Review Article

Antifungal Targets

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Antifungal drugs are most widely used drugs, but suffer from a common drawback of immediate development of resistance. In the recent years, several drugs were developed which cause various toxic effects. Various novel approaches were carried out to the current existing drugs for reducing the fungal resistance. These mainly involve structural modification of existing antifungal agents to increase fungal intracellular concentration of drug, and thereby to increase antifungal activity. The incidence of systemic fungal infections has been increasing dramatically due to an increase in the number of immunocompromised hosts.

Key words: Antifungal, Azoles, resistance, Candida spp., Triazole

INTRODUCTION

The developments of resistance to currently available antifungal azoles in Candida spp., as well as clinical failures in the treatment of fungal infections have been reported. Triazole may be considered as a bioisostere of imidazole that is incorporated into the structures of many antifungal compounds.[1]

Pathogenic (disease-causing) microorganisms have repeatedly altered the course of human history. For example, the data from the National Noscomial Infections Surveillance System conducted in the United States showed a 487% increase in Candida bloodstream infections between 1980-1989.[2]

Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents. Immunocompromised patients are particularly at risk of developing these infections, with *Candida* and *Aspergillus* spp. being the mycoses most commonly identified. Patients who develop candidemia have a mortality rate as high as 60%.

In addition, the prevalence of *Candida* spp. that are resistant to triazole antifungal agents is increasing, making treatment options a concern. Aspergillosis carries a 100% mortality rate if left untreated. Although there are numerous treatment options, no broad-spectrum antifungal agents with an acceptable safety profile and

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with both intravenous and oral formulations are available at this time.[3]

The incidence and severity of fungal diseases has increased due to impaired immunity. The growing number of cases of fungi implicated in sepsis has been shown to be a consistent trend and Candida is the third or fourth most common isolate in Noscomial blood stream infections in the United States. The invasive Candidosis is a problem of critically ill non-immunosuppressed patients. The mortality rate due to invasive aspergillosis has increased by 357% between 1980 and 1997 in the United States.[4]

Until the 1970s, fungal infections had generally been considered curable and thus the demand for new antifungal drugs had been very low. To this day, superficial fungal infections of the skin and nails are common and for the most part treated successfully with existing antifungal agents. However, at the same time, serious invasive fungal infections caused by Candida spp., Cryptococcus neoformans, Aspergillus spp., Pneumocystis carinii and Histoplasma capsulatum are becoming a growing danger to human health.[5]

With the increase in incidence of systemic mycosis in recent years, there has been an increasing emphasis on the importance of antifungal chemotherapy. Since the introduction of fluconazole for clinical use, aspergillosis has replaced Candidosis as the most important issue in the control of mycosis. Thus the development of broad-spectrum antifungal agents with a potent anti-Aspergillus activity and good safety is needed.[6]

Several clinical drugs, such as azoles, amphotericin B, 5-fluorocytosine, and caspofungin, have been developed to reduce the impact of fungal diseases. Among those, azoles, especially triazole antifungal agents, were used widely and efficiently. For example, fluconazole, voriconazole and itraconazole presently play a leading role in the treatment of invasive fungal infections.[7]

Definition: An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host.

Types of Mycoses:

The fungal infection can be divided into various groups[8]

1. Superficial Infection –

These are the most common type of infection. It includes the various forms of Tinea or ringworm, which are the infections of the hair or hair follicles. These lesions are mild, superficial and restricted. The causative microbes are specialized with the unusual ability to digest keratin. They are frequently transmitted from one host to another. e.g. Athlete's foot.

2. Systemic Mycoses-

The deep-seated, systemic mycoses have a sporadic distribution. The causative organism includes histoplasmosis, sporotrichosis, blastomycosis, coccidomycosis, cryptococcosis. The fungal spores are inhaled into the lung, and a mild, cold like condition may result. The causative agents are soil inhabiting saprophytes with the ability to adopt to the internal environment of their host.

3. Opportunistic Fungal Infections-

These infections include systemic Candidiasis, aspergillosis, and mucormycosis. *Candida albicans* is a particularly common opportunist. Oral Candidiasis is common in poorly nourished persons, inpatients on immunosuppressive drugs.

4. Cutaneous Infection-

These are the superficial infections of the keratinized epidermis and keratinized epidermal appendages (the hair and nails). The Severity of an infection depends largely on the location of the lesion and the species of fungus involved. The ability of these organisms to invade and parasitize the cornified tissues of hair, skin and nails.

5. Subcutaneous Fungal Infections-

Subcutaneous mycosis refers to a group of fungal disease in which both skin and subcutaneous tissues are involved but typically, no dissemination of internal organs occurs. They are primary soil saprophytes of very low-grade virulence and invasive ability. The tissues reaction is a localized lesion.[9]

1.2.3. Classification of antifungal agents**:**

1. Polyenes: [10]

The Polyenes are complex molecules with a large conjugated double bond (-CH=CH-) system in a lactone ring linked to an amino sugar. These large cyclic lactones have lipophillicity and lipophobic portions making solubility in water difficult. They have useful antifungal properties, but are not antibacterial because of absence of sterols in bacterial cell membranes.

2. Azoles:

The azoles represent a class of versatile antifungal agents within an apparently unique mechanism of action. Azoles at high concentrations (micro molar) are fungicidal and at lower concentrations (nanomolar) are fungistatic. The characteristic chemical features of azoles are presence of five-member aromatic ring containing either two or three nitrogen atom.

a) Mechanism of Action:

Azoles interfere with ergesterol biosynthesis by inhibiting lanosterol 14α-demethylase thereby causing accumulation of 14α sterols that disrupt the various sterol functions in the cell. The target protein (lanosterol 14α -demethylase, also known

as CYP-450) is a cytochrome P-450 (CYP-450) enzyme that achieves its effects by three sequential radical mediated hydroxylation's converting from a hydrocarbon through alcohol, aldehyde, and carboxylic acid oxidation states. The methyl group is eliminated as formic acid to create double bond between C-14 and C-15 of the D-ring.[11]

The most significant agent in clinical use is Chlormidazole, Miconazole, Clotrimazole, Econazole, Ketoconazole, Itraconazole and Fluconazole. Voriconazole. The recent advances in azoles include- Posaconazole and Ravuconazole. The latter two are currently in phase III clinical trial.[12]

3. Allylamines[13]

The allylamines are the most prominent of a number of antifungal classes that exert their activity by through inhibition of squalene epoxidase; squalene epoxidase forms an epoxide at the C-2 $ C-3 position of Squalene. The prominent examples of this class are Terbinafine, Naftifine, and Butenafine.

4. Candins: [14]

Echinocandins are presumed to act as non-competitive inhibitors of (1, 3)-2-D glucan synthase, an enzyme complex that forms glucan polymer in the fungal cell wall. E.g.:- Anidulafungin (LY 303366), V-Echinocandin, (V-002). Cilofungin is an antifungal cyclopeptide which inhibits cell wall (1, 3)-β-glucan biosynthesis in fungal organisms.

5. Miscellaneous drugs:-

A. Other inhibitors of ergosterol biosynthesis, Thiocarbamates: [15]

These are reversible, non-competitive inhibitors of squalene epoxidase with inherent selectivity for the fungal enzymes. The most significant member of the class is Tolnaftate.

B. Flucytosine: [16]

Flucytosine is a fluorinated pyrimidine related to the anticancer agent flurouracil.

C. Griseofulvin: [17]

It acts by arresting cell division in metaphase by binding with the tubuline dimer required for microtubule assembly. It may also interfere directly with DNA replication.

D. Antifungal Antibiotics:-

a) Polyoxins and Nikkomycins[18]

These are naturally occurring nucleoside peptide antibiotics that inhibit chitin synthase, an enzyme that catalyses the polymerization of N-acetyl glucosamine.

b) Sordarins: [19]

A unique tetracylic diterpene core including a norbornene system inhibits protein synthesis in fungi by stabilizing the ribosome/EF2 complex.

c) Galbonolide B: [20]

It is natural products developed against fungal resistance work by inhibiting

sphingolipid biosynthesis.

d) Aureobasidins

Aureobasidins A is a cyclic depsipeptide produced by *Aureobasidium pullan*, inhibits inositol phosphorylceramide synthase, an enzyme essential and unique in fungal sphingolipid biosynthesis.

1.2.4Mechanisms of antifungal resistance:

Resistance to azoles has become a significant problem since their use in the treatment of systemic fungal infections has increased. Target modifications is clearly common contributor to clinical resistance to azoles therapy and has been implicated directly for *C. albicans* and by interference for other Candida spp. Azoles resistance also occurs when compensatory changes exist in other enzymes in the ergesterol biosynthesis pathway notably inactivation of $\Delta^{5,6}$ desaturase. This lead to the build of non-toxic 14α-methylated sterols in fungal membranes.

The change in membrane composition can lead to cross-resistance to Polyenes. Probably the most prevalent cause of resistance to azoles therapy is caused by reduction in intracellular drug concentration ascribed to active efflux. In C. albicans, two types of efflux pump have been shown to be clinically relevant: a member of the major facilitator super family known as MDR1 and ATP binding cassette (ABC) -type transporters CDR1 and CDR2.

Table 1: Discovery of antifungal drugs [21]

Decade	Drug
1950s	Amphotericin
1960s	Griseofulvin
1970s	Flucytosine, clotrimazole, miconazole
1980s	Ketoconazole, fluconazole, itraconazole
1990s	Terbinafine, naftifine,
2000s	Caspofungin
Under development	Voriconazole, posaconazole, ravuconazole, micafungin(FK-463), sordarins, pramidicin, nikkomycin

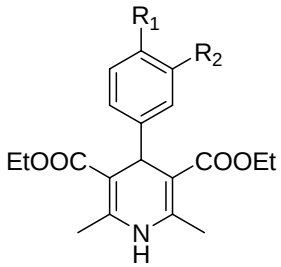
Table 2: Drugs of choice in the treatment of systemic fungal infections [22]

Disease	Fungus	Effective antifungal agents
Actinomycosis	Actinomyces israelii	Penicillin G,
Aspergillosis	Aspergillus fumigatus,	Amphotericin B, Rifampin
Blastomycosis	Blastomyces dermatidis	Amphotericin B, Rifampin
Candidiasis	Candida albicans	Amphotericin B, Nystatin
Chromoblasto-mycosis	Cladosporium	Amphotericin B, Flucytosine
Coccidiodo-mycosis	Coccidodes immitis	Miconazole, ketoconazole
Cryptoco-ccosis	Cryptococcus neoformans	Amphotericin B, Flucytosine

Dihydropyridine as antifungal agents:

1. Sharma GL ***et al.*** synthesized the ten 4-aryl-1, 4- dihydropyridine and three 4-

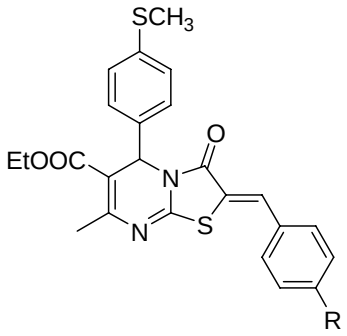
aryl-1, 2, 3, 4-tetrahydropyrimidin-2-one derivatives and examined for their activity against pathogenic strains of *Aspergillus fumigatus* and *Candida albicans* by disc diffusion, micro broth dilution and percent spore germination inhibition. The two of the compounds of dihydropyridine series exhibited significant activity against A. fumigatus. The most active diethyl dihydropyridine derivative exhibited a MIC value of 2.92 µg/disc in discdiffusion and 15.62 µg/ ml in micro broth dilution assays. [23]



R_1 = Br, OMe, OH

R_2 = Cl, OH, OMe

2. Kumari N. S. *et al.* synthesized a series of new 2-(arylidene/5-arylfurfurylidene)-5-(4-methylthiophenyl)-5H-thiazolo [2,3-b] pyrimidin-3(1H)-ones 2 and 3 have been synthesized by a three component (MCR) reaction involving 4-(4-methylthiophenyl)-5-carbethoxy-6-methyl-3,4- dihydro-pyridine-2(1H)-thione, monochloroacetic acid and arylaldehydes/arylfurfuraldehydes, respectively. The newly synthesized compounds were screened for their antibacterial and antifungal activities and exhibited moderate to excellent growth inhibition of bacteria and fungi.[24]



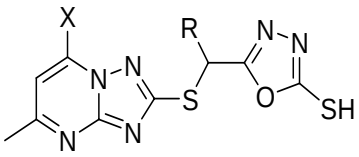
R = 4-SCH_3, 4-OCH_3, 4-Cl, 4-F, 4-OH, 2, 4-Cl_2, 4-OH 3-OCH_3, 4-F-3-OPh

3. Ablordeppey S. Y *et al.* synthesized and evaluated the isosteres of cryptolepine for their anti-infective activities. Both the carbon and oxygen isosteres were less potent than cryptolepine. The evaluation of the activities of 5b compared with standard antifungal /anti-protozoal agents suggests that the benzothienoquinoline scaffold could serve as a lead for optimization.[25]

X = NH, S

4. Yang G.F, *et al.* designed and synthesized, a series of new 1, 2, 4-triazolo [1, 5] pyrimidine derivatives bearing 1, 3, 4-oxadiazole moieties in order to search novel agrochemicals with higher antifungal activity. By determining the EC_{50} values of all the newly synthesized compounds 2-((5-(sec- butylthio)- 1, 3, 4- oxadiazo - 2- yl)-

methylthio)-5-dimethyl-1,2,4- triazolo-[1,5-a] pyrimidine, was found to display the highest antifungal activity (EC_{50} = 6.57 µg /mL).[26]



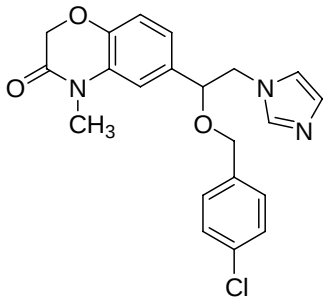
X = H, CH_3,
R = H, CH_3

5. Singh OM, *et al*., efficiently catalyses the synthesis of dihydropyrimidinones (80–96% yields) by the Biginelli reaction in presence of Copper (II) chloride in the absence of any solvent. Six compounds were selected and examined their antifungal activities against the radial growth of three fungal species viz., Trichoderma hammatum, Trichoderma koningii and Aspergillus niger.[27]

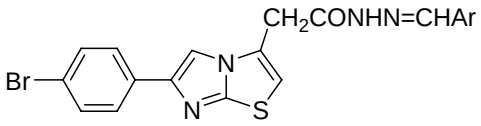
R^1 = C_6H_5, 2-HO C_6H_4, 4-Cl C_6H_4
R^2 = C_2H_5, C_2H_5, C_2H_5

Biological Profiles of Azoles / Imidazoles:

1. Fringuelli R. *et al.* synthesized the 1, 4-benzoxazine analogues and examined their possible antifungal activity to further analyze the structure–activity relationships. Results of in vitro and in vivo experiments showed that 1, 4-benzoxazine analogues show appreciable antifungal activity.[28]

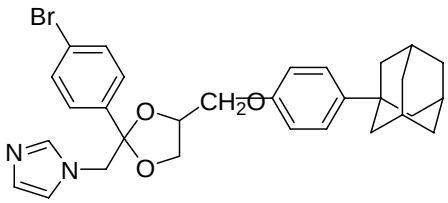


2. Gursoy E. *et al.* synthesized a series of arylidenehydrazides (3a-3i) from [6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetic acid hydrazide. The synthesized compounds were evaluated in the National Cancer Institute's 3-cell line against the full panel of 60 human tumors cell lines at a minimum of five concentrations at 10-fold dilutions.[29]



Ar = C_6H_5, 2-OHC_6H_4, 4-OHC_6H_4, 4-$OCH_3C_6H_4$, 4-F C_6H_4, 4-BrC_6H_4

3. **Plachta D.A**. *et al.* synthesized the 1-{4-[4-(adamant-1-yl) phenoxymethyl]-2-(4-bromophenyl)-1, 3-dioxolan-2-methyl}imidazole with expected antifungal and antibacterial activity. A multistep synthesis of a new analogue of ketoconazole has been described.[30]



4. Botta M. *et al.* designed and synthesized the homochiral azole antifungal agents by rationality. The first synthesis of both enantiomers of the antifungal drug bifonazole and related imidazole compounds is described, starting from enriched amines, to predict the inhibitory activity of azoles compounds against *C. albicans* $P450_{14DM}$.[31]

R_1 = CN, Cl, SMe, CF_3, NO_2, Ph
X = CH, N, CH

5. **Sztanke K**. *et al.* designed 3-unsubstituted and 3-substituted-7-aryl-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazoles were and obtained from biologically active 1-aryl-2-hydrazonoimidazolidines by cyclocondensation reaction with triethyl orthoformates, phenoxyacetic acid derivatives and carbon disulfide, respectively. Compounds were evaluated for their cytotoxic activity against three cancer cell lines. [32]

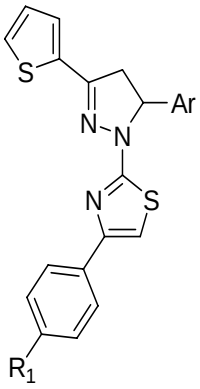
R = 4-OCH_3, 4-Cl, 3, 4-Cl_2, H, 4-CH_3
R' = 4-Cl, 2, 4-Cl_2, SH, H, 2-CH_3; 4-Cl

Different moieties with antifungal activity:

1. **Zhou Y.J.** *et al*. designed and synthesized novel tetralin compounds on the three-dimensional model of lanosterol 14α-demethylase of Candida albicans. All of the lead compounds exhibited potent antifungal activities. They interact with the amino acid residues in the active sites and avoid the serious toxicity arising from azoles.[33]

R = H, $(CH_2)_3CH_3$, C_6H_4-*o* -NO_2, $(CH_2)_7CH_3$, *p* –Cl C_6H_4
R' = 5-OH, 6-OH, 7-OH, 8-OH

2. **Ozdemir A.** *et al.* synthesized several 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives by reacting substituted 3-(2-thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines with phenacyl bromides in ethanol. Their antifungal activities against *Candida albicans* and *Candida glabrata* were investigated.[34]



CONCLUSION:

The development of new potential drugs, which will be devoid of side effect profile of currently available drugs, will be one of the possible solutions to treat various infectious diseases with multi-drug regimen over a long period. Extensive efforts have been made to find more effective antifungal agents and to decrease the fungal resistance to the current existing drugs.

REFERENCE:

1. Zahra R, Soghra K, Keyvan P, Zahra H, Amiri F, Elham A. Design, synthesis and antifungal activity of triazole and benzotriazole derivatives. Eur J Med Chem 2008, 1-4.
2. Abraham, D J; In Burgers Medicinal Chemistry and Drug Discovery, Vol-5 Chemotherapeutic agents, A John Wiley and Sons, Inc., Publication, 2003**,** 881-911.
3. Greer N. D. Voriconazole: the newest triazole antifungal agent, BUMC PROCEEDINGS, 2003; 16(2):241-248.
4. Pinto E, Kirsch G, Begouin JM, et al. Antifungal activity of synthetic di(hetero)arylamines based on the benzo[b]thiophene moiety, Bioorg. Med. Chem., 2008; 16:8172-8177.
5. Borelli C, Korting HC, Ruge E, et al. Current state of three-dimensional characterization of antifungal targets and its use for molecular modeling in drug design, Int. J. Antimicrob. Agents. 2005; 26: 427-44.
6. Uchida K, Yokata N, Yamaguchi H, et al. In vitro antifungal activity of posaconazole against various pathogenic fungi, Int. J. Antimicrob. Agents, 2001; 18 :167-172.
7. Jiang Y, Chai X, Zhang J, et al. Design, synthesis, and biological evaluation of novel triazole derivatives as inhibitors of cytochrome P450 14α-demethylase, Eur. J. Med. Chem.,2009; Article in Press:1-8.
8. John M, Beale J, "Anti-infective Agents" In:Block, J. H.; Beale, J. M.; Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. Lippincot williams and wikins, 11th edition, 2004, New York, 230-246.
9. Pedler SJ, Fungal Infections In: Walker R, Whittlesea C, Clinical pharmacy and therapeutics.Churchill Livingstone Elsevier, 4th ed.,2007: 599-610.
10. Barar, F. S. K. In Essentials of Pharmacotherapeutics, S. Chand $ Company Ltd,3rd Edition, 2006**,** 501-507.
11. Ballington DA, Laughlin MM, Pharmacology, CBS Publishers $ Distributors, New Delhi, 3rd ed., 2008: 97-100.
12. Abraham, D. J.; In Burgers Medicinal Chemistry and Drug Discovery, Vol-5 Chemotherapeutic agents, A John

Wiley and Sons Inc. Publication, 2003, 881-911.

13. Tripathi, K. D.; In Essentials of Medical pharmacology, Jaypee Brother's Publication (P) Ltd. New Delhi, 5th edition, 2003, 715-724.

14. Waserman BP, Frost DJ, et al "Use of Cilofungin as Direct Fluoroscent Probe for Monitoring Antifungal Drug-Membrane Interactions" Antimicrobial Agents and Chemotherapy" Vol. 38, No.6, June 1994, 1378-1385.

15. Bennet, J. E. "Antimicrobial Agents: Antifungal Agents"; In Goodman and Gilmon's The pharmacological basis of therapeutics, McGraw-Hill Publication, 11th Edition, 2006, 1029-1057.

16. Harvey R A; Champe P.C; Finkel R; Lippincott's Illustrated Reviews: Pharmacology, Lippincot Williams and Wikins, New Delhi, 4th edition, 2009,407-415.

17. Dollery C. Therapeutic Drugs, 2 nd ed. Vol-1(A-H), Churchill Livingstone, F62-F65.

18. Matsuda A, Ebisu H, Sugimoto O, Iwase K, et al; " Synthesis and structure-activity relationships of novel nikkomycin analogs: Inhibitors of the fungal cell wall biosynthesis enzyme chitin synthase" Nucleic Acids Symposium Series No. 42, 1999, Oxford University Press, 13-14.

19. Liang H, "Sordarin, an antifungal agent with a unique mode of action" Beilstein Journal of Organic Chemistry, 2008, (4):31.

20. Pennicott L, Parsons P et al, "A total synthesis of the antifungal agent Galbonide B" Life Sighs, Issue 7 Autumn 2006.

21. Rang, H. P.; Dale, M. M.; Riter, J. M.; In pharmacology, Churchill Livingstone, 5th edition, 2003, 666-671.

22. Kadam, S.S; Mahadik, K.R; Bothra, K.G; "Principles of Medicinal Chemistry" 6th edition, 2008, 115-124.

23. Sharma GL. et al. Microwave-assisted synthesis of antimicrobial dihydropyridines and tetrahydropyrimidin-2-ones: Novel compounds against aspergillosis, Bioorganic & Medicinal Chemistry, 2006; 14: 973–981.

24. Kumari NS, Ashok M, Holla BS et al. Convenient one pot synthesis of some novel derivatives of thiazolo[2,3-b]dihydropyrimidinone possessing 4-methylthiophenyl moiety and evaluation of their antibacterial and antifungal activities, Eur J Med Chem, 2007;42:380-385.

25. Ablordeppey SY, Jacob M, Khan S, Walker L et al.Synthesis and evaluation of isosteres of N-methyl indolo[3,2-b]-quinoline cryptolepine) as new antiinfective agents, Bioorganic & Medicinal Chemistry, 2007; 15: 686–695.

26. Yang GF, Chen Q, Zhu XL, Jiang LL, Liu ZM., Synthesis, antifungal activity and CoMFA analysis of novel 1, 2, 4-triazolo[1,5-a]pyrimidine derivatives. European Journal of Medicinal Chemistry 43 (2008) 595-603.

27. Singh OM, Singh SJ, Devi MB. Synthesis and in vitro evaluation of the antifungal activities of dihydropyrimidinones, Bioorganic & Medicinal Chemistry Letters 2008; 18: 6462–6467.

28. Fringuelli R, Pietrella D, Schiaffella F. Anti-Candida albicans Properties of Novel Benzoxazine Analogues, Bioorganic and Medicinal Chemistry 2002;10: 1681–1686.

29. Gursoy E, Guzeldemirci NU. Synthesis and primary cytotoxicity evaluation of new imidazo[2,1-b] thiazole derivatives, Eur. J. Med. Chem., 2007; 42: 320-326.

30. Plachata DA, Baranowski AM, Laudy AE. et al. Synthesis of 1-{4-[4-(adamant-1-yl) phenoxymethyl]-2-(4-bromophenyl)-1, 3-dioxolan-2-methyl}imidazole with expected antifungal and antibacterial activity, Acta Poloniae Pharmaceutica – Drug Research, 2007; 64 (6):535-540.

31. Botta M, Corelli F, Manetti F, et al. Rational design and synthesis of homochiral azole antifungal agents, Pure Appl. Chem., 2001;73(9):1477-1485.

32. Sztanke K, Tuzimski T, Rzymowska J. et al. Synthesis, determination of the lipophilicity, anticancer and antimicrobial properties of some fused 1,2,4-triazole derivatives, Eur. J. Med. Chem., 2008;43:404-419.

33. Zhou YJ, Yao B, Zhu J,. Design, Synthesis and Biolgical Evaluation of Non-azole Inhibitors of Lanosterol 14α-Demethylase of Fungi, Chinese Chemical Letters, 2006;17(9):1189-1192.

34. Ozdemir A, Zitouni GT, Kaplancikli ZA. et al. synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives, Eur. J. Med. Chem., 2007; 42: 403-409.