Effect of new synthesized piperazine derivative [1] containing 1, 2, 4 –triazole ring on the growth of some pathogenic microorganisms

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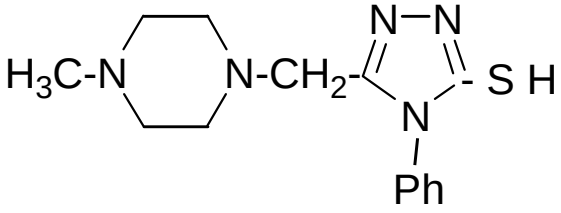
الخلاصة

يهدف البحث الحالي الى وصف الفعالية المضادة للجراثيم لمركبات مخلقة مثيل بايبروين. ان مشتق البايبرزين تم تخليقه من ناتج وسطي منتج من تفاعل بين Acetic hydrizide-4-methyl piperazine و phenyl iso thiosyanate اظهر الاختيار (خارج الجسم الحي) للمركب فعالية ضد نوعين من البكتريا الممرضة *Staphylococcus aureus* (موجبة لصبغة غرام) و *Escherichia coli* و (gram positive) سالبة لصبغة غرام (gram negahive) اضافة الى فعاليته ضد الفطريات الجلدية الممرضة *Trichophyton rubrum* .
اوضحت النتائج ان التركيز 50-ملغم/ مل اظهراعلى فعالية تثبيطية لبكتريا الـ E.coli تليها الـ Staph. aureus في حين ان التركيز 25ملغم/ مل من المركب اظهر اعلى فعالية تثبيطية على الفطر *Trichophyton rubrum*.

ABSTRACT

This work describes the antimicrobial activity of newly synthesized 4-methyl (3-mercapto -4-phenyl -4H- 1,2,4-Triazole –5-yl) methyl piperazine. [1]. The newly synthesized piperazine derivative was obtained from basic cyclization of the intermediate product obtained from the reaction between acetic hydrizide –4-methyl piperazine and phenylisothiosyanate(1) .



[1]

The *in vitro* tests showed antimicrobial activity against two pathogenic bacteria viz; *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative), in addition to the dermatophyte pathogenic fungi *Trichophyton rubrum*. The screening antibacterial results illustrated below indicate that concentration [50mg/ml] showed the highest inhibitory effect against *Escherichia coli*, while the same concentration has a moderate activity against *Staphylococcus aureus*.
While the highest inhibitory effect against *Trichophyton rubrum* was achieved using the concentration [25 mg/ml].

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INTRODUCTION:

Triazole derivatives had been found to exhibit wide spectrum of biological activities and most of molecules studied contain substituents.

On the 2 and 5 positions of the triazole ring and of special interest are the derivatives of 2-mercapto – 1,2,4 – triazole due to the presence of toxophoric -N – C = S moiety which included in many basic structure of drugs. Triazole containing this moiety have been reported as antibacterial[2], fungicidal[3], pesticidal[4],or anti-anxiety[5] agents. Several workers to incorporate structural modification in order to obtain new triazole of potential activity have carried out many attempts. Extensive work has been reported on the synthesis of new derivatives of these compounds[6].

The field of antifungal chemotherapy is presently rapidly moving. It began in 1903, a new approach has been developed to complex the drug with lipids or entrap it in liposomes. Itraconazole is a broad-spectrum oral triazole whose greatest advantages over the imidazoles are in its activity against aspergillosis and cryptococcosis, though it is also efficacious against the endemic deep mycoses. Fluconazole is a broad-spectrum triazole. It has been shown to be efficacious in various forms of superficial candidosis, including esophageal disease[7].

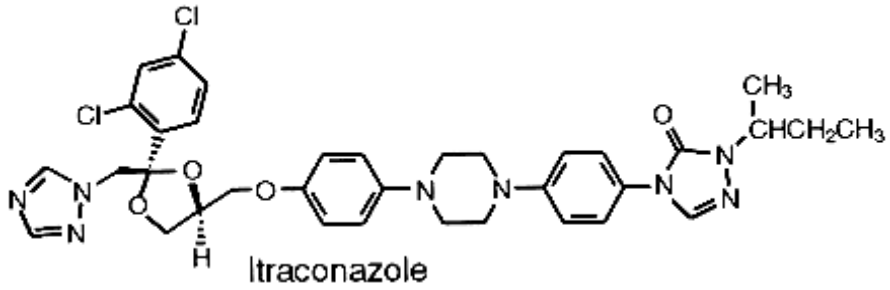
The first generation antifungal agent triazoles, fluconazole and itraconazole, have revolutionized the treatment of serious fungal infections such as mucosal and invasive candidiasis and cryptococcal meningitis. However, the treatment of some fungal infections, particularly aspergillosis, is still far from satisfactory and thus there is an important requirement for new broad-spectrum antifungal agents. The new second-generation triazoles voriconazole and SCH-56592 show considerable promise in achieving this goal in the near future[8].

A series of new nitrogen-carbon-linked (azolylphenyl) oxazolidinone antibacterial agents has been prepared in an effort to expand the spectrum of activity of this class of antibiotics to include Gram-negative organisms. Triazole moiety has been used to replace the morpholine ring of linezolid. These changes resulted in the preparation of compounds with good activity against the fastidious Gram-negative organisms Haemophilus influenzae and Moraxella catarrhalis. The unsubstituted pyrrolyl analogue 3 and the 1H-1,2,3-triazolyl analogue 6 have MICs against H. influenzae. Various substituents were also placed on the azole moieties in order to study their effects on antibacterial activity *in vitro* and *in vivo*. Interesting differences in activity were observed for many analogues that cannot be rationalized solely on the basis of sterics and position/number of nitrogen atoms in the azole ring. Differences in activity rely strongly on subtle changes in the electronic character of the overall[9].

Piperazine ring has been found to exhibit wide spectrum of biological activities and it is used in many drugs against different diseases. Some are known to exhibit antihypertensive[10], anti-inflammatory[11], antiallergenic[12], antitussive[13], antibacterial[14], antiserotonic[15], antipsychotic[16], anti-influenza[17], anticancer[18], antischizophernia[19], or central nervous system CNS-depressant activity[20]. And since substitute effects antibacterial activity of nitrogen-carbon-linked agents for example (azolylphenyl) oxazolidinones the activity expanded against the fastidious gram-negative organisms Haemophilus influenzae and Moraxella catarrhalis[21].

To explore the possibility of obtaining biologically useful compound that contain 1,2,4-triazol ring system, such biological activities promote us to prepare the new piperazin derivatives containing the above mentioned triazol unit.

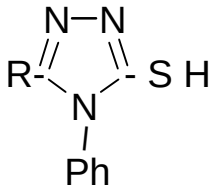
A new study revealing the strong antifungal activity of a newly synthesized derivative containing piperazine and triazole moieties (Itraconazole)[22].



Itraconazole

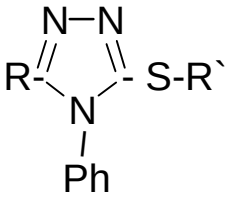
Some studies[23,24] indicates the ability of some synthesized 1,2,4-triazole derivatives [I] to inhibit the growth of some pathogenic microorganisms and the using of these derivatives as bactericidal and fungicidal agents.

[I]



Other studies[25,26] examined the ability of some thiol-substituted 1,2,4-triazole [II], which showed a moderate antibacterial activity.

[II]



EXPERIMENTAL :

Study of the Antimicrobial Activities:
The microorganism used for antimicrobial activities are listed in table (1):

Table 1 . The tested microorganisms and their origin

No.	Microorganism	Origin
1	Staphylococcus aureus	Biotechnology Dept., Al-Nahrain University
2	Escherichia coli	
3	Trichophyton rubrum	

Sterilization Methods[27]:

- Cultured media were sterilized by autoclaving at 121Ċ 15 pounds / in ^{2} for 15 minutes.
- Glassware were sterilized in the electric oven 180 – 200Ċ for 2 hours.

Preparation of Culture Media:

The following culture media were used routinely in our study :

1. Modified Sabouraud Dextrose agar

Fungi were cultured on modified Sabouraud Dextrose agar prepared by mixing the following ingredients :

Peptone	10 gm
Glucose	20 gm
Agar	20 gm
Cyclohexamide	500 gm
Cephalexin	500 gm
Distilled water	1000 ml

Cephalexin was added to prevent the growth of bacteria while cyclohexamide was added to prevent the growth of saprophytic fungi[28].

2. Nutrient Agar

The bacteria were cultured on nutrient agar by mixing the following ingredients[29] :

Nutrient broth	8 gm
Agar	20 gm
Distilled water	1000 ml

Preparation of antifungal samples:

Different concentrations of the tested derivatives as shown in table (2) were added to modified sabouraud dextrose agar containing cephalexin and cycloheximide at the ratio 3:1.5 ml, all petri dishes were inoculated with fungal spore and incubated at 30ċ for 7 days

Preparation of Antibacterial Samples:

For all the tested derivatives [1a-d] the stock solution was prepared by dissolving 0.2gm of each derivative in 2ml of 70% ethanol.

Nutrient agar media were prepared for controlling plates. The derivatives [1a-d] were prepared at different concentrations as shown in table (2).

The medium was mixed well, poured in petri dishes (25ml) and left to be solidified. 10µl overnight culture (O.D. About 0.2-0.4) was spotted on the top of the agar medium and left without spreading. The inoculated plates were placed at room temperature for 30 minutes to allow absorption of excess moisture. These plates were incubated for 24 hours at 37Ċ, after incubation, the diameter of inhibition zones were measured[30].

Our results were compared with the control, which were represented by a Petri dish containing media with ethanol 70% and inoculated with the microorganism (fungi and bacteria).

RESULTS AND DISCUSSION:

Microorganisms cause different kinds of diseases to humans and animals; discovery of antibacterial agents played a very important role in controlling and preventing such diseases. For this reason searching for new antimicrobial agents is a continues process and great efforts have been employed to find new antibiotics or synthesis of new chemical compounds with good antimicrobial activity, so this work has been established to find out the activity of the above synthesized compound which belongs to the triazoles class of heterocyclic compounds that constitute an important group having a wide spectrum of biological activity.

The antifungal, antibacterial and anti-inflammatory properties exhibited by various N-bridged heterocycles derived from 4-amio-5-mercapto-1, 2, 4-triazolees, have made them an important class of chemotherapeutic agents. Certain 4H-1, 2, 4-triazole derivatives have been reported to possess bactericidal, fungicidal and insecticidal[9] agents.
The results of our study indicated some variations in the ability of the used different concentrations to inhibit fungal growth, the percentage of inhibition listed in table (2) depends on the Concentration of the inhibitor.

Table 2 . Effect of 4-methyl (3-mercapto -4-phenyl -4H- 1,2,4-Triazole –5-yl) methyl piperazine on the growth of fungi

Compound	Concentration (mg/ml)	Trichophyton rubrum	
		Average of colonial diameter (mm)	Percentage of inhibition (%)
Control	——	90	0
The tested Compound	10	63.5	29.4
	15	29	67.7
	25	0	100

The diameter of fungal colonies was determined after the period of incubation then the inhibition percentage was calculated according to the equation :

$$\% \text{ of Inhibition} = \frac{\text{Av. of fungal growth in cont. plate} - \text{Av. of fungal growth in treated plate}}{\text{Average of fungal growth in control plate}} * 100$$

The synthesized novel[1] triazole was tested for antifungal activity and it was shown that it has affected the growth of the fungus [*Trichophyton rubrum*] at different concentrations shown in table (2) and figure (1), and the highest antifungal activity could be shown at the concentration (25 mg/ml) with percentage of inhibition [100 %].

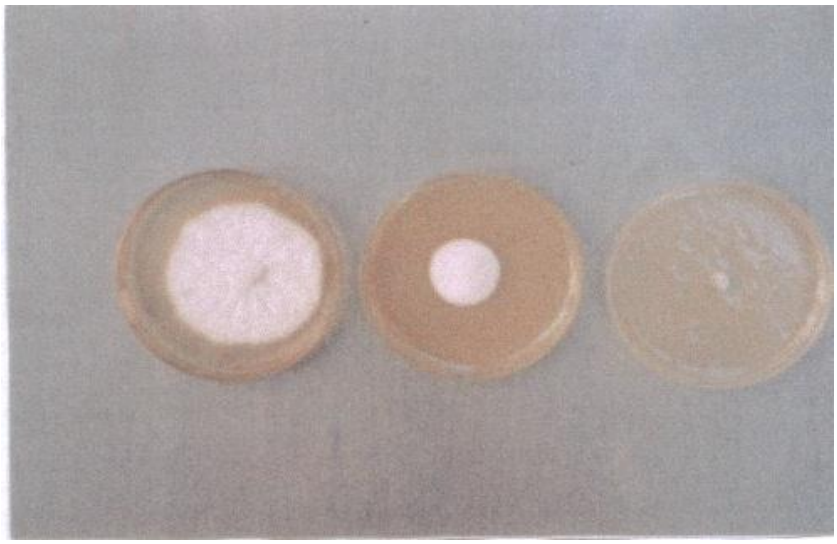


FIG 1 . EFFECT OF COMPOUND [1] ON THE GROWTH OF TRICHOPHYTON RUBRUM

While the other used concentrations of the tested compound (15 and 10mg/ml) showed a moderate growth of the same fungi, and with percentage of inhibition [67.7 and 29.4%] respectively.
The growth inhibition by this triazole derivative could be attributed to the presence of both piperazine and 4H-1, 2, 4 Triazol units which both posses high inhibitory effects towards fungi[31].
After inoculation and incubation no inhibition was seen using 70% with the media (Fig. 2).

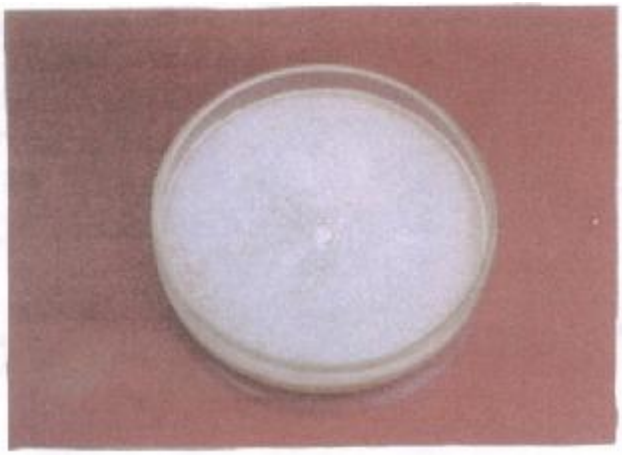


FIG 2 . CONTROL PLATE

The antibacterial activity for the prepared compounds was determined *in vitro* using paper disc method (agar plate diffusion method)[32], a standard 5mm diameter sterilized filter paper impregnated with the tested doses of the compounds was placed on agar plate seeded with the tested organisms. The plates were incubated for 24 hours at 37Ċ, the zone of inhibition of bacterial growth around the disc was observed.
The zones of inhibition formed were measured in mm and are represented by (+), (++), (+++) depending upon the diameter and clarity as shown in table (3).

Table 3 . Effect of 4-methyl (3-mercapto -4-phenyl -4H- 1,2,4-Triazole –5-yl) methyl piperazine on the growth of bacteria

The Novel Compound	Concentration Mg/ml	Inhibition Zone	
		Staph. aureus	E. coli
The tested Compound	30	0(0.35 cm)	0(0.45 cm)
	40	0(0.5 cm)	+(1 cm)
	50	+(1 cm)	++(1.5 cm)

The activity of antibacterial agents are related to :

a) Inhibition of cell wall synthesis such as cycloserine[33], penicillin[34] and bacitracin[35] .
b) Alteration of cell membrane permeability or inhibition of active transport across cell membrane such as surfactants[36].
c) Inhibition of protein synthesis [i.e. inhibition of translation and transcription of genetic material] such as erythromycin[37], streptomycin[38] and tetracycline[39].
d) Inhibition of nucleic acid synthesis such as sulfonamide[40].

The different tested concentrations (30, 40 and 50 mg/ml) showed measurable activity against the used microorganisms. The highest activity was observed using the concentration (50mg/ml) of the tested compound, the *in vitro* tests showed antibacterial activity against the pathogenic bacteria *Escherichia coli* (gram negative) while least activity could be observed viz; *Staphylococcus aureus* (gram positive), that could be attributed to the least sensitivity of the triazole derivatives towards the pathogenic species *Staphylococcus aureus*[41]. (Fig.3).

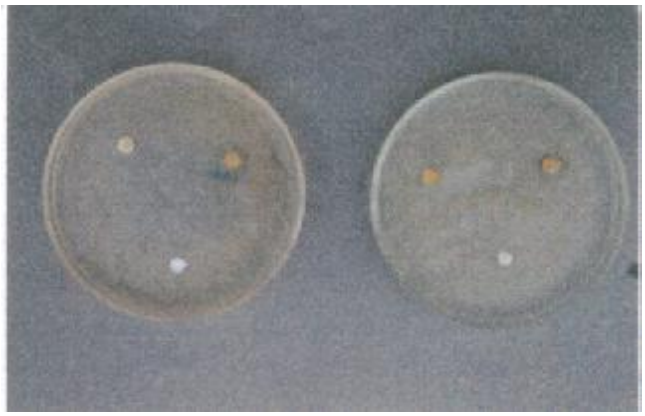


FIG 3 . EFFECT OF COMPOUND [1] ON THE GROWTH OF BACTERIA [ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS]

The differences in effectiveness of such drugs between the two types of bacteria might be attributed to the differences in cell wall structure, in which bacterial cell wall consists of a network of polysaccharide chains containing alternating units of N-acetyl muramic acid connected by poly peptide cross linkage, this network is called the glycol peptide[42].
In gram (+ve) bacteria this basic layer is covered with teichoic acid (which is a Ribitol hposphate), N-acetyl glucose amine polymer and glycine , making up to 20% of cell weight. While in gram (-ve) bacteria, lipo polysaccharide with lipoproteins were external to glycopeptides, which makes about 80 % of the cell wall weight[43].
This lipid containing layer of gram (-ve) cell wall keeps various small molecules of chemical compound or drug from reaching the membrane, in addition to the fact that gram (+ve) cell wall is more permeable to molecules than gram (-ve) cell wall[43].
After inoculation and incubation no inhibition was seen using 70% with the media (Fig. 4).

FIG 4 . CONTROL PLATE

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