

Original Research Article

A Novel Mechanistic Approach to Identify New Antifungal Lead Compounds Based on Amphotericin B Molecular Architecture

Marzieh Ferdosian and Soroush Sardari *

Drug Design and Bioinformatics Unit, Medical Biotechnology Department, Biotechnology Research Center, Pasteur Institute, Tehran, Iran 13164

*For correspondence: E-mail: ssardari@hotmail.com; Tel: +982166953311

Received: 30 April 2012

Revised accepted: 25 February 2013

Abstract

Purpose: To identify new antifungal lead compounds based on amphotericin B (AmB) molecular architecture.

Methods: The strategy employed was molecular similarity search and screening based on the molecular constraints of polyene macrolide antibiotics, as well as docking experiments. Several new compounds were analyzed for their general inhibitory effect against indicator microbial strains. Interaction of the antifungal compounds with ergosterol and cholesterol was studied by UV-Vis spectroscopy and their effect on lipid/polydiacetylene (PDA) vesicles identified. Furthermore, the cytotoxicity of the compounds was evaluated and compared with that of amphotericin B.

Results: In silico screening of 20,000 compounds obtained from the similarity search yielded seven candidates for in vitro antifungal test. The MIC of the more effective compounds, delta-decalactone and mandelonitrile, against three fungi - *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger* - was in the range of < 46.8 to 750.0 µg/ml. By comparing peak position shifts for the absorbance of mandelonitrile and delta-decalactone individually and in combination with sterols, it was found that mandelonitrile has a more selective interaction with ergosterol. The color change intensity of lipid/PDA vesicles indicated that delta-decalactone potently disturbed simulated membrane structure. Furthermore, cytotoxicity data for mandelonitrile and delta-decalactone on HepG2 and MCF7 show that mandelonitrile is less cytotoxic, with IC_{50} of 1095.04 and 2010.34 µg/ml, and more selective against fungal cells.

Conclusion: This study presents a new insight into algorithmic discovery of novel antifungal agents by in silico methodology based on a mechanistic approach.

Keywords: Mandelonitrile, Delta-decalactone, Amphotericin B, Virtual screening, Antifungal lead compounds, Cytotoxicity, Mechanistic approach.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Systemic infections have increased dramatically in the last few decades and they remain a major direct cause of death in patients being treated for malignant diseases and emerging immune deficiency diseases [1]. Management of fungal

infections is notably limited by problems of drug safety, resistance and effectiveness drug forms [2].

Current therapy for invasive mycoses uses a relatively reduced number of antifungal drugs that belong to three major groups: polyenes,

azoles and pyrimidines. The polyene antibiotics are among the most important ones and are produced by several different species of *Streptomyces*. The structure of these drugs consists of a lactone ring (20 - 24 carbons). The ring contains a series of conjugated double bonds ($n = 3 - 8$) which often have a glycoside residue.

The target for the biological actions of these antibiotics are related to the cell membrane sterols [3]. AmB, one of these polyene antibiotics used to treat systemic fungal infections, forms membrane channels in fungal cells. These channels are thought to be made from antibiotic/sterol and are responsible for the leakage of ions, which cause cell destruction [4]. The chemotherapeutic use of AmB is based on its higher affinity for ergosterol (principal sterol in fungal cell membrane) than cholesterol (principal sterol in mammalian cell membrane). Since AmB also has a high affinity for cholesterol-containing membranes, it is quite toxic and its application in clinical treatment is limited to rather severe cases. AmB exhibits several crucial positive features as an effective antifungal drug, such as high antifungal activity, broad antifungal spectrum and very rare induction of fungal resistance as well as the ability to overcome multidrug-resistant fungi [5].

Rational Drug Design (RDD) helps to facilitate and speed up the drug design process, which involves a variety of methods to identify novel compounds. One of these methods is virtual screening which is increasingly used as a cost-effective complement to high-throughput screening [6]. There are two principal types of virtual screening system: 1) structure-based approaches, such as docking, which allows the scientist to virtually screen a database of compounds by fitting two molecules together in 3D space for predicting the strongest binding based on various score functions; and 2) ligand-based approaches, such as descriptor matrices and similarity methods. Similarity methods have been underdeveloped for decades and are based on Similar Structure-Property Principle that states the molecules which are structurally similar and are likely to have similar properties. In 2D fingerprints, an attempt is made to map one molecule onto another; this means treating molecules A and B as graphs and finding any association between atoms in A and B [7,8].

In this context, the purpose of the present work was to find new antifungal lead compounds by using *in silico* methods.

EXPERIMENTAL

In silico methods

The focus was on screening of biological databases such as Enhanced NCBI database, Chemical Biology Laboratory (CBL, NCI, NIH), Pubchem (National Center for Biotechnology Information, Bethesda, United States), ChemIDplus (National Institutes of Health, Bethesda, Maryland, United States) and ChemBank-welcom (Broad Institute of Harvard & MIT/Chemical Biology Program, Cambridge, United States), by utilizing 2D fingerprints to compute a measure of molecular similarity between AmB, as a known reference structure, and each of the molecules in these databases. The 2D fingerprints encode the presence or absence of substructural fragments for the measurement [9]. Three similarity coefficients; Tversky, Euclidian and Tanimoto ($> 70\%$) were used to study the 2D fingerprint-based similarity. Among the molecules obtained from similarity search, the small molecules which had less molecular complexity than AmB were selected to dock, as ligands, with ergosterol and cholesterol, as receptors. Docking various ligands to the target of interest was followed by scoring to determine the affinity of binding and the strength of interactions. In general, it is important to visualize the docked poses of high-scoring compounds because many ligands are docked in different orientations and may often miss interactions that are known to be important for the target receptor [10].

The docking analysis of the selected compounds were evaluated by Hex applicatoin (version 5.1, 2008, LORIA, France). Default parameters were selected to obtain the best binding energy per ligand-receptor. SDfiles (.sdf) of receptors and ligands were converted to PDB (Protein Data Bank) format with MarvinSketch (version 5.0.4, ChemAxon, Budapest, Hungary) application software. Due to the large amount of the selected compounds, besides the docking experiments, an alternative approach to eliminate unpromising compounds was established. Hence, about 60 polyene macrolid antibiotics, which form pores in the membrane, were gathered from literature [11]. Several physicochemical properties such as "logP, H-bond acceptor, H-bond donor, rotatable bond count and topological polar surface area", which are important in drug design process, were gained from system (<http://pubchem.ncbi.nlm.nih.gov/search/>) to create a scoring system for filtering the selected compounds. Also, the mentioned properties for the selected compounds were measured in the same way as the polyene macrolid antibiotics. A datafile of the

docking results and physicochemical properties of the selected compounds were prepared. Using these data, the compounds that had the nearest interaction energy values with ergosterol in connection to AmB and also more similar properties to the computed ranges of the polyene macrolide antibiotics were selected. As many novel compounds, that could be found, were purchased for assay of their antifungal, simulated membrane activity and cytotoxic activities.

***In vitro* methods**

Antifungal activity assay

The antifungal activity of the purchased compounds were examined on three strains, viz, *C. albicans* (ATCC10231), *S. cerevisiae* (PTCC 5052) and *A.niger* (A420), using broth microdilution assay. The broth microdilution method was performed according to NCCLS' proposed guidelines [12]. All strains were sub-cultured on Sabouraud maltose agar medium (SMA, Merck, Germany). *C. albicans* and *S. cerevisiae* were suspended in sterile 0.9 % NaCl solution; to make the inoculum of *A.niger*, the spores were dispersed in 0.1 % solution of Tween 20 (Merck, Germany). The turbidity of the suspensions of *C. albicans*, *S. cerevisiae* (75 – 77 %) and *A. niger* (80 – 82 %) were adjusted using a spectrophotometer (Cecil, CE1021, Germany) at 530 nm. All inoculum suspensions were diluted (1:1000 for yeast and 1:50 for filamentous organisms) in Sabouraud maltose broth medium to obtain the final test inoculum.

All the test compounds, namely, AmB, mandelonitrile, retinyl acetate, delta-decalactone, ergocalciferol (Sigma, Germany) and 2-aminophenyl B-D-glucuronide hydrochloride (Carbosynth, Compton Berkshire UK), were dissolved in dimethyl sulfoxide (DMSO, Sigma, Germany) but amygdalin and retinyl palmitate (Sigma, Germany) were dissolved in water and acetone respectively. The concentrations of these compounds and AmB (positive control) were 10 mg/ml and 3.2 mg/ml, respectively. Serial dilution of these stock solutions were prepared with SMB into microdilution wells. The diluted suspensions (100 µl) were added to 100 µl of the diluted solutions to obtain the desired concentrations for the experiment. The final test samples of the compounds and amphotericin B were diluted, from 1000 to 1.9 and 32 to 0.06 µg/ml, respectively, by addition of culture media and diluted microbial suspensions to all the wells. Microdilution trays were incubated at 35 °C and examined after 48 h to determine MIC (minimum inhibitory concentration) values.

Study of interactions in solution

Among the test compounds, mandelonitrile and delta-decalactone show moderate antifungal activity, and so the interaction (in solution) of these compounds with ergosterol and cholesterol (Sigma, Germany) were studied by UV-Vis spectroscopy. Mandelonitrile, delta-decalactone and AmB (as positive control) were dissolved separately in DMSO and diluted with water to obtain 50-µM stock solutions; they were always freshly prepared the same day they were used. The final concentration of DMSO in the stock solutions was 1 % v/v. The sterols were solubilized in *n*-propanol (Sigma, Germany). The samples used were prepared from the stock solutions by adding the required quantity of sterol solution. The final concentration of these compounds was set at 6.5 µM and the solution contained 6.5 %v/v of *n*-propanol [13]. This concentration for AmB was chosen because it represents the concentration of AmB in circulation in the blood after administration. It has been observed that in sterols:AmB molar ratio of 5:1, the selectivity of interaction of AmB with ergosterol and cholesterol is increased [14]. Therefore, this concentration and molar ratio were selected for the preparation of mandelonitrile and delta-decalactone solutions. UV-Vis spectra were recorded within 30 min of their preparation, and the absorbance against a 6.5 % v/v solution of *n*-propanol in water was taken using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA) in the region of 300-750 nm. This solvent was used to ensure proper solubility of the sterols and, it has been shown that at this concentration, *n*-propanol has no effect on interactions of the antibiotic with the sterols.

Membrane interaction study

In order to determine the effect of mandelonitrile and delta-decalactone on lipid membrane, phospholipid/PDA vesicles were used as a membrane module. Polydiacetylene polymers have some important characteristics such as blue-red color transition under external perturbation that can be visible by the naked eye. To prepare polymerized vesicles, the stock solutions (1 mg/ml) of dimyristoylphosphatidylcholine (DMPC, Sigma, USA), and the diacetylenic monomer, 10,12-tricosadiynoic acid (TCDA, Sigma, USA), were prepared in dichloromethane. A working solution comprising DMPC- TCDA (40:60, v/v) was obtained prior to use. The tube was dried under nitrogen. The residue was dissolved in 1ml deionized water by bath sonicator for 8-9 min. The final solution was kept at 4 °C overnight and when needed, final

solution polymerized by UV irradiation at 254 nm for 60 s, resulting in intense blue color appearance due to polymerization of the diacetylene units [15]. Mandelonitrile, delta-decalactone and AmB (positive control) were dissolved in DMSO and diluted with water. The molar ratio of the compound/vesicle solutions were kept the same and the final concentrations were similar to that used for UV-Vis spectroscopy experiments.

***In vitro* toxicity study**

HepG2 (Human hepatocellular liver carcinoma) and MCF7 (Human breast adenocarcinoma) cell lines were obtained from National Cell Bank of Pasture Institute of Iran. The cells were grown in Roswell Park Memorial Institute (RPMI) 1640, culture medium (Gibco, Grand Island, NY) supplemented with fetal bovine serum 10 % (FBS, Gibco, Grand Island, NY) and incubated for 3 days at 37 °C in a humidified 5 % CO₂ atmosphere.

In vitro cytotoxicity of mandelonitrile and delta-decalactone were evaluated by MTT ([3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide]) assay. Harvested cells with trypsin (0.25 %, Sigma, Germany) were counted by Neubauer slide. The cells were seeded into 96-well plates in RPMI, 10 % FBS (10⁴ cells in 100 µl per well) and incubated for 48 h. Thereafter, the medium was replaced with 100 µl of various dilutions of the test compounds. In the first step, the stock solutions of the compounds were prepared in DMSO and sterilized using membrane filters of 0.45µm pore size [16]. Different concentrations of the stock solutions were prepared by serial dilution using RPMI, 10 % FBS. Mandelonitrile and delta-decalactone (in 750, 375, 187 and 93 µg/ml concentrations) and AmB (positive control, in 2, 1, 0.5 and 0.025 µg/ml concentrations) were prepared. DMSO solution was applied as solvent control in concentrations ranging from 1 to 0.12 %. The plates were incubated for 24 h at 37 °C in a humidified 5 % CO₂ chamber. A single dose of each compound was evaluated in triplicate in the cytotoxicity assay. Following, the incubation period, 0.01 ml of MTT solution, (Sigma, Germany) at a final concentration of 5 mg/ml in phosphate-buffered saline (PBS, Sigma, Germany) was added per well. The plates were then incubated for an additional period of 3 - 5 h at 37 °C. Thereafter, the culture medium including MTT solution in each well, was removed, 150 µl of DMSO was added to each well and then mixed to dissolve the blue formazan crystals. The absorbance of the samples was immediately read at 570 nm using

a 96-well plate reader (ELISA reader, Organon Tekninka, Netherlands). Cytotoxicity (%) was calculated according to Eqs 1 and 2.

$$\text{Viability (\%)} = (A_t/A_u)100 \dots\dots\dots (1)$$

$$\text{Cytotoxicity (\%)} = 100 - \text{Viability (\%)} \dots\dots\dots (2)$$

Data analysis

The 50 % inhibition concentration (IC₅₀) values of mandelonitrile and delta-decalactone on MCF-7 and HepG2 cells at 24 h were determined by probit analysis using the GraphPad InStat (DATASET1.ISD) software. To determine the cytotoxic effect of test compounds, paired t-test ($p < 0.05$) was applied.

RESULTS

***In silico* results**

Approximately 20,000 compounds were obtained from AmB structural similarity search. In total, a subset of about 400 compounds, which had less molecular complexity compared with AmB, were selected for further screening by docking experiments and molecular constraints (property based search). It has been shown that AmB does not only target ergosterol as a receptor in the fungal cell membrane, but also has less affinity towards cholesterol in mammalian cell membrane. Therefore, the selected compounds were docked onto the target receptors, i.e., ergosterol and cholesterol by Hex application and were evaluated for the binding compatibility to these receptors based on docked energy. The docking tool generated 100 poses for each docked pair of molecules. AmB on docking with ergosterol and cholesterol produced an energy value of -186.0 and -170.9 kcal/mol, respectively. Depending on the docked energy values and property filter approach, a library of 40 specific and diverse ligand candidates was formed. Ultimately, seven of these compounds were commercially available. The structures of these compounds are presented in Figure 1.

Antifungal activity

The MIC values of the test compounds against the test fungal strains are presented in Table 1. The MICs of delta-decalactone and mandelonitrile were lower than those of the other test compounds.

UV-Vis spectra

UV-Vis spectroscopy was used to study the interactions in solution between the antifungal

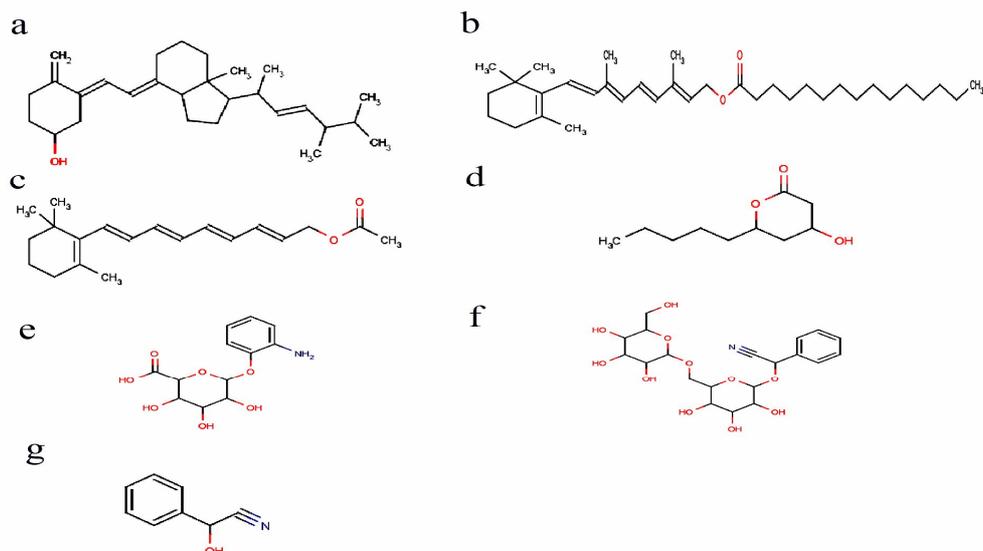


Figure 1: Structure of compounds that were obtained by virtual screening: a = ergocalciferol; b = retinyl palmitate; c = retinyl acetate; d = delta-decalactone; e = 2-aminophenyl glucuronide; f = amygdalin; g = mandelonitrile

Table 1: *In vitro* activity (MIC) of test compounds against two strains of yeasts and one strain of filamentous fungus after 48 h.

Compound	MIC ($\mu\text{g/ml}$)		
	C. <i>albicans</i>	S. <i>cerevisiae</i>	A. <i>niger</i>
AmB	0.5-1.0	0.25-0.50	2.0-4.0
Ergocalciferol	N.I.	N.I.	N.I.
Retinyl acetate	N.I.	N.I.	N.I.
Retinyl palmitate	N.I.	N.I.	N.I.
Delta-decalactone	93.7	< 46.8	187.5
Mandelonitrile	750	187.5	750
Amygdalin	N.I.	N.I.	N.I.
2 -Aminophenyl glucuronide	1000	1000	2000

N.I = No Inhibition up to the highest test concentration

compounds and the sterols. The interactions resulted in different spectra on the basis of the difference in their structures. Ergosterol has an additional double bond at position 7 in the molecule, compared to cholesterol, and an additional methyl and double bond on the side chain that makes it susceptible to maximum hydrophobic interactions (compared to cholesterol). [13].

The spectra recorded for AmB (reference) alone shows 4 specific bands in the regions of 335, 364, 368 and 409 nm. The interactions between AmB and the sterols could be followed through a shift in absorbance intensity and wavelength. When AmB was mixed with sterols, the spectra shifted to the right (red shift) and showed 4 specific bands approximately in the following regions: 353, 367, 388 and 415 nm. The band at

415 nm has been identified in the literature as characteristic of AmB interaction with the sterols [13]. The absorbance intensity of AmB mixed with ergosterol is higher than that for cholesterol. The spectra of either mandelonitrile alone, or mixed with cholesterol, does not present any specific band in the region 220 - 750 nm; however, the spectra of mandelonitrile mixed with ergosterol presents two additional bands, compared with ergosterol alone. It indicates that cholesterol, unlike ergosterol, did not noticeably interact with mandelonitrile. Also, the spectra of delta-decalactone alone did not show any specific band in this region, but the spectra recorded for delta-decalactone mixed with sterols indicate three bands in the regions 271, 283 and 298 nm that have near identical absorbance. They, therefore, may have the same tendency towards these sterols. The absorbance values obtained at these wavelengths are shown in Table 2.

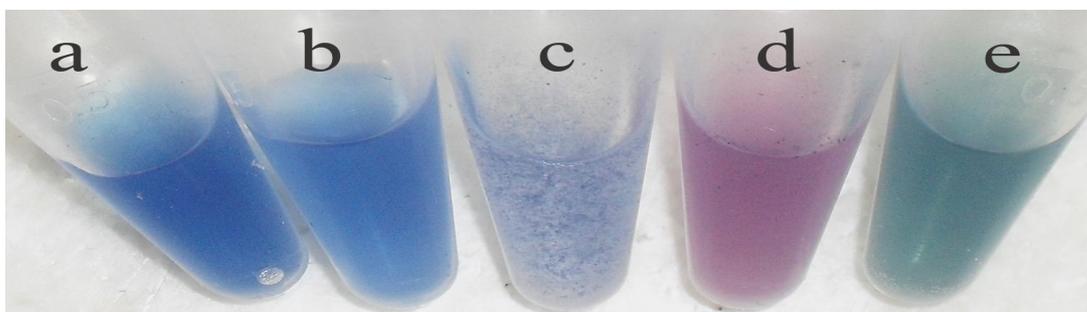
Membrane activity

Lipid/PDA vesicles were used to study the impact of the antifungal compounds on simulated lipid membrane. By interacting tested compounds with vesicles and disrupting them, blue-red color transition can be recognized with the naked eye [15]. As shown in Figure 2, the color change intensity of delta-decalactone was greater than that of AmB (positive control) and mandelonitrile. This means that delta-decalactone was more potent on lipids of membrane simulated vesicles.

Table 2: The regions of the specific bands for the UV-Vis spectra of antifungal compounds individually and in combination with sterols

Compounds	Band I		Band II		Band III		Band IV	
	λ	A	λ	A	λ	A	λ	A
AmB	335	0.04	364	0.023	386	0.022	409	0.017
Cholesterol	---	---	---	---	---	---	---	---
Ergosterol	262	0.023	276	0.024	---	---	---	---
Mandelonitrile	---	---	---	---	---	---	---	---
Delta-decalactone	---	---	---	---	---	---	---	---
AmB:Ergosterol	353	0.038	367	0.052	388	0.043	415	0.031
AmB:Cholesterol	353	0.019	367	0.035	388	0.024	415	0.017
Mandelonitrile:Ergosterol	262	0.031	273	0.035	283	0.032	296	0.025
Mandelonitrile:Cholesterol	---	---	---	---	---	---	---	---
Delta-decalactone:Ergosterol	271	0.027	283	0.023	298	0.016	---	---
Delta-decalactone:Cholesterol	271	0.035	283	0.029	298	0.022	---	---

Key: --- = Absence of any specific band in the region 220-750 nm ; λ : (lambda = wavelength in nm A = absorbance (mAU)

**Figure 2:** Color changes produced by interaction of lipid vesicles with test antifungal compounds after adding (a) deionized water (control solution); (b) DMSO (control solvent); (c) mandelonitrile; (d) delta-decalactone; (e) AmB (positive control)

Cytotoxicity of antifungal compounds

Both cell lines (HepG2 and MCF7) showed nearly the same pattern of dose-dependent responses in the presence of each test compound. The concentrations of AmB, delta-decalactone and mandelonitrile that inhibited 50 % growth of cell lines (IC_{50}) are shown in Table 3. To determine if the cytotoxic effect of the solvent those of test compounds, paired t-test ($p < 0.05$) was applied to the data and the results (Table 4) show that cytotoxic activity of all the test compounds on the two cell lines was independent of that of the solvent, DMSO.

Table 3: Cytotoxic activity of the test antifungal compounds on two cancer cell lines

Cell line	IC_{50} ($\mu\text{g/ml}$)		
	AmB	Mandelonitrile	Delta-decalactone
HepG2	2.06	1095.04	750.79
MCF7	4.96	2010.34	1601.48

Table 4: P -value to determine whether cytotoxic activity of the test antifungal compounds was influenced by that of the solvent (DMSO)

Cell line	P -value ($p < 0.05$)*		
	Am B	Mandelonitrile	Delta-decalactone
HepG2	0.0016	0.0006	0.0258
MCF7	0.0051	0.0003	0.0212

* P -value was calculated by GraphPad Instat (version 3.00) using paired t-test

DISCUSSION

There is still a need to develop suitable antifungal lead compounds for the drug development pipeline. Similarity lead finding studies using 2D fingerprints, which came into wide use in the late 1990s, is one of the simplest virtual screening tools and can be useful in the early stages of any lead-discovery project. Some advantages of this method include the fact that there is no need to know the active conformation of the query molecule, searching large databases

can be routinely performed and this method is much more cost-effective than others [6].

In this study, we proposed a combination of methods involving 2D similarity search, docking and filtering the similar compounds based on the molecular properties of the polyene macrolide antibiotics in order to reduce the dataset size to be finally selected. Based on the results obtained, two compounds were identified that may be worth studying further in terms of their antifungal activities, delta-decalactone and mandelonitrile, with the former more effective than the latter, but the activity of the latter was more targeted towards ergosterol. These differences may be due to differences between their structures.

Mandelonitrile is naturally found in the millipede, *Apheloria corrugate*, as a defense weapon [17]. On the other hand, the special flavor of ripe peach is mainly due to lactones, especially delta-decalactone. It is also present in apricot, *Prunus armeniaca*, and nectarine, *Prunus persica* var. *nucipersica* [18]. The skin of ripe nectarines smells powerfully of these lactones. It is applied in industry as a feed additive and flavoring agent. It is important to note that the structures of these compounds are rather simpler than that of AmB. However, they both exhibit antifungal activity and a similar mode of antifungal action as AmB, based on the findings of the present study.

The interactions of AmB with ergosterol and cholesterol have previously been studied [13]. It is also well-known that the UV-Vis spectrum of AmB in the region of 300-450 nm is very sensitive to the environment around the antibiotic molecule. One can also conclude that the affinity of AmB towards ergosterol is higher than cholesterol. The higher UV-Vis absorption demonstrated by delta-decalactone solution indicates that the compound has a lower affinity than mandelonitrile to ergosterol. However, mandelonitrile has almost no affinity towards cholesterol, which in general, translates to more selectivity towards ergosterol.

The results for the lipid/PDA vesicles indicate that delta-decalactone disrupts the membrane's phospholipids more actively. Since, mandelonitrile is less cytotoxic than delta-decalactone on the selected cell lines, could the latter is likely to show lower adverse effects on mammalian cell membranes.

In addition to the compounds tested in this study, other molecules present in the filtered subset structures, such as fumifungin and glucolanomycin, that have previously been

reported as antifungal compounds. Fumifungin and glucolanomycin were isolated from Y-83,0405 culture obtained from soil and fermentation liquids of *Pycnidophora dispersa*, respectively [19,20]. The presence of such antifungal agents among the filtered library of compounds would further aid in the elucidation of the capacity of the screening algorithm applied in our study.

CONCLUSION

The *in silico* experimental data obtained in the present study indicate that the selection algorithm used is efficient and the process is less time-consuming and more cost-effective, and therefore can speed up the identification of suitable novel antifungal lead compounds. Furthermore, mandelonitrile and delta-decalactone are suitable antifungal lead compounds that can be further developed to improve their potency, and thus, may find a potential place in the drug development pipeline.

ACKNOWLEDGMENT

The first-named author would like to thank the Drug Design and Bioinformatics Unit at Pasteur Institute of Iran for supporting this work.

REFERENCES

1. García-Ruiz JC, Amutio E, Pontón J. Invasive fungal infection in immunocompromised patients. *Rev Iberoam Micol.* 2004; 21: 55-62.
2. Dismukes WE. Introduction to antifungal drugs. *Clin Infect Dis.* 2000; 30: 653-657.
3. Hammond SM, Lambert PA, Kliger BN. Classification of Polyene Antibiotics According to Chemical Structure and Biological Effects. *Prog Med Chem.* 1977; 14: 105-179.
4. Baginski M, Sternal K, Czub J, Borowski E. Molecular modelling of membrane activity of amphotericin B a polyene macrolide antifungal antibiotic. *Acta Biochimica Polonica.* 2005; 52: 655-658.
5. Gallis HA, Drew RH, Pickard WW. Amphotericin B: 30 years of clinical experience. *Rev Infect Dis.* 1990; 12: 308-329.
6. Wilton D, Willett P, Lawson K, Mullier G. Comparison of ranking methods for virtual screening in lead discovery programs. *J Chem Inf Comput Sci.* 2003; 43: 469-474.
7. Downs GM, Willett P, Fisanick W. Similarity searching and clustering of chemical structure databases using molecular property data. *J Chem Inf Comput Sci.* 1994; 34: 1094-1102.
8. Halperin I, Ma B, Wolfson H, Nussinov R. Principles of docking: an overview of search algorithms and a guide to scoring functions. *Proteins.* 2002; 47: 409-443.
9. Charifson PS, Walters WP. Filtering databases and chemical libraries. *J Comput Aided Mol Des.* 2002; 16: 311-323.
10. Ritchie DW, Kemp G JL. Protein docking using spherical polar Fourier correlations. *Proteins.* 2000; 39: 178-194.

11. Hamilton-miller JMT. Chemistry and biology of the polyene macrolide antibiotics. *Bacteriol Rev.* 1973; 37: 166-196.
12. Cuenca-Estrella M, Lee-Yang W, Ciblak MA, Arthington-Skaggs BA, Mellado E, Warnock DW, Rodriguez-Tudela JL. Comparative evaluation of NCCLS M27-A and EUCAST broth microdilution procedures for antifungal susceptibility testing of *Candida* species. *Antimicrob Agents Chemother.* 2002; 46: 3644-3647.
13. Charbonneau C, Fournier I, Dufresne S, Barwicz J, Tancrede P. The interactions of amphotericin B with various sterols in relation to its possible use in anticancer therapy. *Biophys Chem.* 2001; 91: 125-133.
14. Fournier I, Barwicz J, Tancrede P. The structuring effects of amphotericin B on pure and ergosterol- or cholesterol-containing dipalmitoylphosphatidylcholine bilayers; a differential scanning calorimetry study *Biochim Biophys Acta.* 1998; 14: 76-86.
15. Mehravar M, Sardari S. Screening of antimicrobial membrane-active metabolites of soil microfungi by using chromatic phospholipid/polydiacetylene vesicles. *J. Mycmed* 2011; 21: 188-197.
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Meth.* 1983; 65: 5-63.
17. Roncadori RW, Duffey SS, Blum MS. Antifungal Activity of Defensive Secretions of Certain Millipedes. *Mycologia J.* 1985; 77: 185-191.
18. Noriaki K, Satoshi S, Kyo M, Tokio F. In vitro antifungal and antiviral activities of GAMMA and DELTA-Lactone analogs utilized as food flavoring. *Biocontrol Sci.* 2005; 10: 31-36.
19. Kluepfel D, Bagli J, Baker H, Charest MP, Kudelski A. Myriocin, a new antifungal antibiotic from *Myriococcum albomyces*. *J Antibiot.* 1972; 25: 109-115.
20. Philipson W, O'sullivan, Johnson JH, Bolgarm S, Kahle AD. Lanomycin and glucolanomycin, antifungal agents produced by *Pycnidophora dispersa*. II. Structure elucidation. *Antibiotics J.* 1992; 45: 313-319.