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Antifungal Potential of 1,2-4Triazole Derivatives and Therapeutic Efficacy of Tinea Corporis in Albino Rats

Anees Ahmad Siddiqui*[1], , Hamad M.I.Hasan[2], Mojahidul Islam[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, India*
[2]*Faculty of Pharmacy, Albeida, Libya*

ABSTRACT

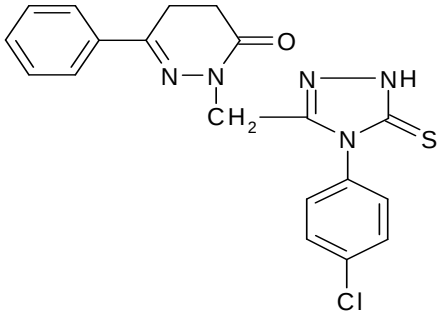
New triazole 6-substituted-2[4'-(substituted phenyl-5'-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazine-3-one derivatives possessing antifungal activity have been developed to meet the rising incidence of invasive fungal infections and the emergence of fungal resistance. The therapeutic efficacy of novel triazole derivatives for albino rat models with Trichophyton rubrum infection was investigated. Animals with developing infection were divided into five groups. The results show a marked decrease in the fungal burden on the skin. Hence, these novel triazole derivatives can be considered a promising candidate as a new anti-dermatophytic agent for topical use.

Key words: antifungal and triazole.

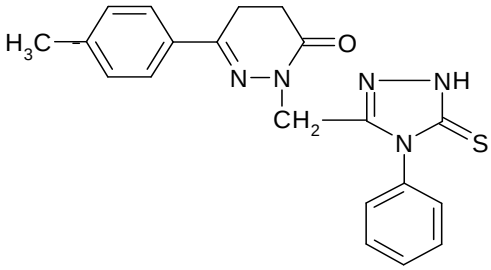
INTRODUCTION

Dermatomycoses are among the most widespread and common superficial and cutaneous fungal infection in humans caused by filamentous fungi that have a high affinity for keratin. Causative fungi were recognized as pathogens long ago but development of antifungal agents has been disappointingly slow. With the increase in the number of patients compromised by the human immunodeficiency virus, cancer chemotherapy, organ transplants and long-term antimicrobial therapy, the incidence of opportunistic fungal infections have taken increasing (1-2) especially during last two decades. Diseases caused by fungi including topical infections of the skin by dermatophytes are *Microsporum, Trichophyton* or *Epidermophyton* genera. These include infections like *Tinea pedis* (athlete's foot), *Tinea corporis* (ringworm), *Tinea cruris* (jock itch), *Tinea captis* (scalp), *Tinea barbae* (beard/hair) and *Tinea unguium* (nails) (3).

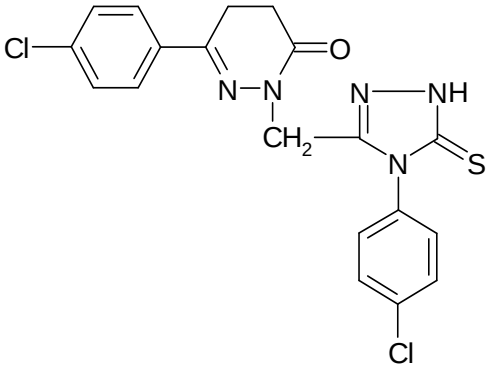
The antifungal agents with azole nucleus are the largest and effective class of synthetic antimycotics .(4). Antifungal activity stems from the presence of an aromatic five-member heterocyclic either benzylamine (butenafine) (5) and imidazole (clotrimazole, miconazole, ketoconazole) (6) or triazole (lanoconazole, itraconazole, fluconazole, voriconazole) (7-10). These drugs inhibit ergosterol biosynthetic pathway (11-12). Dermatophytes represented by *Trichophyton* parasitize the keratinized tissues of the horny layer of the epidermis, hair and nail because they utilize keratin for their growth (13). In the present study, we describe the *in vitro* and *in vivo* activity of three novel triazole derivatives (Fig 1). These antifungal drugs exhibit comparable activity against various fungal species. The efficacy of 1% cream preparation of these compounds shows comparable results with standard drug fluconazole. This study was made to achieve a mycological cure of *Tinea* infection in albino rats.



Test Compound-I 6-phenyl-2[{(4‘-p-chlorophenyl-5’-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazin-3-one



Test Compound-II6-tolyl-2[{(4’-phenyl-5’-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazin-3-one



Test Compound-III 6-(4′-chlorophenyl)-2[{(4’-p-chlorophenyl-5’-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazin-3-one

Fig 1 . Structure of antifungal triazole derivatives

MATERIALS AND METHODS

Experimental

In this paper, we describe the synthesis and antifungal activity of the novel antifungal compounds. The Test compounds I, II and III were prepared by following steps.

To the dry anhydrous aluminium chloride (24.5 g) was added in appropriate hydrocarbon (50 ml) and the contents were refluxed on a water bath under anhydrous condition. Succinic anhydride (10 g) was then added in small portions through the three-neck flask. After complete addition, the reaction mixture was refluxed for 3 h and then allowed to rest for 24 h. A cold solution of concentration hydrochloric acid (2.5% v/v) was then added to the reaction mixture and the contents were subjected to distillation to remove the unreacted benzene. The contents were concentrated on water bath and cooled to separate out the crystalline compound, which was filtered and crystallized from hot water to get a compound (1).

The β-benzoyl propionic acid (1) (1g) was dissolved in absolute ethanol (15 ml). To it, hydrazine hydrate (99%, 2 ml) was added and the contents were refluxed for 8-10 h. After the completion of reaction, ethanol was distilled off and the residue was poured into ice-cold water. A solid, which separated out was filtered and crystallized from ethanol to give pure needle shape compound (2).

Appropriate pyridazinone (2) (0.02M) were added in ethanolic solution (50 ml) of sodium (0.46 g). The mixture was refluxed for 30 min. Then ethyl bromoacetate (3.34 g, 0.02M) was added drop by drop to the cooled solution, which was refluxed for 24 h. The solvent was evaporates off under in vacuum and the residue was triturated with di-isopropyl ether and the solid which was formed was collected by filtration and dried (3).

To above compound (1g) (3) was dissolved in absolute ethanol (50 ml). Hydrazine hydrate (99%, 4 ml) was added in the solution and the reaction mixture was refluxed for 8-10 h under anhydrous condition using guard tube. After evaporating the resulting solution was added in the crushed ice and the precipitate, which was separated, was filtered off and the compound was crystallized from ethanol (4)

Ethanolic solution of hydrazide (4) (0.01M) and respective phenyl isothiocynates (0.01M) was refluxed for 5 h. The contents were concentrated and poured into crushed ice to get intermediate thiosemicarbazide. Thiosemicarbazide (0.05M) was refluxed in sodium hydroxide solution for 5 h, cooled and poured into excess of water and containing crushed ice. On acidification with glacial acetic acid yielded a solid. The solid obtained was crystallized from ethanol to get a compound (5)

Test Compound-I 6-phenyl-2[{(4'-p-chlorophenyl-5'-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazin-3-one

IR: (KBr) cm^{-1}: 3439.45 (NH), 1679 (C=O), 1642 (C-N), 1415 (C=S)

^{1}HNMR (DMSO-d6) ppm : 2.4 (t, 2H, CH_2), 2.44 (s, 2H, CH_2), 2.94 (t, 2H, CH_2), 7.41-7.75 (m, 9H, Ar-H), 10.96 (s, 1H, CSNH)

Test Compound-II 6-tolyl-2[{(4'-phenyl-5'-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazin-3-one
IR: (KBr) cm^{-1}: 3504 (NH), 1670 (C=O), 1548 (C-N), 1420 (C=S)
^{1}HNMR(DMSO-d6)ppm:2.08 (s, 3H, CH_3), 2.43 (t, 2H, CH_2), 2.5 (t, 2H, CH_2), 2.92 (t, 2H, CH_2CO), 7.21-7.23 (dd, J=7.8, H-3', H-5'), 7.34-7.40 (m, 5H, Ar-H), 7.61-7.64 (dd, J=7.8, H-2', H-6'), 10.66 (s, 1H, CSNH)

Test Drug-III 6-(4'-chlorophenyl)-2[{(4'-p-chlorophenyl-5'-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazin-3-one
IR: (KBr) cm^{-1}: 3400 (NH), 1684 (C=O), 1600 (C-N), 1454 (C=S)
^{1}HNMR (DMSOd6) ppm: 2.42 (t, 2H, CH_2), 2.50 (s, 2H, N-CH_2), 2.50 (s, 2H, N-CH_2), 2.939 (t, 2H, CH_2CO), 7.32-7.83 (dd, J=8.0, 4H, Ar-H), 10.98 (s, 1H, CSNH)

Biological Screening
***Antifungal drugs* Test,** drugs I, II and III were synthesized and structurally elucidated on the basis of IR and NMR spectral data. A 1% cream preparation of test Drugs I, II and III were prepared in the Department of Pharmaceutics, Jamia Hamdard, New Delhi was used as a topical antifungal agent.

1% cream of standard fluconazole and test antifungal compounds was prepared by using this formula

Cetyl Alcohol	- 10 %
Na cetyl sulphate	- 2.0 %
Stearic Acid	- 10 %
Steryl alcohol	- 2 %
Glycerol	- 8 %
Sodium Lauryl Sulphate	- 1 %
Water	- make 100 ml

Animals 4-5 week old female albino rats were used in the study. These were donated by the Animal house (approved by CPSCEA), Jamia Hamdard, New Delhi. The experiments were conducted with five groups of six animals each. They were housed individually in cages and placed in the separate room in temperature range between 25-30^0 C. Food and water was given once daily during experiment procedure.

Test Organism and Preparation of fungal inoculums- The fungal strains of *Trichophyton rubrum, Candida albicans, Candida glabrata, Aspergillus niger and Aspergillus flavus* were collected from fresh human samples from Maulana Azad Medical College, New Delhi. Cultures were grown on Sabouroud Dextrose Agar at 27^0 C for 15 days and the conidial suspension of dermatophytes was prepared in sterile saline containing 0.05% (w/v) Tween 80 (14). The MIC of test drugs I, II and III were determined by standard micro dilution method was shown in Table 1.

Production of tinea corporis model- Hair of the albino rats on the back portion was removed by electronic shaver and a clean patch of 5cm x 4cm was exposed. The skin was disinfected by a cotton swab of alcohol kept for about 5 min. Scars were made on the dorsal region of the skin and the conidial suspension applied with the swab containing fungal spores. The exposed skin

was covered with adhesive bandage and kept in a separate room. After being reared in the infected animal room for two weeks until the infection has fully developed, 30 infected albino rats were randomized in 5 groups, six animals each in one group. Group I was control, Group II for the Standard and Groups III, IV and V were treated with the test drugs. These were then transferred to sterilized cages and moved to another room where they were reared until the day of the culture study.

Topical treatment of tinea corporis The 1% cream of test compound I,II III and standard drug was applied with the help of sterile spatula on the infected area on the day first of the third week consequently to the end of the sixth week. The control group was not received any treatment.

Evaluation of therapeutic efficacy 3, 4, 5 and 6 weeks after the infection one animal of each group was sacrificed respectively using chloroform for culture study. After the sacrifice of all the animals in their respective weeks, the skin was cut into small pieces. These tissues were implanted on SDA agar plates. All plates were incubated at 27^0 C for 5 days. Tissue blocks yielding *T. rubrum* and other fungal species colony was considered positive. The fungal burdens of all the plates were calculated.

Table 1 shows the MICs of the three antifungal compounds in µ gm/ml against five fungal species *Trichophyton rubrum, Candida albicans, Candida glabrata, Aspergillus niger and Aspergillus flavus.* The MIC for Test compound I against *Trichophyton rubrum, Candida albicans, Candida glabrata, Aspergillus niger and Aspergillus flavus* was 1.25, 1.0, 1.0, 2.0 and 1.0 µgm/ml respectively. The MIC for Test compound II against the same species was 1.0, 1.5, 2.0, 1.0 and 2.0µgm/ml respectively. The MIC for Test compound III was 0.75, 1.0, 2.0, 4.0 and 1.5µgm/ml respectively. The compounds show comparable results with the standard drug Fluconazole.

The results of culture study with skin tissues with both treated and untreated (control) animal groups are shown in Table 2. It shows the average fungal burden on female albino rat. For the test groups, the days of culture study correspond to 3, 4, 5, 6 weeks after the completion of drug treatment. At 3 weeks post infection when drug treatment in the treated animal group had not yet been initiated, the infected sites of all animals became culture positive with the highest fungal burden score at the level of 10. All animals were also culture positive, although fungal burden scores gradually decreased, by the end of the experimental period, which was as long as 6 weeks post infection. This shows that in this albino rat model of Tinea, intensive local infection persisted for at least 6 weeks after inoculation without spontaneous healing. All animals show significant cure by applying the cream after 3 weeks post infection. In control group the infection persist after 6 week of infection.

All the three drugs produced significant results as compared to the standard are shown in Table 3. The inhibitory effect of standard with the zone of inhibition was *Trichophyton rubrum* 30 mm, *Candida albicans* 28 mm, *Candida glabrata* 28 mm, *Aspergillus* niger 25 mm, *Aspergillus flavus* 26 mm. Drug III was found to be more effective against all the fungal species with a zone of inhibition of 22 mm for *Trichophyton rubrum*, 21mm for *Candida albicans*, 20 mm for *Candida glabrata*, 19 mm for *Aspergillus niger and* 18 mm for *Aspergillus flavus*.

Tinea caused superficial dermatomycosis in humans and consists of various clinical forms depending on the site of infection, depth of invasion and the degree of inflammatory responses during infection (15) .The albino rat model of Tinea corporis is regarded as extremely useful for studying the pathogenesis of the disease and responses to the antifungal treatment. *In vivo* study was carried out on the female albino rat skin by removing the hairs on the dorsal region (16) and then the inoculums of *Trichophyton rubrum* is spread on the skin with the help of cotton swab (17). It takes 2 weeks to develop patches on skin. The confirmation of fungal infection was done by microscopic slide of the scar of fungal species stained in Gimsa dye.

Tinea model was used to evaluate *in vivo* anti-dermatophytic activity of triazole derivatives on various fungal species. The drug fluconazole was used as standard. Fluconazole is a triazole antifungal medication used to treat serious fungal infections both topical and systemic. Other antifungal agents including clotrimazole (18), tolnaflate (19), omoconazole (20), terbinafine (21), butenafine (22-23) and lanoconazole were particularly effective in eradicating the tinea infection. In conclusion Test compound I, II and III may be a promising topical antimycotic candidate because it possesses comparable activity against standard drug fluconazole.

Table 1. MIC of compounds against various organisms in (µgm/ml)

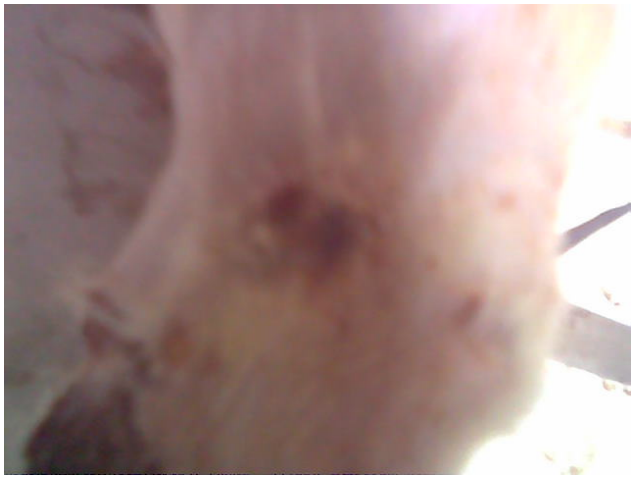
Fungal species	Std	Test Drug I	Test Drug II	Test Drug III
Trichophyton rubrum	0.20	1.25	1.00	0.75
Candida albicans	0.15	1.00	1.25	1.00
Candida glabrata	0.20	1.00	2.00	2.00
Aspergillus niger	0.30	2.00	1.00	4.00
Aspergillus flavus	0.50	1.00	2.00	1.25

Table 2. Average fungal burdens on Female Albino rats on ten-pointer scale.

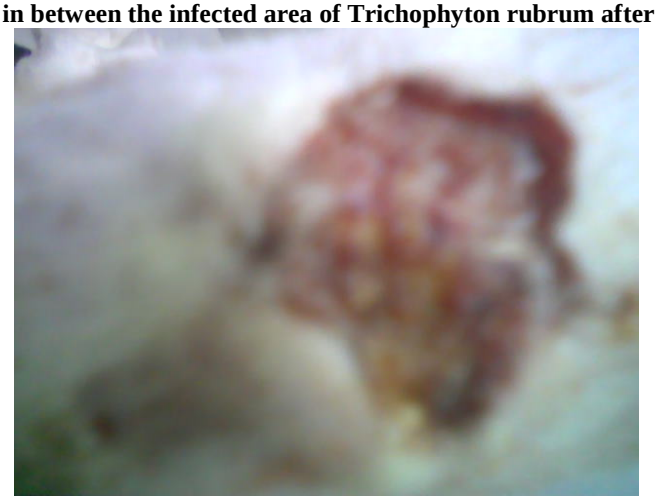
Test Drug	Time after infection (3 weeks)	Time after infection (4 weeks)	Time after infection (5 weeks)	Time after infection (6 weeks)
I	(8/10)	(4/10)	(3/10)	(2/10)
II	(9/10)	(6/10)	(5/10)	(4/10)
III	(9/10)	(7/10)	(6/10)	(3/10)
Std	(5/10)	(3/10)	(0/10)	(0/10)
Control	(10/10)	(10/10)	(10/10)	(10/10)

Table 3. Zone of inhibition of 1% cream preparation of the compounds by Cup Plate Method

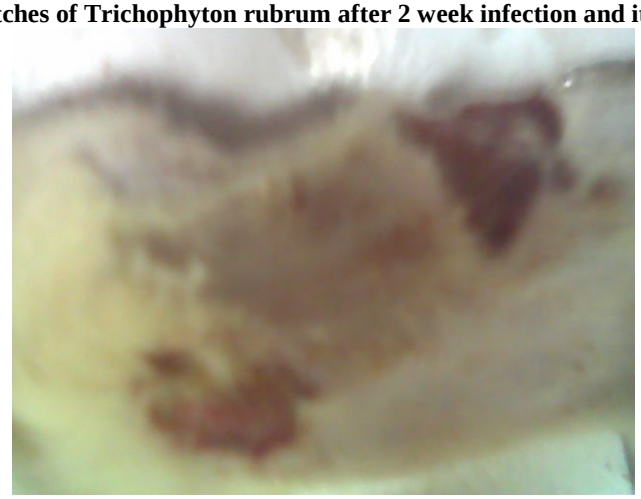
Fungal species	Standard Zone of inhibition (mm)	Drug I Zone of inhibition(mm)	Drug II Zone of inhibition(mm)	Drug III Zone of inhibition(mm)
Trichophyton rubrum	30	21	19	22
Candida albicans	28	19	20	21
Candida glabrata	28	18	19	20
Aspergillus niger	25	17	18	19
Aspergillus flavus	26	16	17	18



Slide 1. The hole in between the infected area of Trichophyton rubrum after 2 week infection

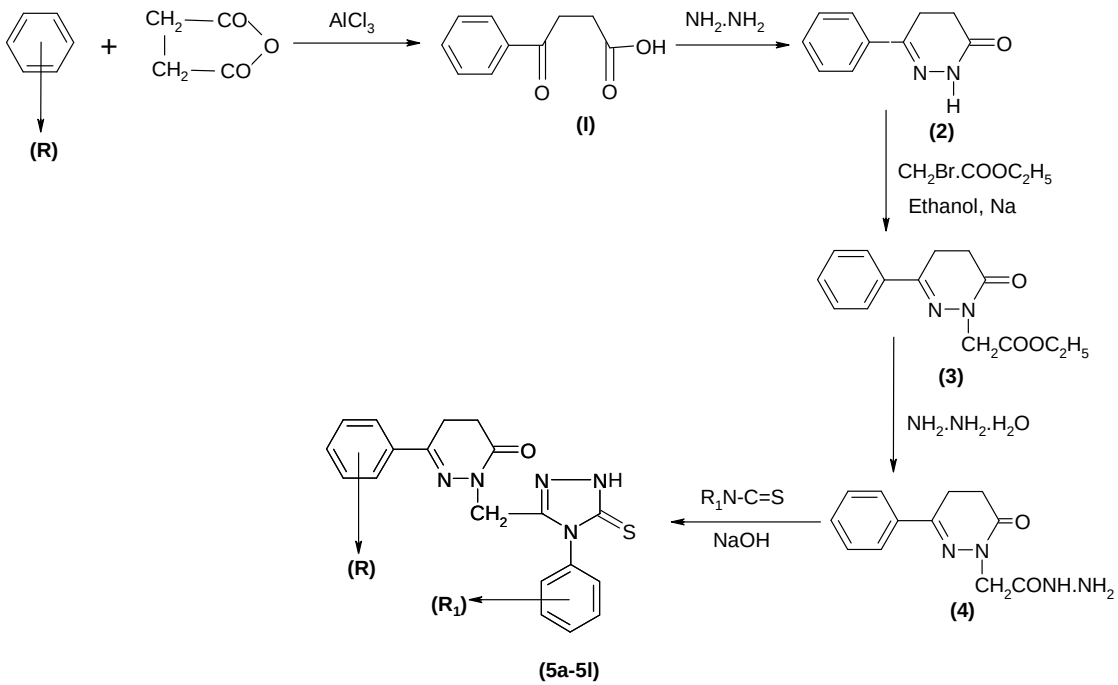


Slide 2. The skin patches of Trichophyton rubrum after 2 week infection and it was fully developed



Slide3.The skin patches of Trichophyton rubrum after 4 week post infection treatment of Test drug III.

Scheme



a): R= H, CH_3, Cl
b): R_1= H, p-Cl, p-Br
Scheme-1 Synthesis of 6-substituted-2[4'-(substituted phenyl-5'-thioxo)-1,2,4-triazol-3-yl}-methyl]-2,3,4,5-tetrahydropyridazine-3-one.

REFERENCES

[1] T Arika, .; M Yokoo.;H Yamaguchi ;. *Antimicob. Agents Chemother*. **1992** 36, 2523-2525
[2] R Traboulsi .;Z A Kanafani.; S Kanj, *J. Med. Liban*, **2004**. 52, 202-209
[3] M Mohrenschlager.;H Seidl .;J Ring.; D Abeck, *American J. Clin. Dermatol*. **2005**, 6, 203-213,
[4] T Itoyoma.; K Uchida.;H Yamaguchi, *J. Antimicrob. Chemother*. 1997, 39, 825-827,
[5] T Arika; M Yokoo.; T Hase.; T Maeda.; K Amemia.; *Antimicrob. Agents Chemother*. 1990, 34, 2250-2253.
[6] K Uchida.; H Yamaguchi; *J. Antibiot*.; **1999**, 52, 68-74
[7] T Ohmi.; S Konaka.; M Uchida.; H Yamaguchi; *Drug Res*. **1991**, 41, 847-851
[8] H Oka.; Y Niwano.; T Tanaka.; M Uchida.; H Yamaguchi; *Drug Res*. **1992,** 42, 345-349.
[9] G Aperis.; E Mylonakis;. *Expert Opin Investig Drugs*. **2006**, 15, 579-602.
[10] A K Gupta.; Y Kohli.; R Batra; *Med. Mycol*; **2005**, 43, 179-185.
[11] M Borgers.; H Degreef.; G Cauwenbergh;. *Curr Drug Targets*; **2005**, 6, 849-62
[12] R A Fromtling; *Clinical Microbiology Reviews*; **1988**, 45, 187-217,
[13] T Arika, .; M Yokoo.;H Yamaguchi ;. *Antimicob. Agents Chemother*. **1992** 36, 2523-2525
[14] K Uchida.; T Tanaka.; H Yamaguchi; *Microbiol Immunol*;. **2003**, 47, 143-146.
[15] A A Padhye.; I Weitzman;. Medical Mycology. In Ajello, L., and Hay, R. J. (eds), Topley and Wilson's microbiology and microbial infection, The Hodder Headline Group, London; **1998**, 215-236.

[16] K Uchida.; T Tanaka.; H Yamaguchi; Microbiol Immunol; **2003**, 47, 143-146.
[17] Y Tatsumi.; M Yokoo.; T Arika.; H Yamaguchi; *Antimicrob. Agents Chemother*; **2001**, 45, 1493-1499.
[18] K Uchida.; H Yamaguchi;. **1999**, J. *Antibiot*; 52, 68-74.
[19] H Yamaguchi.; K Uchida.; T Tanaka.; T Yamaguchi;. *Jpn. J. Antbiot*.; **2001**, 54, 323-330.
[20] T Itoyoma.; K Uchida.; H Yamaguchi;. *J. Antimicrob. Chemother*; **1997**, 39, 825-827,
[21] K Uchida.; M Kudho.; H Yamaguch; *Jpn. J. Antibiot*;. **1994**, 47, 1407-412.
[22] T Arika.; M Yokoo.; T Hase.; T Maeda.; K.;Yamaguchi; *Antimicrob. Agents Chemother*; **1990**, 34, 2250-2253.
[23] T Arika.; M Yokoo.; H Yamaguchi; *Antimicob. Agents Chemother*; **1992,** 36, 2523-2525.