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ORIGINAL RESEARCH ARTICLE

Synthesis, Characterization and Antifungal activity of Certain(E)-1-(1-(substitutedphenyl) ethylidene) - 2 -(6-methylbenzo[d]thiazol-2-yl) hydrazine analogues

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ABSTRACT

In the present study, five new derivatives (R1 to R5) of benzothiazoles were synthesized and were evaluated against four fungal strains: *Candida albicans (MTCC 183), Aspergillus niger (MTCC 228), Candida tropicalis (MTCC 6192), and Fusarium oxysporium (MTCC 3656). p-T*oluidine on treatment with ammonium thiocynate formed 2-benzothiazolamines, which on reacted with hydrazine hydrate formed a hydrazino derivative. Compounds (R1 to R5) were synthesized by reacting hydrazine derivative with different substituted acetophenones. All the synthesized compounds were identified by IR, ^{1}H-NMR and antifungal activity was performed on the synthesized compounds. The results obtained justify the importance of benzothiazoles in the field of therapeutics.

Keywords: Benzothiazole, Substituted Acetophenones, Antifungal activity.

INTRODUCTION

Benz-fused compounds have been employed in the synthesis of number of pharmaceutical compounds because of the significant activities possessed by them (benzotriazole, benzodiazole, benzoxazoles, benzimidazole and benzothiazole etc.) Benzothiazole, a multifaceted nucleus, has been under research for the last two decades. Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization[1]. Many dyes, such as thioflavin, and pharmaceutical drugs, such as riluzole, zolantidine (**Fig.** 1) have benzothiazoles as a structural motif. From the literature survey, it has been found that extensive work has been reported on 2-substituted benzothiazole derivatives in past and evaluated for different activities like antibacterial[1], anticancer[2], antiviral[3], antitumor[4], antifungal [5], antiinflammatory [6]

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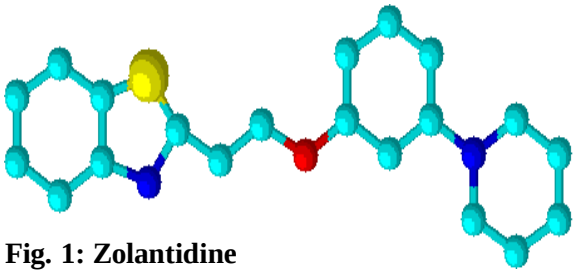
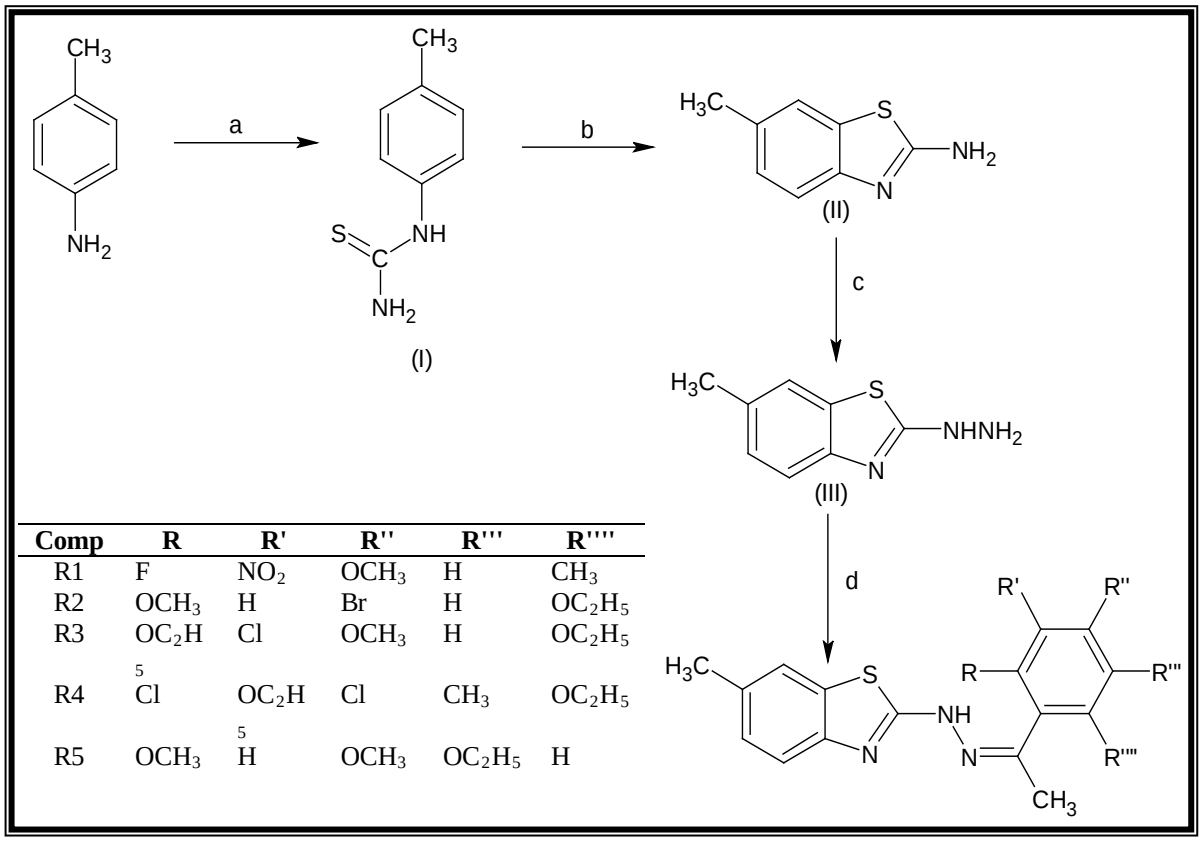


Fig. 1: Zolantidine

antioxidative and radioprotective [7] antidiabetic [8,9], anthelmintic [10], anti-leishmanial [11], anticonvulsant [12], neuroprotective [13]. Taking this into view, certain new derivatives were synthesized taking benzothiazole as the basic moiety.

EXPERIMENTAL

All the chemicals and solvents used during the experimental studies were of analytical grade and procured form CDH, New Delhi and Sigma Chemicals, Mumbai. Melting points of all synthesized compounds were determined using open capillary tube and are uncorrected. IR data were recorded in KBr disks on Perkin Elmer R-IX FTIR spectrophotometer and H^1 NMR spectra on Bruker AC 30 of NMR spectrometer 400 MHz. Scheme for synthesis shown in **Fig 2**



Comp	R	R'	R''	R'''	R''''
R1	F	NO_2	OCH_3	H	CH_3
R2	OCH_3	H	Br	H	OC_2H_5
R3	OC_2H	Cl	OCH_3	H	OC_2H_5
R4	^{5}Cl	OC_2H	Cl	CH_3	OC_2H_5
R5	OCH_3	^{5}H	OCH_3	OC_2H_5	H

Fig 2: Reagents and Conditions; a) ammonium thiocyanate, HCl, H_2O, reflux, 22h; b) HBr, H_2SO_4, reflux, 2h; c) $NHNH_2$, ethylene glycol, reflux, 4h; d) appropriate substituted acetophenones, glacial CH_3COOH, EtOH.The structures of the compounds (R1-R5) were determined by using spectroscopic methods including IR and ^{1}H-NMR

CHEMISTRY

p-Tolylthiourea (I)

p-Toluidine (5.35g) was dissolved in a mixture of conc. HCl (4.3ml) and water (11.6ml) by heating on water bath. Cooled the contents and added solid ammonium thiocyanate (3.5g), heated the mixture on water bath for about 22 hours. Cooled the precipitated product and filtered. Washed with water 3-4 times and dried. Recrystallized with aqueous methanol to get cream colour crystals. IR: 3435 (N-H), 2999 (Aliphatic C-H), 1612 (N-H), 1462

(Aromatic C=C), 1310 (AromaticC-H). ^{1}H-NMR: 3.35 (s, 2H, NH2), 7.24-7.11 (dd, 4H, Ar-H), 2.30 (s, 3H, CH_3).

2-Amino-6-methylbenzothiazole (II)

15ml of conc. H_2SO_4 was added to *p-Tolylthiourea* **(I)** (8.3g) and the temperature of mixture was raised to 80^0C on water bath. Then 48% hydrobromic (0.5g) acid was added slowly and the reaction mixture was stirred for 2 hours at 80^0C and cooled to room temperature. The reaction mixture was slowly introduced to cold water and then adjusted to pH 9 or 10 by adding ammonia water. The whole mixture was stirred for 1 hour by heating at 70^0C and then cooled to room temperature. The mixture was extracted 2 times with dichloromethane and the combined extract was dried with anhydrous sodium sulphate and evaporated to obtain the title compound. IR: 3395 (N-H), 3261 (N-H), 1462 (AromaticC=C), 1326 (AromaticC-N), 1253 (C-S). ^{1}H-NMR: 3.45 (s, 2H, NH_2), 7.32-7.26 (m, 3H, Ar-H), 2.34 (s, 3H, CH_3).

2-Hydrazino-6-methylbenzothiazole (III)

2-amino-6-methybenzothiazole **(II)** (20g) [0.82mmol] and hydrazine hydrate (85%) [0.11mmol] in 50ml of ethylene glycol, refluxed by stirring for 4 hours (60^0C). The colour of reaction changed to green and the homogeneous solution appeared. A white solid was precipitated at the end of the reflux period. The mixture was cooled and the product was filtered and then washed with water several times. Air dried and recrystallized from ethanol. IR: 3434 (NHNH), 3162 (Aromatic C-H), 3000 (Aliphatic C-H), 1611 (N-H). ^{1}H-NMR: 9.59 (s, 1H, NH), 7.34-7.11 (m, 5H, Ar-H), 3.37 (s, 2H, NH_2), 2.26 (s, 3H, CH_3).

(E)-1-(1-(2-fluoro-4-methoxy-6-methyl-3-nitrophenyl) ethylidene) – 2 - (6 - methylbenzo[d]thiazol-2-yl) hydrazine (R1)

2-Hydrazino-6-methylbenzothiazole **(III)** (1.5mmol) and 2-fluoro-4-methoxy-6-methyl-3-nitroacetophenone (2.2mmol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20ml) and refluxed on water bath for 8 hours till a different spots on TLC may appears. On cooling, solid get separated which was filtered and wash with little water and recrystallized with absolute ethanol. IR: 3428 (N-H), 3087 (Aromatic C-H), 1613 (C=N), 823 (Aromatic C-N). ^{1}H-NMR: 8.11-7.92 (m, 7H, Ar-H), 7.02 (s, 1H, NH), 6.6 (t, 3H, CH), 3.73 (s, 3H, CH), 2.35 (s, 3H, CH_3).

(E) – 1 - (1 - (4 – bromo – 2 – methoxy – 6 - ethoxyphenyl) ethylidene) – 2 - (6 – methylbenzo [d] thiazol-2-yl) hydrazine (R2)

2-Hydrazino-6-methylbenzothiazole **(III)** (1.5mmol) and 4-bromo-2-methoxy-6-ethoxyacetophenone (2.2mmol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20ml) and refluxed on water bath for 8 hours till a different spots on TLC may appears. On cooling, solid get separated which was filtered and wash with little water and recrystallized with absolute ethanol. IR: 3434 (NH) 3164 (Aromatic-CH), 1612 (C=N), 1581 (NH), 699 (C-Br). ^{1}H-NMR: 8.10-7.94 (m, 7H, Ar-H), 7.00 (s, 1H, NH), 6.5 (t, 3H, CH), 3.98 (s, 3H, CH_2), 1.33 (s, 3H, CH_3).

(E) – 1 - (1 - (3 – chloro – 2, 6 – diethoxy - 4 – methoxyphenyl) ethylidene) – 2 - (6 - methylbenzo[d]thiazol-2-yl) hydrazine (R3)

2-Hydrazino-6-methylbenzothiazole **(III)** (1.5mmol) and 3-chloro-2,6-diethoxy-4-methoxyacetophenone (2.2mmol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20ml) and refluxed on water bath for 8 hours till a different spots on TLC may appears. On cooling, solid get separated which was filtered and wash with little water and recrystallized with absolute ethanol. IR: 3435 (N-H), 3165 (Aromatic C-H), 1612 (C=N), 1581 (N-H), 1285 (Aromatic C-N). ^{1}H-NMR: 8.07-7.96 (m, 7H, Ar-H), 7.00 (s, 1H, NH), 5.8 (t, 3H, CH), 3.91 (s, 3H, CH_2), 2.35 (s, 3H, CH_3).

(E) – 1 - (1 - (2, 4 – dichloro - 3,6 – diethoxy – 5 – methylphenyl) ethylidene) -2

- (6-methylbenzo[d]thiazol-2-yl) hydrazine (R4)

2-Hydrazino-6-methylbenzothiazole **(III)** (1.5mmol) and 2,4-dichloro-3,6-diethoxy-5-methylacetophenone (2.2mmol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20ml) and refluxed on water bath for 8 hours till a different spots on TLC may appears. On cooling, solid get separated which was filtered and wash with little water and recrystallized with absolute ethanol. IR: 3434 (N-H), 3164 (Aromatic C-H), 1612.1 (C=N), 1582 (N-H), 800.8 (Aromatic C-Cl). ^{1}H-NMR: 8.12-7.93 (m, 7H, Ar-H), 7.02 (s, 1H, NH), 6.6 (t, 3H, CH), 3.98 (s, 3H, CH_2), 1.33 (s, 3H, CH_3).

[d]thiazol-2-yl) hydrazine (R5)

2-Hydrazino-6-methylbenzothiazole **(III)** (1.5mmol) and 5-ethoxy-2,4-dimethoxyacetophenone (2.2mmol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20ml) and refluxed on water bath for 8 hours till a different spots on TLC may appears. On cooling, solid get separated which was filtered and wash with little water and recrystallized with absolute ethanol. IR: 3433 (O-H and N-H), 3163 (Aromatic C-H), 1610 (C=N), 1415 (Aromatic C=C). ^{1}H-NMR: 8.12-7.93 (m, 7H, Ar-H), 7.02 (s, 1H, NH), 6.9 (s, 3H, CH), 6.2 (s, 3H, CH), 3.98 (s, 3H, CH_2), 1.33 (s, 3H, CH_3).

(E) – 1 - (1 - (5 – ethoxy - 2, 4 –dimethoxy phenyl) ethylidene) – 2 - (6 –methylbenzo

Table 1: Some Physical Characteristics of the Synthesized Compounds.

Comp.	m.p. (°C)	Yield (%)	Mol. Form. & Wt.	Elemental analysis calcd. /found (%)							
				C	H	F	Br	N	O	S	Cl
R1	625.8	67	$C_{18}H_{17}FN_4O_3S$ (388)	55.6 55.4	4.41 4.26	4.89 4.52	- -	14.4 14.2	12.3 12.1	8.26 8.13	- -
R2	671.8	63	$C_{19}H_{20}BrN_3O_2S$ (434)	52.5 52.3	4.64 4.45	- -	- -	9.67 9.52	7.37 7.23	7.38 7.26	- -
R3	599.0	53	$C_{21}H_{24}ClN_3O_3S$ (433)	58.1 58.0	5.57 5.42	- -	18.4 18.3	9.68 9.52	11.0 10.9	- -	8.17 8.07
R4	551.9	69	$C_{21}H_{23}Cl_2N_3O_2S$ (452)	55.7 54.9	5.12 5.01	- -	- -	9.29 9.11	7.07 6.95	- -	15.6 15.4
R5	580.3	62	$C_{20}H_{23}N_3O_3S$ (385)	62.3 62.1	6.01 5.91	- -	- -	10.9 10.78	12.4 12.2	8.32 8.11	- -

RESULTS AND DISCUSSIONS

The efficient synthetic route for the synthesis of benzothiazole derivatives was shown in Fig 1. *p-T*oluidine on treatment with ammonium thiocyanate formed p-Tolylthiourea **(I)** which on reaction with hydrobromic acid yields 2-benzothiazolamines **(II)**, which on reaction with hydrazine hydrate formed a hydrazino derivative **(III)**. Compounds (R1 to R5) were synthesized by reacting hydrazine derivative with different acetophenones.

ANTIFUNGAL ACTIVITY

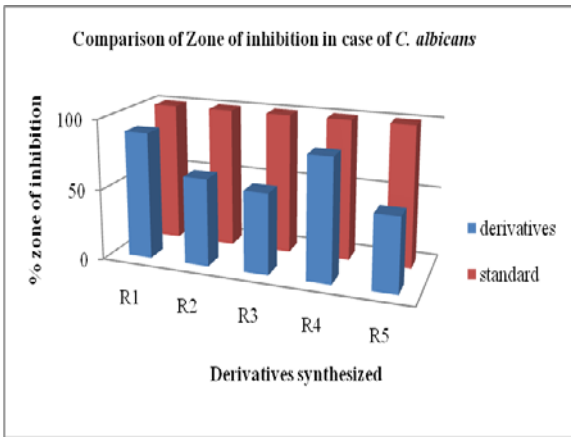
For present work efficacy of five compounds were detected against four fungal strains *Candida albicans (MTCC 183), Aspergillus niger (MTCC 228), Candida tropicalis (MTCC 6192), and Fusarium oxysporium (MTCC 3656)*. The concentration of the test compound used was 1mg/ml Clotrimazole were taken as the standard drug (Table1 and Table 2). Acetone was used as solvent control. The zones of

inhibition obtained in different strains of fungi are shown graphically of *Candida albicans (MTCC 183), Aspergillus niger (MTCC 228), Candida tropicalis (MTCC 6192), Fusarium oxysporium (MTCC 3656) and Candida tropicalis (MTCC 6192)* (Graphs 1-4).

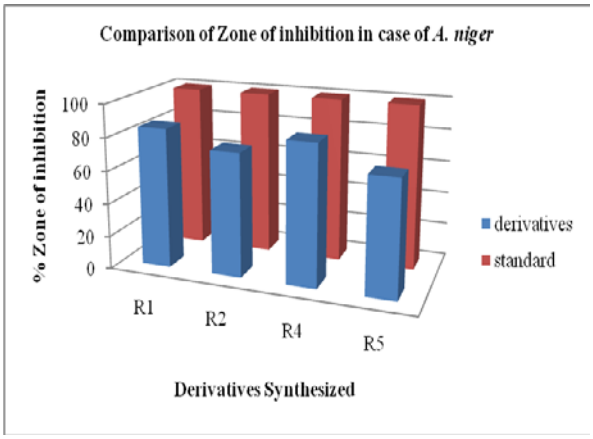
Table 2: Comparison of Zone of Inhibition of various compounds derivatives

Comp.	Antifungal activity (in mm)			
	C. albicans	***A. niger***	***F. oxysporium***	***C. Tropicalis***
STD	21	20	20	21
R1	19	17	17	16
R2	13	15	12	11
R3	12	-	13	15
R4	18	17	13	11
R5	11	14	-	14

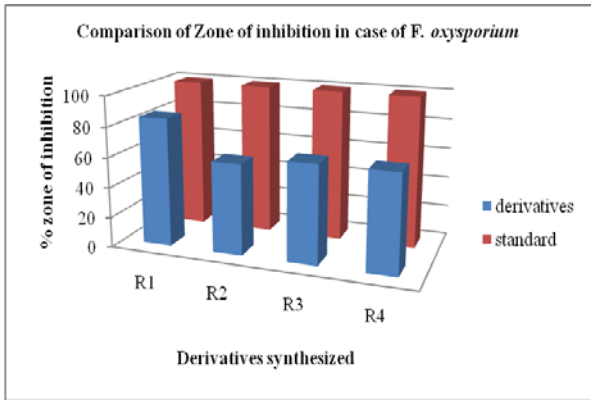
Graph 1: Comparison of Zone of inhibition in case of *C. albicans* with synthesized derivatives



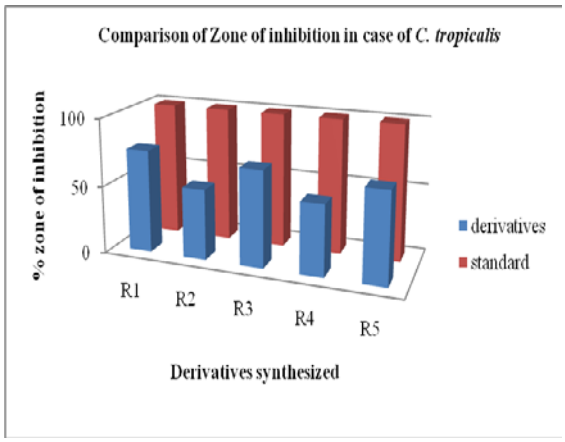
Graph 2: Comparison of Zone of inhibition in case of *A. niger* with synthesized derivatives



Graph 3: Comparison of Zone of inhibition in case of *F. oxysporium* with synthesized derivatives



Graph 4: Comparison of Zone of inhibition in case of *C.Tropicalis* with synthesized derivatives



Compounds R1 showed significant activity against *Candida albicans (MTCC 183), Aspergillus niger (MTCC 228), and Fusarium oxysporium (MTCC 3656)* while R4 showed comparable activity against *Candida albicans (MTCC 183), Aspergillus niger (MTCC 228)* when tested at 1mg/ml concentration taking Clotrimazole as the standard. The rest of the synthesized derivatives showed low to moderate activity. From the above results, it may be concluded that the derivatives of benzothiazoles posses moderate to potent antifungal activity[1,5] when compared to standard Clotrimazole. Therefore, the experimental study justifies the therapeutic application of the benzothiazole moiety in the present era.

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