

Synthesis and antifungal activity of benzimidazole, benzotriazole and aminothiazole derivatives

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Abstract

In recent years, the use of antifungal drugs in human medicine has increased, especially with the advent of AIDS epidemic. Efforts have focused on the development of new, less toxic and more efficacious antifungal drugs with novel mechanism of action. The purpose of this study was to synthesize of some new benzimidazole, benzotriazole and aminothiazole derivatives and to evaluate their activity against some species of *Candida*, *Aspergillus* and dermatophytes. The desired compounds were synthesized by the reaction of benzimidazole and benzotriazole with bromoalkanes and also by the reaction of an amide derivative of aminothiazole with 2-piperazino-1-ethanol in an efficient solvent in the presence of tetraethyl ammonium bromide or triethylamine) as catalyst. Chemical structures of all the new compounds were confirmed by spectrophotometric methods. Antifungal activities of the new compounds were evaluated by broth micro dilution method as recommended by CLSI. Among the tested compounds, 1-nonyl-1*H*-benzo[*d*]imidazole and 1-decyl-1*H*-benzo[*d*]imidazole exhibited the best antifungal activities. Of the examined synthetic compounds in different categories, benzimidazole derivatives established better antifungal activities than benzotriazole derivatives, and the piperazine analogue had no significant antifungal effect.

Keywords: Benzimidazole; Benzotriazole; Aminothiazole; Antifungal activity

INTRODUCTION

During the past two decades, the frequency of invasive and systemic fungal infections has increased dramatically due mainly to *Candida* species (1). The growing number of immunocompromised patients as a result of cancer chemotherapy, organ transplantation, and HIV infection are the major factors contributing to this incidence (2). Recently, the expansion of antifungal drug research has occurred because there is a critical need for new antifungal agents to treat these life-threatening invasive fungal infections (3). Since *Candida albicans* and *Aspergillus fumigatus* are the main causative fungi of the systemic mycosis, antifungal drugs for treating patients of deep mycosis should have a broad antifungal spectrum including at least these microorganisms. Unfortunately, none of the antifungal drugs which are currently available

for the treatment of systemic mycoses are ideal in terms of efficacy, antifungal spectrum or safety (2).

Azole compounds are divided into the older imidazoles and the new triazoles (4). They inhibit fungal cytochrome P₄₅₀ 3A-dependent C14- α -demethylase which is responsible for the conversion of lanosterol to ergosterol, thereby depleting ergosterol in the fungal cell membrane (3). Interaction of triazoles with cytochrome P₄₅₀ may cause disturbances of hepatic enzymes (4). Azole antifungal activity varies with each compound and clinical efficacy may not coincide with *in vitro* activity (3). Newer and less toxic antifungal agents are available for clinical use, but their clinical efficacy in some invasive fungal infections, is not optimal (5).

The incorporation of the benzimidazole nuclei is an important synthetic strategy in drug discovery. The high therapeutic

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properties of the related drugs have encouraged the medicinal chemists to synthesize the large number of novel chemotherapeutic agents (6).

In general, nitrogen and sulfur containing organic compounds and their metal complexes display a wide range of biological activity as antitumor, antibacterial, antifungal and antiviral agents (7).

In the previous reports, we described the preparation of a number of imidazole, benzimidazole and benzotriazole derivatives with biological interest (8-10). In the current study, we have synthesized some new derivatives of benzimidazole, benzotriazole and aminothiazole as antifungal agents. In these new analogues an alkyl chain is substituted at the position 1 of benzimidazole and benzotriazole rings. A new compound of 2-aminothiazole also was prepared by the reaction of a piperazine moiety with an amide intermediate of 2-aminothiazole.

MATERIALS AND METHODS

All chemicals and solvents were purchased from Merck, Germany. ¹HNMR spectra were obtained on a Bruker Avance DPX 500 MHz instrument, USA. Thin-layer chromatography (TLC) was performed using 250 μm silica gel GF plates, Merck, Germany. All reactions were carried out under nitrogen gas. Microorganisms were obtained from Mycology and Parasitology department of the Shiraz University of Medical Sciences.

Chemistry

In the benzimidazole and benzotriazole categories, theazole rings reacted with appropriate bromoalkane in a suitable solvent at reflux temperature. 1,2,4-Triazoles can be alkylated on N1 or N4 and 1,2,3-benzotriazoles on N1 or N2 positions. The controlled regioselective alkylation of them is a difficult task. Our strategy was to modify the reported methods to get N1-substituted of the azoles in higher yield. Therefore, we employed acetonitrile as the solvent, and tetra ethyl ammonium iodide, anhydrous potassium carbonate, and sodium hydroxide as catalysts (10). The reaction mixture was worked up to

get the crude product. Purification was carried out by plate chromatography. Chemical structures of the final compounds were confirmed by spectroscopy methods. For the aminothiazole analogue in the first step 3-chloropropionyl chloride was reacted with 2-aminothiazole to get an amide intermediate and in the second step, 2-piperazino-1-ethanol was reacted with the intermediate to afford the final compound.

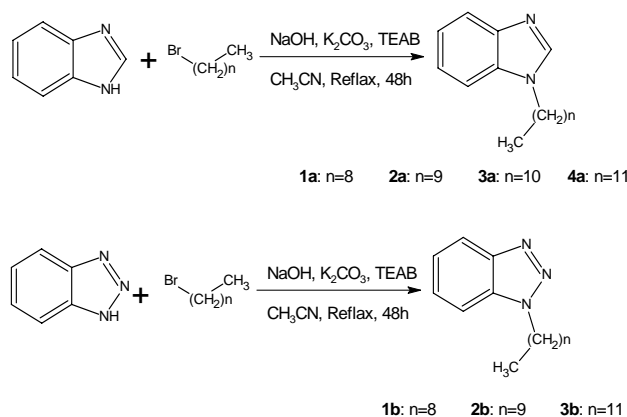
General procedure

Benzimidazole and benzotriazole series

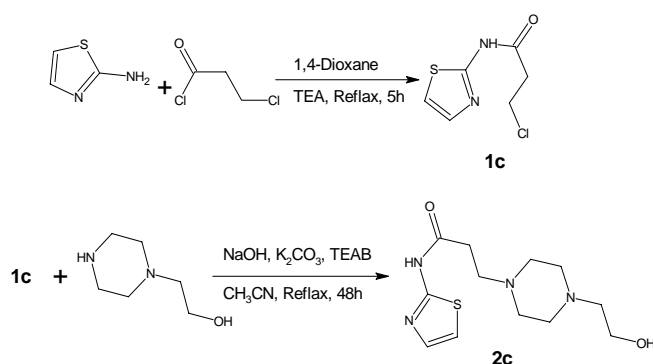
Benzimidazole or benzotriazole ring, bromoalkane, tetraethyl ammonium bromide (TEAB), sodium hydroxide and potassium carbonate in acetonitrile were refluxed for 48 h. The reaction mixture was then filtered and concentrated in vacuo. Water and chloroform was added, the organic layer was dried over Na₂SO₄, and concentrated in vacuo. The crude compound was purified by plate chromatography on silica gel using petroleum ether and ethyl acetate as solvent to obtain the final product (Scheme 1).

Aminothiazole analogue

In the first step, 3-chloropropionyl chloride and triethylamine (TEA) were added dropwise to a solution of 2-aminothiazole in 1,4-dioxane and reacted at reflux temperature. The crude product filtrated and extracted by dichloromethane and water. The organic layer quenched with saturated solution of NaCl in water and evaporated to get the amide



Scheme 1. Synthesis of benzimidazole and benzotriazole derivatives.



Scheme 2: Synthesis of aminothiazole analogues.

intermediate. In the second step, piperazine 2-ethanol was reacted with the amide intermediate in the similar condition as benzimidazole and benzotriazole compounds reacted with bromoalkanes (Scheme 2).

Synthesis of benzimidazole series (series a)

General procedure for the synthesis of benzimidazole series (series a)

A Solution of 590 mg benzimidazole (5 mmol), 0.95 ml of bromononane (5 mmol), 200 mg of NaOH (5 mmol), 690 mg of potassium carbonate (5 mmol), and 42 mg of TEAB (5 mmol) in 25 ml acetonitrile were heated under reflux for 48 h. Compounds **1a**, **2a**, **3a** and **4a** were purified by preparative thin layer chromatography using chloroform/methanol: 95 ml/5ml.

1-Nonyl-1 H-benzo[d]imidazole (1a)

670 mg (55%) compound **1a** was obtained as brown oily liquid. ^1H NMR (DMSO- d_6 , 500 MHz): (δ ppm) 8.20 (s, 1H, imidazole), 7.56 (d, 2H, phenyl), 7.16-7.24 (m, 2H, phenyl), 4.21 (t, $J=7.07$ HZ, 2H, N- CH_2), 1.76 (m, 2H, N- CH_2 - CH_2), 1.20 (m, 12H, N- CH_2 - CH_2 - $(\text{CH}_2)_6$), 0.81 (t, $J=7.12$ HZ, 3H, CH_3). ^{13}C -NMR: (125 MHz), δ (ppm) = 14.74, 22.92, 26.96, 29.37, 29.44, 29.73, 30.23, 32.08, 44.91, 111.15, 120.28, 122.14, 122.97, 134.66, 144.38, 144.82. MS (EI): M/Z (%): 243 (100 M^+), 227 (2.2), 213 (3), 199 (4.5), 130 (14), 117 (8).

1-Decyl-1 H-benzo[d]imidazole (2a)

900 mg (69%) compound **2a** was obtained as yellow liquid. ^1H NMR (DMSO- d_6 , 500MHz): (δ ppm) 8.20 (s, 1H, imidazole),

7.56 (d, 2H, phenyl) 7.16-7.24 (m, 2H, phenyl), 4.21 (t, $J=7.07$ HZ, 2H, N- CH_2), 1.76 (m, 2H, N- CH_2 - CH_2), 1.16 (m, 14H, N- CH_2 - CH_2 - $(\text{CH}_2)_7$), 0.82 (t, $J=7.09$ HZ, 3H, CH_3). ^{13}C -NMR: (125 MHz), δ (ppm) = 14.76, 22.94, 26.96, 29.36, 29.51, 29.74, 29.77, 30.23, 32.13, 44.91, 111.14, 120.283, 122.14, 122.96, 134.66, 144.38, 144.81. MS (EI): M/Z (%), 257 (100), 242 (1), 172 (10), 131 (11), 118 (9).

1-Undecyl-1 H-benzo[d]imidazole (3a)

890 mg (64%) compound **3a** was obtained as light cream crystals, m.p. 38-40°C. ^1H NMR (DMSO- d_6 , 500MHz): (δ ppm) 8.20 (s, 1H, imidazole), 7.55 (d, 2H, phenyl), 7.16-7.24 (m, 2H, phenyl), 4.20 (t, $J=7.09$ HZ, 2H, N- CH_2), 1.76 (m, 2H, N- CH_2 - CH_2), 1.22 (m, 16H, N- CH_2 - CH_2 - $(\text{CH}_2)_8$), 0.82 (t, $J=7.09$ HZ, 3H, CH_3). ^{13}C -NMR: (125MHz), δ (ppm) = 14.75, 22.95, 26.97, 29.37, 29.56, 29.77, 29.80, 29.82, 30.23, 32.15, 44.91, 111.12, 120.28, 122.13, 122.95, 134.66, 144.39, 144.80. MS (EI): M/Z (%): 271 (100), 228 (6), 172 (8), 131 (13), 118 (6).

1-Dodecyl-1 H-benzo[d]imidazole (4a)

1100 mg (77%) compound **4a** was obtained as cream liquid. ^1H NMR (DMSO- d_6 , 500 MHz): (δ ppm) 8.19 (s, 1H, imidazole), 7.55 (d, 2H, phenyl), 7.15-7.23 (m, 2H, phenyl), 4.20 (t, $J=7.08$ HZ, 2H, N- CH_2), 1.74 (m, 2H, N- CH_2 - CH_2), 1.22 (m, 18H, N- CH_2 - CH_2 - $(\text{CH}_2)_9$), 0.82 (t, $J=7.09$ HZ, 3H, CH_3). ^{13}C -NMR: (125MHz), δ (ppm)=14.75, 22.96, 26.97, 29.38, 29.58, 29.78, 29.79, 29.79, 29.87, 30.23, 32.16, 44.91, 111.11, 120.28, 122.12, 122.94, 134.66, 144.38, 144.79. Ms (EI); M/Z (%): 285 (100), 256 (4), 228 (6), 214 (4), 200 (6), 186 (6), 172 (7), 131 (10), 117 (6).

Synthesis of benzotriazole series (series b)

General procedure for the synthesis of benzotriazole series (series b)

A Solution of 595 mg benzotriazole (5 mmol), 0.95 ml of bromononane (5 mmol), 200 mg of NaOH (5 mmol), 690 mg of potassium carbonate (5 mmol), and 42 mg of TEAB (0.20 mmol) in 25 ml acetonitrile were heated under reflux for 48 h. Compound **1b**, **2b**, and **3b** were purified by preparative TLC using petroleum ether /ethyl acetate: 90 ml/10ml.

1-Nonyl-1H-1,2,3-benzotriazole (1b)

460 mg (37%) compound **1b** was obtained as light cream crystals, m.p. 32-35 °C. ¹H-NMR (DMSO-*d*₆, 500 MHz): (δppm) 8.02 (d, 1H, phenyl), 7.87 (d, 1H, phenyl), 7.53 (t, 1H, phenyl), 7.38 (t, 1H, phenyl), 4.69 (t, J=6.98 HZ, 2H, N-CH₂), 1.89 (m, 2H, N-CH₂-CH₂), 1.21 (m, 12H, N-CH₂-CH₂-(CH₂)₆), 0.81 (t, J=7.01 HZ, 3H, CH₃). ¹³C-NMR: (125MHz), δ(ppm) = 14.75, 22.90, 26.85, 29.22, 29.37, 29.63, 30.03, 32.04, 48.26, 111.40, 119.96, 124.68, 127.92, 133.67, 196.07. MS (EI): M/Z (%) 244 (100), 187 (18), 174 (37), 145 (58), 132 (81), 118 (37), 91 (40), 77 (21).

1-Decyl-1H-1,2,3-benzotriazole (2b)

550 mg (42%) compound **2b** was obtained as white crystals, m.p. 35-37°C. ¹H-NMR (DMSO-*d*₆, 500 MHz): (δppm) 8.02 (d, 1H, phenyl), 7.08 (d, 1H, phenyl), 7.52 (t, 1H, phenyl), 7.37 (t, 1H, phenyl), 4.68 (t, J=6.97 HZ, 2H, N-CH₂), 1.90 (m, 2H, N-CH₂-CH₂), 1.20 (m, 14H, N-CH₂-CH₂-(CH₂)₇), 0.80 (t, J=7.11 HZ, 3H, CH₃). ¹³C-NMR: (125 MHz), δ(ppm) = 14.74, 22.91, 26.85, 29.22, 29.46, 29.68, 29.68, 30.03, 32.10, 48.25, 111.37, 119.95, 124.65, 127.89, 133.66, 146.02. MS, M/Z (%): 258 (100), 187 (11), 173 (12), 145 (30), 132 (46), 118 (7).

1-Dodecyl-1H-1,2,3-benzotriazole (3b)

640 mg (44%) compound **3b** was obtained as light cream crystals, m.p. 40-42°C. ¹H-NMR (DMSO-*d*₆, 500 MHz): (δppm) 8.02 (d, 1H, phenyl), 7.86 (d, 1H, phenyl), 7.52 (t, 1H, phenyl), 7.37 (t, 1H, phenyl), 4.68 (t, J=6.98 HZ, 2H, N-CH₂), 1.88 (m, 2H, N-CH₂-CH₂), 1.21 (m, 18H, N-CH₂-CH₂-(CH₂)₉), 0.82 (t, J=7.09 HZ, 3H, CH₃). ¹³C-NMR: (125 MHz), δ(ppm) = 14.76, 22.94, 26.86, 29.23, 29.54, 29.67, 29.73, 29.73, 29.82, 30.03, 32.14, 48.25, 111.37, 119.95, 124.65, 127.89, 133.66, 146.02. MS: M/Z (%): 286 (100), 257 (11), 216 (14), 187 (15), 173 (21), 145 (50), 132 (44), 118 (33), 91 (20), 77 (10).

Aminothiazole analogues (series c)*N1-(1,3-thiazol-2-yl)-3-Chloropropaneamide (1c)*

1,3-Thiazol-2-amine (5 mmol, 500 mg) was dissolved in 1,4-dioxane (25 ml); then

3-chloropropanoylchloride (7.5 mmol, 0.198 ml) and TEAB (5.5 mmol, 0.8 ml) were added dropwise and the reaction mixture were refluxed at 102°C for 5 h. the reaction mixture were filtrated and extracted with dichloromethane and water. The organic layer was saturated by NaCl solution, dried using NaSO₄ and evaporated to get **1c** as brown liquid (650 mg, 70%). ¹H-NMR (DMSO-*d*₆, 500 MHz): (δppm) 11.9 (1H, NH), 7.50 (d, J=5.00 HZ 1H, N-CH), 7.06 (d, J=5.00 HZ, 1H, S-CH), 3.94 (CH₂Cl), 3.02 (CO-CH₂). Ms (EI); M/Z (%): 190 (80), 154 (46), 126 (17), 100 (100).

N1-(1,3-thiazol-2-yl)-3-[4-(2-hydroxyethyl)piperazino]propaneamide (2c)

N1-(1,3-thiazol-2-yl)-3-Chloropropaneamide (**1c**) (3.5 mmol, 650 mg), 2-piperazino-1-ethanol (3.5 mmol, 0.43 ml), potassium carbonate (3.5 mmol, 483 mg) and TEAB (0.15 mmol, 31.5 mg) in acetonitrile (25 ml) were refluxed for 48 h. The reaction mixture were filtrated and extracted by dichloromethane and water. The organic layer was saturated by NaCl solution, dried using NaSO₄ and evaporated to get **2c** brown liquid (600 mg, 60%). ¹H-NMR (DMSO-*d*₆, 500 MHz): (δppm) 7.98 (d, 1H, NH), 7.43 (d, J=5.00 HZ, 1H, N-CH), 7.15 (d, J=3.15 HZ 1H, S-CH), 3.46 (m, 5H, OH, CH₂-OH, CO-CH₂), 2.56 (m, 4H, CH₂-CH₂-OH, CO-CH₂-CH₂), 2.35 (m, 8H, N-[CH₂-CH₂]₂-N). ¹³C-NMR: (125 MHz), δ(ppm) = 33.34, 53.23, 54.00, 54.00, 54.18, 59.36, 61.08, 114.06, 138.39, 158.74, 163.85, 171.05. MS (EI); M/Z (%), 283 (100), 251 (27), 183 (31), 153 (40), 142 (30), 111 (23), 99 (33).

Biological assay*Microorganisms*

The antifungal activities of the synthesized compounds were determined against eleven standard strains of fungi including *Candida albicans* (ATCC 10261, CBS 1912), *Candida krusei* (ATCC 6258), *Candida glabrata* (ATCC 90030), *Candida parapsilosis* (ATCC 4344), *Candida dubliniensis* (CBS 8500), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformans* (ATCC 9011), *Aspergillus flavus* (ATCC 64025), *Aspergillus oryzae* (ATCC) and *Aspergillus fumigatus* (ATCC 14110) as

well as nine clinical isolates of yeasts identified by PCR-RFLP (11). Moreover, the inhibitory activities of the mentioned compounds against dermatophytes (*Microsporum canis* and *Epidermophyton floccosum*) which were identified by morphological and physiological tests were also examined. The susceptibility of the examined isolates of fungi against flocunazole (for *Aspergillus* species and yeasts) or griseofulvin (for dermatophytes) were examined by broth microdilution method (12). The interpretative breakpoints for susceptibility testing of fluconazole as recommended by clinical and laboratory standards institute (CLSI) were: Susceptible ≤ 8 $\mu\text{g/ml}$, Susceptible Dose Dependent 16-32 $\mu\text{g/ml}$ and Resistant ≥ 64 $\mu\text{g/ml}$ (12).

Determination of minimum inhibitory and fungicidal concentrations

Minimal inhibitory concentrations of the synthesized agents and selected drugs against standard and clinical species of the fungi were determined by broth microdilution method as recommended by CLSI, with some modifications (12).

Briefly, the RPMI-1640 (with l-glutamine and phenol red, without bicarbonate) (Sigma, USA) was prepared and buffered at pH 7.0 with 0.165 mol 3-(N-morpholino) propane sulfonic acid (MOPS) (Sigma-Aldrich, Steinheim, Germany). Serial dilutions of the selected compound (0.5-256.0 $\mu\text{g/mL}$) were prepared in 96-well microtitre trays using RPMI-1640 media (Sigma, St. Louis, USA) buffered with MOPS (Sigma, St. Louis, USA). Double dilutions of fluconazole were also prepared for each of the tested *Candida* with the final concentration of 0.25-128 $\mu\text{g/mL}$.

Stock inoculums were prepared by suspending three colonies of the examined yeasts in 5 ml of sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standard at 530 nm wavelength (this yields stock suspension of $1-5 \times 10^6$ cells/ml). For moulds (*Aspergillus* spp. and dermatophytes), conidia were recovered from the 7-day old cultures grown on potato dextrose agar by a wetting loop with Tween 20. The collected conidia were transferred in sterile saline and their turbidity was adjusted

to OD=0.09-0.11 that yields $0.4-5 \times 10^6$ conidia/ml. Working suspension was prepared by making a 1/50 and 1/1000 dilution with RPMI of the stock suspension for moulds and yeasts, respectively. After addition of 0.1 ml of the inoculums to the wells, the trays were incubated at 30°C for 24-48 h in a humid atmosphere. 200 μl of the uninoculated medium was included as a sterility control (blank).

In addition, growth controls [medium with inoculums and 5% (v/v) DMSO (maximum DMSO concentration) but without the synthetic compounds] were also included. The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of the compounds that produced ≥ 50 growth inhibitions. Each experiment was performed in triplicate. MFCs were of the lowest concentration that showed either no growth or fewer than 4 colonies, which corresponded to 98% killing activity of the initial inoculums. MFCs were also determined by culturing 10 μl from the wells showing no visible growth onto Sabouraud dextrose agar plates.

RESULTS

During the past two decades, a number of different classes of antifungal agents have been discovered. Since the discovery of amphotericin B, there has been much progress in this field (5). Recently the developments of resistance to currently available antifungal azoles have been reported. The azole compounds have also some clinical failures in the treatment of fungal infections (13). Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities (14). In the course of our search for therapeutically useful antifungal azoles, we synthesized some new derivatives of benzimidazole, benzotriazole and aminothiazole (Table 1).

The biological results are presented in Tables 2 and 3. As shown in Table 2, compounds **1a** and **2a** had the most antifungal effects against tested clinical species of *Candida* at 0.5-256 and 2-256 $\mu\text{g/ml}$. These two compounds also were effective against

resistant species to fluconazole. Compounds **3a** and **4a** had moderate activity against tested clinical species of *Candida* at 0.5-256 $\mu\text{g/ml}$. As shown in Table 3, compounds **1a** and **2a** had the most antifungal effects against

Aspergillus species at 16-256 $\mu\text{g/ml}$. Compounds **1a**, **2a** and **3a** had the most antifungal effects against dermatophytes species at 0.5-256 $\mu\text{g/ml}$. Other compounds showed desirable antifungal activity at 16-128 $\mu\text{g/ml}$.

Table 1. Synthesis of the new azole compounds.

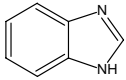
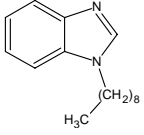
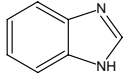
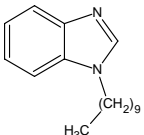
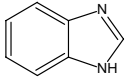
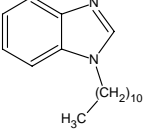
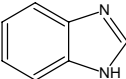
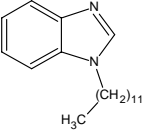
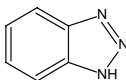
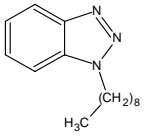
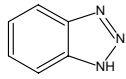
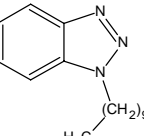
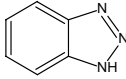
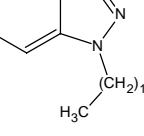
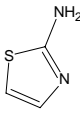
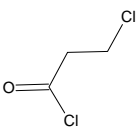
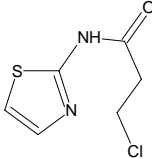
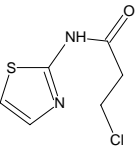
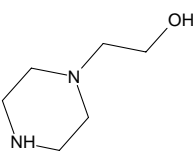
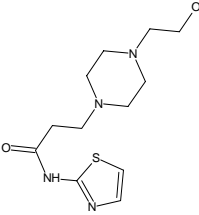
Compounds	Azole Ring	Substitution	Product	Time (h)	Yield (%)
1a		$\text{Br}-\text{CH}_2-\text{CH}_3$		48	55
2a		$\text{Br}-\text{CH}_2-\text{CH}_3$		48	69
3a		$\text{Br}-\text{CH}_2-\text{CH}_3$		48	64
4a		$\text{Br}-\text{CH}_2-\text{CH}_3$		48	77
1b		$\text{Br}-\text{CH}_2-\text{CH}_3$		48	37
2b		$\text{Br}-\text{CH}_2-\text{CH}_3$		48	42
3b		$\text{Br}-\text{CH}_2-\text{CH}_3$		48	44
1c				5	70
2c				48	60

Table 2: MIC ($\mu\text{g/ml}$) of the new azole compounds against different species of *Candida*.

Fungi species	Compounds								
	1a	2a	3a	4a	1b	2b	3b	2c	Fluconazole
<i>Candida albicans</i> (ATCC10261)	1	8	64	G	G	G	G	G	G
<i>Candida albicans</i> (CBS1912)	32	G	G	G	G	G	G	G	G
<i>Candida albicans</i> (10 _M)	16	G	G	G	G	G	G	G	G
<i>Candida albicans</i> (34 _{D2})	0.5	G	G	G	G	G	G	G	G
<i>Candida albicans</i> (46 _M)	32	256	G	G	G	G	G	G	G
<i>Candida albicans</i> (35 _D)	256	G	G	G	G	G	G	G	G
<i>Candida albicans</i> (27 _{M1})	32	G	G	G	G	G	G	G	G
<i>Candida glabrata</i> (ATCC90030)	4	32	G	G	G	G	G	G	4
<i>Candida parapsilosis</i> (ATCC4344)	32	256	G	G	G	G	G	G	2
<i>Cryptococcus neoformans</i> (ATCC9011)	0.5	<0.5	<0.5	128	2	G	G	G	4
<i>Candida krusei</i> (14 _{M2})	G	G	G	G	G	G	G	G	0.5
<i>Candida krusei</i> (ATCC6258)	2	G	G	64	G	G	G	G	1
<i>Candida dubliniensis</i> (48 _{M2})	<0.5	1	32	G	G	G	G	G	G
<i>Candida dubliniensis</i> (CBS8500)	8	G	G	G	G	G	G	G	16
<i>Candida tropicalis</i> (8 _{D2})	64	G	G	256	G	G	G	G	32
<i>Candida tropicalis</i> (ATCC750)	16	64	256	G	G	G	G	G	G
<i>Candida tropicalis</i> (15 _{M2})	32	G	G	G	G	G	G	G	4

G=Growth

Table 3: MIC ($\mu\text{g/ml}$) of the new azole compounds against different species of *Aspergillus* and dermatophytes.

Fungi species	Compounds									
	1a	2a	3a	4a	1b	2b	3b	2c	Fluconazole	Grisofulvine
<i>Aspergillus oryzae</i>	>256	G	G	G	G	G	G	G	G	-
<i>Aspergillus fumigatus</i>	64	>256	G	G	G	G	G	G	G	-
<i>Aspergillus flavus</i>	>256	>256	G	G	G	G	G	G	G	-
<i>Microsporum gypseum</i>	0.5	1	0.5	32	128	32	G	256		G
<i>Trichophyton mentagrophytes</i>	>256	G	G	G	G	G	G	G		G
<i>Epidermophyton floccosum</i>	0.5	0.5	0.5	128	256	256	G	256		G

G=Growth

DISCUSSION

Previously we reported that benzimidazole derivatives are more potent than benzotriazole compounds in the point of antifungal activity (15). The results also showed that alkylbenzimidazoles are more active than alkylbenzotriazole. In the benzimidazole category **1a** and **2a** affected all species of fungi. Compounds **3b** had no antifungal effect. All tested compounds in this study except **3b** and **2c** showed desirable activity on *C. neoformans*. Some of *Candida* species such as *C. albicans*, *C. krusei* and *C. tropicalis* which are resistant to fluconazole and itraconazole were affected by the synthesized compounds (**1a** and **2a**).

Che X and coworkers tested some phenoxy propyl piperazine derivatives and reported that these compounds showed good antifungal effect on some pathogenic and resistant fungi (16). In this study compound **2c** (piperazine analogue) was affective only on the dermatophytes.

Although Ram Janam Singh showed that some benzimidazole and benzotriazole compounds were effective on *C. albicans*, *Aspergillus nigeri* and *Trichophyton rubrum* in solid agar technique (17) but in our study *Aspergillus* species were affected only by **1a** and **2a** and not by the other compounds.

Hakan Goker and coworkers showed that addition of a 4-carbon atom chain with a phenyl moiety to benzimidazole ring increases

Table 4: MIC ($\mu\text{g/ml}$) of the new azole compounds on the resistant species of fungi.

Fungi species	Compounds							
	1a	2a	3a	4a	1b	2b	3b	3h
<i>C. albicans</i>	++++	++	G	G	G	G	G	G
<i>E. floccosum</i>	++++	++++	++++	++	+	++	G	+
<i>A. fumigates</i>	+++	+	G	G	G	G	G	G

G=Growth, +=128-256 $\mu\text{g/ml}$, ++=32-64 $\mu\text{g/ml}$, +++=8-16 $\mu\text{g/ml}$, ++++=0.5-4 $\mu\text{g/ml}$

the antifungal activity (18). Our results showed that antifungal activities of the benzimidazole derivatives were optimum with 9 carbon atoms in the alkyl chain and as the number of carbon atoms increases, the antifungal activities will decrease.

CONCLUSION

Some fungi which are resistant to fluconazole and itraconazole were affected by the compounds synthesized in this study (Table 4). We suggest further *in vivo* studies on these compounds to elucidate their effects and toxicity.

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