



DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME BENZIMIDAZOLE-BENZTHIAZOLE CARBOHYDRAZIDE DERIVATIVES

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ABSTRACT

Benzimidazole and benzthiazole derivatives play vital role in biological field such as antimicrobial, antiviral, antidiabetic, and anticancer activity. Therapeutic significance of these clinically useful drugs in treatment of microbial infections encouraged the development of some more potent and significant compounds. New chemical entities of benzimidazole condensed with benzothiazole may show enhanced antimicrobial activity and antifungal activity. A series comprising benzimidazole-benzthiazole carbohydrazide substituted by aromatic system were prepared. Investigation of antimicrobial activity of the compounds was done by using Gram-positive (*S. aureus*), Gram-negative (*E. coli*, and *P. aeruginosa*) bacteria and minimum inhibition concentration (MIC) values were determined.

Key words: Benzimidazole-benzthiazole, Carbohydrazide, Antimicrobial activity, Antifungal activity.

INTRODUCTION

There is a growing interest over the past years for the synthesis of benzimidazole-based heterocycles due to the crucial role of benzimidazole unit in the functions of biologically important molecules. In view of biological significance of benzothiazole and benzimidazole moiety, it is yet to be explored synthetically and biologically with several other important heterocyclic systems. Benzothiazoles, azetidinones and thiazolidinones possess wide range of biological and pharmacological activities. In this paper, some benzimidazole-benzthiazole carbohydrazide derivatives have been synthesized with pharmacological activity such as antibacterial and antifungal properties. However, very few references are available on the synthesis and evaluation of condensed benzimidazole-benzthiazole. In continuation of our work on synthesis and evaluation of benzimidazole-benzthiazoles, we have made an attempt to synthesize some novel compounds comprising benzimidazole-benzothiazole moiety

Prompted by these observations and in continuation of our work on biologically active nitrogen heterocycles for the present work the syntheses and biological activity of a series of 2-((benzo[d]thiazol-2-ylthio)methyl)-6-nitro-1H-benzo[d]imidazole-1-carbohydrazide have been carried out. For the this work, 2-(chloromethyl)-6-nitro-1H-benzo[d]imidazole and mercapto benzthiazole have been selected as synthons.

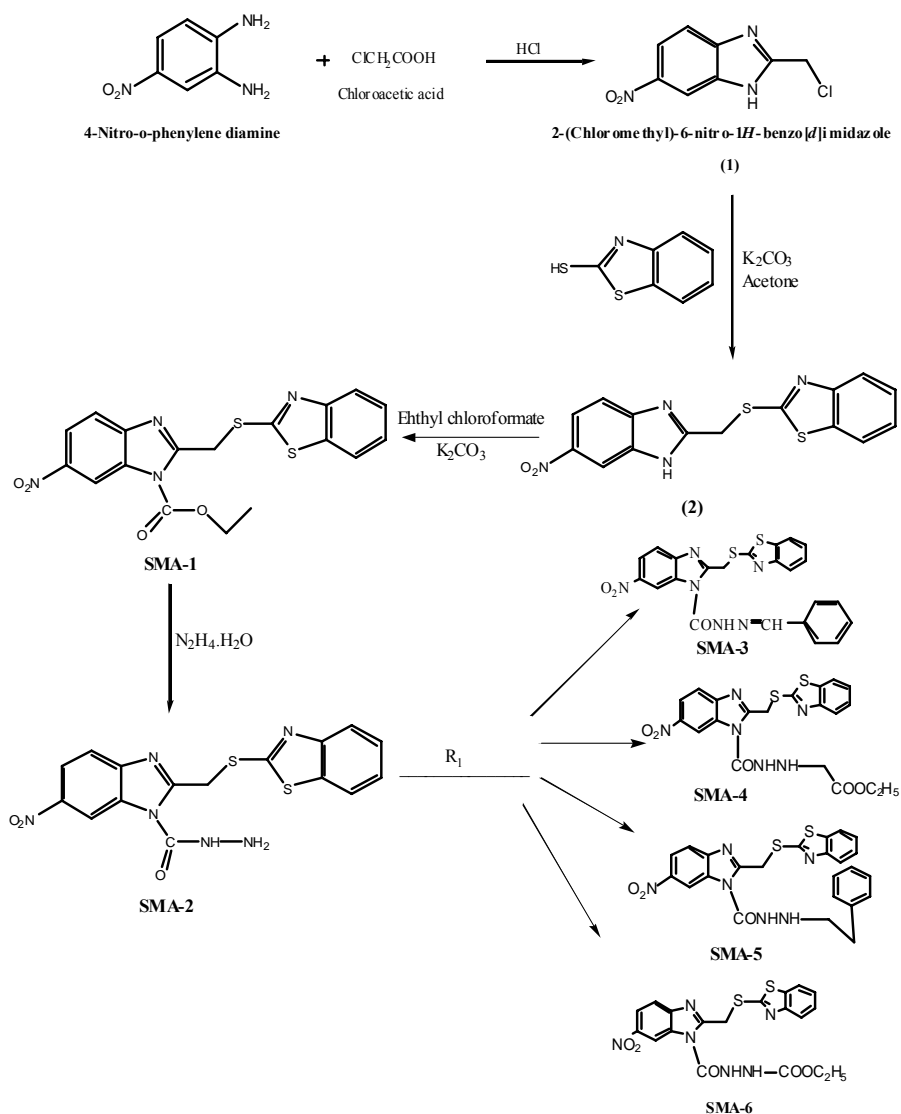
Some novel compounds were prepared by the reaction of **Scheme 1**. The structures of the newly synthesized compounds were confirmed on the basis of analytical, ^1H NMR and mass spectral data. The characterization data of the compounds are given in Tables 1-7.

EXPERIMENTAL

Materials and methods

Melting points were determined in open capillaries with the help of VEGGO melting point apparatus and IR spectra (KBr) were recorded on Shimadzu IR spectrophotometer. ^1H NMR spectra were recorded by Bruker WM 400 FT instrument using D_2O as solvent and tetramethylsilane (TMS) as internal reference standard. All chemical shifts (δ) are in ppm. The purities of the compounds were checked by thin layer chromatography (TLC) on silica gel-G plates. The major chemicals were purchased from Aldrich Chemical Corporation.

Synthetic route for the preparation of title compounds (SMA-2)



R_1 = Benzaldehyde or substituted aldehyde, ethylchloroacetate, or other chlorinated nonaromatic compound, 2-phenylethyl bromide or phenylhalogenated compound, ethyl chloroformate etc.

EXPERIMENTAL

Preparation of 2-(chloromethyl)-6-nitro-1H-benzo[d]imidazole (1)

To a solution of 60 mL 6 N hydrochloric acid, 10 g (0.065 mol) 4-nitro-o-phenylenediamine and 10 g (0.10 mol) chloro acetic acid were added. The reaction mass was heated at 85-90°C for 16-18 hrs. Reaction was monitored by TLC. It was cooled to 25-30°C and the reaction mass was diluted by 10 mL water. The reaction mass was then neutralised by ammonia solution and stir red for 60 min for complete crystallization. Solid mass was separated by filtration. The solid material was purified in DMF and water. It was dried at 70-75°C. Yield – 120 g (86.0%).

Preparation of 2-((benzo[d]thiazol-2-ylthio) methyl)-6-nitro-1H-benzo[d]imidazole (2)

A mixture of 10 g (0.047 mol) (1), acetone (400 mL), 13.0 g potassium carbonate (0.094 mol) and 15.5 g mercaptobenzothiazole (0.092 mol) was stir red for four hrs at reflux temperature. Reaction was monitored by TLC to check completion of reaction. The reaction mass was filtered in hot condition. It was concentrated mass by vacuum and isolated by methanol (60 mL) and water (60 mL). Then the reaction mass was stir red for 120 min for complete crystallization. The material was dried at 60-65°C for 12 hrs. Yield – 12.0 g (80.0%).

Preparation of ethyl 2-((benzo[d]thiazol-2-ylthio)methyl)-6-nitro-1H-benzo[d]imidazole-1-carboxylate (SMA-1)

To a solution of 10 g (0.029 mol) (2), acetone (300 mL), anhydrous potassium carbonate 5.0 g (0.036 mol) and ethyl chloroformate 4.5 g (0.041 mol) were added. Then reaction mass was refluxed for 60-90 min. Reaction was monitored by TLC, filtered in hot condition and concentrated under vaccun. The material was isolated by ethanol and filtered to separate out the solid material. It was dried at 60-65°C for 6.0 hrs. Yield – 10.0 g (83.0%).

Preparation of 2-((benzo[d]thiazol-2-ylthio)methyl)-6-nitro-1H-benzo[d]imidazolecarbohydrazide (SMA-2)

A mixture of ethanol 300 mL, 10 g (0.024 mol) (SMA-1) and hydrazine hydrate 2.5 g (0.05 mol) was heated and stirred for 60 min at reflux temperature for completion of reaction. Reaction was monitored by TLC. It was concentrated by vaccum and isolated by 200 mL binary mixture of MDC and Hexane (2:8). The reaction mass was filtered and the product was dried at 60-65°C for 8.0 hrs. Yield – 8.0 g (83.0%).

Preparation of 2-((benzo[d]thiazol-2-ylthio)methyl)-N'-benzylidene-6-nitro-1H-benzo[d]imidazole-1-carbohydrazide (SMA-3)

A mixture of 10 g (0.25 mol) (SMA-2), 200 mL ethanol, 100 mL toluene with 4-5 drops of acetic acid and 3.0 g (0.028 mol) benzaldehyde was taken. It was heated at reflux condition for 12.0 hrs. The reaction was monitored by TLC (only 30-40% conversion is possible). Maximum solvent was distilled under vacuum. The material was isolated with 40 mL with methanol and the reaction mass was filtered to separate out unreacted material. Filtrate was concentrated and purified by column chromatography with hexane and ethyl acetate mobile phase. Yield – 2.0 g.

Preparation of ethyl 2-(2-((benzo[d]thiazol-2-ylthio)methyl)-6-nitro-1H-benzo[d]imidazole-1-carboamido) acetate (SMA-4)

To solution of 10 g (0.025 mol) (SMA-2), acetone (300 mL), and anhydrous potassium carbonate 8.0 g were added (0.036 mol) and stirred for 10-15 min. Then ethylchloro acetate 6.0 g (0.048 mol) was added. Reaction mixture was refluxed for 60-90 min. Reaction was monitored by TLC. It was filtered in hot

condition and concentrated under vacuum and isolated by ethanol. Then it was filtered to separate out the solid material and dried at 60-65°C for 6.0 hrs. Yield – 8.0 g (66.6%).

Preparation of 2-((benzo[d]thiazol-2-ylthio)methyl)-6-nitro-N'-phenethyl-1H-benzo[d]imidazole-1-carbohydrazide (SMA-5)

To a solution of 10 g (0.025 mol) (SMA-2), acetone (300 mL), and anhydrous potassium carbonate 8.0 g were added (0.036 mol) and stirred for 10-15 min. Then 2-phenylethyl bromide 5.0 g (0.027 mol) was added and refluxed for 24.0 hrs. Reaction was monitored by TLC. It was filtered in hot condition and concentrated under vacuum and isolated by acetonitrile (40 mL). The solution was filtered to separate out the solid material and dried at 60-65°C for 6.0 hrs. Yield – 5.6 g (46.0%).

Preparation of ethyl 2-(2-((benzo[d]thiazol-2-ylthio)-6-nitro-1H-benzo[d]imidazole-1-carboamido)acetate (SMA-6)

A mixture of 10 g (0.025 mol) (SMA-2), dichloromethane (200 mL) and triethylamine 4.0 g (0.036 mol) was stirred for 10-15 min and then ethyl chloroformate 3.5 g (0.048 mol) was added. It was stirred for 4.0 hrs. Reaction was monitored by TLC. After completion of reaction, water was added to separate the organic phase. It was concentrated by vacuum and the material was isolated by methanol - ethanol 100 mL. The solid material was separated by filtration and dried at 60-65°C for 6.0 hrs. Yield – 8.5 g (72.0%).

Analysis of the product

Table 1: Physical data of the compounds

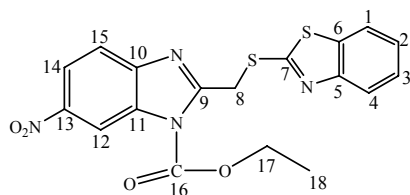
Compd.	Structure formula	Molecular Mass	Appearance	Solubility	Melting point
SMA-1	C ₁₈ H ₁₄ N ₄ O ₄ S ₂	414.6	Off white powder	Soluble in chloroform	140-146°C
SMA-2	C ₁₆ H ₁₂ N ₆ O ₃ S ₂	400.43	Light brown powder	Soluble in dimethyl sulfoxide	150-155°C
SMA-3	C ₂₃ H ₁₆ N ₆ O ₃ S ₂	488.5	Light yellow powder	Soluble in methanol	127-134°C
SMA-4	C ₂₀ H ₁₈ N ₆ O ₅ S ₂	486.5	Light brown powder	Soluble in chloroform	119-125°C
SMA-5	C ₂₄ H ₂₀ N ₆ O ₃ S ₂	504.5	Yellow powder	Soluble in dimethyl sulfoxide	135-140°C
SMA-6	C ₁₉ H ₁₆ N ₆ O ₅ S ₂	472.5	Light brown powder	Soluble in dimethyl sulfoxide	155-160°C

Antimicrobial activity test

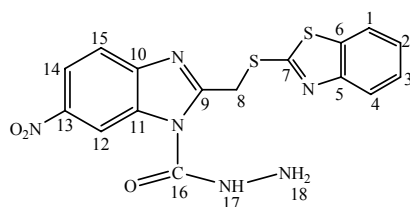
The compounds (SMA-3, SMA-4, SMA-5 and SMA-6) were tested by Broth dilution method (It is one of the non-automated in vitro bacterial susceptibility tests). The bacterial strains used were *Staphylococcus aureus* MTCC 96 (Gram-positive) and *Escherichia coli* MTCC 442, *S. Pyogenus* MTCC 443 and *Pseudomonas aeruginosa* MTCC 441 (all Gram-negative).

For testing the antifungal activity of the synthesized compounds, the fungal strains *Candida albicans* MTCC 227 and *Aspergillus niger* were used. The inhibition zones of synthesized compounds were determined using by Broth dilution method. In this method, each synthesized drug was diluted to obtain 2000 microgram /mL concentration, as a stock solution.

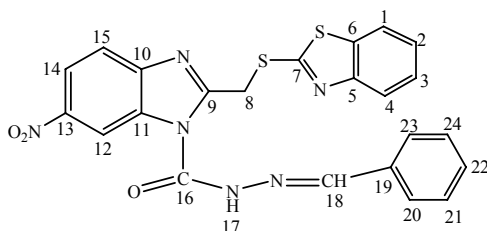
Primary screen: In primary screening, 1000 microgram/mL, 500 microgram/mL, and 250 microgram/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Table 2: Spectral data of SMA-1

¹ H NMR in CDCl ₃ (d ⁶)			Molecular mass	
Chemical shift (δ) value (ppm)	Multiplicity	Assignments of proton	Theoretical	Observed
8.872-8.578	<i>d</i>	12		
8.275-8.258	<i>d</i>	4		
8.093-7.757	<i>m</i>	14,15,1		
7.432-7.301	<i>t</i>	2,3	414.6	414.0
5.212-5.166	<i>d</i>	17		
4.693-4.676	<i>s</i>	8		
1.623-1.605	<i>s</i>	18		

Table 3: Spectral data of SMA-2

¹ H NMR in DMSO (d ⁶)			Molecular mass	
Chemical shift (δ) value (ppm)	Multiplicity	Assignments of proton	Theoretical	Observed
8.457-8.4525	<i>s</i>	12		
8.116-8.122	<i>d</i>	4		
8.0943-8.0889	<i>d</i>	17		
8.036-8.017	<i>d</i>	14		
7.896-7.876	<i>d</i>	1		
7.726-7.703	<i>d</i>	15		
7.495-7.454	<i>t</i>	3	400.43	400.0
7.3936-7.3536	<i>t</i>	2		
4.9805	<i>s</i>	8		

Table 4: Spectral data of SMA-3

¹ H NMR in CDCl ₃ (d ⁶)			¹³ C NMR in DMSO		Molecular mass	
Chemical shift (δ) value (ppm)	Multiplicity	Assignments of proton	Chemical shift (δ) value	Assignments of carbon	Theoretical	Observed
8.886-8.883	<i>s</i>	12	165.04	16		
8.362-8.335	<i>d</i>	4	164.02	7		
8.023-8.003	<i>d</i>	14	153.42	5		
7.906-7.886	<i>d</i>	1	150.04	10		
7.629-7.590	<i>t</i>	17	147.04	18	488.5	489
7.537	<i>t</i>	18,2	143.75	13,9		
7.516-7.494	<i>d</i>	3	138.81	6		
7.494-7.457	<i>d</i>	15	134.38	19		
7.425-7.367	<i>m</i>	23	129.47	11,22		
7.280-7.243	<i>t</i>	20	128.69	24,21		
6.626-6.607	<i>d</i>	24	126.55-126.23	23,20		
5.974	<i>d</i>	21,22	124.27	2,3		
5.134	<i>s</i>	8	122.27	1,4		
			120.27	14		
			119.76	15		
			117.70	12		
			31.27-30.32	8		
IR (cm⁻¹)	NO₂	1556.55				
	C=O	1635.64				
	C=N	1693.50				

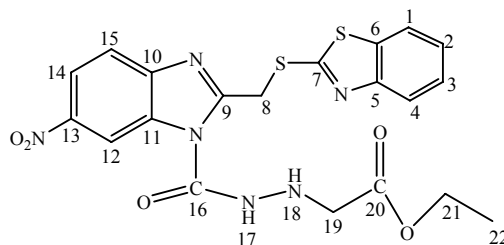
Secondary screen: The drugs found active in primary screening were similarly diluted to obtain 200 microgram/mL, 100 microgram/mL, 50 microgram/mL, 25 microgram/mL, 12.5 microgram/mL, and 6.250 microgram/mL, concentrations.

The highest dilution showing at least 99% growth inhibition was taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 10⁸ organism/mL.

This is often used to determine the smallest amount of antibiotic necessary to inhibit a test organism. This amount is known as the minimum inhibitory concentration (MIC). A set of tubes with different

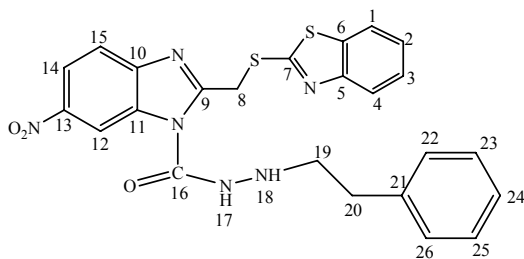
concentrations of a particular antibiotic are prepared. The tubes are inoculated with the test organism, incubated, and examined for growth of bacteria.

Table 5 Spectral data of SMA-4

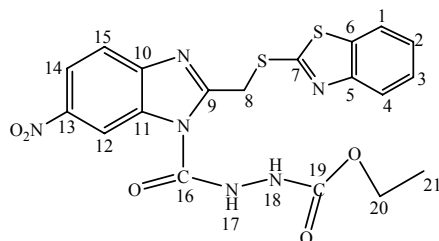


¹ H NMR in CDCl ₃ (d ⁶)			¹³ C NMR in DMSO		Molecular Mass	
Chemical shift (δ) value (ppm)	Multiplicity	Assignments of proton	Chemical shift (δ) value	Assignments of carbon	Theoretical	Observed
8.573-8.569	<i>s</i>	12	167.71-167.55	20		
8.157-8.108	<i>d</i>	14	165.02-164.98	7		
7.799-7.779	<i>d</i>	15	156.08	16		
7.731-7.678	<i>dd</i>	2,3	154.69	5		
7.401-7.363	<i>t</i>	4	152.22	10		
7.279-7.194	<i>m</i>	1	143.09-143.02	13	486.5	486.0
5.300-5.225	<i>s</i>	8	140.95-140.09	9		
4.891-4.877	<i>d</i>	21	135.15-134.92	6		
4.126-4.056	<i>m</i>	19	126.41	11		
1.174-1.110	<i>m</i>	22	124.69	2		
			121.93	3		
			121.21	4		
			119.26	1		
			118.35	14		
			117.78	15		
			115.13	12		
			61.54-61.51	21		
			45.35-45.30	19		
			28.45	8		
			13.85	22		
IR (cm⁻¹)	NO₂	1565.55				
	C=ONH	1635.64				
	NH	3219.19				

Table 6: Spectral data of SMA-5



$^1\text{H NMR}$ in CDCl_3 (d^6)			$^{13}\text{C NMR}$ in DMSO		Molecular Mass	
Chemical shift (δ) value (ppm)	Multiplicity	Assignments of proton	Chemical shift (δ) value	Assignments of carbon	Theoretical	Observed
8.795-8.790	<i>s</i>	12	165.13-165.09	7		
8.520-8.515	<i>d</i>	4	155.40	16		
8.220-8.172	<i>m</i>	14,1	154.16	5		
8.014-7.991	<i>d</i>	17	152.26	10		
7.824-7.803	<i>d</i>	15	146.27	13		
7.734-7.671	<i>m</i>	2,3,24	142.75-142.64	9		
7.366-7.325	<i>t</i>	23,25	140.97	21	504.5	505.0
7.253-7.191	<i>m</i>	22,26	139.46	6		
5.128-5.102	<i>s</i>	8	137.86-137.64	11		
4.664-4.564	<i>m</i>	19,20	128.98	23,25		
2.099	<i>s</i>	18	128.44-18.37	22,26		
			126.72-126.64	2,24		
			124.64	3		
			121.90	1		
			121.16	4		
			119.00	14		
			117.88-117.35	15		
			115.03	12		
			45.46-45.38	19		
			35.16-35.15	20		
			28.57	8		
IR (cm^{-1})	NO₂	1558.55				
	C=ONH	1716.65				
	NH	3649.32				

Table 7: Spectral data of SMA-6

¹ H NMR in DMSO(d ⁶)			¹³ C NMR in DMSO		Molecular Mass	
Chemical shift (δ) value (ppm)	Multiplicity	Assignments of proton	Chemical shift (δ) value	Assignments of carbon	Theoretical	Observed
8.564-8.558	<i>s</i>	12	167.81-167.58	7		
8.322-8.241	<i>dd</i>	4	165.82-164.97	16		
8.150-7.912	<i>m</i>	1,14	156.07	19		
7.880-7.860	<i>d</i>	15	154.69	5		
7.501-7.364	<i>m</i>	2,3	151.21	10		
5.232-5.215	<i>d</i>	8	143.07-143.01	13		
4.642-4.569	<i>m</i>	20	141.95-140.07	9	472.5	472
1.527-1.479	<i>m</i>	21	135.15-134.82	6		
			126.41	11		
			124.67	2		
			121.83	3		
			121.21	4		
			119.26	1		
			118.35	14		
			117.88	15		
			115.13	12		
			61.57-61.51	20		
			28.45	8		
			13.75	21		
IR (cm⁻¹)	NO₂	1525.69				
	C=ONH	1755.22				
	NH	3649.32				

Growth was diminishing as the concentration of antibiotic was increased, and eventually an antibiotic concentration may be observed at which growth fails to occur. This is the minimum inhibitory concentration-MIC zone of inhibition.

The principle used here is that antibiotic will diffuse from a paper disc or small cylinder into agar medium that contains test organisms. Inhibition was observed as a failure of the organism to grow in the region of the antibiotic. A common application of this method is the Kirby Bauer test, developed in the 1960s. The procedure is used to determine the sensitivity of an organism isolated from a patient to a series of antibiotics.

The results serve as a guide to physician to prescribe a drug. An agar medium such as Mueller Hinton medium is inoculated with the organism and poured to the plate. Paper discs containing known concentrations of antibiotics are applied to the surface, and the plate was incubated. The appearance of a zone of inhibition surrounding the disc is indicative of sensitivity. By comparing the diameter of the zones to a standard table, one may determine if the test organism is susceptible, or resistant to the antibiotic. If the organism is susceptible, it is likely to be killed in the blood stream of the patient if that concentration of the drug is reached. Resistance indicates that the antibiotic will not be effective at that concentration in the blood stream.

The data on antimicrobial activity of compounds ((SMA-3, SMA-4, SMA-5 and SMA-6)) are shown in below table with standard.

Table 8: Antibacterial activity

Minimal inhibition concentration				
Code No.	<i>E. Coli</i>	<i>P. Aeruginosa</i>	<i>S. Aureus</i>	<i>S. Pyogenus</i>
	MTCC 442	MTCC 441	MTCC 96	MTCC 443
(Microgram/mL)				
SMA-1	200	250	100	125
SMA-4	125	200	200	250
SMA-5	50	200	62.5	100
SMA-6	62.5	125	250	200

Table 9: Antifungal activity

Minimal fungicidal concentration		
Code No.	<i>C. albicans</i>	<i>A. niger</i>
	MTCC 227	MTCC 282
(Microgram/mL)		
SMA-1	1000	> 1000
SMA-4	1000	500
SMA-5	250	> 1000
SMA-6	500	1000

Table 10: Activity of standards drugs

Drugs	Minimal inhibition concentration			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenus</i>
	MTCC 442	MTCC 441	MTCC 96	MTCC 443
(Microgram/mL)				
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	--	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table 11: Minimal fungicidal concentration

Drugs	<i>C. albicans</i>	<i>A. niger</i>
	MTCC 227	MTCC 282
(Microgram/mL)		
Nystatin	100	100
Griseofulvin	500	100

RESULTS AND DISCUSSION

With the purpose of finding new chemical entities, benzimidazole was condensed with benzothiazole. It shows the enhanced antimicrobial activity as compared to standard drug chloramphenicol and antifungal activity as compared to standard drug griseofulvin against *C. Albicans*.

CONCLUSION

Benzimidazole-benzthiazole nucleus has been reported to possess several medicinal properties such as antibacterial, antiviral, anticancer, anticonvulsant, antihistaminic, anthelmintic, antidepressant, antiasthmatic, antidiabetic activity etc. However; more experimental and clinical researches should be conducted to support its therapeutic use. In conclusion, synthesis at different positions of benzimidazole give a wide variety of compounds with broad spectrum of pharmacological activity as benzimidazole itself is present as a novel nucleus

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