Synthesis and Antimicrobial Screening of Three Triazole Derivatives

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ABSTRACT: Due to the medicinal importance of triazole derivatives, the antimicrobial property of three synthesized triazole derivatives were screened *in vitro* against some Gram positive and Gram negative pathogenic bacteria and some pathogenic fungi. In this study, three compounds 2-(5-mercapto-4-phenyl-4H-[1,2,4] triazole-3-yl]-cyclohexa-1,5-dienol (S_1), 2-[5-mercapto-4-(2-mercapto-phenyl)-4H- [1,2,4] triazole-3-yl]-phenol (S_2) and 4-[3-(2-hydroxy-phenyl)-5-mercapto-[1,2,4] triazole-4-yl]-benzene sulfonamide (S_3) have been synthesized. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were assessed by serial dilution technique. The compounds showed moderate antimicrobial activity against all the tested organisms and the results are comparable to standard antibiotic kanamycin. The MIC values of the compounds were found to be in the range of 16~64µg/ml. The low MIC and MBC values and high sensitivity of pathogenic microorganisms to the compounds led to conclude that the triazole derivatives have potential antimicrobial properties.

Key words: Antimicrobial, MIC, MBC, Triazole derivatives.

INTRODUCTION

One of the main causes of morbidity and mortality in immunocompromised patients in the developing countries is mainly due to the frequent life threatening infective diseases originated from various pathogenic microorganisms. A large number of drugs have been discovered so far to combat such situation. But none of these drugs could completely destroy such microorganisms in some cases. It is mainly because these organisms are developing resistance towards such drugs. As a result, the drugs already in use are gradually losing their effectiveness. The discovery of novel antibiotics which are much more effective against such microorganisms is essential. The 1,2,4-triazoles and their derivatives are found to be associated with various biological activities such as anticonvulsant[1], antifungal[2], anticancer[3], antiinflammatory[4] and antibacterial properties.[5] Also several compounds containing 1,2,4-triazole rings are well known as drugs. For example, fluconazole is used as an antimicrobial drug, while vorozole, letrozole and anastrozole are non-steroidal drugs used for the treatment of cancer. Furthermore, in recent years some Schiff base derivatives of 1,2,4-triazoles and their reduced derivatives have been also found to possess important pharmacological activities.[6-8] In this context, these biological data prompted to investigate the antimicrobial activity of synthesized 2-(5-mercapto-4-phenyl-4H-[1,2,4] triazole-3-yl]-cyclohexa-1,5-dienol (S_1), 2-[5-mercapto-4-(2-mercapto-phenyl)-4H- [1,2,4] triazole-3-yl]-phenol (S_2) and 4-[3-(2-hydroxy-phenyl)-5-mercapto-[1,2,4] triazole-4-yl]-benzene sulfonamide (S_3).

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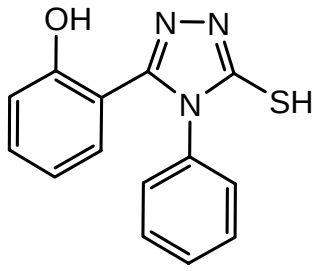

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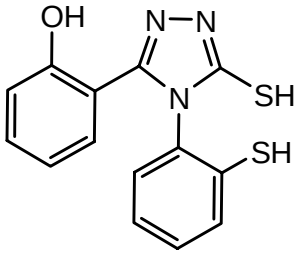

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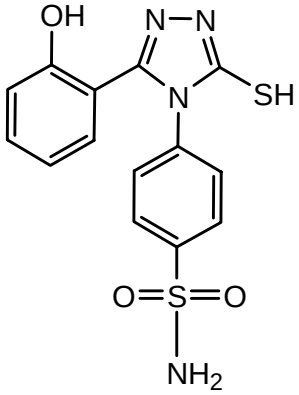

Structure S_3

MATERIALS AND METHODS

The organisms used were collected from the Microbiology Laboratory of the Institute of Nutrition and Food Sciences, Dhaka University, Bangladesh. All chemicals used throughout the research were purchased from BDH (England) and used without further purification.

Preparation of inoculums. Suspension of organism was prepared as per McFarland nephelometer standard. A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted so that it contained approximately 1.5×10^8 cells/ml. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05 ml of 1.175% of barium chloride and 9.95 ml of 1% sulphuric acid.

Antimicrobial screening. The synthesized compounds were screened *in vitro* for their antimicrobial activity against three gram-negative *(Escherichi coli, Shigella shiga* and *S. sonnei*) and three gram-positive *(Bacillus megaterium, B. subtilis, and Sarcina lutea*) bacterial strains using disc diffusion method. Briefly, three calculated amount (5 mg, 10 mg and 20 mg) of the compounds S_1, S_2 and S_3 were dissolved in 1 ml of DMSO in three different vials for getting solutions having concentrations of 50 μg/disc, 100 μg/disc and 200 μg/disc respectively. They were then applied on filter paper disc. Standard kanamycin (30 μg/disc) was used as positive control and DMSO as negative control. Both experimental and control discs were placed in petridishes seeded with organism in nutrient agar medium. The petridishes were kept in a refrigerator at 4°C for 24 hours to ensure diffusion of the test materials. Finally, they were incubated at 37±1°C for 24 hours and all experiments were done as triplicates. The antibacterial activity was determined by measuring the diameter of zone of inhibition in mm.

Determination of MIC and MBC. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of S_1, S_2, and S_3 were determined by serial dilution technique against the above mentioned pathogenic bacteria. S_1, S_2 and S_3 were used from a concentration of 1 μg/ml to 512 μg/ml. A control test-tube containing only medium (nutrient broth medium) was used to confirm the sterility of the medium. Bacterial suspension (10 μl) containing 10^7 cells/ml was inoculated into all tubes. All of the test tubes were incubated at 37±1°C and observed for bacterial growth for 24 hours for MIC and 96 hours (4 days) for MBC determinations

After inoculation for 24 hours, the test tube with no visible growth of the microorganism was taken to represent the MIC value of the sample in μg/ml. MBC, in which no viable organism occurred was determined by keeping the test tubes which was used for MIC determination for four days. After four days, bacterial growth was observed and MBC was determined at lowest concentrations where no bacterial growth was observed.

Antifungal activity. For antifungal screening, each sample was tested at concentrations of 100, 200, 400 μg/disc. The experimental protocol was almost same as antibacterial screening except the plates were incubated at 37±1˚C for 48h and Nystatin disc was used as positive control. All experiments were done in triplicates.

Statistical analysis. The experimental results have been expressed as the mean ± SEM (Standard Error of Mean). Statistical analysis was performing with SPSS software of 10 versions.

RESULTS AND DISCUSSION

The results for the antibacterial activity of S_1, S_2, and S_3 as well as the Kanamycin have been presented in Table 1. The compounds showed a significant antibacterial activity against all test organisms. The diameter of zone of inhibition of S_1 at the dose of 50 μg/disc against *B.megaterium, B. subtilis, E. coli, S. lutea, S. shiga,* and *S. sonnei* were found to be 18 ± 1.2, 19 ± 1.6, 20 ± 1.0, 17 ± 1.7, 20 ± 2.1 and 24 ± 2.5 mm, respectively. These results indicate that compound S_1 is more effective against *Bacillus megaterium* (24±2.5 mm) and all these values were quite comparable with those obtained with kanamycin at dose 30 μg/disc in Table 1. The values of zone of inhibition for the compounds S_2 and S_3 at 50 μg/disc were also quite comparable with kanamycin at 30 μg/disc. Compound S_2 showed highest inhibition of growth of *S. lutea* and *B. megaterium* (20 ± 1.2 mm and 19 ± 1.0 mm respectively) whereas compound S_3 revealed highest activity against *Shi. shiga* (20 ± 3.6 mm). All these compounds showed higher zone of inhibition when tested with higher doses (Table 1).

Table 1. Antibacterial activity of S_1, S_2, S_3 and Kanamycin

Name of bacteria	Category of bacteria	Diameter of zone of inhibition (mm)			Name of Sample	Standard (Kanamycin) 30 μg/disc
		50 μg/disc	100 μg/disc	200 μg/disc		
Shigella sonnei	Gram negative bacteria	18 ± 1.2	19 ± 1.5	22 ± 1.0	2-(5-mercapto-4-phenyl-4H-[1,2,4] triazole-3-yl]-cyclohexa-1,5-dienol	23 ± 0.4
Escherichia coli		19 ± 1.6	20 ± 2.2	21 ± 2.6		24 ± 1.0
Shigella shiga		20 ± 1.0	21 ± 1.5	22 ± 0.5		26 ± 0.5
Bacillus subtilis	Gram positive bacteria	17 ± 1.7	19 ± 1.2	20 ± 1.2		26 ± 0.5
Sarcina lutea		20 ± 2.1	23 ± 1.0	29 ± 2.1		27 ± 1.0
Bacillus megaterium		24 ± 2.5	26 ± 2.8	28 ± 2.5		36 ± 0.4
Shigella sonnei	Gram negative bacteria	14 ± 1.0	15 ± 1.9	16 ± 1.4	2-[5-mercapto-4-(2- mercapto-phenyl)-4H-[1,2,4] triazole-3-yl]-phenol	23 ± 0.5
Escherichia coli		15 ± 0.8	17 ± 2.0	19 ± 2.0		24 ± 0.4
Shigella shiga		17 ± 2.5	19 ± 1.1	21 ± 1.6		26 ± 0.5
Bacillus subtilis	Gram positive bacteria	17 ± 2.5	19 ± 2.0	20 ± 0.4		26 ± 1.5
Sarcina lutea		20 ± 1.2	21 ± 2.5	29 ± 3.1		27 ± 0.5
Bacillus megaterium		19 ± 1.0	22 ± 1.2	24 ± 1.1		36 ± 1.3
Shigella sonnei	Gram negative bacteria	14 ± 1.0	16 ± 2.6	19 ± 1.0	4-[3-(2-hydroxy-phenyl)-5-mercapto-[1,2,4] triazole-4-yl]-benzene sulfonamide	23 ± 1.1
Escherichia coli		15 ± 3.2	17 ± 1.3	19 ± 2.1		24 ± 1.5
Shigella shiga		20 ± 3.6	19 ± 0.3	21 ± 2.5		26 ± 1.0
Bacillus subtilis	Gram positive bacteria	17 ± 1.8	19 ± 1.2	20 ± 0.4		26 ± 1.2
Sarcina lutea		17 ± 3.5	21 ± 1.0	29 ± 3.1		27 ± 0.5
Bacillus megaterium		16 ± 2.8	19 ± 3.4	23 ± 2.0		36 ± 2.1

Table 2. Minimum inhibitory concentration MIC and MBC of S_1, S_2 and S_3.

Test organism	S_1		S_2		S_3	
	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
Shigella sonnei	32	128	64	256	32	256
Escherichia coli	32	64	16	256	16	64
Shi. shiga	16	32	32	64	32	128
Bacillus subtilis	16	32	16	128	64	128
Sarcina lutea	32	128	32	128	32	128
B. megaterium	16	64	32	64	64	256

Table 3. Antifungal activity of S_1, S_2, S_3 and Nystatin

Name of fungi	Diameter of zone of inhibition (mm)			Name of Sample	Standard (Nystatin) 100 µg/disc
	100 µg/disc	200 µg/disc	400 µg/disc		
Candida albicans	R	13 ±1.6	14 ± 2.1	2-(5-mercapto-4-phenyl-4H-[1,2,4] triazole-3-yl]-cyclohexa-1,5-dienol	25 ± 2.3
Aspergillus fumigatus	R	12 ± 1.2	14 ± 1.8		26 ± 1.1
Vasinfactum sp.	R	R	13 ± 1.2		23 ± 0.6
Human-3 sp.	R	11 ± 2.1	14 ± 1.0		21 ± 1.0
Aspergillus flavus	R	15 ± 1.4	17 ± 0.4		29 ± 0.7
Aspergillus niger	R	16 ±2.2	19 ± 1.3		31 ± 1.3
Candida albicans	R	R	08 ± 0.3	2-[5-mercapto-4-(2-mercapto-phenyl)-4H-[1,2,4] triazole-3-yl]-phenol	22 ±1.2
Aspergillus fumigatus	R	10 ±0.8	14 ± 1.3		26 ± 3.1
Vasinfactum sp.	R	R	11 ± 0.4		24 ± 2.2
Human-3 sp.	09±1.3	12 ±1.5	14 ± 1.5		21 ± 1.0
Aspergillus flavus	R	R	11 ± 2.3		29 ± 1.5
Aspergillus niger	R	R	12 ± 0.2		30 ± 0.5
Candida albicans	R	13 ±1.0	17 ± 1.4	4-[3-(2-hydroxy-phenyl)-5-mercapto-[1,2,4] triazole-4-yl]-benzene sulfonamide	24 ± 2.0
Aspergillus fumigatus	R	R	11 ± 1.1		26 ± 2.5
Vasinfactum sp.	08±1.7	11 ±1.8	15 ± 2.5		31 ± 0.5
Human-3 sp.	R	R	09 ± 1.0		33 ±1.6
Aspergillus flavus	R	R	11 ± 1.2		26 ± 2.4
Aspergillus niger	R	R	12 ± 0.4		29 ±1.3

R= Resistance

The MIC values of S_1, S_2, and S_3 were determined against early cited six pathogenic bacteria and the results are shown in Table 2. The MIC values of S_1 were found to be between 16-32 µg/ml and the MBC values for S_1 were 32-128 µg/ml. On the other hand the MIC and MBC values of S_2 and S_3 were also found to be between 16-64 and 64-256 µg/ml in Table 2. From the above results, it is said that there is evident that there is no concrete correlation between antimicrobial activity, MIC and MBC values.

Antifungal activity of the compounds were also determined at three different doses (100, 200, 400 µg/disc) against six pathogenic fungi such as *Aspergillus flavus, A. fumigatus, A. niger, Candida albicans, Human-3* sp, and *Vasinfctum* sp. At lower doses all these organisms were almost insensitive to the fat compounds but at higher doses the compounds showed mild to moderate antifungal activity which are given in Table 3. Compound S_1 showed highest activity against *Aspergillus niger* (zone of inhibition 16 ± 2.2 mm at 200 µg/disc) whereas S_2 and S_3 were effective to same extent against *Human-3* sp. (zone of inhibition 09 ±1.3 mm at 100 µg/disc) and *Vasinfctum sp*. (zone of inhibition 08±1.7 mm at 100 µg/disc) respectively.

In the present study we observed almost similar antimicrobial characteristics of 2-(5-mercapto-4-

phenyl-4H-[1,2,4] triazole-3-yl]-cyclohexa-1,5-dienol (S_1), 2-[5-mercapto-4-(2- mercapto-phenyl)-4H- [1,2,4] triazole-3-yl]-phenol (S_2) and 4-[3-(2-hydroxy-phenyl)-5-mercapto-[1,2,4] triazole-4-yl]-benzene sulfonamide (S_3). It is clear that the synthesized compounds have broad spectrum of antimicrobial activity. Further experiments are required to investigate the actual mechanism of bioactivities and their probable effects on animal model.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Biochemistry and Molecular Biology, Rajshahi University for kindly providing laboratory facilities to carry out the work..

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