



Journal of Medical Sciences

ISSN 1682-4474

science
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Fungicidal Effects of Some Derivatives of 2-Ferrocenyl-Benzimidazoles: A Possible Template for Antifungal Drug Design

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The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) for a group of newly synthesized derivatives of 2-ferrocenyl-5-fluoro-6-(4-substituted-1-piperazinyl) benzimidazoles were determined using a broth macrodilution method following clinical and laboratory standards institute (CLSI) recommendation for medically important *Candida* sp. The data demonstrate that compounds 4-fluoro-5-(4-methyl-1-piperazinyl)-2-nitroaniline(5a), 4-Fluoro-5-(4-phenyl-1-piperazinyl)-2-nitroaniline(5b), 2-ferrocenyl-5-fluoro-6-(4-methyl-1-piperazinyl) benzimidazole (7a), 2-ferrocenyl-5-fluoro-6-(4-phenyl-1-piperazinyl) benzimidazole (7b) and 2-ferrocenyl-5-fluoro-6-[4-(4-fluorophenyl)-1-piperazinyl] benzimidazole (7e) were found to have potent *in vitro* antifungal activity with MICs at which 80% of the strains were inhibited (MIC_{80s}) of 15-125 µg mL⁻¹. The active compounds were further screened in order to establish their mode of action on the basis of inhibitory effects on growth, budding, germ-tube formation and leakage of cytoplasmic content release after treatment. In comparison to control drugs (e.g., nystatin, miconazole nitrate and ketoconazole). Some derivatives of the 4-fluoro-5-(4-substituted-1-piperazinyl)-2-nitroanilines (series 5) and 2-ferrocenyl-5-fluoro-6-(4-substituted-1-piperazinyl)-[1H] benzimidazoles (series 7) were found to be more potent in lower micro-molar concentrations (15-500 µg mL⁻¹). It is clear from the data presented here that further studies on the structure-activity relationships, mechanisms of action and *in vivo* efficacies of these compounds are warranted to determine their clinical potential.

Key words: Antifungal susceptibility, benzimidazoles derivatives, drug design, *Candida* sp., virulence factors

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INTRODUCTION

Invasive fungal infections, particularly in immunocompromised patients, are continuously increasing during the past 20 years and are now significant causes of morbidity and mortality (Bodey *et al.*, 2002; Krcmery and Barnes, 2002). This is particularly severe in patients with hematological malignancies undergoing induction or consolidation chemotherapy, in immunosuppressed organ transplant recipients and in patients with acquired immunodeficiency secondary to infection by human immunodeficiency viruses (HIV). More than half of the patients who die from malignancies are infected with *Candida* sp., approximately one-third with *Aspergillus* sp. and increasing numbers with *Cryptococcus* sp. and/or other fungi such as *Fusarium* sp. (Roilides *et al.*, 2007; Sampathkumar and Paya, 2001). *Candida albicans*, *C. glabrata* and *C. tropicalis* represent more than 80% of isolates from clinical infections (Safdar *et al.*, 2001). The problems caused by this increase in fungal pathogenesis are of great importance because of the relative lack of antifungal agents which are both, effective and free of side effects (Abu-Elteen and Hamad, 2005; Ghannoum and Rice, 1999; Speller, 1980). During the latter half of the last century, particularly during the past two decades, a number of different classes of antifungal agents have been discovered (Abu-Elteen and Hamad, 2007). It is consequently very desirable to investigate thoroughly for any new drug with antimycotic potential to establish its mode of action and clinical applications.

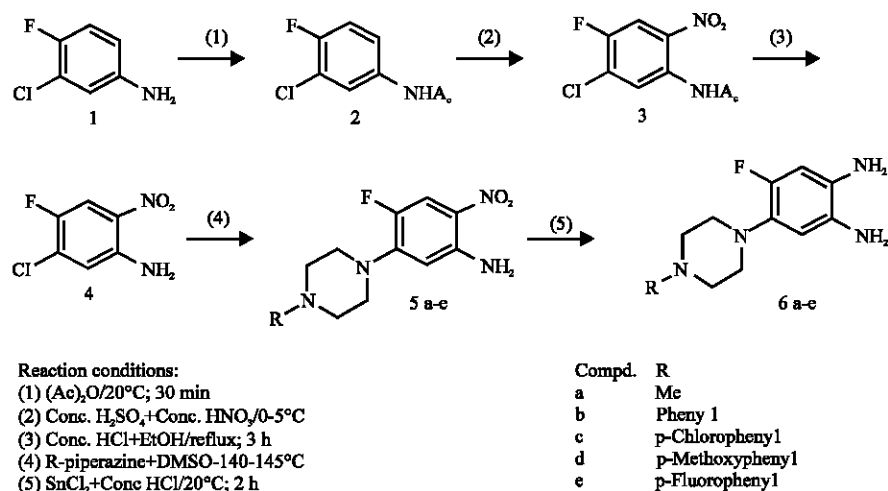
Aromatic dicationic compounds are widely used in medicine and related fields of biology. The most important examples of these aromatics include dication-substituted

carbazoles, furans and benzimidazoles which exhibit good antimicrobial activities (Andriole, 1999; Del Poeta *et al.*, 1998a). Some are quite active *in vitro* against the *Candida* species (including azole-resistant strains) *C. neoformans*, *A. fumigatus* and *Fusarium* species (Del Poeta *et al.*, 1998b, 1999). Furthermore, carbendazim, a benzimidazole derivative was used as an agricultural fungicide, but unfortunately, as recently demonstrated, it causes experimental histoplasmosis (Bartlett *et al.*, 1998).

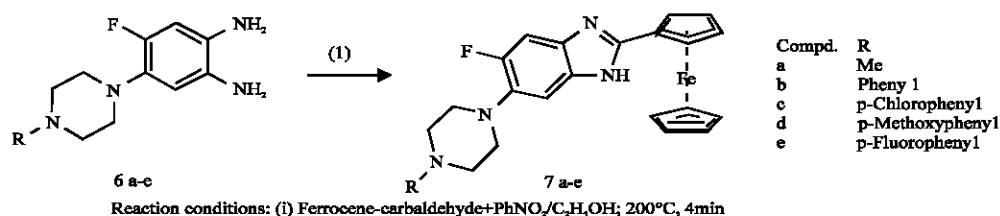
The increasing clinical importance of drug-resistant fungal pathogens has urged additional needs to fungal research and new antifungal compound development (Kauffman, 2004; Ullmann and Cornely, 2006). For this purpose, this study describe some studies related to the antimycotic effects of some newly synthesized derivatives of 2-ferrocenyl-5-fluoro-6-(4-substituted-1-piperazinyl) benzimidazoles. This series of compounds was chosen to establish the structural correlation with their antimycotic activities screened by several well-established bioassays.

MATERIALS AND METHODS

Drugs: All compounds were synthesized and characterized in the laboratories of three of the researchers at Interfakultäres Institut für Biochemie der Universität Tübingen and chemistry department-Hashemite University in 2004. The synthesis and physical properties of the compounds (Scheme 1 and 2) have been described previously by Abdel-Jalil and Voelter (2005). The diamine derivatives 6a-e were obtained from the commercially available 3-chloro-4-fluoroaniline (1) using several sequential steps involving acetylation, nitration, deacetylation and piperazinylation followed by reduction



Scheme 1



Scheme 2

(Scheme 1). Benzimidazole derivatives (7a-e) were prepared by direct coupling of the corresponding diamines (6a-e) with ferrocene-carbaldehyde in nitrobenzene (Scheme 2) (Abdel-Jalil and Voelter, 2005). The hydrochloride salts of compounds 5-7(a-e) were prepared by bubbling their ethereal solutions with hydrogen chloride gas at low temperatures.

IR spectra were acquired on Nicolet-MAGNA-IR-560 (Waltham, MA, USA) spectrophotometer. ¹H NMR spectra were recorded on a Bruker AM 250 FT (Reinstetten-Forchheim, Germany) spectrophotometer operating at 300 K in CDCl₃ using TMS as internal standard. Mass spectra (electron impact) were obtained on a Varian CH-7 spectrophotometer (Darmstadt, Germany) at 70 eV with an ion source temperature of 200°C. Melting points were determined on a Büchi (Flawil, Switzerland) 510 apparatus and are uncorrected. Elemental analysis were determined on a Perkin-Elmer (Wellesley, MA, USA) elemental analyzer model 240.

Ferrocene-carbaldehyde, acetic anhydride, ethanol, dimethylsulfoxide and 3-chlorofluoroaniline and all reagents (HCl, H₂SO₄, HNO₃ and SnCl₂) were purchased from Aldrich (Steinheim, Germany) and used without further purification. Substituted piperazines were ordered from Acros Organics (Geel, Belgium).

Stock solutions of 5 mg mL⁻¹ of all compounds were made in dimethyl sulphoxide (DMSO) and then filter sterilized by passage through a 0.22 µm millipore membrane filter (Millipore, MA, USA) and stored at -20°C until used.

Test organisms and clinical isolates: *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*, (two strains for each) were used in this study. Members of this collection were obtained from oral cavity, vaginal swabs and from sputum specimen taken from diabetic and cancer patients. The isolation and identification techniques used have already been described (Abu-Elteen, 2001; Abu-Elteen *et al.*, 2007). *C. albicans* (ATCC 36082), *C. glabrata* (ATCC 22553) and *C. parapsilosis* (ATCC 22019), kindly provided by Dr. Ghannoum, Center for Medical Mycology, University Hospital of Cleveland, Ohio, USA, were used as a reference strains and were

included in each run of the experiments. All the organisms were maintained on sabouraud dextrose agar (SDA) (Difco, Detroit, Mich, USA), stored at 4°C and subcultured routinely.

In vitro susceptibility testing: Antifungal susceptibility testing was performed at Hashemite University, Department of Biology and Biotechnology during 2007 by using RPMI-1640 medium (Sigma Chemical Co., St., Louis, MO, USA) with glutamine, without sodium bicarbonate and buffered at pH 7.0 with 0.165 M morpholine propane-sulphonic acid (MOPS). Drug dilutions were prepared at 10 times the strength of the final drug concentrations (1000-0.09 µg mL⁻¹) by a serial drug dilution scheme for minimizing systematic pipetting errors (Abu-Elteen *et al.*, 2007). Experiments for determination of minimum inhibitory concentrations (MICs) were performed by the broth macrodilution method as described earlier (Abu-Elteen *et al.*, 2007). Dilutions were dispensed as 0.1 mL volumes into sterile polystyrene tubes (12×75 mm; Falcon, Becton Dickinson, Lincoln Park, NJ, USA) and stored at -20°C until used. Yeast isolates were grown on yeast extract peptone dextrose agar (YEPD) (Difco, Detroit, Mich, USA) at 30°C and subcultures twice to ensure viability. For each test organism, an inoculum concentration of 0.5×10³-2.5×10³ cells mL⁻¹ was standardized spectrophotometrically and by quantitative plate counts. A 100 µL yeast inoculum was added to each macrodilution tube. All tubes were incubated at 35°C and were read after 48 h of incubation. The MIC is defined as the lowest drug concentration that resulted in a visual turbidity less than or equal to 80% (≤80%) inhibition compared to that produced by the growth in the control tube (0.2 mL of growth control plus 0.8 mL of un-inoculated RPMI 1640) (Abu-Elteen *et al.*, 2007). The methods used in experiments for the determination of minimum fungicidal concentration (MFC) were adapted from a method by Ghannoum *et al.* (1990). Briefly, one loopful (approx. 100 µL) of the broth tubes showing no visible growth was further subcultured on to a SDA plate. The plates were incubated at 37°C for 24 h and then checked for growth. The MFC was recorded as the lowest drug concentration yielding no colonies.

Effect on growth: Inocula of yeast isolates were grown overnight at 37°C with rotary agitation (160 rpm) in YEPD broth. The cells were centrifuged and re-suspended in a small volume of fresh medium. This suspension was used to inoculate (10^5 cell mL^{-1}) 4×100 mL of fresh medium containing zero (control), 1/4×MIC, 1/2×MIC and 1×MIC of the drugs. The flasks were incubated at 37°C, samples withdrawn at 60 min intervals and the rate of growth of shaken cultures was followed by determining of the optical density at 420 nm (Genesys 5 spectrophotometer, Milton Ray, USA).

Effect on budding cells and germ tube formation: *C. albicans* ATCC 36082 and two clinical isolates of *C. albicans* were maintained on YEPD broth at 37°C for 48 h. The cells were centrifuged and washed three times with sterile phosphate buffer saline (PBS pH 7.2). Yeast cells (10^7 cells mL^{-1}) were inoculated in flasks containing 20 mL of Eagle's medium (which supports bud formation of yeasts) with and without drugs and shaken at 25°C on a rotary shaker at 200 rpm. Samples were taken at different time intervals and scored for bud formation by counting the mean number of buds in every 300 yeast cells. Similarly, *C. albicans* ATCC 36082 and two clinical isolates of *C. albicans* were grown with and without drugs and washed three times with sterile PBS. Inocula (10^7 cells mL^{-1}) from each preparation were added to tubes containing calf serum (Gibco) and incubated in a shaking water bath at 37°C. Samples were taken at different time intervals and added to an equal volume of 1% glutaraldehyde in PBS for fixation. The number of yeasts with germ tubes was determined microscopically (Abu-Elteen, 2000).

Leakage of intracellular material: Equal volumes (5 mL) of drug solutions (zero (control), 1/4×MIC, 1/2MIC and 1×MIC) and cell suspension of *C. albicans* ATCC 36082 and two clinical isolates of *C. albicans* were mixed to give a final cell concentration of 1 mg (wet weight)/mL and incubated at 24°C. At different time intervals, cells were removed by centrifugation (7000 rpm for 5 min). Cellular exudates were determined by direct spectrophotometric measurement of the material absorbing at 260 nm in the supernatant (Ghannoum *et al.*, 1990).

Statistical analysis: Data analysis was carried out by means of one-way Analysis of Variance (ANOVA) and by multiple comparison using computer statistical analysis software (STATISTICA for Windows (1995), Stat Soft Inc., OK, USA), differences were considered as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Antifungal activities: Preliminary screening of the synthesized derivatives (as hydrochloride salts), 2-ferrocenyl-5-fluoro-6-(4-substituted-1-piperazinyl)-[1H]benzimidazoles (7a-e) as well as their corresponding intermediates, 1, 2-diamino-5-fluoro-6-(4-substituted-1-piperazinyl)benzenes (6a-e) and 4-fluoro-5-(4-substituted-1-piperazinyl)-2-nitro anilines (5a-e) (Scheme 1, 2), was carried out against five medically important *Candida* sp. Nystatin, miconazole nitrate and ketoconazole were used as a positive control (Table 1, 2). Compounds 5a-b, 7a, b and e which showed most potent antimycotic activities were selected and subjected to further in-depth analysis to establish their mode of inhibitory effects on growth,

Table 1: Antimycotic effects of selected derivatives of 2-ferrocenyl-benzimidazoles in terms of their MICs and MFCs

<i>Candida</i> sp.	5a	5b	5c	5d	6b	6d	6e	7a	7b	7c	7d	7e
MIC values ($\mu\text{g mL}^{-1}$)												
<i>C. albicans</i>	62.5	250	1000	>1000	500	250	1000	125	125	500	250	600
<i>C. parapsilosis</i>	60	60	500	250	500	500	500	30	150	500	250	125
<i>C. glabrata</i>	30	30	1000	250	250	1000	30	15	300	500	250	250
<i>C. krusei</i>	250	250	500	250	500	1000	500	250	75	500	250	600
<i>C. tropicalis</i>	300	500	<30	500	1000	>1000	500	500	125	<30	250	250
MFC values ($\mu\text{g mL}^{-1}$)												
<i>C. albicans</i>	600	500	1000	>1000	500	250	1000	500	>1000	500	250	600
<i>C. parapsilosis</i>	600	1000	500	250	500	500	500	60	1000	500	250	600
<i>C. glabrata</i>	600	250	500	250	>1000	500	1000	30	>1000	1000	250	600
<i>C. krusei</i>	500	500	>1000	1000	1000	>1000	1000	250	>600	1000	>1000	600
<i>C. tropicalis</i>	600	1000	>1000	1000	>1000	>1000	>1000	500	>1000	1000	500	600

Table 2: Positive control values in comparison to antimycotic effects of selected derivatives of 2-ferrocenyl-benzimidazoles in terms of their MICs and MFCs

<i>Candida</i> sp.	MIC values ($\mu\text{g mL}^{-1}$)			MFC values ($\mu\text{g mL}^{-1}$)		
	Miconazole	Ketoconazole	Nystatin	Miconazole	Ketoconazole	Nystatin
<i>C. albicans</i>	<30	125	<30	<30	125	<30
<i>C. parapsilosis</i>	<30	<30	<30	125	60	60
<i>C. glabrata</i>	<30	<30	<30	<30	<30	<30
<i>C. krusei</i>	<30	<30	<30	<30	<30	<30
<i>C. tropicalis</i>	<30	<30	<30	<30	<30	<30

budding, germ-tube formation and leakage of cytoplasmic contents. Table 1 shows the minimum inhibitory and fungicidal concentrations (MIC and MFC, respectively) of the compounds for each of the tested *Candida* sp. Interestingly, different compounds often revealed different activity profiles (MICs) for the series of *Candida* sp. tested. For example, the MIC values of the most potent compound 5a against *C. albicans*, *C. parapsilosis* and *C. glabrata* were found to be 62.5, 60 and 30 $\mu\text{g mL}^{-1}$, respectively, but were 250 and 300 $\mu\text{g mL}^{-1}$ for *C. krusei* and *C. tropicalis*, respectively. Despite a high potency of compound 7a against *C. glabrata*, *C. parapsilosis* and *C. albicans* (15, 30 and 125 $\mu\text{g mL}^{-1}$, respectively), it was

less active against *C. krusei* and *C. tropicalis* (250 and 500 $\mu\text{g mL}^{-1}$, respectively) as compared with the more susceptible strain (Table 1; Fig. 1A-H, 2A-H). In a general comparison of MIC data for compounds 5a, b, 7a, b and e versus nystatin, miconazole nitrate and ketoconazole for *C. glabrata* strains, a statistically significant difference was found only with compound 7a ($p = 0.043$). This suggests different drug resistant and/or susceptibility patterns of the various fungal species. This is further supported by the observation that 5c is least active against most of the fungal sp. tested, but shows a very potent ($<30 \mu\text{g mL}^{-1}$) effect on the strains of *C. tropicalis* (Table 1). Accordingly, the possibility of designing highly

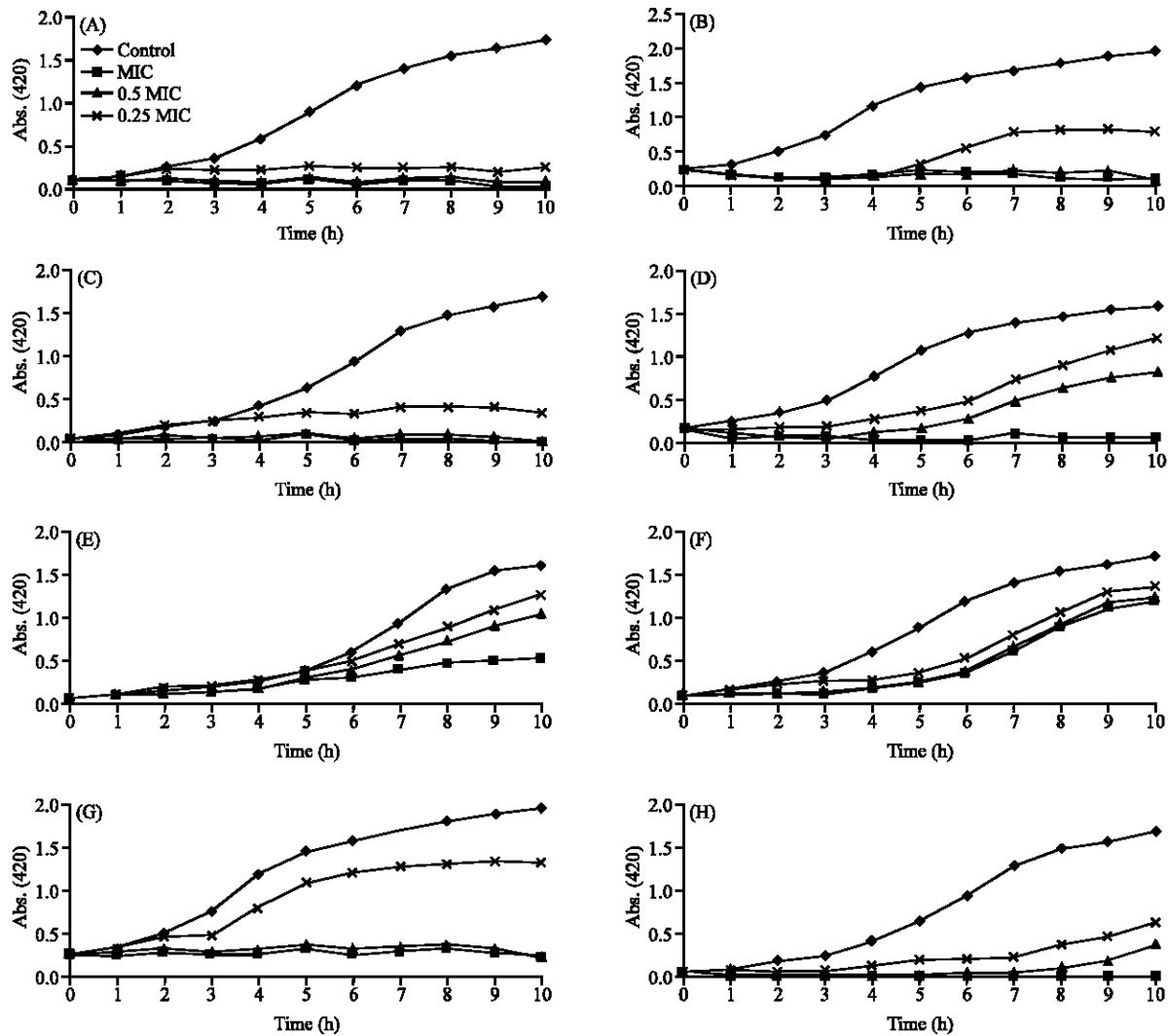


Fig. 1: Effect of three different concentration of MICs (1/4x, 1/2x and 1xMIC) of compounds 5a, b on various cultures of *Candida* sp. (A) *C. glabrata* (5a), (B) *C. tropicalis* (5b), (C) *C. krusei* (5a), (D) *C. albicans* (5b), (E) *C. parapsilosis*, (F) *C. glabrata* (5b), (g) *C. tropicalis* and (H) *C. krusei* (5b)

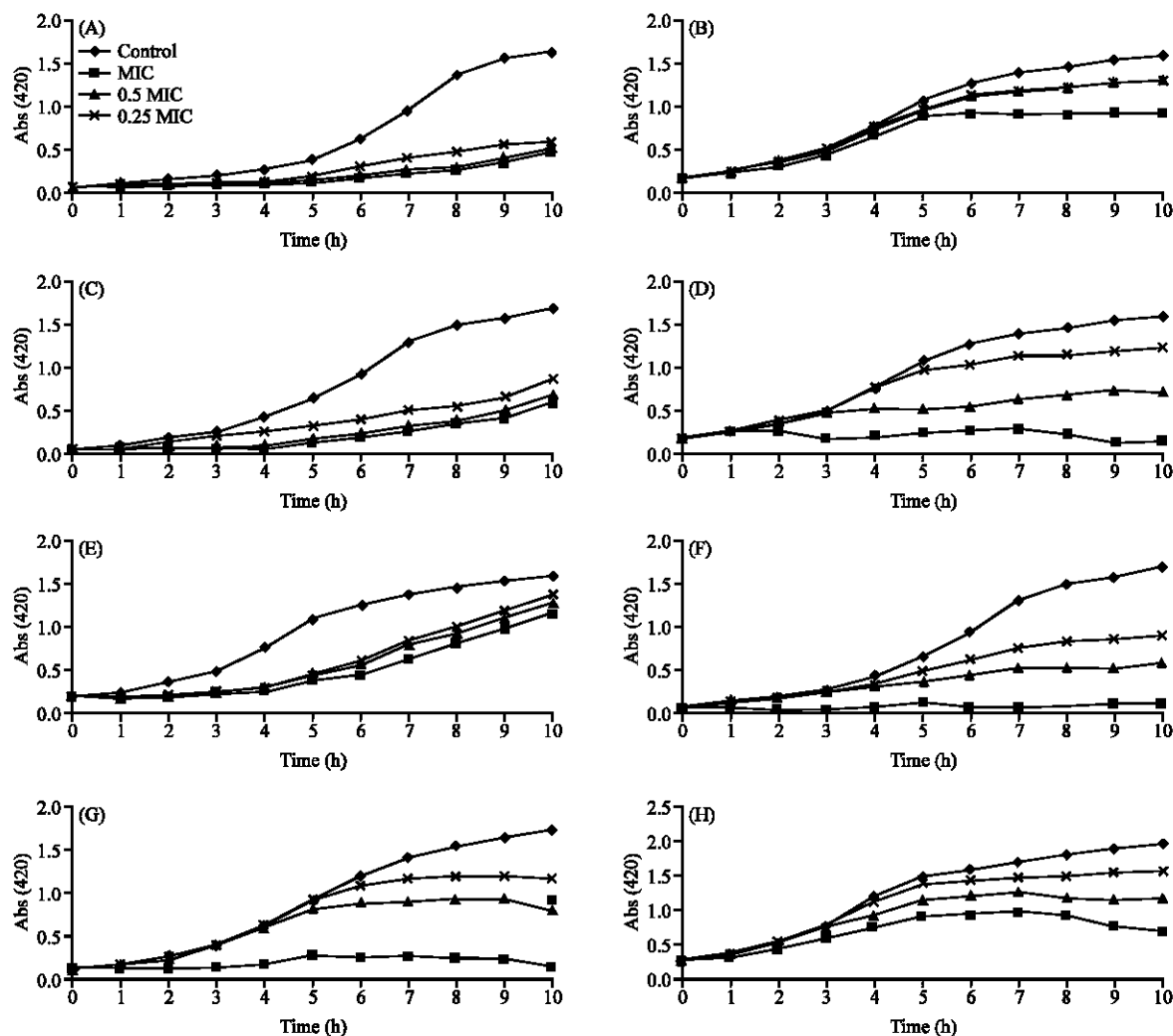


Fig. 2: Effect of three different concentration of MICs (1/4x, 1/2x and 1xMIC) of compounds 7a, b and e on various cultures of *Candida* sp. (A) *C. parapsilosis* (7a), (B) *C. albicans* (7b), (C) *C. krusei* (7a), (D) *C. albicans* (7e), (E) *C. albicans* (7a), (F) *C. krusei* (7e), (G) *C. glabrata* (7b) and (H) *C. tropicalis* (7e)

specific drugs against specific strains of *Candida* sp. may have some merit. The remaining organisms tested, manifested higher resistance with MIC values ranging 500-1000 $\mu\text{g mL}^{-1}$.

All compounds, in particular, 5a-c and 7b, showed higher MIC values (<30 and 500 $\mu\text{g mL}^{-1}$) against the *Candida* sp. compared to the control drugs (nystatin, miconazole nitrate and ketoconazole). Much variation was observed in the MFCs of the mentioned compounds compared to their MICs. This becomes quite obvious in the case of compounds 7a, b and 5a and b (Table 1). Such an observation is well within the known patterns of activity of antifungal compounds (Abu-Elteen and Hamad, 2005).

The effects of different concentrations of the compounds 7a-c (2-ferrocenyl-5-fluoro-6-(4-phenyl-1-piperazinyl)-[1H] benzimidazoles) on growth curves of various *Candida* sp. showed that in all cases growth inhibition was concentration-dependent (Fig. 1A-H, 2A-H). All compounds had a significant effect on the growth curves of the various strains tested. Figure 1 shows the growth curves obtained with 4-fluoro-5-(4-methyl-1-piperazinyl)-2-nitroaniline (5a), 4-fluoro-5-(4-phenyl-1-piperazinyl)-2-nitroaniline (5b) and various *Candida* sp., with exponential growth in the presence of the compounds but increased doubling times. Qualitatively similar results were obtained with 2-ferrocenyl-5-fluoro-6-(4-methyl-1-piperazinyl) benzimidazole (7a), 2-ferrocenyl-5-fluoro-6-(4-phenyl-1-

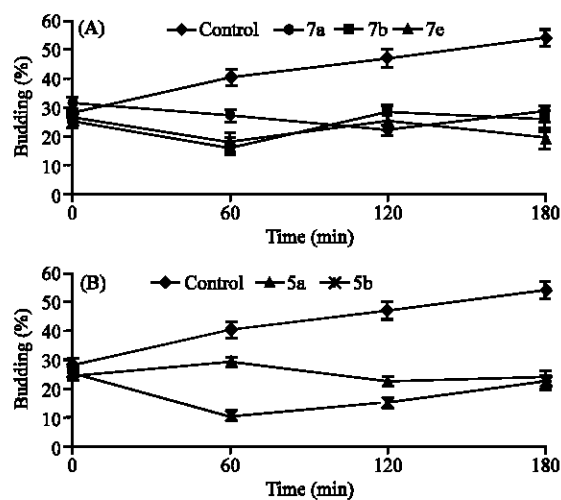


Fig. 3: Effect of (A) 7a, b and e and (B) 5a and b on budding of *C. albicans* at 1xMIC concentration

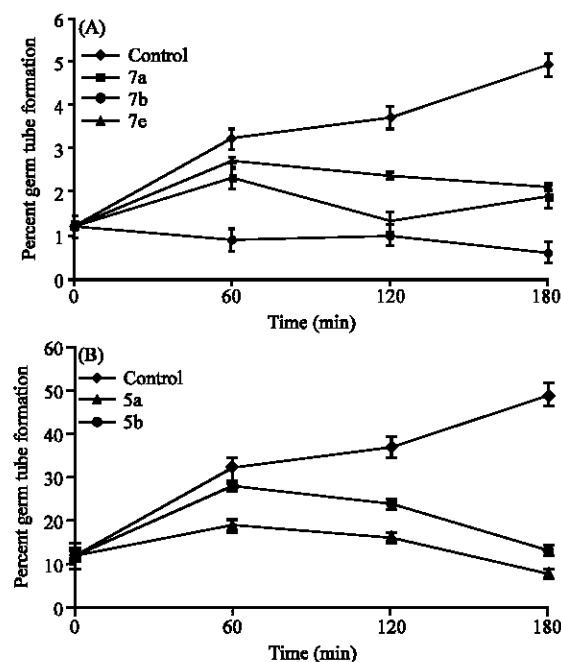


Fig. 4: Effect of (A) 7a, b and e and (B) 5a and b on germ tube formation of *C. albicans* at 1xMIC concentration

piperaziny] benzimidazole (7b) and 2-ferrocenyl-5-fluoro-6-[4-(4-fluorophenyl)-1-piperaziny]benzimidazole(7e) and with various yeast species (Fig. 2). No pseudohyphal formation was noticed at MIC or in control flasks containing YEPD broth only. Conversely, compound 7e (2-ferrocenyl-5-fluoro-6-(4-(4-fluorophenyl)-1-piperaziny)-[1H] benzimidazole) at an MIC concentration of 1xMIC, 600 $\mu\text{g mL}^{-1}$, inhibited the extensive budding initiated on

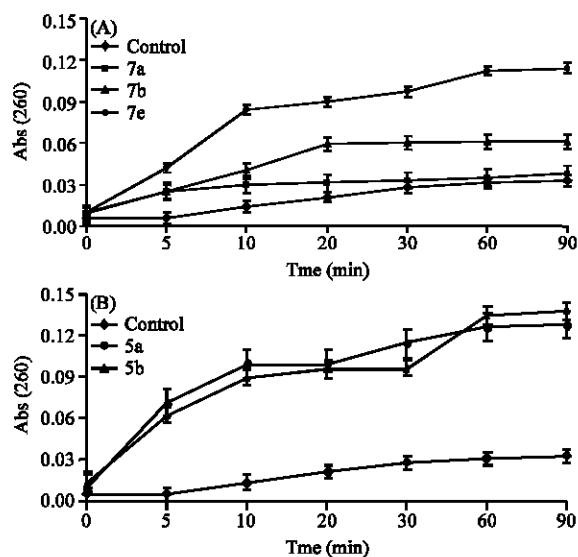


Fig. 5: Effect of (A) 7a, b and e and (B) 5a and b on *C. albicans* at 1xMIC concentration followed by leakage of the cytoplasmic material released

transfer of *C. albicans* to Eagle's medium as compared with the control culture. Similar observations were made, when compounds 5a, b were tested for their budding effect (Fig. 3A, B).

Furthermore, compounds 5a and b at 1xMIC concentrations (62.5 and 250 $\mu\text{g mL}^{-1}$, respectively) also block germ tube formation in *C. albicans* on incubation in calf serum (Fig. 4A, B). Furthermore, leakage of cytoplasmic material (another indicator of growth arrest) was also determined (Fig. 5A, B) in *C. albicans* cells treated with 1xMIC concentrations for compounds 7a-e and 5a, b. All of these compounds showed a significant effect on leakage of cellular contents. More pronounced effects were observed for compounds 7e, 5a and b compared to 7a and b. In qualitative terms, similar results were observed for a number of other compounds, although less UV-absorbing material was released suggestive of a lower antimycotic activity (Ghannoum *et al.*, 1990). As the results demonstrate, several compounds of the novel series, in particular 5a, b, 7a, b and e are highly potent against a number of *Candida* sp. Although the mode of action of the various 4-fluoro-5-(4-substituted-1-piperaziny)-2-nitroanilines can not be readily ascertained at this point, it is obvious that the potency of the active compounds is differential and seems to target different molecular entities within the fungal cells. Further studies are still needed in order expand our findings and get more insight into the mode of action of the described compounds.

In general, many dicationic-substituted bis-benzimidazole derivatives have shown good *in-vivo* activity for *Pneumocystis carinii* pneumonia in the rat model (Boykin *et al.*, 1998) and also excellent *in-vitro* fungicidal activity for *C. albicans* and *C. neoformans* (Del Poeta *et al.*, 1998b). Moreover, Del Poeta *et al.* (1999) showed that two bis-benzimidazoles compounds showed excellent *in vitro* fungicidal activity against *C. krusei*, *C. glabrata*, *C. lusitaniae* and *Cryptococcus neoformans* in addition to fluconazole-sensitive and fluconazole-resistant isolate of *C. albicans*.

A potential key to further antifungal development of these compounds will be to establish their mechanism(s) of action. The effects of these compounds on the growth and leakage of cell content begin at an early stage, which may suggest the possibility that the primary site of attack of the drugs is at the level of the cell wall or plasma membrane. Inhibition of germ tube formation by the drugs is of interest as germ tube formation has been implicated in the adherence and pathogenicity of *C. albicans* (Abu-Elteen, 2000) which highlights their potential for clinical control of yeast infection. It is clear that these compounds warrant further studies on structure-activity relationships, mechanism(s) of action and toxicity to determine their clinical potential as a class of antifungal agents.

ACKNOWLEDGMENTS

This study was supported by research grant (grant No. Abu-Elteen and Abdel-Jalil, 2004), Deanship of Graduate Studies and Scientific Research, Hashemite University. R.J. A.-J expresses his gratitude to Deutsche Gemeinschaft Forschung (DFG) for fellowship.

REFERENCES

- Abdel-Jalil, R.J. and W. Voelter, 2005. Synthesis of new 2-ferrocenyl-5-fluoro-6-(4-substituted-1-piperazinyl)-1H-benzimidazoles of potential biological interest. *J. Heterocyclic Chem.*, 42: 67-71.
- Abu-Elteen, K.H., 2001. Increased incidence of vulvovaginal candidiasis caused by *Candida glabrata* in Jordan. *Jap. J. Infect. Dis.*, 54: 103-107.
- Abu-Elteen, K.H. and M. Hamad, 2005. Antifungal Agents for Use in Human Therapy. In: *Fungi: Biology and Applications*, Kavanagh, K. (Ed). Wiley and Sons, UK., pp: 191-217.
- Abu-Elteen, K.H. and M. Hamad, 2007. Novel Antifungal Therapies. In: *New Insights in Medical Mycology*, Kavanagh, K. (Ed.). Springer, The Netherlands, pp: 69-98.
- Abu-Elteen, K.H., 2000. Effects of date extract on adhesion of *Candida* species to human buccal epithelial cells *in vitro*. *J. Oral Pathol. Med.*, 29: 200-205.
- Abu-Elteen, K.H., M. Hamad and K. Kavanagh, 2007. Epsilon-meter test-based determination of susceptibility of clinically important *Candida* isolates to conventional antifungal agents. *J. Med. Sci.*, 7: 374-382.
- Andriole, V.T., 1999. Current and future antifungal therapy: New targets for antifungal agents. *J. Antimicrob. Chemother.*, 44: 151-162.
- Bartlett, M.S., T.D. Edline, M.M. Shaw and L.J. Wheat, 1998. Carbendazim cures histoplasmosis in mice. Program and Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, Sept. 24-27, American Society for Microbiology, San Diego, CA, J58 Washington DC, USA., pp: 467-468.
- Bodey, G., M. Mardani, H. Hanna, M. Boktour and J. Abbas *et al.*, 2002. The epidemiology of *Candida glabrata* fungemia in immunocompromised patients with cancer. *Am. J. Med.*, 112: 380-385.
- Boykin, D.W., A. Kumar, G. Xiao, W.D. Wilson and B.C. Bender *et al.*, 1998. 2,5-Bis[4-(N-alkylamidino)phenyl]furans as anti *Pneumocystis carinii* agents. *J. Med. Chem.*, 41: 124-129.
- Del Poeta, M., A.S. Bixel, F. Barchiesi, R.R. Tidwell, D. Boykin, G. Scalise and J.R. Perfect, 1999. *In vitro* activity of dicationic aromatic compounds and fluconazole against *Cryptococcus neoformans* and *Candida* sp. *J. Antimicrob. Chemother.*, 44: 223-228.
- Del Poeta, M., W.A. Schell, C.C. Dykstra, S.J. Jones and R.R. Tidwell *et al.*, 1998a. *In vitro* antifungal activities of a series of dicationic-substituted carbazoles, furans and benzimidazoles. *Antimicrob. Agents Chemother.*, 42: 2503-2510.
- Del Poeta, M., W.A. Schell, C.C. Dykstra, S.J. Jones and R.R. Tidwell *et al.*, 1998b. Structure-*in vitro* activity relationships of pentamidine analogues and dicationic-substituted bis-benzimidazoles as new antifungal agents. *Antimicrob. Agents Chemother.*, 42: 2495-2502.
- Ghannoum, M.A. and L.B. Rice, 1999. Antifungal agents: Mode of action, mechanisms of resistance and clinical correlation of these mechanisms with bacterial resistance. *Clin. Microbiol. Rev.*, 12: 501-517.
- Ghannoum, M.A., K.H. Abu-Elteen, M. Ellabib and P.A. Whittaker, 1990. Antimycotic effects of octenidine and pirtenidine. *J. Antimicrob. Chemother.*, 25: 237-245.

- Kremery, V. and A.J. Barnes, 2002. Non-albicans *Candida* sp. causing fungemia: Pathogenicity and antifungal resistance. *J. Hosp. Infect.*, 50: 243-260.
- Roilides, E., J. Dotis and A. Katragkou, 2007. *Fusarium* and *Scedosporium*: Emerging Fungal Pathogens. In: *New Insights in Medical Mycology*, Kavanagh, K. (Ed.). Springer, The Netherlands, pp: 267-285.
- Safdar, A., V. Chaturvedi, E.W. Cross, S. Park, E.M. Bernard, D. Armstrong and D. Perlin, 2001. Prospective study of *Candida* species in patients at a comprehensive cancer center. *Antimicrob. Agents Chemother.*, 45: 2129-2133.
- Sampathkumar, P. and C.V. Paya, 2001. *Fusarium* infection after solid organ transplantation. *Clin. Infect. Dis.*, 32: 1237-1240.
- Speller, D.C.E., 1980. Other Antifungal Agents. In: *Antifungal Chemotherapy*, Speller, D.C.E. (Ed). John Wiley Chichester, UK., pp: 183-210.