Biological Activity of Thiazole Derivatives on Some Pathogenic Microorganisms

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Abstract

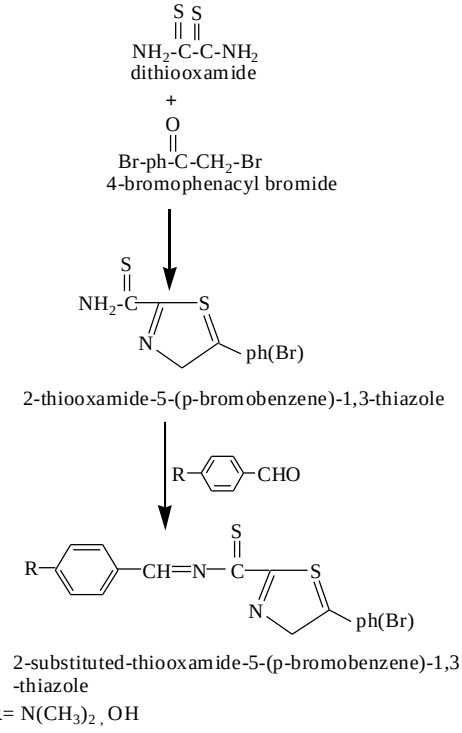
The thiazole derivatives were prepared by the reaction of benzaldehyde derivatives with (2-thiooxamide-5-(p-bromobenzene)-1,3-thiazole) that was readily prepared by heating dithiooxamide with 4-bromophenacylbromide. Within *invitro* study the the effect of the prepared compounds on the growth of two types of fungi *geotrichum cadidum* and *Trichophyton rubrum* and the bacteria *Escherichia coli, pseudomonas aeruginosa, Staphylococcus aureus,* and *Klebsiella pneumoniae* was evaluated. Incubation time and concentration were studied to find the optimal conditions for the activity. Compound II with the concentration 20 mg/ml found to be the most active compound toward *geotrichum* and *Trichophyton rubrum*. Statistical analysis of the values of colonial diameter shows low percentage error for the studied fungi with regard to incubation time.

Keywords: Thiazole derivatives, antibacterial activity, antifungal activity, statistical analysis.

Introduction

Dermatophytes are infections of keratinized tissue, that is, the epidermis, hair and nails, caused by a group of specialized fungi. The dermatophytes do not invade subcutaneous or deep tissue. *Dermytophyte-Trichophyton schoenleinii* was the first microorganism that was proven to cause an infections disease of humans [1]. The dermatophytes species can be categorized as an ecological basic as being geophilic, zoophilic or anthrophilic [2]. The geophilic species are natural habitats in the soil, natural habitats of the zoophilic dermatophytes are domestic and wild animals [3]. *Geotrichum candidum* was believed to be part of the normal flora of human skin and gastrointestinal tract. *Geotrichum* is frequently isolated from milk and is recorded as a spoilage organism on dairy products [4]. Some fungi are parasitic, especially on plants and others are symbiotic with roots and algae [5]. Fungi cells are quite different from plant cells not only by lacking chloroplasts but also by having a cell wall that contains chitin and not cellulose [6].

The thiazole compounds have a common mode of action involving the interaction of the lone pair of electrons on the ring nitrogen with the hem group of the cytochrome P450 of the enzyme catalyzing the C-14 demethylation reaction [6]. Azole antifungal include imidazole compounds with a five membered ring containing two nitrogens, and triazoles, with three nitrogen in the ring [7]. With various compounds of azoles, activity has been demonstrated in experimental animals or patients with *candidiasis, histoplasmosis* and *tinea* versicolor [8]. This recent results prompt us to examine some 5-membered, thiazole derivatives on above fungi and bacteria. The following scheme displays the reaction route to the examined thiazole derivatives in this work.



Scheme (1) Reaction route of the prepared thiazole derivatives [3].

Materials and Method

Chemistry part:

Thiazole derivatives were prepared according to method reported [8]. All chemicals used where of laboratory**.**

Instruments:

TLC was run on Merk silica gel coated aluminium plates and melting points were taken in open capillary tubes and are uncorrected.IR spectra in KBr pellets were recorded on FT-IR.8300 Shimadzu spectrophotometer**.**

Methods:

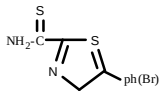
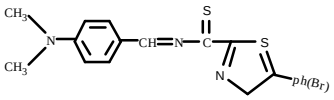
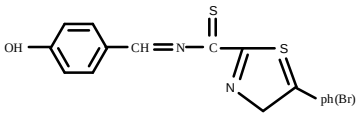
Synthesis of 2-thiooxamide-5-(P-bromobenzene) –1, 3- thiazole :

To a solution of (dithiooxamide) 1.02g, 0.002 mol dissolved in 30ml ethanol, (4-bromophenancylbromide) 0.55g, 0.002 mole was added. The reaction mixture was refluxed for 8 hrs. Cooled and neutralized with ammonium hydroxide solution. The precipitate was used for recrystallization with absolute ethanol, the physical properties were found in the Table (1).

Synthesis of 2-substituted-thiooxamide-5-(p-bromobenzene)-1, 3-thiazole (I, II):

A mixture of (2-thiooxamide-5-(p-bromobenzene)-1,3-thiazole) 1.33g, 0.01 mole, absolute ethanol 20ml and 0.01 mole of aldehydes such as (N, N-dimethyl benzaldehyde or p-hydroxybenzaldehyde) was refluxed for 6 hrs. After cooling to room temperature the precipitate was filtered recrystallization by ethanol, the physical properties were found in the Table (1).

Table (1)
Physical data of prepared compounds.

Name of compound	*structure*	*Percentage yield%*	*m.p °C*	*Color*
2-thiooxamide-5-(P-bromobenzene)–1, 3-thiazole		78	201-203	Dark brown
2-[N,N-dimethylbenzylidine] -thiooxamide-5-(p-bromobenzene)-1, 3-thiazole (I)		80	124-126	Redish-brown
2-[p-hydroxybenzylidine]-thiooxamide-5-(p-bromobenzene)-1, 3-thiazole (II)		62	188-190	Orange

Biological part:

Invitro results of fungi work from the procedure described by cappuccino et. al. [9]. A suspension of fungal spores was made by dissolving 0.01g agar in 5 ml distilled water, autoclaved & loopful of spores was added to it. A loopful of spore suspension was taken & put at the middle of each plate and incubated at 30°C for 48 and 72 hrs. After each incubation period plates were checked for fungal growth by determining the average of fungal colonies (AFG) and then the inhibition percent [10].

Fungi were cultured on modified *sabouraud dextrose agar* prepared by mixing the following ingredients [10]:

Peptone	10g
Glucose	20g
Agar	20g
Cycloheximide	500ml
Cephalexin	500ml
Distilled water	1000ml

The bacteria were cultured on nutrient agar by mixing the following [11]:

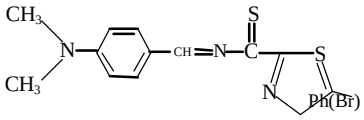
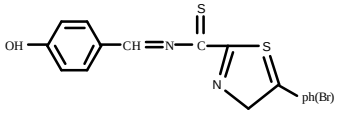
Nutrient Broth	8g
Agar	20g
D.W.	1000ml

Nutrient agar media were prepared at concentration 75mg/ml for all used controlling plates. Different concentration of thiazole compounds in different incubation times was examined.

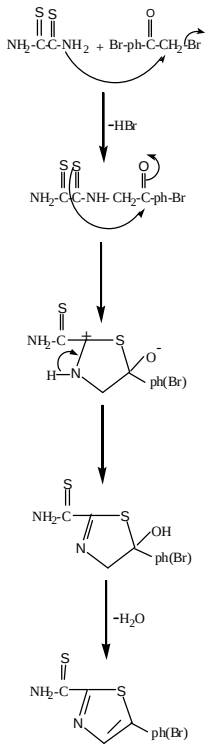
Results and Discussion

The reaction of (2-thiooxamide-5-(p-bromobenzene)-1,3-thiazole) with different aromatic aldehydes give (2-substituted-thiooxamide-5-(p-bromobenzene)-1,3-thiazole) (I, II) that showed disappearance of NH_2 bands appearance of a characteristic bands in the FTIR spectum, Table (2) showed the FTIR absorption bands of compounds (I, II).

Table (2)
FTIR absorption band of prepared compounds.

No	*Structure*	*υ (C-H) Aromatic cm^{-1}*	*υ (C=C) cm^{-1}*	*υ (C=S) cm^{-1}*	*υ (C=N cm^{-1}*	*υ (C-H) AliphatiC cm^{-1}*	*υ (O-H) cm^{-1}*
I		3105	1529	1336	1604	2950-2809	___
II		3134	1505	1320	1583	___	3425

The reaction of dithiooxamide with (4-bromophenancylbromide) under refluxing condition affected on intermolecular cyclization through S_N^2 mechanism giving the (2-thiooxamide-5-(p-bromobenzene)-1,3-thiazole). The following scheme shows the mechanism of the formation of (2-thiooxamide-5-(p-bromobenzene)-1,3-thiazole). These two compounds of thiazole derivatives that were prepared in this research showed different inhibition effect in growth of bacteria and animals fungi.



Scheme (2) mechanism of the formation of 2- thiooxamide-5-(p-bromobenzene)– 1, 3-thiazole [3].

Antibacterial Studies

Thiazole derivatives were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonus aeruginosa*, and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method [10]. Antibacterial activity was determined by measuring the diameter of the inhibition zone. Ampicillin was used as standard drug. The results of such studies are given in Table (3).

The compound (1) was found to be the most active against *S. aureus* and *K. pneumoniae*, while compound (2) showed moderate activity against these bacteria. *E. coli* was found to be resistant to all the tested compounds.

Table (3)
Effects of compound (1 and 2) on the growth of bacteria and yeast.

Inhibition zone (mm)				
compound	***K.pneumoniae***	***Pseudo aeruginosa***	***E. coli***	***Staph. Aureus***
1	20	12	6	18
2	8	-	7	12
Ampicillin	13	-	20	28

1-5 (mm) weak effect, 6-10 (mm) moderate effect, 11-20 (mm) strong effect.

Antifungal studies

Thiazole derivatives were screened for their antifungal activity against *Trichophyton*, and *Geotrichum* (recultured) in DMSO by serial plate dilution method [10]. Antifungal activity was determined by measuring the diameter of colonial of fungi. The results of these studies are given in Table (2). Compound 1 was found to be the most active against Geotrichum in concentration 25mg/ml.

Compound (2) emerged as the most active against *Trichophyton* and *Geotrichum* in concentration 20 mg/ml and 25 mg/ml as shown in Table (4).

The compound (1) was not effective against the fungi *Trichophyton rubrum* and *Geotrichum candidum* at the concentration (5, 10 mg/ml) and optimum inhibition of both species was achieved at the concentration (20 and 25mg/ml) that give percentage 100% as shown in Table (5).

Table (4)
Effects of prepared compounds on the diameter of fungal colonies (mm) throughout 48hrs. of incubation in PDA and sabouraud agar, pH 7.1 at 30°C.

Conc. mg/ml	***Geotrichum candidum***				***Trichophyton rubrum***			
	Percentage of inhibition		***Average of colonical diameter(mm)***		***Percentage of inhibition***		***Average of colonial diameter(mm)***	
	Comp.1	***Comp.2***	***Comp.1***	***Comp.2***	***Comp.1***	***Comp.2***	***Comp.1***	***Comp.2***
Cont.	0	0	87	75	0	0	85	85
5	8.04	6.66	80	70	5.85	5.85	80	80
10	16.06	42.66	73	43	17.64	52.94	70	40
20	19.54	100	70	0	11.76	100	60	0
25	25.28	100	65	0	17.64	100	54	0

Table (5)
Effects of prepared compounds on the diameter of fungal colonies (mm) throughout 72hrs. of incubation in PDA and sabouraud agar, pH 7.1 at 30℃.

Conc. mg/ml	Geotrichum candidum				Trichophyton rubrum			
	Percentage of inhibition		Average of colonical diameter(mm)		Percentage of inhibiti on		Average of colonial diameter(mm)	
	Comp.1	Comp.2	Comp.1	Comp.2	Comp.1	Comp.2	Comp.1	Comp.2
Cont.	0	0	40	45	0	0	45	50
5	25	24.4	30	34	44.4	40	25	30
10	50	44.4	20	25	60	54	18	23
20	100	100	0	0	100	100	0	0
25	100	100	0	0	100	100	0	0

The outer wall of the fungi cell is a complex multilayered structure where amorphous, granular and fibrillar structures interact with one another to give the cell a rigid shape and to confer osmotic stability. The cell wall of fungi do not contain peptidoglycan so that the B-lactam antibiotics have no effect [12].

Azole compounds have a common mode of action involving the involving the interaction of the lone pair of electrons on the ring nitrogen with the heme group of the cytochrome P_{450} of the enzyme catalyzing the C-14 demethylation reaction [13,14].

Statistical Analysis

Results obtained from at least three replicate trials were analyzed from significant differences using duncans multiple range test and general linear model **(GLM)** procedures of **SAS** software. All treatment of significant are based on the probability level of 0.05 [9], as shown in Table (6).

Table (6)
Colonial Diameter (Mm) Of Trichophyton Rubrum &Geotrichum Candidum That Incubation In PDA And Sabouraud Agar , PH 7.1 At 30℃ .

Concentration mg/ml	Average of colonial diameter (mm)			
	Trichophyton Rubrum		Geotrichum Candidum	
	48 hrs	72 hrs	48 hrs	72 hrs
Control	40^{a}±0.16	45^{a}±0.498	45^{a}±0.61	50^{a}±0.238
5	30^{a}±0.5	34±0.712	25^{a}±0.732	30^{b}±0.352
10	20^{a}±0.426	25^{a}±0.186	18^{b}±1.321	23^{b}±0.567

a,b letter represent significant differences (P>0.05) between means of the same column.

Conclusion

Compound 1 emerged as a promising antibacterial agent and compound 2 emerged as a promising antifungal agent. Compound 2 with the concentration 20 mg/ml found to be the most active compound toward *geotrichum* and *Trichophyton*.

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الخلاصة

تم تحضير مشتقات الثـــايزول مـــن تفاعـــل مشـــتقات البنزلدهايد مع (2-ثايواوكســـامايد-5-بـــارابروموبنزين-1,3-ثايواوكســـامايد) المحضـــر مـــن تســـخين(داي ثايواوكسامايد4-بروموفنسايل برمايد). تم دراسة المركبات المحضرة خارج جسم الكائن الحي على نمـــو الفطريـــات *Trichophyton rubrum* و *Geotrichum Candidum* وعلـــى نمـــو البكتريـــا *E.Coli*, *Staphylococcus aureus*, *Pseudomonus aeruginosa*, *Klebsiella Pneumoniae*

تم دراسة اوقات الحضن وتركيز المركبـــات لايجـــاد الفعالية المؤثرة. المركب الثاني وجـــد لـــه فعاليـــة ضـــد الفطريات المذكوره اعلاه في تركيز 20 mg/ml. التحليل الاحصائي اظهر ان هناك نسبة خطا قليلـــة فـــي انصـــاف الاقطار للفطريات مع اوقات الحضن.

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