EVALUATION OF ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF SOME NEW BENZIMIDAZOLE DERIVATIVES

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***Abstract*– The extensive use of antifungal drugs and their resistance against fungal infections have led to discover new antimicrobial compounds. We previously described synthesis of some new derivatives of 2-methylbenzimidazole (1a-5a) and 5,6-dimethylbenzimidazol (1b-5b). Here we evaluated the antimicrobial activities of these compounds against different species of micro organisms including gram positive and gram negative bacteria as well as fungi. Broth micro-dilution method as recommended by clinical and laboratory standard institute (CLSI) was used for this purpose. The results show compounds 2-Methyl-1-(3-methylbenzyl)-1H-benzo [d]imidazole (5a) and 5,6-Dimethyl-1-(3-methyl benzyl)-1H-benzo[d]imidazole (5b) had the best antifungal activity against the examined fungi and gram positive bacteria. Moreover these two compounds inhibited the growth of azole resistant strains. By comparison the relationship between the structures and activities of the tested compounds revealed that the presence of methyl residue in meta position of benzyl group enhance the antifungal activity. Regarding a broad spectrum antifungal activities of some of the tested compounds, they might be a good candidate for further in vivo studies to evaluate their pharmacological activity and toxicity as a novel antifungal agents.**

***Keywords*– Benzimidazole; Antifungal Agents; Anti-Bacterial Agents.**

I. INTRODUCTION

During the past two decades, resistance to established antimicrobial drugs has increased dramatically (Adibpour *et al*., 2014; Badiee and Alborzi, 2011a, Badiee and Alborzi, 2011b). These resistant strains cause failure in the treatment and enhance the mortality risks, and sometimes contribute to complications. Unlike antibacterial agents, the variety of antifungal drugs is restricted due to the similarity of structure and metabolism of eukaryotic fungal cells to those of mammalian cells. On the other hand, the limited diversity of antifungal agents and recent resistance of fungi to the known antifungal drugs created substantial medical need for new classes of antifungal agents.

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 (Rezaei *et al.*, 2009). However, the emergence of azole resistant strains has spurred the search for new antimycotic compounds (Zampieri *et al.*, 2007). Therefore, design and synthesis of novel antimicrobials will always remain an area of immense significance (Badiee and Alborzi, 2011a).

Among the important pharmacophores responsible for antimicrobial activity, the azole scaffold is still considered as a practicable lead structure for synthesis of proficient and broad spectrum antimicrobial agents. Novel azole compounds are presented for the treatment of superficial and systemic antimicrobial agents. Of the azole compounds benzimidazoles and their derivatives demonstrate a large range of biological properties depending on the substituent pattern in the benzimidazole rings. These compounds are considered as a promising class of bioactive heterocyclic compounds surrounding a diverse range of biological activities such as antihypertensive, anticoagulant, anti-inflammatory, anti-HIV, anti-tumor and antimicrobial (Badiee and Alborzi, 2011b; Badiee *et al.*, 2011; Khabnadideh *et al.*, 2012; Pakshir *et al.*, 2011; Zomorodian *et al.*, 2011a).

We previously reported the synthesis of different azole compounds including: metronidazole (Khabnadideh *et al.*, 2007; Shahriari *et al.*, 2015), imidazole (Khabnadideh *et al.*, 2009; Khabnadideh *et al.*, 2003; Rezaei *et al.*, 2011), triazole (Rezaei *et al.*, 2009; Rezaei *et al.*, 2011), benztriazole (Rezaei *et al.*, 2009), and benzimidazole (Zamani *et al.*, 2014), derivatives (Khabnadideh *et al.*, 2014) as antifungal and antibacterial agents. Of these mentioned compounds, benzimidazole derivatives established better antifungal activities (Zamani *et al.*, 2014).

Recently we synthesized some novel derivatives of 2-methyl and 5,6-dimethyl benzimidazole in organic and ionic solvents and compared the rate of reactions in these two media (Khabnadideh and Harper, 2014). In this study, we decided to investigate their antifungal and antibacterial activities against different species of micro organisms.

II. MATERIALS AND METHODS

New synthesized compounds were prepared in the school of chemistry, University of New Sought Wales, Sydney, Australia (Table 1) (Khabnadideh and Harper,

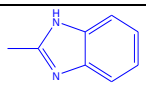
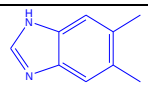
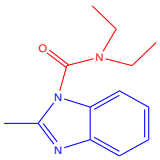
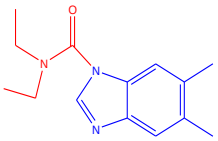
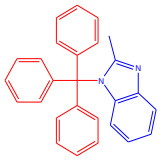
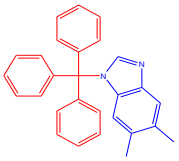
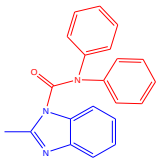
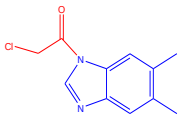
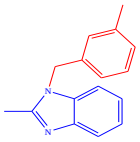
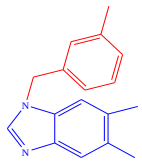
Compounds	Chemical name	Chemical structure	Compounds	Chemical name	Chemical structure
1a	2-Methyl-1*H*-benzoimidazole		1b	5,6-Dimethyl-1*H*-benzoimidazole	
2a	*N*,*N*-Diethyl-2-Methyl-1*H*-benzoimidazole-1-carboxamide		2b	*N*,*N*-Diethyl-5,6-Dimethyl-1*H*-benzoimidazole-1-carboxamide	
3a	2-Methyl-1-trityl-1*H*-benzoimidazole		3b	5,6-Dimethyl-1-trityl-1*H*-benzoimidazole	
4a	2-Methyl-*N*,*N*-diphenyl-1*H*-benzoimidazole-1-carboxamide		4b	2-Chloro-1-(5,6-dimethyl-1*H*-benzoimidazol-1-yl)-ethanone	
5a	2-Methyl-1-(3-methyl-benzyl)-1*H*-benzoimidazole		5b	5,6-Dimethyl-1-(3-methyl-benzyl)-1*H*-benzoimidazole	

Table 1. Synthesized benzimidazole derivatives, which were tested against fungi and bacteria.

2014). The RPMI-1640 media were used from Sigma, St. Louis, USA. All chemicals and solvents were purchased from Merck. Serial dilutions (0.5-256 µl/mL) were prepared in the Muller-Hinton media (Merck, Darmstadt, Germany) and Sabouraud dextrose agar was produced from Merck, Darmstadt, Germany.

A. Biological activity

A.1. Microorganisms

Antifungal activities of the synthetic compounds against some standard strains of fungi, including *Candida albicans* (ATCC 10261, ATCC 5982, ATCC 2730, ATCC 562, CBS 1912), *C. tropicalis* (ATCC 750), *C. glabarata* (ATCC 2192, ATCC 863, ATCC 6146, ATCC 90030), *C. krusei* (ATCC 6258), *C. dubliniensis* (ATCC8501, ATCC 7987, CBS 8500, ATCC 7988), *C. parapsilosis* (ATCC 4344), *Cryptococcus neoformance* (ATCC 9011), *Exophiala dermatitidis* (CBS 120433), *Psuedoalscheria boydii* (CBS 329.93), *Aspergillus fumigates* (ATCC 14110), *A. flavous* (ATCC 6402) and *A. clavatus* (CBS514.65) as well as three clinical isolates of each azole resistant and azole sensitive yeasts were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). In addition, antimicrobial properties of the synthesized compounds against 40 clinical isolates of yeasts by PCR-RFLP were also tested (Khabnadideh *et al.*, 2007; Zomorodian *et al.*, 2011b). The antibacterial activities of the synthetic compounds against standard species of *Staphylococcus aureus* (ATCC 2592, ATCC 700698), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), Vancomycin Resistant *Enterococcus fecalis* (ATCC 51299), and clinical isolates of *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa* collected from the Dr. Faghihi Hospital (Shiraz, Iran) were also evaluated in this work. Microdilution and disk diffusion methods were used to determine the susceptibility of all clinical isolates of bacteria and fungi against select compounds (Wayne, 2006a; Wayne, 2006b; Adibpour *et al.*, 2014).

Gram positive bacteria with a tick peptidoglycan layer give a positive result in the Gram stain test. They take up the crystal violet stain. But the peptidoglycan layer of Gram negative bacteria is much thinner and they cannot retain the violet stain after the decolorization.

A.2. Determination of Minimum Inhibitory Concentrations

MICs were determined by using the broth microdilution method recommended by the CLSI (Clinical & Laboratory Standards Institute) with some modifications. In order to determination of antimicrobial activities against fungi, serial dilutions of the synthetic compounds (1–1024 µg/mL) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St. Louis, MO, USA) buffered with MOPS (Sigma). Stock inoculums were prepared by suspending three colonies of the examined yeast in 5 mL sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standards at 530 nm wavelengths (this yields stock suspension of 1-5 × 106 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 × 106 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. Working inoculums (0.1 mL) were added to the microtiter plates, which were incubated in a humid environment at 30ºC for 24–48 h. Uninoculated medium (200 µL) was included as a sterility control. In addition, growth controls (medium with inoculums but without antibiotics or the synthetic compounds) were also included.

The growth in each well was compared with that of the growth in the control well. MICs were visually determined and defined as the lowest concentration of the compounds produced ≥95% growth reduction compared with the growth in the control well. Each experiment was performed in triplicate.

In addition, media from the wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC). MFCs were determined as the lowest concentration yielding no more than 4 colonies, which resulted in mortality of 98% of the microbes in the initial inoculums.

III. RESULTS AND DISCUSSION

In this study, all compounds in two categories (2-methylbenzimidazole (1a-5a) and 5,6-dimethylbenzimidazol (1b-5b) were estimated against various species of fungi.

Antifungal activities of the tested compounds are presented in Table 2. For antifungal activities, compounds 2-Methyl-1-(3-methylbenzyl)-1*H*-benzo[*d*] imidazole (5a) and 5,6-Dimethyl-1-(3-methylbenzyl)-1*H*-benzo[*d*]imidazole (5b) exhibited the best antifungal activities against all standard and clinical strains including fluconazole resistant strains.

Of the tested benzimidazole derivatives, compounds 2-methyl- 1- (3-methylbenzyl)- 1*H*-benzo [*d*]imidazole (5a) and 5,6-Dimethyl-1-(3-methyl-benzyl)-1*H*-benzoimidazole (5b) and showed inhibitory effect on gram positive bacteria at concentrations ranging from 8 to 256µg/mL (Table 3). Comparison of MIC values for 2-methylbenzimidazole derivatives (1a-5a) with 5, 6-dimethylbenzimidazol derivatives (1b-5b) demonstrated that, series b were more active against microorganisms than series a. With evaluated MIC values of two basic compounds, 2-methylbenzimidazole (1a) and 5, 6-dimethylbenzimidazol (1b), compound (1b) was more active compound against microorganisms than compound (1a). Additionally, compounds 2-Chloro-1-(5, 6-dimethyl-1H-benzo[d]imidazol-1-yl)ethanone (4b) and *N,N*-Diethyl-5,6-dimethyl-1*H*-benzo[*d*]imidazole-1-carboxamide (2b) exhibited both inhibitory and fungicidal activities against the examined fungi at concentrations ranging from 16 to ≤512 µg/mL.

Compound (5a) inhibited the growth of all examined Candida species (*C. glabrata* and *C.dubliniensis*) at concentrations ranging from 0.5 to 32 µg/mL (Geometric mean MICs = 4.7 µg/mL). Replacement of hydrogen at *N*-position of benzimidazole ring with diethylcarboxamide residue (2a and 2b), with trityl group (3a and 3b) or with diphenylcarboxamide residue (4a) reduced their antifungal activity in compared to the base compounds especially (1a). *o*-Chloro-ethanone substitute of benzimidazole ring provided compound (4b) which exhibited better activity against the studied fungi than the base compound (1b). But in the compound (4a) with diphenylcarboxamide residue at *N*-position of benzimidazole ring, reduced its antifungal activity compared to base compound (1a).

The best inhibitory effect of the compounds (5b) and (5a) on positive gram of bacteria, might be due to the replacement of 3-methyl-benzyl group in the benzimidazole ring in comparison the other compounds, although compound (5b) more effective than compound (5a). Also, compounds (5a) and (5b), had significant enhancement of the inhibitory activity and they were effective against two azole-resistant strains of *C. albicans*.

Of the tested compounds 2-methyl-1-(3-methylbenzyl)-1*H*-benzo[*d*]imidazole (5a) and 5,6-Dimethyl-1-(3-methyl-benzyl)-1*H*-benzoimidazole (5b) showed inhibitory effects on the gram positive microorganisms (concentrations ranging from 8 to 256µg/mL) respectively. These two compounds also had the most antifungal activates even against fluconazole resistant strains including *C. parapsilosis, C. kruzei, C. tropicalis* and *E. dermatitidis*.

IV. CONCLUSIONS

2-Chloro-ethanone substitute of benzimidazole ring provided compound (4b) which exhibited a better activity against the studied fungi than (1b). But compound (4a) with diphenylcarboxamide residue on the benzimidazole ring, showed less antifungal activity compared to (1a), perhaps this is due to low solubility in aqueous media.

The best inhibitory effect of the compounds (5a) and (5b) on gram positive bacteria, might be due to the replacement of 3-methyl-benzyl group in the benzimidazole ring in comparison the other substitutions. According to the mechanism of azole compounds which act by inhibition of 14-α-demethylase enzyme we suggest the same mechanism for our benzimidazole derivatives as well. But further studies are needed to establish the exact way for their activity.

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Table 2. MIC and MFC (µg/mL) of the 2-methylbenzimidazole and 5,6-dimethylbenzimidazole derivatives against fungi

	Compound	1a		2a		3a		4a		5a	
	Fungi(names of strains)	MIC50	MFC	MIC 50	MFC	MIC50	MFC	MIC50	MFC	MIC50	MFC
Yeast	*C.albicans*	32	>256	>256	>256	>256	>256	>256	>256	8	128
	Azole-sensitive C.albicans	128	>256	>256	>256	>256	>256	>256	>256	4	256
	Azole-resistant C.albicans	>256	>256	>256	>256	>256	>256	>256	>256	4	128
	C.dubliniensis	64	>256	>256	>256	>256	>256	>256	>256	1	32
	C.glabrata	16	>256	>256	>256	>256	>256	>256	>256	0.5	16
	C.kruzei	>256	>256	>256	>256	>256	>256	>256	>256	8	64
	C.parapsilosis	128	>256	>256	>256	>256	>256	>256	>256	8	128
	Azole-sensitive C.parapsilosis	>256	>256	>256	>256	>256	>256	>256	>256	64	128
	C.tropicalis	128	>256	>256	>256	>256	>256	>256	>256	16	256
	Azole-resistan C.tropicalis	>256	>256	>256	>256	>256	>256	>256	>256	64	>256
	C. neoformans	128	>256	>256	>256	>256	>256	>256	>256	16	128
Filamentusss	*E. dermatitidis*	>256	>256	>256	>256	>256	>256	>256	>256	16	256
	A. famigatus	256	>256	>256	>256	>256	>256	>256	>256	64	>256
	A. clavatus	256	>256	>256	>256	>256	>256	>256	>256	32	256
	P. boydii	128	>256	>256	>256	>256	>256	>256	>256	64	>256
	A. flavus	>256	>256	>256	>256	>256	>256	>256	>256	64	256
	Compound	**1b**		**2b**		**3b**		**4b**		**5b**	
	Fungi(names of strains)	MIC50	MFC	MIC 50	MFC	MIC50	MFC	MIC50	MFC	MIC50	MFC
Yeast	*C.albicans*	64	>256	64	>256	>256	>256	64	>256	4	32
	Azole-sensitive C.albicans	64	>256	64	>256	>256	>256	64	>256	2	256
	Azole-resistant C.albicans	64	>256	128	>256	>256	>256	64	128	4	128
	C.dubliniensis	64	>256	64	>256	>256	>256	64	>256	4	32
	C.glabrata	16	>256	64	>256	>256	>256	16	>256	1	>256
	C.kruzei	128	256	128	>256	>256	>256	64	256	4	32
	C.parapsilosis	32	256	256	>256	>256	>256	32	256	2	>256
	Azole-sensitive C.parapsilosis	128	>256	>256	>256	>256	>256	256	>256	64	>256
	C.tropicalis	32	>256	256	>256	>256	>256	32	256	8	>256
	Azole-resistan C.tropicalis	128	>256	256	>256	>256	>256	64	>256	32	>256
	C. neoformans	128	>256	64	>256	>256	>256	128	>256	16	128
Filamentusss	*E. dermatitidis*	64	>256	128	>256	>256	>256	64	>256	8	>256
	A. famigatus	128	>256	128	>256	>256	>256	128	>256	32	>256
	A. clavatus	128	>256	128	>256	>256	>256	128	>256	16	>256
	P. boydii	128	>256	128	>256	>256	>256	128	>256	32	>256
	A. flavus	128	>256	256	>256	>256	>256	128	>256	32	256

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration

Table 3. MIC and MBC (µg/mL) of the 2-methylbenzimidazole and 5, 6-dimethylbenzimidazole derivatives against bacteria

	Compound	1b		2b		3b		4b		5b	
	Bacteria (names of strains)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram positive	*S. aureus*	32	>256	>256	>256	>256	>256	>256	>256	8	128
	Methicillin-sensitive S.aureus (3)	128	>256	>256	>256	>256	>256	>256	>256	4	256
	Methicillin-resistant S.aureus (3)	>256	>256	>256	>256	>256	>256	>256	>256	4	128
	E. fecalis	64	>256	>256	>256	>256	>256	>256	>256	1	32
Gram negative	*E.coli*	16	>256	>256	>256	>256	>256	>256	>256	0.5	16
	Third-generation cephalosporin-sensitive E.coli (3)	>256	>256	>256	>256	>256	>256	>256	>256	8	64
	Third-generation Cephalosporine- resistant E.coli (3)	128	>256	>256	>256	>256	>256	>256	>256	8	128
	P.aeruginosa	>256	>256	>256	>256	>256	>256	>256	>256	64	128
	Sensitive strain P.aeruginosa	128	>256	>256	>256	>256	>256	>256	>256	16	256
	Multidrug-resistant P.aeruginosa	>256	>256	>256	>256	>256	>256	>256	>256	64	>256
	Compound	**1b**		**2b**		**3b**		**4b**		**5b**	
	Bacteria (names of strains)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram positive	*S. aureus*	>256	>256	>256	>256	>256	>256	>256	>256	32	>256
	Methicillin-sensitive S.aureus (3)	>256	>256	>256	>256	>256	>256	>256	>256	8	256
	Methicillin-resistant S.aureus (3)	>256	>256	>256	>256	>256	>256	>256	>256	64	256
	E. fecalis	>256	>256	>256	>256	>256	>256	>256	>256	64	256
Gram negative	*E.coli*	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
	Third-generation cephalosporin-sensitive E.coli (3)	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
	Third-generation Cephalosporine- resistant E.coli (3)	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
	P.aeruginosa	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
	Sensitive strain P.aeruginosa	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
	Multidrug-resistant P.aeruginosa	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256

MIC: Minimum inhibitory concentration, MBC: Minimum bacetricidal concentration

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