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Research Article

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF SOME NOVEL TRIAZOLE DERIVATIVE

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ABSTRACT

In recent year nitrogen containing azole ring and their analoug have attracted strong attention for the discovering of novel and potent anti-microbial agents. Triazole are important class of heterocyclic compounds, found in many potent biologically active molecules such as Rizatriptan, Vorozole, Posaconazole, Fluconazole, and Anastrozole. The increasing clinical importance of drug-resistant fungal and bacterial pathogens has lent additional urgency to microbiological research and new antimicrobial compound development. For this purpose new series of triazole derivative were synthesized and evaluated their Minimum inhibitory concentration. Minimum inhibitory concentration (MIC) may be defined, as the lowest concentration of antimicrobial agent requires to inhibit the growth of microorganism.The minimum inhibitory concentration (MIC) values of the synthesized compound were evaluated against (+ ve bacteria) Streptococus pyrogen (Sp), Bacillus aureus (Ba), Micrococus leuteus (Ml), Streptococus epidermis (Se), Clostridium sporogen (Cs), (- ve bacteria) Klebsiella pneumonia (Kp), Salmonella typhimurium (St), Pseudomonas aeruginosa (Pa), Serratia marcesens (Sm) and Proteus vulgaris (Pv) and fungal strain such as Gibberella fujikuroi (Gf), Rhizopus oligosporus (Ro), Neurospora crassa (Nc), Aspergillus niger (An), Candida albican (Ca). The studies show that compounds a, g, A1, B1 exhibited promising good antimicribial activity.

Keywords: Triazole, Gram(+) bacteria, Gram(-) bacteria, Fungi and MIC ($\mu g/ml$).

INTRODUCTION

It is well known that the emergence of multi resistant strains of bacteria is most commonly connected with the misuse and excessive use of antibiotics, in human and in veterinary medicine alike. Even though there are over 200 kinds of antibiotics and chemotherapeutics on the market nowadays, including 50 kinds of penicillin, 70 kinds of cephalosporine and 20 kinds od quinolone, the problem of multi resistance of bacteria to antibiotics is at its peak. Multi resistant strains serve as fatal infections worldwide in animals and humans. Azole moiety is of great importance to chemists as well as biologists as it is found in a large variety of naturally occurring compounds and also chemically useful molecules having diverse biological activities. Triazole compounds are an important class of antimicrobial agents because of their generally broad antimicrobial spectrum, high potency and low toxicity[1]. Triazole derivatives displace

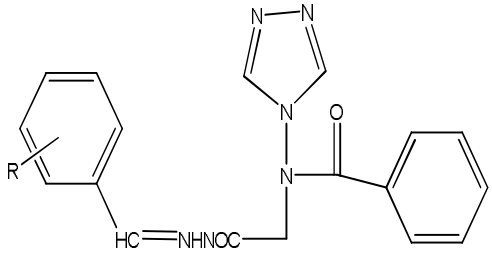
lanosterol from lanosterol 14-demethylase, a cytochrome P450-dependent enzyme, and block the biosynthesis of an essential component of the fungal cell membrane, ergosterol[2]. In recent years a significant portion of research work in heterocyclic chemistry has been devoted to 1, 2, 4-Triazole containing different aryl groups as substituent[3]. Triazole moiety has also been found to have other important activities such as analgesic[4], antimicrobial[5-6], antitubercular[7], anticonvulsant[8], antitumor[9], antiinflammatory[10], HIV-RT inhibitory activity[11] and plant-growth regulatory activities[12] activities.

MATERIALS AND METHODS

minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. This procedure is a standard assay for antimicrobials. In the continuation of global effort for the determination of new and potent derivative of triazole, the present study has focused on the determination of minimum inhibitory concentration (MIC) of triazole derivative of two different series.

Series i : N-(substituted benzylidene)- 2-(N-(4H-1,2,4 triazole-4-yl)benzamido) acetohydrazide

4-Amino triazole (0.01mole) reacts with Benzoyl chloride (0.01mole) and form 4H-(1,2,4-triazole-4-yl)benzamide which reacts with sodium ethoxide(0.01mole) and ethyl chloro acetate (0.01mole). The resulted product reacts with hydrazine hydrate (0.01 mole) and produced N-(substituted benzylidene)- 2-(N-(4H-1,2,4 triazole-4-yl) benzamido) acetohydrazide which reacts with different aromatic aldehyde (0.01mole) in ethanol (30 ml) for 8 hrs in presence of few drops of conc H_2SO_4 acid to get the final product.

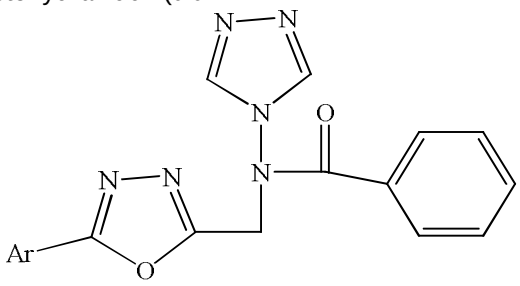


N-(substituted benzylidene)-2-(N-(4H-1,2,4-triazole-4-yl)benzamido) acetohydrazide

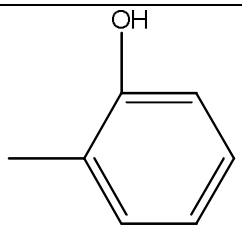
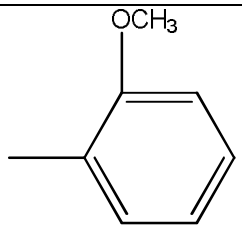
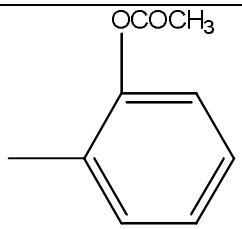
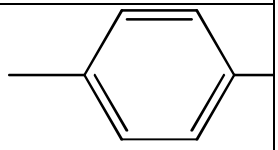
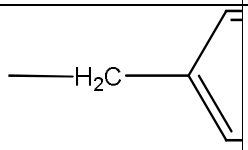
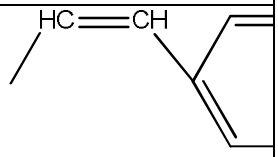
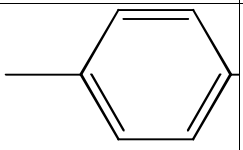
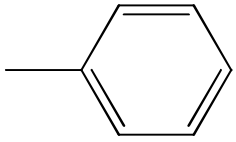
S. No.	R	S. No.	R
a	H	f	4-Cl
b	4-CH_3	g	4-$N(CH_3)_2$
c	4-OCH_3	h	4-OH
d	3,4-OCH_3	i	3-NO_2
e	2-Cl	j	4-NO_2

Series ii: N-((5-(substituted aryl)-1,3,4-oxadiazole -2-yl)methyl-N-(4H-1,2,4 triazole-4-yl)benzamide

N-((5-(substituted aryl)-1,3,4-oxadiazole -2-yl)methyl-N-(4H-1,2,4 triazole-4-yl)benzamide obtained by the reaction of 2-(N-(4H-1,2,4 triazole-4-yl)benzamido)acetohydrazide (0.01 mole) with different aromatic acid (0.01mole) in the presences of phosphorus oxy chloride (10 ml) for 8 hrs and bacify with sodium bicarbonate solution . The separated precipitate was filtered and recrystallised from ethanol to get the final product.



N-((5-(substituted aryl)-1,3,4- oxadiazole-2-yl) methyl-N-(4H-1,2,4- triazole-4-yl) benzamide

S. No.	Ar	S. N.	Ar
A_1		E_1	
B_1		F_1	
C_1		G_1	
D_1		H_1	

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. This procedure is a standard assay for antimicrobials.

Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

Preparation of stock solution

Stock solutions of standard compound and synthesized compound having concentration 250 µg/ml prepared by dissolving 25mg of synthesized compound in 2ml of DMSO and make upthe volume 100 ml with sterile distilled water. From this stock solution different concentration such as 100 µg/ml , 50 µg/ml, 25 µg/ml, 12.5 µg/ml , 6.25 µg/ml ,3.12 µg/ml ,1.56 µg/ml were prepared.

Preparation of Nutrient broth (ph 7.03±0.1)

S.N.	Ingredient	Quantity
1	Peptone	10 g
2	Beef Extract	10 g
3	NaCl	5 g
4	Distilled water	Up to 1000 ml

Name of Microorgnism

S. No.	Gram(+)	Gram(-)	Fungi
1	*Streptococus pyrogen* (NCIM-2608)	*Klebsiella pneumonia* (NCIM-2957)	*Gibberella Fujikuroi* (NCIM-655)
2	*Bacillus aureus* (NCIM-2797)	*Salmonella Typhimurium* (NCIM-2501)	*Rhizopus- oligosporus* (NCIM-1215)
3	*Micrococus leuteus* (NCIM-2704)	*Pseudomonas aeruginosa* (NCIM-2863),	*Neurospora- crassa* (NCIM-908),
4	*Streptococus epidermis* (NCIM-2493)	*Serratia marcesens* (NCIM-2078)	*Aspergillus niger* (NCIM-618)
5	*Clostridium sporogen* (NCIM-2559)	*Proteus vulgaris* (NCIM-2813)	*Candida albican* (NCIM-3557)

Antibacterial assay

The newly prepared compounds were screened for their MIC activity in DMSO by serial plate dilution method.[13] Sabourand's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. The Nutrient Broth, which contained logarithmic serially two fold diluted amount of test compound and controls, was inoculated with approximately 10^5 c.f.u/ml (colony forming unit/mL) of actively dividing bacteria cells. The cultures were incubated for 24 h at 37^0C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (MIC).

Antifungal assay

Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. The Nutrient Broth, which contained logarithmic serially twofold diluted amount of test compound and controls, was inoculated with approximately 10^5 c.f.u/ml (colony forming unit/mL) was used. The cultures

were incubated for 48 h at 35^{0}C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentrations.

RESULT and DISCUSSION

In the present study, MIC was determined by using **"Serial plate dilution technique".** In this technique the plates of broth medium, containing graded doses of compounds are inoculated with test organisms. After suitable incubation, growth will occur in those plates where the concentration of compound is below the inhibitory level and the culture will become turbid (cloudy). Therefore, growth will not occur above the inhibitory level and the plates will remain clear. Triazole derivative such as a, g, A1, B1 showed highest activity against tested microorganisms. It appears that triazole derivative shown promising antibacterial and antifungal activity.

Table 1: mics (µg/ml) value of n-(substituted benzylidene)-2-(n-(4h-1,2,4 triazole-4-yl)benzamido) acetohydrazide derivative against gram (+ ve bacteria) & gram (- ve bacteria)

S.N.	Sp	Ba	Mi	Se	Cs	Kp	St	Pa	Sm	Pv
a	12.5	25	25	25	12.5	3.12	12.5	50	12.5	50
b	12.5	12.5	6.25	6.25	50	6.25	6.25	6.25	25	25
c	6.25	50	6.25	12.5	12.5	6.25	50	-	50	12.5
d	25	25	12.5	50	12.5	12.5	25	25	6.25	25
e	50	100	6.25	6.25	25	25	6.25	6.25	12.5	50
f	6.25	6.25	25	25	50	50	12.5	12.5	-	12.5
g	12.5	25	-	50	25	-	6.25	3.12	25	25
h	6.25	12.5	50	12.5	12.5	50	50	25	50	-
Standard	1.56	3.12	6.25	1.56	3.12	6.25	1.56	6.25	3.12	1.56

Streptococus pyrogen (Sp), Bacillus aureus (Ba), Micrococus leuteus (Ml), Streptococus epidermis (Se), Clostridium sporogen (Cs), Klebsiella pneumonia (Kp), Salmonella typhimurium (St), Pseudomonas aeruginosa (Pa), Serratia marcesens (Sm) and Proteus vulgaris (Pv).

— Indicates bacteria are resistant to the compounds >100 µg/ml.
MIC (µg/ml) = minimum inhibitory concentration, that is lowest concentration to completely inhibit bacterial growth.

Table 2: mics (µg/ml) value of n-(substituted benzylidene)- 2-(n-(4h-1,2,4 triazole-4-yl) benzamido) acetohydrazide derivative against fungi

S.N.	Gf	Ro	Nc	An	Ca
a	12.5	25	25	25	12.5
b	25	6.25	25	50	50
c	-	50	25	12.5	-
d	25	12.5	12.5	25	6.25
e	50	50	6.25	12.5	100
f	6.25	25	25	25	6.25
g	25	-	-	12.5	12.5
h	1.56	25	12.5	12.5	6.25
Standard	1.56	6.25	3.12	1.56	6.25

Streptococus pyrogen (Sp), Bacillus aureus (Ba), Micrococus leuteus (Ml), Streptococus epidermis (Se), Clostridium sporogen (Cs), Klebsiella pneumonia (Kp), Salmonella typhimurium (St), Pseudomonas aeruginosa (Pa), Serratia marcesens (Sm) and Proteus vulgaris (Pv).
— Indicates bacteria are resistant to the compounds >100 µg/ml.
MIC (µg/ml) = minimum inhibitory concentration, that is lowest concentration to completely inhibit bacterial growth

Table 3: mics (μg/ml) value of n-(5-(substituted aryl)-1,3,4-oxadiazole -2-yl)methyl-n-(4h-1,2,4 triazole-4-yl)benzamide derivative against gram (+ ve bacteria) & gram (- ve bacteria)

S.N.	Sp	Ba	Mi	Se	Cs	Kp	St	Pa	Sm	Pv
A1	6.25	100	6.25	25	12.5	12.5	6.25	50	-	25
B1	12.5	12.5	6.25	6.25	50	6.25	12.5	6.25	50	50
C1	50	50	50	50	25	6.25	50	-	50	-
D1	50	12.5	12.5	50	12.5	3.12	50	25	6.25	25
E1	25	6.25	6.25	-	50	25	6.25	6.25	12.5	12.5
F1	6.25	25	25	25	12.5	50	12.5	12.5	12.5	50
G1	12.5	25	25	12.5	25	50	25	25	25	25
H1	6.25	25	-	6.25	12.5	-	6.25	-	25	12.5
Standard	1.56	3.12	6.25	1.56	3.12	6.25	1.56	6.25	3.12	1.56

Streptococus pyrogen (Sp), Bacillus aureus (Ba), Micrococus leuteus (Ml), Streptococus epidermis (Se), Clostridium sporogen (Cs), Klebsiella pneumonia (Kp), Salmonella typhimurium (St), Pseudomonas aeruginosa (Pa), Serratia marcesens (Sm) and Proteus vulgaris (Pv).

— Indicates bacteria are resistant to the compounds >100 μg/ml.

MIC (μg/ml) = minimum inhibitory concentration, that is lowest concentration to completely inhibit bacterial growth

Table 4: mics (μg/ml) value of n-(5-(substituted aryl)-1,3,4-oxadiazole -2-yl) methyl-n-(4h-1,2,4 triazole-4-yl)benzamide derivative against fungi

S.N.	Gf	Ro	Nc	An	Ca
A1	12.5	6.25	-	50	12.5
B1	6.25	25	12.5	25	50
C1	1.56	50	25	12.5	12.5
D1	-	25	25	12.5	12.5
E1	50	50	6.25	-	-
F1	25	25	-	25	6.25
G1	25	1.56	25	12.5	50
H1	25	12.5	-	25	6.25
Standard	1.56	6.25	3.12	1.56	6.25

Streptococus pyrogen (Sp), Bacillus aureus (Ba), Micrococus leuteus (Ml), Streptococus epidermis (Se), Clostridium sporogen (Cs), Klebsiella pneumonia (Kp), Salmonella typhimurium (St), Pseudomonas aeruginosa (Pa), Serratia marcesens (Sm) and Proteus vulgaris (Pv).

— Indicates bacteria are resistant to the compounds >100 μg/ml.

MIC (μg/ml) = minimum inhibitory concentration, that is lowest concentration to completely inhibit bacterial growth.

CONCLUSIONS

Recent advances in antimicrobial chemotherapy, Triazole play important role in the prevention of fungal infection. The work has approached towards the synthetic and biological approach of these wonder molecule. The investigation of antimicrobial screening data reveals that among the compounds of two series were screened and result shows that substitution at various position at triazole moiety, have shown remarkable antimicrobial properties with average inhibition properties.

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