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SYNTHESIS OF NOVEL *N*-SUBSTITUTED 2-(1*H*-BENZOTRIAZOL-1-YL) - ACETOHYDRAZIDE DERIVATIVES AS ANTIMICROBIAL AGENTS

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Abstract

As a part of research project on the synthesis of number of substituted benzotriazole derivatives with electron donating as well as electron withdrawing groups was done and evaluated them for antibacterial and antifungal activity. First of all benzotriazole was prepared using o-phenylene diamine and sodium nitrite in acidic conditions. Now benzotriazole was reacted with ethyl chloroacetate to form benzotriazole ethyl acetate which was then reacted with hydrazine hydrate to produce benzotriazole acetohydrazide. Finally it was reacted with different sulfonyl chlorides and benzoyl chlorides to give various derivatives. The purity of all compounds have been checked by the TLC monitoring and the confirmation of the structure is checked by different spectral analysis like UV, IR, Mass and NMR and evaluated as antibacterial agent by using sulfacetamide as standard drug and for antifungal activity by using clotrimazole as standard drug.

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Key words:
Benzotriazole derivatives, Benzotriazole acetohydrazide, sulfonation, benzoylation, antifungal activity, antibacterial activity.

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INTRODUCTION:

Benzotriazole and its derivatives are important nitrogen containing heterocyclic compounds with biologically interesting properties and some pharmaceutical applications. Benzotriazole moiety has distinct property on biological system as antifungal, antibacterial, diuretics etc. Imidazole, benzimidazole and benzotriazole moiety and to

screen their diverse activity like anti-inflammatory, antifungal and antimicrobial activity. For the above reasons novel series of benzotriazole derivatives had been planned to synthesize.

EXPERIMENTAL SECTION

The entire chemicals were supplied by S.D. Fine Chem. (Mumbai), Finar Chem. Ltd (Ahmedabad) and Loba Chemie Pvt. Ltd. (Mumbai). Melting points were determined by open tube capillary method and were uncorrected. Purity of compounds was checked by thin layer chromatography (TLC) on silica gel G in solvent system hexane-ethyl acetate (1:2), the spots were located under iodine vapours or UV light. IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr. Mass spectra were obtained using 2010EV LCMS Shimadzu instrument.

General procedure for the Synthesis of 1*H*-benzotriazole:

(0.01mole) of o-phenylene diamine was dissolved in acetic acid and kept under stirring in ice-bath. Now (0.01mole) sodium nitrite was added. Temperature of reaction mixture raised to 80-85°C and automatically went down to 35°C. Then, allowed to stir for 15min. Brown coloured precipitates appeared in cold condition. The precipitates were filtered using vacuum pump and recrystallized with hot water.

Compound: 2

General procedure for the synthesis of ethyl 1*H*-benzotriazol-1-yl-acetate:

To a solution of benzotriazole (0.01mol) in acetone, ethyl chloroacetate (0.01mol) was added. In the presence of dry K_2CO_3, the mixture was kept for stirring for 6 hrs. Filteration was done and the filterate was evaporated on waterbath. The resulting precipitates were recrystallized from ether. Brown needle shaped crystals were obtained.[1]

Compound: 4

General procedure for the synthesis of 2-(1*H*-benzotriazol-1-yl)-1-(hydrazinyloxy)-ethanone:

To the solution of (0.05 mole) of ethyl 1*H*-benzotriazol-1-yl-acetate in ethanol was taken in a clean and dry round bottom flask. Now 0.075 mole of hydrazine hydrate was added. After stirring for 2 hrs the reaction mixture was refluxed for 2 hrs. Now the reaction mixture was cooled in ice bath. White fibre like crystals was obtained.[2]

Compound: 6

General procedure for the synthesis of 2-(1*H*-benzotriazol-1-yl)-*N*'-[(4-substituted phenyl)-sulfonyl]-acetohydrazide:

Ethyl 1*H*-benzotriazol-1-yl-acetate (0.001 mole) was dissolve in 10ml of pyridine in cold condition (0°C) with stirring. The substituted sulfonyl chlorides (0.001 mole) were added. After addition, the reaction mixture was stirred at room temperature for 5 hrs at the end of the reaction period; the reaction mixture was poured in crushed ice. Obtained precipitates were filtered and crude was washed with cold water and cold dilute HCl. Crude was recrystallized from Ethanol.[3]

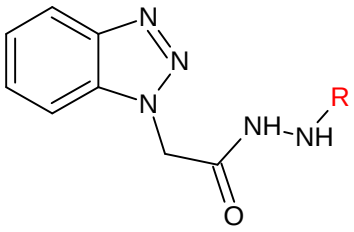
Compound: 7a, 7b, 7c

General procedure for the synthesis of *N*'-(1*H*-benzotriazol-1-yl-acetyl)-4-substituted benzohydrazide:

Ethyl 1*H*-benzotriazol-1-yl-acetate (0.001 mole) was taken in a clean and dry beaker. Now 0.2 ml of substituted benzoyl chloride was added. The mixture was triturated for about 10-15 mins with the help of glass rod. Now crushed ice was added and trituration was continued. Solid product was obtained which was washed with cold water. After filtration recrystallization was done in Ethanol.[4]

Compound: 7d, 7e, 7f

Following compounds were synthesized:



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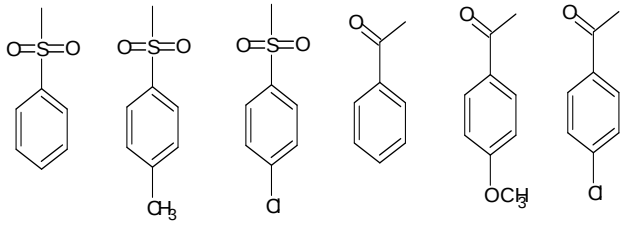
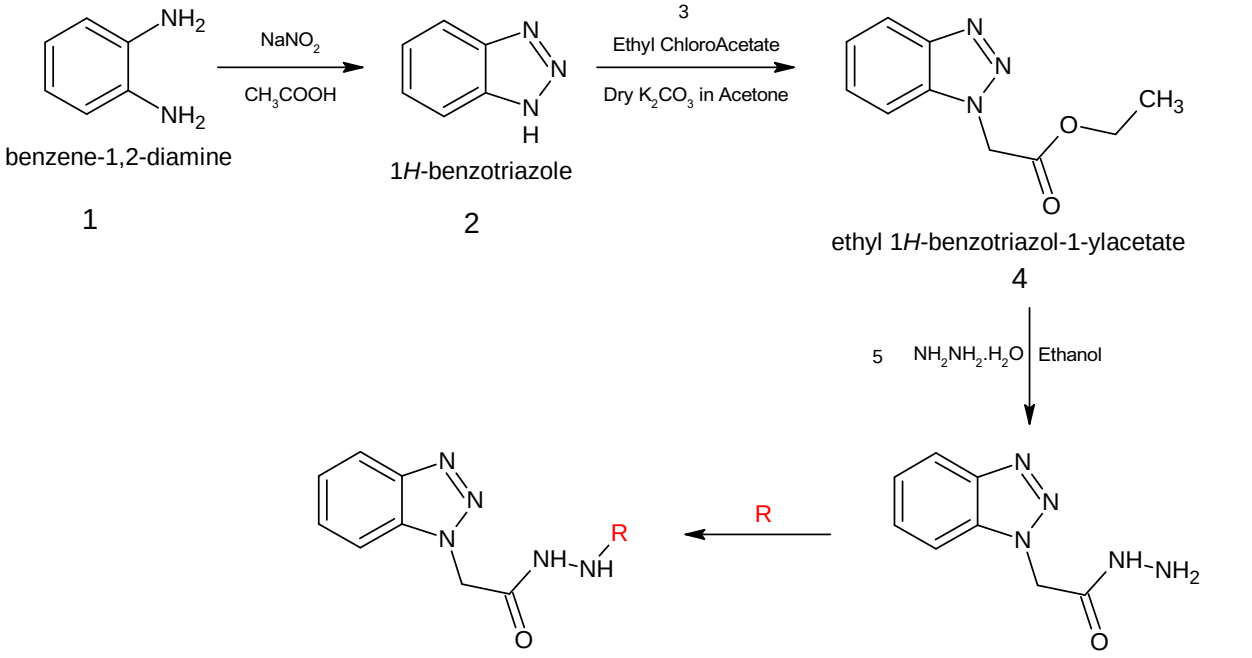
N-Substituted 2-(1*H*-benzotriazol-1-yl)-acetohydrazide

Where R=



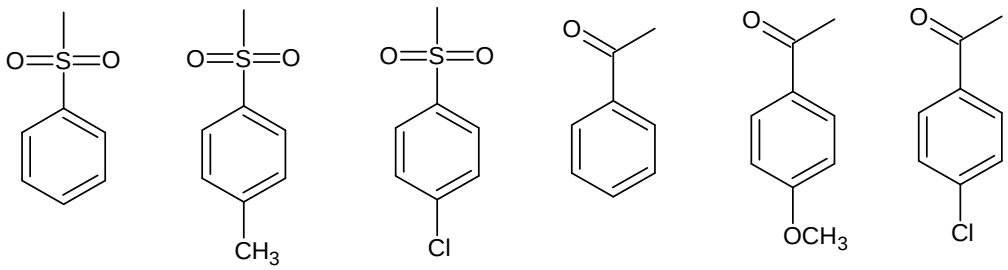
a b c d e f

Scheme 1:



benzene-1,2-diamine (1) → 1*H*-benzotriazole (2) → ethyl 1*H*-benzotriazol-1-ylacetate (4) → 2-(1*H*-benzotriazol-1-yl)acetohydrazide (6) → N-Substituted 2-(1*H*-benzotriazol-1-yl)acetohydrazide (7)

Where R =



a b c d e f

Table 1: Physical Characteristics of synthesized compounds

Compound Code	Molecular Formula	Molecular Weight (g/mol)	Melting Point (°C)	% Yield (%w/w)	Rf
7a	$C_{14}H_{13}N_5O_3S$	331.35	160-162	64	0.55
7b	$C_{14}H_{12}ClN_5O_3S$	365.79	184-186	60	0.63
7c	$C_{15}H_{15}N_5O_3S$	345.38	190-192	58	0.60
7d	$C_{15}H_{13}N_5O_2$	295.29	142-144	71	0.55
7e	$C_{15}H_{12}ClN_5O_2$	329.74	167-169	65	0.65
7f	$C_{16}H_{15}N_5O_3$	325.32	178-181	60	0.60

Table 2: Spectral data of synthesized compounds

Compound Code	UV (λmax, nm)	IR (cm^{-1})	Mass (m/z)	NMR (δ, ppm)
7a	285	NH(3282.12,3391.14),-C=O(1691.14), Ar C-H(3051.63)	331.4[M+]	---
7b	290	NH(3386.77),-C=O(1690),C-Cl(770.31),SO_2(1334.65)	346.4[M+]	---
7c	296	NH(3373.67),-C=O(1663.07),	346.2[M+1]	---
7d	265	NH(3399.05,3289.19),-C=O(1691.10,1651.63), Ar-CH(3065.83)	296.3 [M+1]	---
7e	268	NH(3386.77,3093.61),-C=O(1661.39,1650), Ar-CH(3055.03)	329[M+]	6.86-7.63 (m,8H, Ar**H**), 7.87-7.91 (m,1H,N**H**),4.05-4.09(s,1H,N**H**)
7f	267	NH(3393.65,3286.13),-C=O(1695.05,1654.69),Ar-CH(3062.63)	325.1[M+]	---

Table 3: Log P value of Synthesized Compounds

Compound	Log P
7a	2.44
7b	3.61
7c	4.21
7d	1.90
7e	3.08
7f	3.68
Sulfacetamide	-0.26
Clotrimazole	3.5

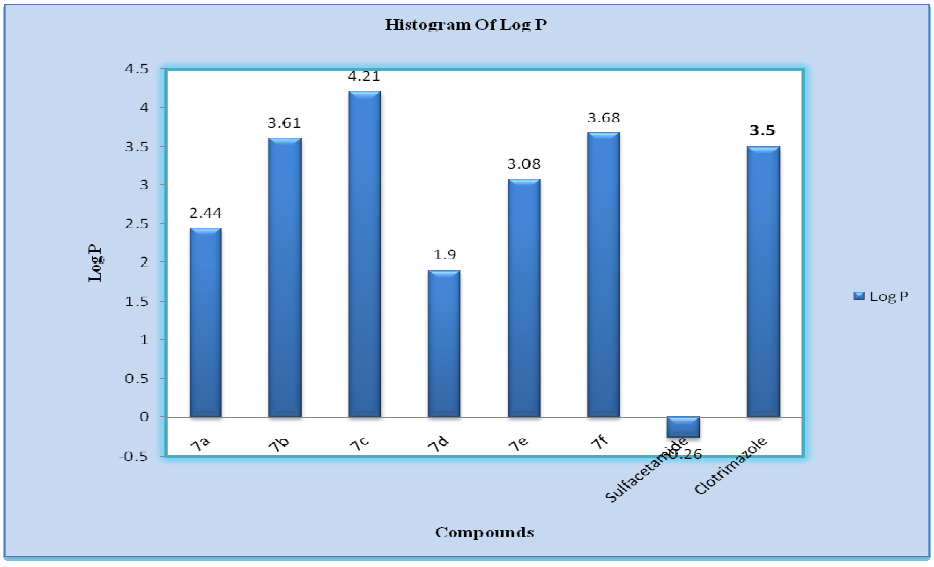


Figure 1: Histogram of Log P

Biological Evaluation:

Biological evaluation holds a great significance in screening the new chemical entities. The newly synthesized compounds were screened for antifungal and antimicrobial activity.

ANTIMICROBIAL ACTIVITY[5-9]

In our current study, evaluation of antimicrobial activity was carried out by using the methods mentioned below. Here responses of microorganisms to the synthesized compounds were measured with that of the standard reference drug. The standard reference drug used was sulfacetamide.

Antibacterial Activity:

The microbiological assay was based upon a comparison of inhibition of growth of micro-organisms by measured concentrations of test compounds with that produced by known concentration of a standard antibiotic. Two methods generally employed were turbidometric (tube-dilution) method and cylinder plate (cup-plate) method. In the turbidometric method inhibition of growth of microbial culture in a uniform dilution of antibiotic in a fluid medium was measured. It was compared with the synthesized compounds. Here the presence or absence of growth was measured. The cylinder plate method depends upon diffusion of antibiotic from a vertical cylinder through a solidified agar layer in a petri dish or plate to an extent such that growth of added micro-organisms was prevented entirely in a zone around the cylinder containing solution of the antibiotics. The cup-plate method was simple and measurement of inhibition of microorganisms was also easy. Here this method was used for antibacterial screening of the test compounds.

Name of Microorganism

- **Gram +Ve microorganisms**

Staphylococcus aureus **(MTCC No. 96)**

Bacillus subtilis **(MTCC No. 121).**

- **Gram -Ve microorganisms**

Escherichia coli **(MTCC No. 521).**

Preparation of medium:-

- Nutrient agar 2%
- Peptone 1%
- Beef extract 1%
- Sodium chloride 0.5%
- Distilled water up to 100ml

All the ingredients were weighed and added to water. This solution was heated on water bath for about one and half-hour till it becomes clear. This nutrient media was sterilized by autoclave.

Apparatus:-

All the apparatus like petri dishes, pipettes, glass rods, test-tubes were properly wrapped with papers and sterilized in hot air oven.

Agar plate disc diffusion method:

- The antibacterial activity was assayed by agar plate disc diffusion method at the concentration of 50 µg per disc.
- All the synthesized compounds were tested in vitro for their antibacterial activity against microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis* (gram positive), *Escherichia coli* (gram negative) strains.
- Each test compounds were dissolved in dimethylsulphoxide (DMSO) to get a concentration of 10 mg/mL.
- The disc (6 mm in diameter) was impregnated with 5 µL of each test solution to get 50 µg/disc, air dried and placed on the agar medium, previously seeded with 0.2 mL of broth culture of each organism for 18 hours.
- The plates were incubated at 37 °C for 24 hours and the inhibition zones measured in mm.
- Discs impregnated with DMSO were used as a control and Sulfacetamide discs as antibacterial reference standard.

ANTIFUNGAL ACTIVITY[5-9]

Principle

The antifungal activity of all newly synthesized benzotriazole derivatives were examined against *Candida albicans*.

Antifungal screening of all the derivatives was done by using filter disc method.

Clotrimazole was used as a standard drug. Activity of the compounds was recorded by measuring the zone of inhibition in mm, and compared with the standard zone of inhibition produced by clotrimazole. This determination indicates whether the organism was sensitive or resistant to the compound.

Materials used

- Test organisms: *Candida albicans* was used for the determination of the activity.
- Growth Media: The activity was conducted on the Sabouraud dextrose agar media.

Composition

- Enzymatic digest of Casein 5g
- Enzymatic digest of Animal Tissue 5g
- Dextrose 20g
- Final pH 5.6 ±0.2 at 25 °C
- Purified water 1000ml

Apparatus:

- Petri plate: Glass plate, which was previously sterilized by Dry Heat Sterilization was used.
- Pipette: Micropipette was used for adding the required concentration of the analogues to the plates.
- Glass wares: 500ml conical flask and test tubes were used.
- Compounds screened: all the synthesized benzotriazole derivatives.
- Solvent used: Dimethyl sulfoxide
- Standard used: Clotrimazole

Preparation of standard solution

The standard drug clotrimazole was dissolved in appropriate quantity of ethanol to obtain the concentration range of 500, 750 and 1000µg/ml and the zone of inhibition was checked.

Preparation of test solution: Specified quantity (100mg) of the compound was accurately weighed and dissolved in 100ml of DMSO to get the 1000µg/ml stock solution. Further dilution was made to obtain the concentration in the range 500µg/ml, 750µg/ml and 1000µg/ml.

Procedure

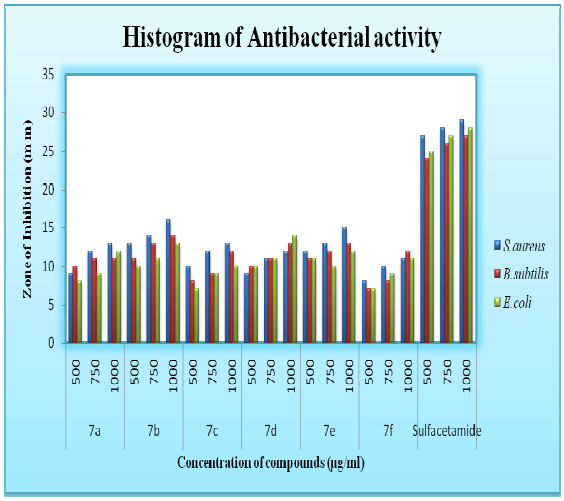
- 30g of the medium was suspended in 1000ml of purified water. The mixture was allowed to boil till it forms a homogeneous solution. The medium was autoclaved at 121°C for 15 minutes at 15psi.
- Media was cooled to the temperature of approximately 40°C temperature and microorganisms were inoculated to the media. 150ml was transferred to a petri plates aseptically. Two such plates were prepared for each organism.
- Plates were allowed to cool for 20 minutes.
- Compounds were dissolved in DMSO and diluted in same to get concentration of 500µg/ml, 750µg/ml and 1000µg/ml.
- Both high and low strength discs were applied for each compound to be tested.
- The organism is reported as being sensitive if clear zone appears around both discs.

Table 4: Screening of Antibacterial activity of synthesized compounds

Compound code	CONC. (µg/ml)	Zone of inhibition (mm)		
		S.aureus	*B.subtilis*	*E.coli*
7a	500	09	10	08
	750	12	11	09
	1000	13	11	12
7b	500	13	11	10
	750	14	13	11
	1000	16	14	13
7c	500	10	08	07
	750	12	09	09
	1000	13	12	10
7d	500	09	10	10
	750	11	11	11
	1000	12	13	14
7e	500	12	11	11
	750	13	12	10
	1000	15	13	12
7f	500	8	7	7
	750	10	8	9
	1000	11	12	11
Sulfacetamide	500	27	24	25
	750	28	26	27
	1000	29	27	28

Table 5: Screening of Antifungal activity of synthesized compounds

Compound code	CONC. (µg/ml)	Zone of inhibition(mm) *C.albicans*
7a	500	10
	750	11
	1000	13
7b	500	11
	750	14
	1000	16
7c	500	7
	750	9
	1000	10
7d	500	8
	750	11
	1000	13
7e	500	12
	750	13
	1000	15
7f	500	9
	750	10
	1000	12
Clotrimazole	500	19
	750	20
	1000	22



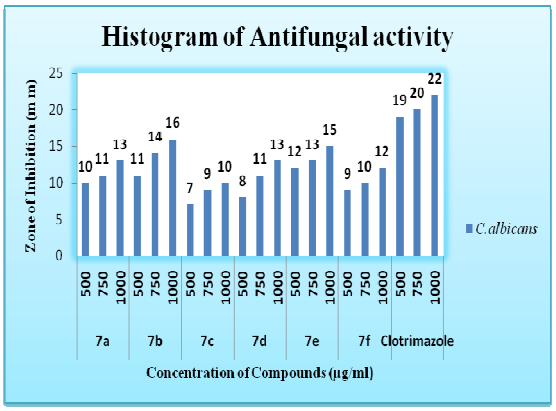


Figure 2

RESULT AND DISCUSSION

All the synthesized compounds were screened for their anti-bacterial activities against *S.aureus, B.subtilis* and *E.coli* and for anti-fungal activities against *Candida albicans*. Compounds ***7a, 7b and 7e*** showed anti-fungal activity but less potent as compared to standard reference drug clotrimazole. Compounds ***7b, 7c and 7e*** showed good anti-bacterial activity but less potent as compared to standard reference drug sulfacetamide. Compounds ***7c, 7d and 7f*** were found to be very less potent towards anti-fungal activity.

Rest all the synthesized compounds were found to be very less potent towards anti-bacterial activity as compared to standard reference drug sulfacetamide.

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