Med Chem Res (2012) 21:1921–1928
DOI 10.1007/s00044-011-9716-z

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Structure–cytotoxicity relationship of a novel series of miconazole-like compounds

Reza Yousefi · Ali Khalafi-Nezhad · Mohammad Navid Soltani Rad · Somayeh Behrouz · Farhad Panahi · Mansoore Esmaili · Sayed Mahmoud Ghaffari · Ali Niazi · Ali Akbar Moosavi-Movahedi

Received: 14 December 2010 / Accepted: 16 June 2011 / Published online: 7 July 2011

Abstract In the current study, two novel classes of the carboacyclic nucleosides having miconazole-like scaffolds as imidazole- and pyrimidine-based compounds were examined for their cytotoxic properties. The aim was to establish a relation between cytotoxic activity and nature of the synthetic compounds. While *Escherichia coli* (DH5α) and human erythromyeloblastoid leukemia cell line (K562) were the target cells, depending on the type of substitution made, ranges of antibacterial and antineoplastic activities were observed. Also the electron-donating and electron-accepting properties of the ligands were proved to play a crucial role in their cytotoxic activities. Accordingly, the substitutions associated with the marked improvement of cytotoxic activities can be considered as the significant point in construction of new generation of either antibacterial or antineoplastic agents.

R. Yousefi (✉)
Protein Chemistry Laboratory (PCL), Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran
e-mail: ryousefi@shirazu.ac.ir

A. Khalafi-Nezhad · F. Panahi
Department of Chemistry, College of Sciences, Shiraz University, Shiraz, Iran

M. N. Soltani Rad · S. Behrouz
Department of Chemistry, Shiraz University of Technology, Shiraz, Iran

M. Esmaili · S. M. Ghaffari · A. A. Moosavi-Movahedi
Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran

A. Niazi
Institute of Biotechnology, Shiraz University, Shiraz, Iran

Keywords Pyrimidine-based compounds · Imidazole-based compounds · Cytotoxic activities

Introduction

The pharmacokinetic properties and cellular permeability of a drug can be modulated by derivatization to bio-reversible forms (Maccari *et al.*, 2002). Hydrazone derivatives are prominent structural motif in numerous pharmaceutically active compounds; consequently several well-known drugs with various chemotherapeutic activities contain a hydrazone moiety in their structure (Kleeman *et al.*, 1999; Szarvasi *et al.*, 1973; Pennington *et al.*, 1953; Sah and Peoples, 1953; Szentesi *et al.*, 2001; Murdock *et al.*, 1982). Azoles are a well-known class of antifungal agents, disrupting biosynthesis of the major sterol of fungal cell membrane (ergosterol) (Turner and Rodrigues, 1996; Bodey, 1992). Miconazole and oxiconazole are the most established antifungal azoles having rational versatility in their structures and family (Fig. 1) (Rossello *et al.*, 2002). Various structurally related miconazole bioactive hydrazones are known as antimicrobial and antifungal agents (Mamolo *et al.*, 2004; Dyer *et al.*, 1983).

In general, there are two basic approaches to develop a new drug for the treatment: (a) synthesis of analogs, modifications, or derivatives of existing compounds for shortening and improving treatment, and (b) searching for novel structures that the organism has never been presented with before. The current study was carried out to examine the biological activities of a novel series of carboacyclic nucleosides having miconazole-like scaffolds as imidazole- and pyrimidine-based compounds. The general structures for the parent compounds are shown in Fig. 1a. In these compounds, the ether and ketoxim-ether linkages were replaced with hydrazone linkages in miconazole and

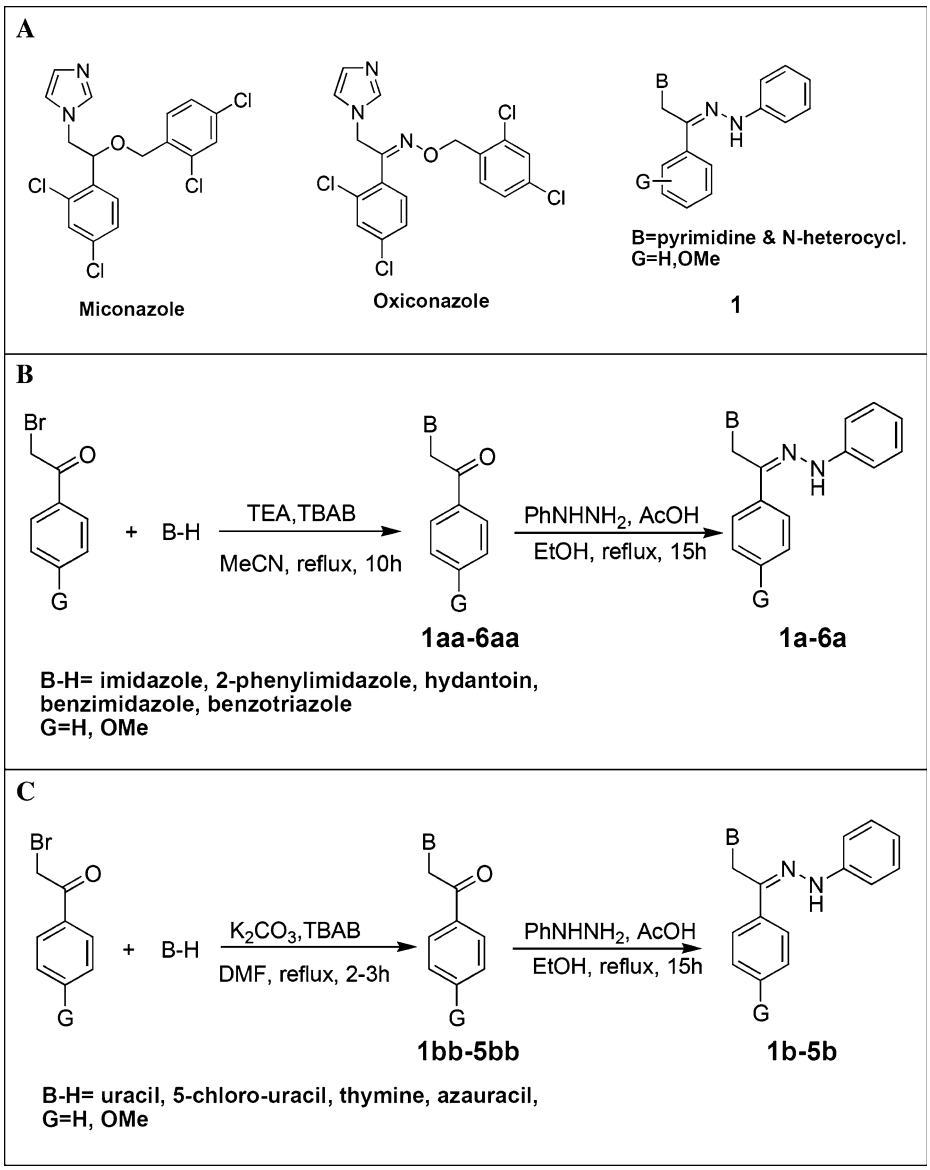

Fig. 1 Chemical structures and synthetic routes for preparation of the hydrazine derivatives. **a** The comparison between the chemical structure of miconazole, oxiconazole, and the parent compounds. The general synthetic routes for the preparation of **b** imidazole-based compounds and **c** pyrimidine-based compounds

oxiconazol, respectively. With the aim to establish a relation between cytotoxic activity and nature of the compounds, the cytotoxic activities of the miconazole-like compounds were examined and compared using *Escherichia coli* (DH5α) and human erythromyeloblastoid leukemia cell line (K562) as the target cells.

Chemistry

Recently, we reported an efficient procedure for preparation of new hydrazon derivatives (Soltani Rad *et al.*, 2010). As shown in Fig. 1b, c, treating the synthesized ethanone derivatives with phenyl hydrazine in refluxing ethanol, resulted in the formation of corresponding hydrazone derivatives. Compounds **1a**–**6a** (Fig. 2) and **1b**–**5b** (Fig. 3) were synthesized using two different strategies according to the procedures used for synthesis of ethanone intermediates considering the differences in chemical behaviors of pyrimidine nucleobases in comparison with azoles family. The ethanone derivatives for ligands **1a**–**6a** were prepared from the substituted 2-bromoacetophenones and the appropriate azole derivatives in the presence of triethylamine in refluxing acetonitrile (Fig. 1b). To synthesize ligands **1b**–**5b**, the corresponding intermediates were obtained by the reaction of substituted 2-bromoacetophenones and pyrimidine nucleobases in refluxing *N*,*N*-dimethyl formamide (DMF) (Fig. 1c).

Biochemistry and pharmacology

Azole antifungals are well known for inhibiting a cytochrome P-450-dependent enzyme which is crucial for ergosterol biosynthesis in fungi, and moreover they involve

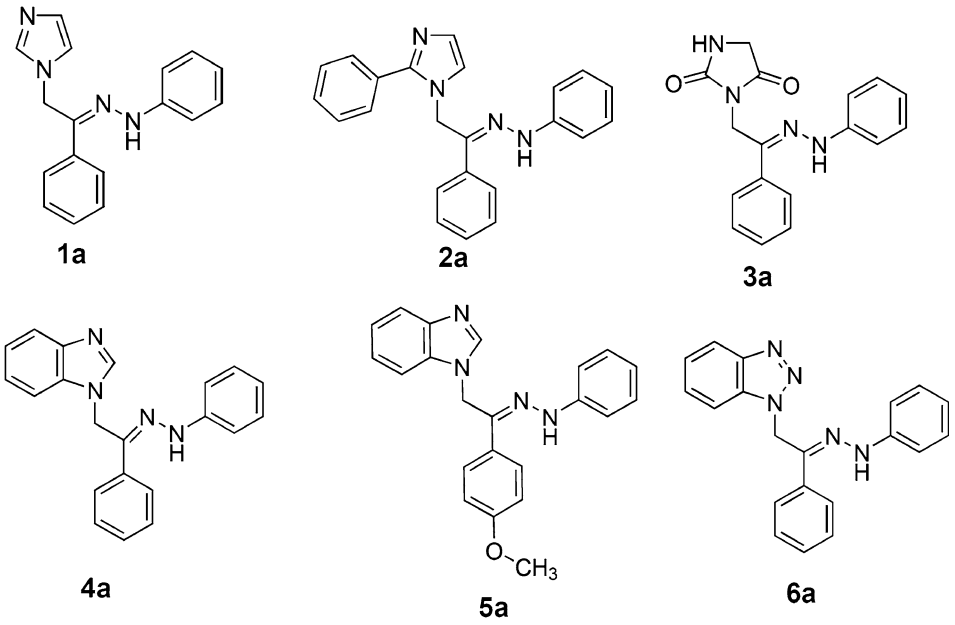

Fig. 2 Chemical structures of the imidazole-based hydrazon derivatives

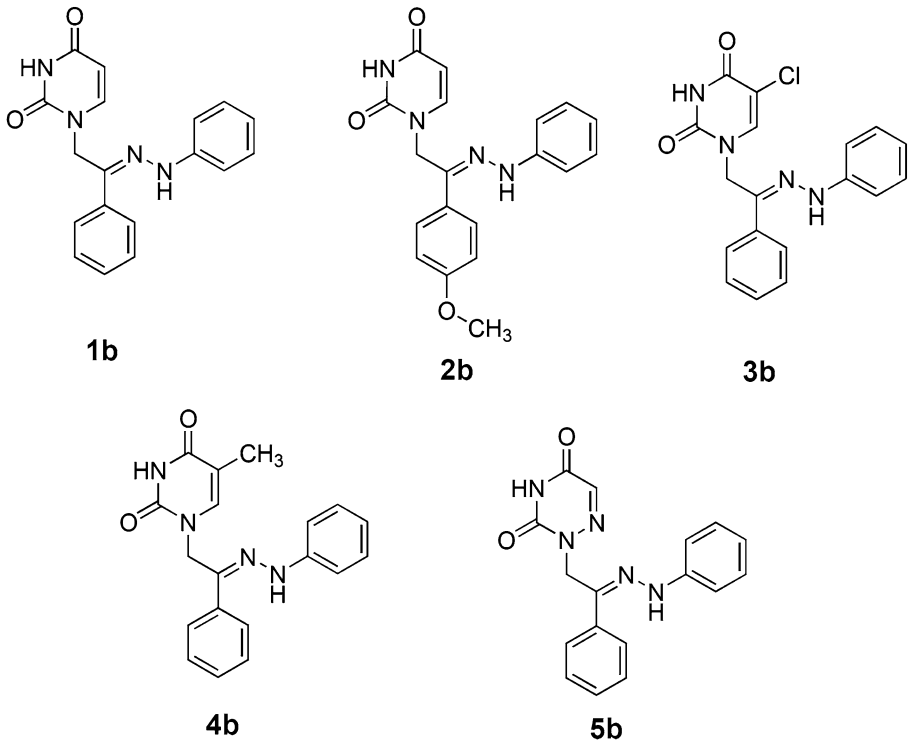

Fig. 3 Chemical structures of pyrimidine-based hydrazone derivatives

in hormone synthesis or drug metabolism in mammalian cells. Furthermore, antifungal azoles have previously been reported to exhibit antibacterial activity, although their mechanism of action is not fully understood (Rossello *et al.*, 2002; Mamolo *et al.*, 2004; Dyer *et al.*, 1983). In this study, two novel classes of miconazole-like scaffolds as imidazole- and pyrimidine-based compounds are examined for their cytotoxic activities. Six imidazole- (**1a–6a)** and five pyrimidine-based compounds (**1b–5b)** were tested for both antibacterial and antineoplastic activities with the aim to establish a relation between chemical structure and cytotoxic activity. The data of antibacterial activities of these compounds are shown in Tables 1 and 2, while Fig. 4 displays those of their antiproliferation activities.

Results and discussion

Antibacterial activities of the miconazole-like compounds

Hydrazide–hydrazone has been demonstrated to possess antibacterial activity (Aussel *et al.*, 1994; Pawelec *et al.*, 1991). In order to evaluate contribution of the new substitution on cytotoxic profile of the miconazole-like compounds, a novel series of imidazole- and pyrimidine-based derivatives were synthesized. In this study, both antimicrobial and antineoplastic activities of the synthetic compounds were examined. The antimicrobial agents applied in this study may be either bacteriostatic or bactericidal. The cytotoxic activities against *E. coli* (DH5α) of the imidazole-based compounds are shown in Table 1. In order to compare the cytotoxic activity among the different compounds of this class, Ligand **1a** with the simplest chemical structure was considered as the reference compound (Fig. 2). As shown in Fig. 2 and Table 1, the addition of electron-donating phenyl moiety to the imidazole (Ligand **2a**), markedly decreases the cytotoxic activity. This finding suggests that the molecule after this modification may gain certain electronic and/or stereo chemical properties which abolish its antibacterial activity. On the other hand, the existence of two carbonyl electron-withdrawing moieties in hydantoin (Ligand **3a**), significantly increases the cytotoxic effect against *E. coli* (DH5α). As the imidazole was replaced with benzimidazole in ligand **4a** and **5a**, the cytotoxic activities against *E. coli* (DH5α) were decreased. Further reduction of the cytotoxic activity was observed in the case of compound **5a** which was bearing an electron-donating methoxy in its structure. Surprisingly, the addition of an electron-withdrawing element such as nitrogen (position 2) in compound **6a** markedly enhances its cytotoxic activity. Overall, these findings show the importance of electronic and chemical properties in the cytotoxicity of the synthetic compounds.

In this study, the different pyrimidine-based compounds were also examined for their cytotoxic activities against *E. coli* (DH5α), and the results are listed as Table 2. In order to compare the cytotoxic activities between different members of this class, ligand **1b** which possesses the simplest chemical structure is considered as the reference compound (Fig. 3). In contrast to the imidazole-based compounds, addition of the electron-donating moieties such as methoxy in compound **2b** and methyl in Ligand **4b** markedly increases their cytotoxic activities as compared with that of the reference compound (Ligand **1b**). Also, the methoxy bearing compound (Ligand **2b**) exhibits slightly higher cytotoxic activity than the compound with methyl moiety (Ligand **4b**). As methyl group was substituted with a halogen (chloride) in ligand **3b**, a significant enhancement in the cytotoxicity was observed. Similar to what was observed for the imidazole-based compound (Ligand **6a**), addition of the nitrogen element to pryimidine ring (position 6) in ligand **5b** noticeably enhanced the cytotoxic activity of this compound, displaying the vital role which might be played by the existence of additional hydrogen-accepting nitrogen element in the cytotoxic compounds. Overall, the electron-donating and electron-accepting properties of the synthetic miconazole-like compounds are identified to be very important for their biological properties.

Table 2 Log P and cytotoxicity of the pyrimidine-based compounds against *E. coli* (DH5α)

Entry	**1b**	**2b**	**3b**	**4b**	**5b**
IC50 (μM)	57.3 ± 3.45	6.2 ± 0.57	4.8 ± 0.71	7.1 ± 0.48	14.6 ± 1.47
Log P	1.84	1.72	1.88	2.19	1.95

Antineoplastic activities of the synthetic miconazole-like derivatives

As shown previously, miconazole-like compounds may slowdown proliferation of the activated human T-cells and human acute myelogenous leukemia (AML) cells (Brusserud, 2001; Krämer *et al.*, 2009; Thomae *et al.*, 2007; Mossman, 1983). In this study, all of the synthetic miconazole-like derivatives (20, 40, and 80 μM) were further examined for their toxicity against K562 cell line. After 24-h exposure of K562 leukemic cells to the different synthetic compounds, viability was assessed on the basis of cellular conversion of 3-(4,5-dimethtl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliun bromide (MTT) into a formazan product, using MTT-based cell proliferation assay (Gurkok *et al.*, 2009; Rufián-Henaresa and Morales, 2008). As the leukemic cells (2×10^4 cells/ml) were cultured in 96-well plates for 24 h the cytotoxic activities were assessed, and a range of antitumor activities was observed for the synthetic compounds (Fig. 4). Among the imidazole-based derivatives, ligand 6a proved to be the most active compound.

Table 1 Log P and cytotoxicity of the imidazole-based compounds against *Escherichia coli* (DH5α)

Entry	**1a**	**2a**	**3a**	**4a**	**5a**	**6a**
IC50 (μM)	7.6 ± 0.81	16.8 ± 1.71	3.1 ± 0.61	12.3 ± 0.23	13.7 ± 1.42	3.4 ± 0.20
Log P	2.13	4.18	1.26	3.60	3.48	4.01

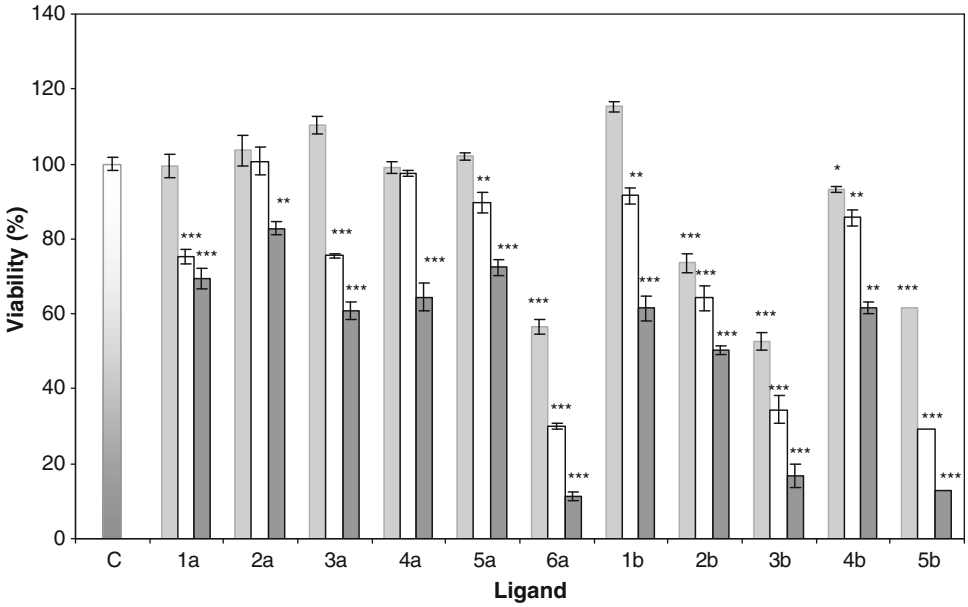

Fig. 4 Antiproliferation activities of the synthetic ligands against K562 cells (2×10^4 cells/ml) were cultured with varying concentrations of the ligands (20, 40, and 80 μM) for 24 h, and cell proliferation was assessed by MTT assay as described in "Materials" Section. Results (Cytotoxicity) are presented relative to cell growth under control conditions (the absence of the ligands) and expressed as percentage of control (as 100%). The vertical bars represent SD of triplicate determinations and asterisks indicate $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ compared to control (the absence of the ligands).

When pyrimidine derivatives were examined for their cytotoxic activity against K562 leukemia cell line, ligand **3b** and **5b** were the most potent of all the compounds tested for their antiproliferation activities. Two ligands **6a** and **5b** possessed additional nitrogen element in the imidazole and pyrimidine rings, respectively (Figs. 2, 3). The obtained results confirm that the addition of further nitrogen element with the ability for hydrogen accepting in the synthetic compounds could significantly enhance the cytotoxic properties of the miconazole-based compounds. As shown in Fig. 3, the substitution of hydrogen (in compound **1b**) with chloride yielded compound **3b** with significantly improved activity against the tumor cells. Thus, electron-donating and electron-accepting natures of the synthetic ligands are proven to be of importance in their antineoplastic activities. Furthermore, the lipophilicity of the synthesized compounds, which varied significantly when compared with the parent compound, might be considered as an important parameter in their cytotoxic activities (Sánchez *et al.*, 2008; Berney *et al.*, 2006). The lipophilicity may render them more capable of penetrating various biomembranes, consequently improving their permeation properties through the cell membranes (Sánchez *et al.*, 2008; Berney *et al.*, 2006). Thus, LogP of the synthetic ligands was calculated using CS Chem Draw Ultra 8/CS Chem 3D Ultra 8 (Cambridge Soft, 2004) and expressed in Tables 1 and 2. The variation of Log P as shown in these tables is more significant in the case of imidazole-based compounds compared to that of pyrimidine-based ligands. However, the results of this study did not show regular pattern of correlation between Log P and antiproliferation activities of the synthetic compounds except in a few cases (Tables 1, 2; Fig. 4).

Conclusion

This study describes the cytotoxic properties of several imidazole- and pyrimidine-based derivatives against both *E. coli* (DH5α) and K562 leukemia cell line. Overall, the addition of the electron-withdrawing elements (N and carbonyl) with the capability for hydrogen being accepted into the imidazole scaffolds displayed a critical role in the enhancement of the antibacterial activities of the studied compounds**.** Conversely, the addition of the electron-donating moieties such as benzyl, methyl, and methoxy to these ligands significantly suppresses their antibacterial activities. The similar pattern of antibacterial properties was not observed in the case of the pyrimidine-based ligands since the methoxy- and methyl-bearing pyrimidine compounds exhibited greater antibacterial activities than that of their corresponding reference ligand. The additional nitrogen element existing in the structure of both imidazole and pyrimidine moieties of the synthetic ligands significantly enhanced their antibacterial activities. As the leukemic cells were used as the target; the synthetic ligands **6a**, **3b,** and **5b** exhibited the highest antiproliferation properties. These ligands also showed higher antibacterial activities than their corresponding reference compounds against *E. coli* (DH5α). Therefore, results of the current study suggest that addition of either halogen or nitrogen elements to the scaffold of the synthetic ligands significantly enhances their cytotoxic activities against both *E. coli* (DH5α) and K562 leukemic cells. In conclusion, the obtained results suggest that the electron-donating and electron-accepting properties and/or stereo chemical features of the ligands may play critical roles in their cytotoxic activities. As several synthetic compounds were screened

for their cytotoxic properties; the compounds with significant activity can also represent a starting point for a new generation of cytotoxic agents. However, additional studies are required to determine whether these synthetic compounds could induce apoptosis in the cancer cells.

Experimental

Synthesis of ligands

The ligands as shown in Figs. 1 and 2 were prepared according to the methods reported in the literatures (Soltani Rad *et al.*, 2010).

General

All the chemicals were purchased from Fluka or Merck chemical companies. The progress of reaction was followed with thin layer chromatography (TLC) using silica gel SILG/UV 254 plates. Silica gel 60, 0.063–0.200 mm (70–230 mesh ASTM) was used for column chromatography. Infrared (IR) spectra were run on a Shimadzu FTIR-8300 spectrophotometer. The ^{1}H NMR (250 MHz) and ^{13}C NMR (62.5 MHz) were run on a Brüker Avanced DPX-250, FT-NMR spectrometer; δ in ppm, J in Hz. Mass spectra were recorded on a Shimadzu GC MS-QP 1000 EX apparatus. Microanalyses were performed on a Perkin–Elmer 240-B microanalyzer. Melting points (mp) were recorded on a Büchi 510 apparatus in open capillary tubes and are uncorrected.

*General procedure for the synthesis of ketones **1aa–6aa***

In a double-neck round-bottomed flask (100 ml) equipped with a condenser, a mixture of appropriate *N*-heterocyle (0.01 mol), 2-bromo acetophenones (0.012 mol), anhydrous tetra ethyl amine (TEA,1.01 g, 0.01 mol), and catalytic amounts of tetra butyl ammonium bromide (TBAB,0.1 g) was dissolved in dry acetonitrile (40 ml). Then, the mixture was heated at reflux for 10 h (TLC control). The solvent was evaporated at reduced pressure, the residue was dissolved in $CHCl_3$ (200 ml), and washed with H_2O (2 × 100 ml). The organic layer was dried (10 g of Na_2SO_4) and concentrated to afford the crude product which was purified by column chromatography on silica gel eluting with proper solvents.

*General procedure for the synthesis of ketones **1bb–5bb***

In a double-neck round-bottomed flask (100 ml) equipped with a condenser, a mixture of appropriate nucleobase (0.01 mol), 2-bromo acetophenones (0.012 mol), K_2CO_3 (1.38 g, 0.01 mol) and catalytic amounts of TBAB (0.1 g) was dissolved in dry DMF (30 ml). Then, the mixture was heated at reflux for 2–3 h (TLC control). The solvent was evaporated at reduced pressure, the residue was dissolved in $CHCl_3$ (200 ml), and washed with H_2O (2 × 100 ml). The organic layer was dried (10 g of Na_2SO_4) and concentrated to afford the crude product which was purified by column chromatography on silica gel eluting with proper solvents.

*General procedure for the synthesis of compounds **1a–6a** and **1b–5b***

In a double-neck round-bottomed flask (100 ml) equipped with a condenser, a mixture of appropriate ketone (0.01 mol), phenylhydrazine (1.62 g, 0.015 mol), and acetic acid (3 drops) was dissolved in ethanol (15 ml), and the mixture was heated at reflux for 15 h (TLC control). Afterward, the reaction mixture was kept at refrigerator overnight. Then, it was filtered and washed with cool ethanol (2 × 5 ml) and recrystallized from MeOH/H_2O to afford pure phenylhydrazone derivatives.

Biology

Materials

Fetal calf serum (FCS) was obtained from veterinary faculty at the University of Tehran. RPMI 1640 medium, Penicillin, Streptomycin, and all other reagents were purchased from the Sigma (Deisenhofen, Germany) and were at least of analytic grade. All solutions were made in double-distilled water.

Determination of antimicrobial activity

The synthetic ligands were dissolved and diluted in ethanol to a given concentration, and from this stock solution serial twofold dilutions were made in the well of microtitre plate. Final concentration ranged from 5 to 80 μM. DH5α, an antibiotic sensitive strain of *E. coli* was grown in LB agar media containing 1% Trypton, 0.5% NaCl, and 0.5% yeast extract. The antibacterial activity of the ligands was assessed on Microtiter plate-based assay according to the method reported previously (El-Ziney *et al.*, 1998). In each well, 200 μl of overnight cultured *E. coli* (DH5α) and 10 μl of each diluted miconazole-like compound were added. As the control experiment, 200 μl of the bacteria and 10 μl of ethanol/ddH_2O (1:1) were inoculated into the wells. The mixture was shaken at 37°C for 16 h, and the optical density at 600 nm was measured every 30 min until the end of the experiment using an Elisa Reader Expert 96 (ASYS Hitech, Eugendorf, Austria). The specific growth

rate (μ) was calculated from the plot of OD_{600} versus time as follows:

$$\mu = \frac{\text{Ln}\, OD_{600}}{\Delta t}$$

where t is the time of incubation (Küçükgüzel *et al.*, 2003; Bruserud, 2001). The lethal concentration 50 (LC50) was determined by probit analysis using the Pharm. PCS statistical package (Springer-Verlage, New York).

Cancer cell culture

K562 Chronic myelogenous leukemia cells were obtained from the cell bank of Pasteur Institute of Iran. K562 cells were grown in RPMI with 10% heat-inactivated fetal calf serum (FCS), supplemented with 4 mM L-glutamine, 100 U penicillin, and 100 μg/ml streptomycin at pH 7.4, in a humidified, 5% CO_2 incubator and at the temperature of 37°C.

K562 proliferation assay

To evaluate the cytotoxicity effect of the ligands on K562, the harvested cells were seeded into 96-well plates (2×10^4 cells/ml) and incubated for 24 h in the presence of 20, 40, and 80 μM of the synthetic compounds. The antiproliferation curve activity was determined based on MTT colorimertic assay (Yousefi *et al.*, 2005; Rufián-Henaresa and Morales, 2008; Gurkok *et al.*, 2009). Four hours before the end of incubations, the cells were washed with PBS three times, changed with fresh medium, and each well loaded with 25 μl MTT solution (5 mg/ml in PBS) followed by 4-h incubation. The insoluble formazan produced by living cells were dissolved in solution containing 10% SDS and 50% DMF (Left for 2 h at 37°C in dark conditions), and optical density (OD) was read against blank with multi-well scanning spectrophotometer (ELISA reader, Model, Multiskan MS) at a wavelength of 540 (OD540). The percentage of cell viability was calculated using equation: [mean optical density (OD) of treated cells/mean OD of control cells] × 100. The final concentration of ethanol never exceeds 2.5%, which has no deleterious effect on the target cells.

Statistical analysis

The experiments were repeated three times for each sample, and the statistical differences were determined by analysis of variance (ANOVA) followed by Turkey–Kramer multiple comparison tests on the instant package. Differences were regarded as significant at $P < 0.05$.

Acknowledgments The authors would like to thank the financial support of Iran National Science Foundation (INSF)—Grant nos. 88001578 and 88001894). Also the financial support of the research council of Shiraz University is gratefully acknowledged.

References

Aussel C, Breittmayer JP, Ticchioni M, Pelassy C, Bernard A (1994) Regulation of T cell activation by cytochrome P450 inhibitors. Cell Immunol 155:436–445

Berney M, Weilenmann HU, Ihssen J, Bassin C, Egli T (2006) Specific growth rate determines the sensitivity of *Escherichia coli* to thermal, UVA, and solar disinfection. Appl Environ Microbiol 72:2586–2593

Bodey GP (1992) Azole antifungal agents. Clin Infect Dis 14:161–169

Bruserud O (2001) Effects of azoles on human acute myelogenous leukemia blasts and T lymphocytes derived from acute leukemia patients with chemotherapy-induced cytopenia. Int Immunopharmacol 1:2183–2195

Dyer RL, Ellames GJ, Hamill BJ, Manley PW, Pope AM (1983) Synthesis of (E)-1-(5-chlorothien-2-yl)-2-(1H-imidazol-1-yl)ethanone 2,6-dichlorophenylhydrazone hydrochloride, a novel, orally active antifungal agent. J Med Chem 26:442–445

El-Ziney MG, Arneborg N, Uyttendaele M, Debevere J, Jakobsen M (1998) Characterization of growth and metabolite production of *Lactobacillus reuteri* during glucose/glycerolcofermentation in batch and continuous cultures. Biotechnology Lett 20:913–916

Gurkok G, Altanlar N, Suzen S (2009) Investigation of antimicrobial activities of indole-3-aldehyde hydrazide/hydrazone derivatives. Chemotherapy 55:15–19

Kleeman A, Engel J, Kutscher B, Reichert D (1999) Pharmaceutical substances, 3rd edn. Thieme, Stuttgart

Krämer SD, Lombardi D, Primorac A, Thomae AV, Wunderli-Allenspach H (2009) Lipid-bilayer permeation of drug-like compounds. Chem Biodivers 6:1900–1916

Küçükgüzel SG, Mazi A, Sahin F, Oztürk S, Stables J (2003) Synthesis and biological activities of diflunisal hydrazide-hydrazones. Eur J Med Chem 38:1005–1013

Maccari R, Ottanà R, Monforte F, Vigorita MG (2002) In vitro antimycobacterial activities of 2'-monosubstituted isonicotinohydrazides and their cyanoborane adducts. Antimicrob Agent Chemother 46(2):294–299

Mamolo MG, Zampieri D, Falagiani V, Vio L, Fermeglia M, Ferreno M, Pricl S, Banfi E, Scialino G (2004) Antifungal and antimycobacterial activity of new N1-[1-aryl-2-(1H-imidazol-1-yl and 1H-1,2,4-triazol-1-yl)-ethylidene]-pyridine-2-carboxamidrazone derivatives: a combined experimental and computational approach. ARKIVOC V: 231–250

Mossman T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Method 65:55–63

Murdock KC, Child RG, Lin Y, Warren JD, Fabio PF, Lee VJ, Izzo PT, Lang SA, Angier RB, Citarella RV, Wallace RE, Durr FE (1982) Antitumor agents. 2. Bisguanylhydrazones of anthracene-9,10-dicarboxaldehydes. J Med Chem 25:505–518

Pawelec G, Ehninger G, Rehbein A, Schaudt K, Jaschonek K (1991) Comparison of the immunosuppressive activities of the antimycotic agents itraconazole, fluconazole, ketoconazole and miconazole on human T-cells. Int J Immunopharmacol 13:299–304

Pennington FC, Guercio PA, Solomons IA (1953) Streptohydrazid. J Am Chem Soc 75:2261

Rossello A, Bertini S, Lapucci A, Macchia M, Martinelli A, Rapposelli S, Herreros E, Macchia B (2002) Synthesis, antifungal activity,

and molecular modeling studies of new inverted oxime ethers of oxiconazole. J Med Chem 45:4903–4912

Rufián-Henaresa JA, Morales FJ (2008) Microtiter plate-based assay for screening antimicrobial activity of melanoidins against *E. coli* and *S. aureus*. Food Chem 11:1069–1074

Sah PPT, Peoples SA (1953) The antitubercular activity of D-galacturonic acid isonicotinyl hydrazone. J Am Chem Soc 75:2512–2513

Sánchez C, Neves AR, Cavalheiro J, dos Santos MM, García-Quintáns N, López P, Santos H (2008) Contribution of citrate metabolism to the growth of *Lactococcus lactis* CRL264 at low pH. Appl Environ Microbiol 74:1136–1144

Soltani Rad MN, Khalafi-Nezhad A, Behrouz S (2010) Synthesis of some novel hydrazono acyclic nucleoside analogues. Beilstein J Org Chem 6:1–8

Szarvasi E, Fontaine L, Betbeder-Matibet A (1973) Antimicrobials: new nitrofuran derivatives. J Med Chem 16:281–287

Szentesi P, Collet C, Sarkozi S, Szegedi C, Jona I, Jacquemond V, Kovacs L, Csernoch L (2001) Effects of dantrolene on steps of excitation-contraction coupling in mammalian skeletal muscle fibers. J Gen Physiol 118:355–376

Thomae AV, Koch T, Panse C, Wunderli-Allenspach H, Krämer SD (2007) Comparing the lipid membrane affinity and permeation of drug-like acids: the intriguing effects of cholesterol and charged lipids. Pharm Res 24:1457–1472

Turner WW, Rodrigues MJ (1996) Recent advances in the medicinal chemistry of antifungal agents. Curr Pharm Design 2:209–224

Yousefi R, Ardestani SK, Saboury AA, Kariminia A, Zeinali M, Amani M (2005) Investigation on the surface hydrophobicity and aggregation kinetics of human calprotectin in the presence of calcium. J Biochem Mol Biol 38:407–413