C=N str.). H^{1} NMR (DMSO-*d6*): δ 10.6, (s, 1H, NH acid hydrazide), 8.2-6.8 (m, 9H, Ar-H including benzylidine proton), 5.2 (s, 2H, -CH_2-), 4.6 (s, 1H, OH). EI-MS: *m/z* = 295(M^+).

Spectral data of (E)-N'-(4-(dimethylamino)benzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (Vg)

m.p.; 230^{o}C - 232^{o}C, yield; 75%.IR (KBr)(cm^{-1}): 3351 (N-H str.), 3135 (Ar-H str.), 1650 (C=O str.), 1625-1569 (C=C str.), 1545 (C=N str.), 762 (C-Cl). H^{1} NMR (DMSO-*d6*): δ 10.2, (s, 1H, NH acid hydrazide), 8.2-7.1 (m, 9H, Ar-H including benzylidine proton), 5.1 (s, 2H, -CH_2-). EI-MS: *m/z* = 313(M^+).



(Va-Vg)

Table 1: Physical data of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va -Vg)

S. No.	Compound	Substituents (R)	Molecular Formula	Molecular Weight	Melting Point ^{o}C	Yield %
1	Va	-H	$C_{15}H_{13}N_5O$	281	232-234	65
2	Vb	4-Cl	$C_{15}H_{12}N_5OCl$	313	236-238	70
3	Vc	4-OCH_3	$C_{16}H_{15}N_5O_2$	311	220-222	65
4	Vd	3-OCH_3 4-OH	$C_{16}H_{15}N_5O_3$	325	210-212	70
5	Ve	4-$N(CH_3)_2$	$C_{17}H_{18}N_6O$	322	224-226	70
6	Vf	2-OH	$C_{15}H_{13}N_5O_2$	295	218-220	60
7	Vg	2-Cl	$C_{15}H_{12}N_5OCl$	313	230-232	75

BIOLOGICAL EVALUATION

Action on central nervous system – gross behavioral studies

Materials

0.1% Sodium CMC, Test compounds

Instruments: Sonicator

Animals: Mice

All the seven newly synthesized compounds were screened for gross behavioral changes, continuously for 5 hrs at 1 hr interval after administration of the compounds. There after the observations were recorded intermittently for 24 hrs and compared with that of control group [10].

In the behavioral profile, the animals have been observed for changes in their

Awareness

- Alertness
- Visual Placing
- Stereotype
- Passivity
- Writhing

Mood

- Grooming
- Vocalization
- Restlessness
- Irritability

The results are presented in Table 2.

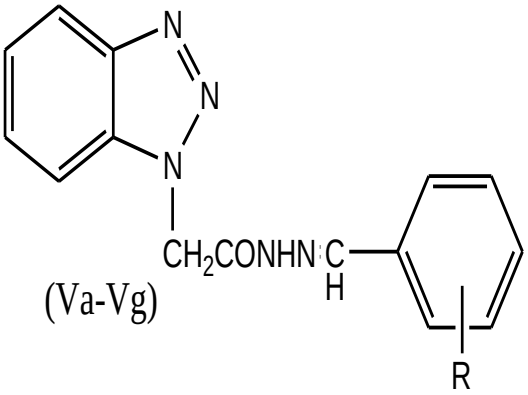


Table 2: Gross Behavioral Studies of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va -Vg)

Compounds	Time (h)	Awareness					Mood			
		Alertness	Visual Placing	Stereotype	Passivity	Writhing	Grooming	Vocalization	Restlessness	Irritability
Va	½	-	-	-	+	-	+	-	-	-
	1	-	-	-	+	-	-	-	-	-
	2	-	-	-	+	-	-	-	-	-
	3	+	-	-	+	-	-	-	-	-
	4	+	+	-	+	-	-	-	-	-
	5	+	+	-	-	-	-	-	-	-
	24	+	+	-	-	-	-	-	-	-
Vb	½	-	-	-	+	-	-	-	-	-
	1	-	-	-	+	-	+	-	-	-
	2	-	-	-	+	-	-	-	-	-
	3	-	-	-	+	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-	-
	24	+	-	-	-	-	-	-	-	-
Vc	½	-	+	-	+	-	-	-	-	-
	1	-	-	-	+	-	-	-	-	-
	2	-	-	-	+	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-
	4	+	-	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-	-
	24	+	-	-	-	-	-	-	-	-
Vd	½	-	+	-	+	-	-	-	-	-
	1	-	-	-	+	-	-	-	-	-

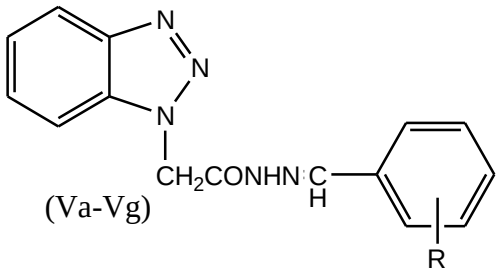
	2	+	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-
	4	+	-	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-	-
	24	+	-	-	-	-	-	-	-	-
Ve	½	-	+	-	+	-	-	-	-	-
	1	-	-	-	+	-	-	-	-	-
	2	-	-	-	+	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-
	4	+	-	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-	-
	24	+	-	-	-	-	-	-	-	-
Vf	½	-	+	-	+	-	-	-	-	-
	1	-	-	-	+	-	-	-	-	-
	2	+	-	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-	-	-
	4	+	-	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-	-
	24	+	-	-	-	-	-	-	-	-
Vg	½	-	-	-	+	-	-	-	-	-
	1	-	-	-	+	-	+	-	-	-
	2	-	-	-	+	-	-	-	-	-
	3	-	-	-	+	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-	-
	24	+	-	-	-	-	-	-	-	-

LOCOMOTOR ACTIVITY

Materials: 0.1% Sodium CMC, Test compounds
Instruments: Sonicator and Actophotometer
Animals: Mice

The locomotor activity was studied by using actophotometer, which operates on photoelectric cells, which are connected in circuit with a counter. When animals cut off beam of light falling on the photocells, a count was recorded. Healthy male mice weighing between 20-25 gm were used. Animals were fasted for overnight and divided into groups of six animals in each group. The test compounds suspended in 0.1% Sodium CMC are administered at a dose of 100 mg/kg body weight i.p. The response (counts) was recorded after 30 min. of administration of drug or test compound [11].

The animals were placed in actophotometer for 10 min. and scores were recorded and the results were compared with the control. The results are presented in Table-3.



(Va-Vg)

Table 3: Locomotor activity of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va -Vg)

S. No.	Compound	Substituents (R)	Locomotor activity (scores) in 10 min, n = 6		% Change in Activity(↓)
			Before Administration	After Administration	
1	Va	-H	408	177	56.6
2	Vb	4-Cl	397	86	78.34
3	Vc	4-OCH_3	429	184	57.16
4	Vd	3-OCH_3 4-OH	373	171	54.27
5	Ve	4-$N(CH_3)_2$	306	95	68.95
6	Vf	2-OH	387	180	53.48
7	Vg	2-Cl	420	115	72.61

n = number of animals

*The compounds were tested at a dose of 100 mg/kg (I.P)

RESULTS AND DISCUSSION

All the compounds 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va - Vg) were schematically synthesized as planned and were authentically identified by their physical and spectral data.

Gross behavioral studies

All the newly synthesized compounds were screened for gross behavioral studies. The gross behavioral studies of the test compounds revealed that all the test compounds exhibited central nervous system depression in the mice.

Table 2 pertaining to the gross behavioral studies of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va - Vg) shows that all the compounds did not show alertness. Among the test compounds, Vb, Vg and Ve showed more depressant activity than the rest of the compounds.

Locomotor activity

Table 3 pertaining to the results of the locomotor activity of the 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va - Vg) in mice showed that all the test compounds reduced the locomotor activity. The locomotor activity was studied by actophotometer. The compound Vb (R = 4-Cl) exhibited more effect among all the compounds with 78.34% reduction activity. The compound Vg (R = 2-Cl) reduced the locomotor activity by 72.61% and the compounds Ve, Vc, Va, Vd and Vf were next in the order of reduction of locomotor activity.

CONCLUSION

The proposed 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N'-(2-substituted benzylidene) acetohydrazide (Va - Vg) derivatives were synthesized successfully as per the planning and as such in all the reactions carried, the expected compounds were obtained with good yield. From the gross behavioral studies and locomotor activity, all the newly synthesized compounds showed CNS depressant activity in mice. The compound Vb with 4-Chloro substitution on the phenyl ring showed more promising depressant activity among all the test compounds.

Acknowledgements

The authors are thankful to the Directors and Principals of Talla Padmavathi College of Pharmacy, Warangal, Sri Shivani Pharmacy College and SVS School of Pharmacy, Warangal, for providing laboratory facilities and financial support.

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