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Synthesis of Some Substituted Triazoles as an Antimicrobial Agents

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ABSTRACT
The substituted triazoles are more important of heterocyclic systems becaus e it is modern agricultural fungicides as well as drugs for fungal diseases in humans. The extra nitrogen atom makes it more like pyridine and so more weakely basic, but it increases its anion is now easy to make. A modern example of an agent used against human fungal infections is Fluconazole, which actually contains two triazoles.
Schiff's bases of certain compounds have shown promising activity against gram positive and gram-negative organisms. Interestingly 1H-benzotriazole when treated with chloracetyl chloride in presence of potassium carbonate to give 1H-benzotriazol-1-ylacetyl chloride [2] The compound [2] further reacts with various amino compounds affordes series of 1H-benzotriazol-1-ylacetyl substituted benzotriazoles [2a-2j] The structure of [2-2a] are confirmed by their IR 1 HNMR and mass spectral data and [2f-2g] by IR spectral data The newly synthesized compounds has been evaluated for their antibacterial and antifungal activities
KEYWORDS:1H-benzotriazole;
Antibacterial activity;Antifungal activity.

I. INTRODUCTION

Humankind has been subject to infection by microorganism since before the dawn of recorded history. One presumes that mankind has been searching for suitable therapy for nearly as long. This was a desperately difficult enterprise given the acute nature of most infections and the nearly total lack of understanding of their origins prevalent until the last century. Although one can find indications in old medical writing of folkloric use of plant and animal preparations, soybean curd, mould bread and cheese, counter infection with other microbes, the slow development of public health measures, and an understanding of the desirability of personal cleanliness, these factors were erratically and inefficiently applied and often failed. Until after the discovery of bacteria 300 years ago, and subsequent understanding of their role in infection about 150 years ago, there was no hope for rational therapy.The modern anti-infective era opened with the discovery of the sulphonamides in France and Germany in 1936 as an offshoot of Paul Ehrlich's earlier achievements in treating infections with organometallics and his theories of vital staining.

Microbes of soil origin remain to this day the most fruitful source of antibiotics, although the specific means employed for their discovery are infinitely more sophisticated today than those employed 50 years ago. Initially extracts of fermentation were screened simply for their ability to kill pathogenic microorganisms in vitro. Those that did were pushed along through ever more complex pathogenic and toxicological tests in attempts to discover clinically useful agents. Today many thousands of such extracts of increasingly exotic microbes are tested each week and the test now include sophisticated assays for agents operating through particular biochemical mechanism or possessing desirable properties. The impact of genomics is expected to have very substantial impact on this effort. As a consequence of this work mankind has now many choices for powerful, effective and specific therapy for some of its most ancient and common bacterial infections1.

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ANTIMICROBIAL AGENTS

Initially the term 'chemotherapeutic agent' was restricted to synthetic compounds, but now since many antibiotics and their analogues have been synthesized, this criterion has become irrelevant; both synthetically and microbiologically produced drugs need to be include together. However, it would be more meaningful to us e the term antimicrobial agent (AMA) to designate synthetic as well as naturally obtained drugs that attenuate microorganisms.

Basis of Antimicrobial Action

Various antimicrobial agents act by interfering with (1) cell wall synthesis, (2) plasma membrane integrity, (3) nucleic acid synthesis, (4) ribosomal function, and (5) folate synthesis.

Biochemical Basis of Antimicrobial Action

Bacterial cells grow and divide, replicating repeatedly to reach the large numbers present during an infection or on the surfaces of the body. To grow and divide, organisms must synthesize or take up many types of biomolecules. Antimicrobial agents interfere with specific processes that are essential for growth and/or division. They can be separated into groups such as inhibitors of bacterial and fungal cell walls, inhibitors of cytoplasmic membranes, inhibitors of nucleic acid synthesis, and inhibitors of ribosome function. Antimicrobial agents may be either bactericidal, killing the target bacterium or fungus, or bacteriostatic, inhibiting its growth. Bactericidal agents are more effective, but bacteriostatic agents can be extremely beneficial since they permit the normal defenses of the host to destroy the microorganisms.

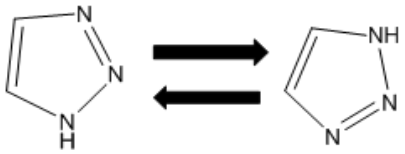
INHIBITION OF BACTERIAL CELL WALL SYNTHESIS

Bacteria are classified as Gram-positive and Gram-negative organisms on the basis of staining characteristics. Gram-positive bacterial cell walls contain peptidoglycan and teichoic or teichuronic acid, and the bacterium may or may not be surrounded by a protein or polysaccharide envelope. Gram-negative bacterial cell walls contain peptidoglycan, lipopolysaccharide, lipoprotein, phospholipid, and protein. The critical attack site of anti-cell-wall agents is the peptidoglycan layer. This layer is essential for the survival of bacteria in hypotonic environments; loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death.

Antimicrobial agents diffuse easily through the loose outer wall of Gram- positive but must go through the narrow channels of the Gram-negative

CHEMISTRY OF TRIAZOLES

Triazoles are five membered rings, which contain two carbons and threenitrogen atoms. There are two Triazoles, and each has one pyrrole like nitrogen andtwo pyridines like nitrogen. Both Triazoles havethe possibility of tautomerism andbothgiverisetoasingleanion



1.2,3- Triazole
(1)

1,2,4- Triazole
(2)

The 1,2,4-triazoles are more stable and possess atomic behavior. Strong oxidising agents attack the side chain of the triazole nucleus, but they do not disrupt the heterocyclic ring. The 1,2,4-triazoles are also very weak base. Triazoles containing an unsubstituted 'NH' group have the capability of forming metal salts.

ANTIMICROBIAL ACTIVITY

The synthesisized compounds were screened against bacteria and fungi to know their antimicrobial activity. To screen these compounds for antibacterial activity bacterias like staphalococcus aureus (gram +ve) and Escherichia coli (gram –ve) and for antifungal activity like Candida albicans and Aspergillus flavus are used.

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ANTIBACTERIAL ACTIVITY

The antibacterial activities are performed by cup plate method (diffusion technique) The fresh culture of bacteria is obtained by inoculating bacteria into peptone water liquid media (Table No.2) and inoculating at 37+2 0 C for 18-24 hours This culture mix ed with nutrient agar media (Table No.3) 20% and poured into Petri dishes by following aseptic techniques. After solidification of the media five bores are made at equal distance by using sterile steel cork borer (8mm diameter) Into these cups different concentrations of standard drug and synthesized compounds are introduced. Dimethyl Formamide is used as a control. After introduction of standard drug and synthesized compounds, the plates were placed in a refrigerator at 8-10 0 C for proper diffusion of drug into the media. After two hours of cold incubation, the Petri plates are transferred to incubator and maintained at 37 0 C+2 0 C for 18-24 hours. After the incubation period, the Petri plates were observed for zone of inhibition by using vernier scale. The result evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drugs. The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated in the Table No.4The standard drugs and synthesized compounds were dissolved in minimum quantity of Dimethyl Formamide (DMF) and adjusted, to made up the volume with distilled water to get 50µg/ml and 100µg/ml concentrations. The procaine penicillin used against staphylococcus aureus and streptomycin used against Escherichia coli as a standard drug.

Table No.2
Preparation of peptone water liquid media

Ingredients	Quantity
Peptone	10gm
Beefextract	10gm
Sodiumchloride	5gm
Distilledwater	Q.S.1000ml

Table No.3
Preparation of assay medium (Indian pharmacopoeia medium)

Ingredients	Quantity
Peptone	6.0gm
Caseinhydroxylatesoyabean	4.0gm
Yeastextract	3.0gm
Beefextract	1.5gm

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Dextrose(dehydrated)	1.0gm
Agar	15.0gm
Distilledwater	Q.S.1000ml

The pH was adjusted to7.4±0.1 at 25^0C temperature.

Table no 4:

Sl. No.	Name of the Compounds	Meanzone of inhibition (inmm)			
		Staphylococcus aureus(+ve)		Escherichia coli(-ve)	
		50µg	100µg	50µg	100µg
1	Procaine penicillin	21	24	+	+
2	Streptomycin	-	-	19	23
3	2a	09	09	15	16
4	2b	09	14	17	16
5	2c	09	12	14	15
6	2d	10	09	12	10
7	2e	15	18	17	14
8	2f	09	11	11	12
9	2g	13	19	13	16
10	2h	09	10	11	13

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11	2i	10	12	09	12
12	2j	09	13	13	17

ANTIFUNGAL SCREENING

The synthesized compounds were screened against two selected fungal strains candida albicans and Aspergillus flavus by using diffusion method. The 48 hours old fungal culture inoculated into nutrient broth (Table No.5) by following aseptic techniques and incubated for 48 hours at 37±20C in a incubator. This culture mixed with potato dextrose agar media (20%) (Table No.6) and poured into Petri plates. After solidification five bores are made at equal distance by using sterile steel cork borer (8mm in diameter). Into these cups different concentration of standard drug and synthesized compounds along with control (Dimethyl Formamide) introduced. After introduction of standard drug and compound, these plates are placed in a refrigerator at 8 0 -10 0 C for two hours for proper diffusion of the drugs. After 2 hours of cold incubation, the Petri plates are transferred to incubator and maintained at 37 0 +2 0 C for 24-36 hours. After the incubation period, the plates were observed for zone of inhibition by using vernier scale. Results evaluated by comparing the zone of inhibition shown by the synthesized compound with standard drug. The results are mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated in Table No. 7 The standard drug and synthesized compounds were dissolved in minimum quantity of DMF and adjusted, to make up the volume with distilled water to get 50µg/ml and100µg/ml concentrations. The Griseofulvin used as a standard drug.

Table No.5
Composition of nutrient broth

Ingredients	Quantity
Peptone	10.0gm
Yeastextract	6.0gm
Potassiumdihydrogenphosphate	3.0gm

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Sodiumchloride	5.0gm
Glucose(anhydrous)	10.0gm
Distilledwater	Q.S.1000ml

pH of the media was adjusted to 7.2($^{\pm}$1) and autoclaved at15Ib/sq.inch.Pressure(121^{0}C) for 15 min.

TableNo.6
Potato–Dextrose agar medium

Ingredients	Quantity
Peeledpotato	200–300.0gm
Dextrose	5.0gm
Agar	20.0gm
Distilledwater	Q.S.1000.0ml

Peeled potato was cut in to piece and boiled for 30 min. to get extract. Dextrose and agar were dissolved in the extract and made up to volume with distilled water and at 15Ib/inch pressure (121 0 C) for 15 min

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Table No.7 Antifungal activity

SI No	Name of the Compounds	Mean of zone inhibition (in mm)			
		Candida albicans		As pergillus flavus	
		50µg	100µg	50µg	100µg
1	Griseofulvin	17	20	18	21
2	2a	12	13	11	15
3	2b	10	10	12	16
4	2c	10	17	14	12
5	2d	13	12	17	15
6	2e	09	11	11	15
7	2f	12	10	12	14
8	2g	10	09	12	11
9	2h	11	10	12	12
10	2i	17	18	15	16
11	2j	16	15	13	17

II. RESULTS

IR spectral data of newly synthesized compounds[2-2f]

Figure No.	Compound	$V_{max}(cm^{-1})$
1	[2]	(C-H)3081,(C=O)1755
2	[2a]	(C-H)3056,(C=O)1632
3	[2b]	(N-H)3362,(Ar-NO_2)1313
4	[2c]	(C-H)3079,(C=O)1790
5	[2d]	(N-H)3327,(C-H)3033,(C=O)1647
6	[2e]	(C-H)3097,(C=O)1746
7	[2f]	(N-H)3452,(C-H)3062,(C=O)1748

^{1}HNMR spectral data of newly synthesized compounds[2-2a]

Figure No.	Compound	δvalue

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1	[2]	5.6(s,2H,CH_2), 8.0-7.3(m,4H,Ar-H),
2	[2a]	4.2(s,1H,NH), 5.79(s,2H,CH_2), 8.10-7.46 (m,8H, Ar-H) 15.74 (s, 1H, COOH

Mass spectral data of newly synthesized compounds[2-2a]

Figure No.	Compound	Mol.Wt (Observed)	Base peaks
1	[2]	191(M^+)	119
2	[2a]	296(M^+)	83

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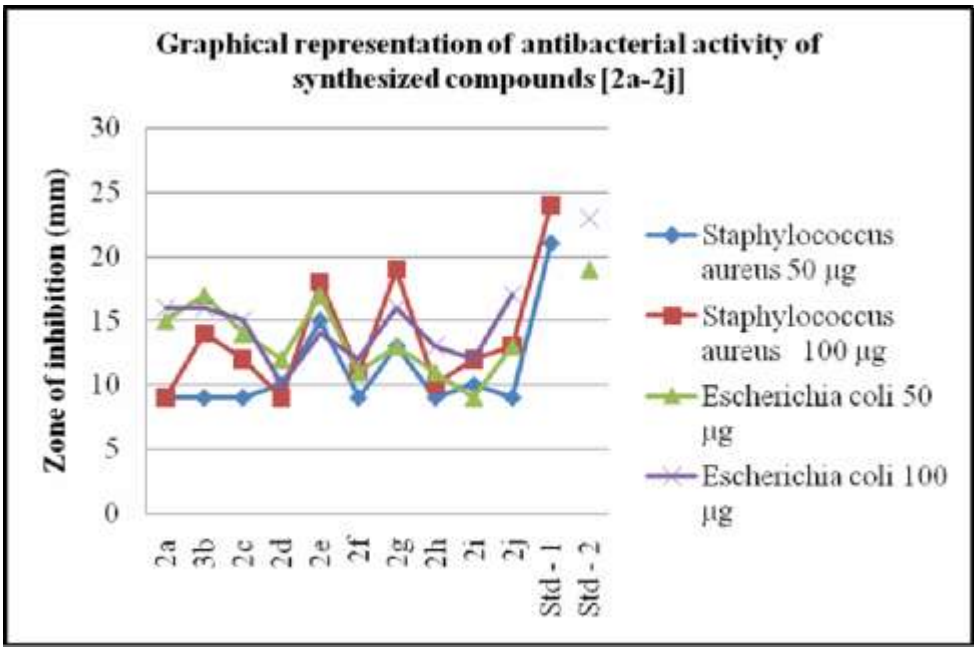
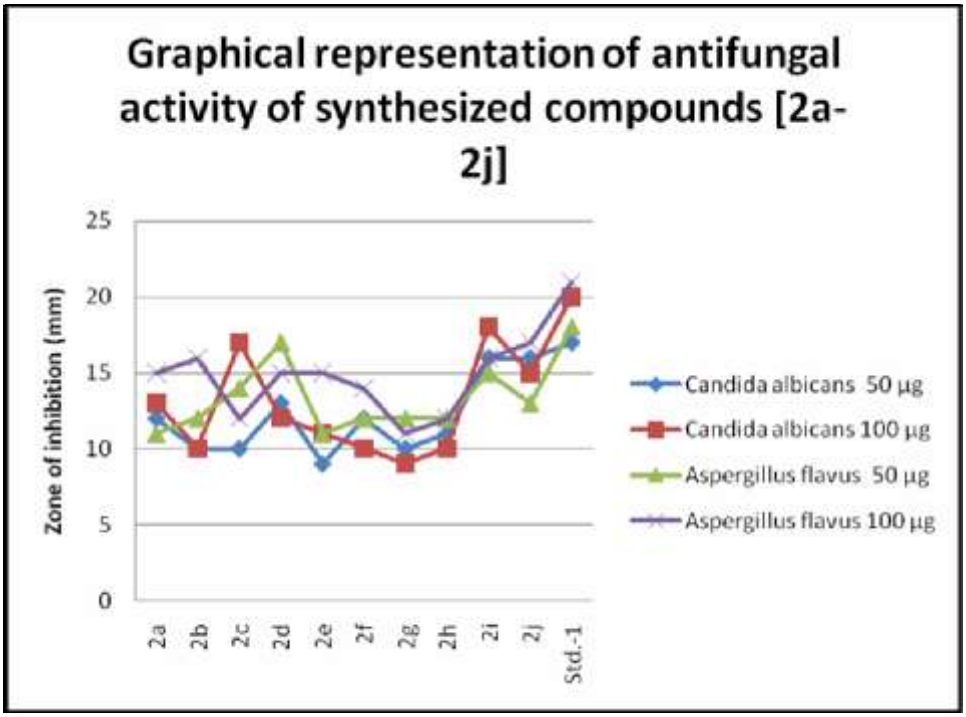
Antibacterial activity of newly synthesized compounds against S.aureus and E.coli [2a-2j]



Antifungal activity of newly synthesized compounds against C. albicans and A. flavus[2a-2j]



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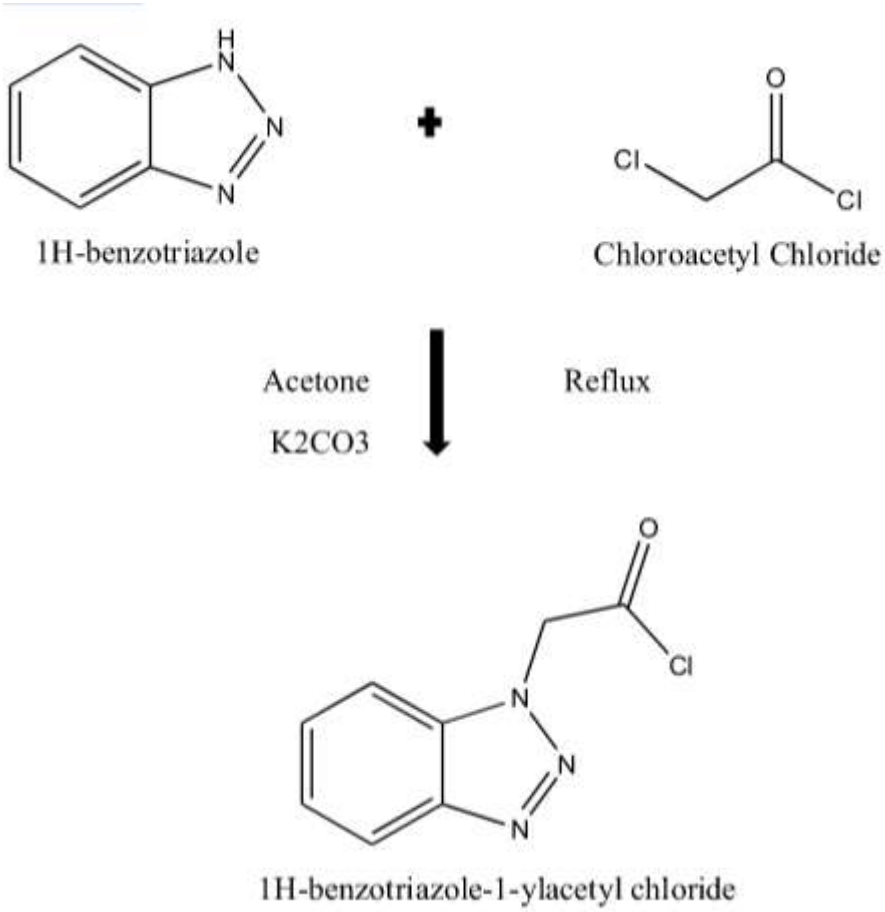
III. DISCUSSION

The structure of new compounds prepared during present investigation have been structurally established by their IR, H-NMR and Mass spectral studies. In the following section the spectral studies of some selected compounds have been dealt.



STEP-A:

The compound 1H-benzotriazol-1-ylacetyl chloride [2] has been prepared by reaction of benzotriazole with chloroacetyl chloride, K 2 CO 3 in the presence of acetone. The formation of [2] has been indicated by the IR spectra. The presence of absorption bands at 1755 cm^{-1} due to C=O chromophore clearly the formation of [2].

The formation of 1H-benzotriazol-1-ylacetyl chloride [2] has been indicated by the H-NMR spectrum. The presence of signals at δ 5.6 (s, 2H, CH 2), 8.0-7.3(m, 4H, Ar-H) [2].

STEP-B:

4[(1H-benzotriazol-1-ylacetyl chloride)amino)]benzoic acid [2a] has been prepared by reaction with 1H-benzotriazol 1ylacetyl chloride and p-amino benzoic acid in dimethyl formamide by refluxing for 2 hours.

The formation of [2a-2e] has also been indicated by the IR spectrum. The presence of Absorption band 1632 cm -1 due to C=o chromophore clearly revealed the formation of [2a]. The formation of 4-[(1H-benzotriazol-1-ylacetyl chloride)amino)]benzoic acid [2a] has been indicated by the 1 H-NMR spectrum. The presence of signals at δ 4.2 (s, 1H, NH), δ 5.79 (s, 2H, CH 2), δ 8.10-7.46 (m, 8H, Ar-H), δ 15.74 (s, 1H, COOH) clearly indicated the formation [2a].

The formation of [2a] has also been indicated by the mass spectrum. The molecular ion peak of [2a]

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has been observed at the 296 (M^+) which is in good agreement with the calculated molecular weight of compound.

1) Anti-bacterial activity:

1H-benzotriazol-1-ylacetyl substituted compounds were synthesized and tested for the antibacterial activity against gram +ve [stephyloccocus aureus] and gram-ve [Echeria coli], the tested compounds, 2e, 2g and 2i showed promising antibacterial activity against S. aureus[gram+ve]. Compound 2a, 2b, 2c, 2e and 2g showed promising antibacterial activity against E. coli [gram-ve].

2) Anti-fungal activity:

1H-benzotriazol-1-ylacetyl substituted compoundswere synthesized and tested forantifungal activity against Candida albicans and aspergillus flavus. Amongthe compound tested; 2a, 2d, 2f, 2i, 2h and 2j showed better activity against both fungus.

IV. CONCLUSION

We designed some new benzotriazole derivatives from different types of substituted amino compounds with an aim to obtain newer and potent bioactive compounds.

The various classes of newly synthesized compounds are given below. i) (2a-2j)

a) The functional groups in the title compounds (2a-2e) are indicated by their IR spectra.

b) The number of protons in the compound (2a) are confirmed by their 1 H NMR spectra.

c) The structure of title compound (2a) is confirmed by their Mass Spectra.

All the synthesized compounds were screened for their antibacterial activity against pathogenic gram-positive and gram-negative microorganisms using Procain penicillin and Streptomycin as a standard reference.

i) 2-(1H-benzotriazol-1-yl)-1-(piperazin-1-yl) ethanone (2e) has shown highest activity against Staphylococcus aureus.

ii) 2-(1H-benzotriazol-1-yl)-1-(piperazin-1-yl) ethanone (2e)) has shown highest activity against Escherichia coli.

But none of them had highest activity against standard reference Streptomycin.

All the synthesized compounds were screened for their antifungul activity against two different strains of fungi Candida albicans and Aspergillus flavus. All the compounds along with standard Griseofulvin were used.

i) 2-(1H-benzotriazol-1-yl)-N-(2-methoxyphenyl) acetamide (2i) has shown highest activity against Candida albicans.

ii) [(1H-benzotriazol-1-ylacetyl) amino] acetic acid (2j)) has shown highest activity against Aspergillus flavus.

V. SUMMARY

1H-benzotriazol-1-ylacetyl chloride [2] and its derivatives [2a-2j] were synthesized. The synthesized compounds were characterized by TLC, IR and HNMR spectral properties.

Synthesized compounds were subjected to antimicrobial activity by following standard procedure Antifungal activity against gram +ve and gram –ve microorganisms at two different (50µg and 100µg) concentration using procaine penicillin, streptomycin and griseoflavin as reference drug.

Respectively compounds 2e, 2g and 2i showed promising antibacterial activiy against S. aureus[gram+ve]. Compound 2a, 2b, 2c, 2e and 2g showed promising anti -bacterial activity against E coli [gram-ve].

While the compounds tested for antifungal activity 2a, 2d, 2f, 2i, 2h and 2j showed better activity against both fungus at lower and higher concentration respectively and was significant activity.

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