Identification of new anti-Candida compounds by ligand-based pharmacophore virtual screening

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SHORT COMMUNICATION

Identification of new anti-*Candida* compounds by ligand-based pharmacophore virtual screening

Maria Concetta Gidaro[1], Stefano Alcaro[1], Daniela Secci[2], Daniela Rivanera[3], Adriano Mollica[4], Mariangela Agamennone[4], Letizia Giampietro[4], and Simone Carradori[4]

[1]Dipartimento Di Scienze Della Salute, Università "Magna Graecia" Di Catanzaro, Campus Universitario "S. Venuta", Catanzaro, Italy, [2]Dipartimento Di Chimica E Tecnologie Del Farmaco, Sapienza Università Di Roma, Rome, Italy, [3]Dipartimento Di Sanità Pubblica E Malattie Infettive, Sapienza University of Rome, Rome, Italy, and [4]Department of Pharmacy, "G. D'Annunzio" University of Chieti-Pescara, Chieti, Italy

Abstract

Candida albicans represents the most prevalent microbial population in mucosal and systemic infections, usually confined to severely immunocompromised people. Considering the increase of resistant strains and the demand for new antifungal drugs endowed with innovative mechanism of action, we performed a ligand-based virtual screening in order to identify new anti-*Candida* compounds. Starting from a large library of natural/semisynthetic products and several published synthesized compounds, three coumarin derivatives were discovered *in silico* as new hit compounds and submitted to the *in vitro* assay in order to confirm their predicted biological activity.

Introduction

The frequency of *Candida* infections has increased in recent years and Sanguinetti et al.[1] recently reviewed the mechanisms of resistance. Clinically, azoles are first-line agents in treating fungal infections; however, there is an emergent demand for the discovery of new antifungal agents to open the possibility of a novel therapeutic approach[2]. As regards natural products, in literature there are several studies investigating the promising antimicrobial activity of chemical compounds content in essential oils and medicinal plants, also traditionally used for the treatment of various health disorders[3–5].

Both structure and ligand-based virtual screening (SBVS and LBVS) represent alternative and innovative approaches for identifying new compounds, but, until now, in the field of anti-*Candida*, computational techniques were used only to explain the structure–activity relationships, to design synthetic derivatives for the lead optimization and to display hypothetic mechanisms of action[6–8]. LBVS often relies on 3D pharmacophore model that is a set of interactions, or chemical features, aligned in three-dimensional space and developed by two possible approaches: one based on the known X-ray or NMR structure of the receptor, and the other one starting from a set of ligands that are supposed to bind to the same unknown target[9]. Therefore, we exploited our synthetic library of (4-aryl-thiazol-2-yl)hydrazine derivatives (Table S1), previously reported in other works as potent anti-*Candida* spp. compounds endowed with low cytotoxicity and synergy with clotrimazole[2,10–12], without a determinate molecular target, to generate a ligand-based pharmacophore model with the purpose to virtually screen a library of natural/semisynthetic products and to identify new potential antifungal drugs. Some 2-thiazolylhydrazine scaffold derivatives were also already[13] studied for their selective human monoamine oxidase inhibitory effects by computational methods.

Ligand-based approach

The 109 structurally similar molecules were ranked according to their MIC values, expressed in µg/mL against several clinical isolates of *Candida albicans*, as follows: compounds with MIC values ≤0.25 µg/mL were classified as "active", while compounds with MIC equal to the value of 256 µg/mL were considered as "inactive". Ligand-based pharmacophore modeling usually requires the "Training-Set" molecules, generally the most active, that are used for the pharmacophore creation and the "Test-Set" ligands, active and inactive, used to verify the resulting pharmacophore hypothesis. Due to the fact that the anti-*Candida* activity of most of the compounds (without asterisk in Table S1) was evaluated using a mixture of *E* and *Z* isomers and the biological activity is usually due to one of the isomers, the molecules **14**, **33**, **36** and **61** (Figure 1) (with asterisk in Table S1 because without isomers) were used as "Training Set" for the pharmacophore creation, into the Ligand Scout's Ligand-Based Modeling Perspective version 3.1[9]. In order to verify the resulting pharmacophore hypothesis, compounds **1–3**, **12–15**, **33–41**, **44** and **59–64** were selected as "Test-Set".

All 2D molecules were built into the graphic interface Maestro of the Scrödinger Suite 2014[14], and saved as .mol2 files, then converted in the .lbd format by the "Idbgen" tool of LigandScout.

Address for correspondence: Dr Maria Concetta Gidaro, Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Campus "S. Venuta", Viale Europa, 88100 Catanzaro, Italy. Tel: +39 0961 3694197. E-mail: mgidaro@unicz.it

Dr Simone Carradori, Department of Pharmacy, "G. D'Annunzio" University of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy. Tel: +39 0871 3554583. E-mail: simone.carradori@unich.it

Figure 1. 2D chemical structures of the ''active'' compounds **14**, **33**, **36** and **61**, with MIC values of 0.25 µg/mL, used as ''Training-Set'' into the LigandScout's Ligand-Based Modeling Perspective, in order to generate the pharmacophore model for the LBVS.

14

33 R= CH₃
36 R= Cl

61
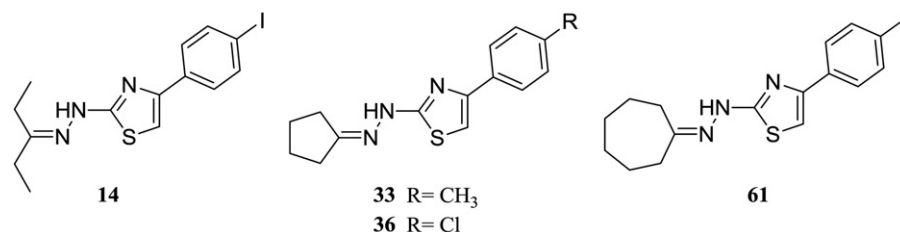
Ligand conformations were generated using the default settings of OMEGA best, which produced a maximum of 500 conformations per molecule. Finally, LigandScout interpolated common pharmacophore features for a configurable number of best alignment solutions. We observed that shared and merged features were located in the same spatial positions, in particular, 10 pharmacophore hypotheses were found with the feature tolerance scale of 1.0 and ranked using the pharmacophore fit and atom overlap as scoring functions. The chemical functionalities of the selected model are all matched by the chemical groups of the ''Training-Set'' molecules, except the aliphatic portion (Figure 2): the thiazole nucleus is located inside an aromatic ring and a hydrophobic feature (AR-HF1), moreover, it fits a region of hydrogen bond acceptor (HBA1) by the nitrogen atom; the phenyl portion at C4 of the thiazole is located inside the second aromatic ring and another hydrophobic feature (AR-HF2), while the substituents at the *para* position of this ring map an additional HF; finally, the hydrazone linker defines a region of hydrogen bond donor (HBD) and the second site of HBA2. The best model (Figure 2) was validated to prospectively evaluate its performance in a VS approach. To this aim, *E* and *Z* isomers of all molecules in Table S1, except those with the asterisk, were included for the construction of two libraries (''active'' and ''inactive'' compounds). Therefore, 42 ''active'' compounds were used to generate 2100 decoys (1:50), downloaded from the DUD-E site[15] and then added to the 21 ''inactive'' for a total of 2121 compounds. The screening process of the built libraries and the receiver-operating curve (ROC) analysis showed a good discrimination of the pharmacophore model; in fact, 34 hits (32 ''active'' and 2 ''inactive'') were found with a sensitivity of about 78% and the AUC value of 88.0% (Figure 3).

The LBVS was performed into the LigandScout's Screening Perspective using the validated pharmacophore model displayed in Figure 2. A library of 152 056 natural compounds was downloaded from the Zinc Database site[16], using all entries of the Natural Products Catalogs, while 105 490 natural/semi-synthetic compounds were available from our *in house* database. Therefore 257 546 ligands were prepared as follows: (*i*) the software Canvas[17] was used to remove the duplicates and to select 237 950 compounds with drug-like properties; (*ii*) the 3D structures were generated by the ''LigPrep'' utility of the Scrödinger package 2014[18]; (*iii*) tautomeric forms and protonated states at the pH range 5–9 were also considered, ending up with a library of 238 377 molecules, further converted in the .ldb format using the same protocol previously reported for the ''active'' and ''inactive'' libraries. The LBVS permitted to early identify three virtual hit compounds, belonging to the coumarin scaffold, with the corresponding ZINC codes **72325331**, **72325699** and **72325788** (Figure 4). The potential anti-*Candida* compounds, only results of the LBVS study, displayed the same alignment, except for the different aliphatic portion, with appropriate chemical functions for a good fit into the six features of the pharmacophore hypothesis. In fact, for each of them, the coumarin portion fulfils the AR-HF2 and the HF features; the
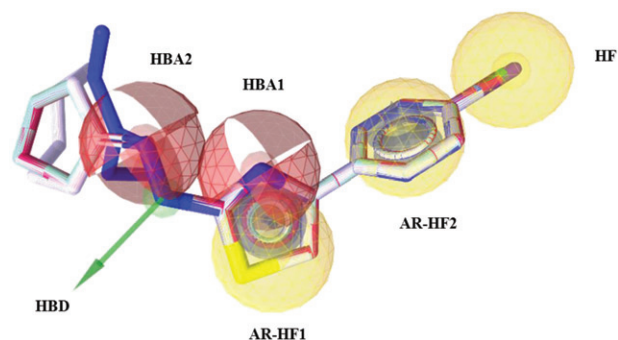

Figure 2. The ''Training-Set'' compounds, rendered as stick with the carbon color indicated in brackets, **14** (blue), **33** (cyan), **36** (red) and **61** (grey) in the best alignment solution, mapped to the different chemical features of the final pharmacophore hypothesis. The feature dictionary used is the following: aromatic ring and hydrophobic features (AR-HF1 and AR-HF2) displayed as grey toroids into the yellow spheres, hydrogen bond acceptors (HBA1 and HBA2) defined as red spheres, hydrophobic feature (HF) as yellow sphere and hydrogen bond donor (HBD) as green arrow. All non-carbon atoms are colored according to atom types. (For the colored version of this Figure, please refer to the online article.).
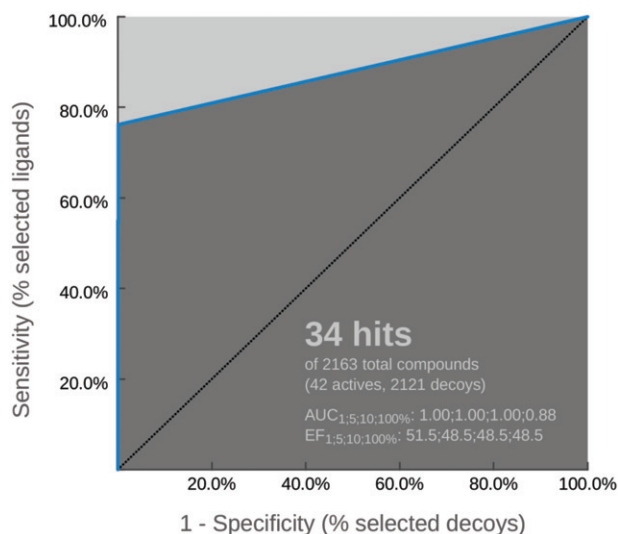
Figure 3. Into the LigandScout, the automated generation of the ROC curve for performance assessments, demonstrated the good discrimination of the selected pharmacophore model. In fact, 34 hits (32 ''active'' and 2 ''inactive'') were found, starting from a library of 2163 compounds, with a sensitivity of about 78% and the AUC value of 88.0%.

pyridine ring maps the AR-HF1 and, in addition, the HBA1 region by the nitrogen atom; finally, the amide group is located within the HBA2 feature, by the carbonylic portion, and within the HBD by the protonated nitrogen atom (Figure 4). In order to verify a possible therapeutic use of these bioactive molecules, the
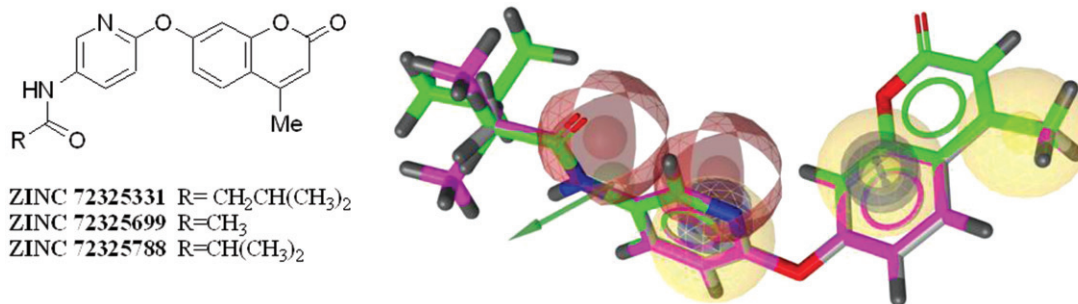
Figure 4. 2D chemical structures of the coumarin derivatives, with the respective ZINC codes, identified as new potential *C. albicans* inhibitors, by the LBVS performed from a large library of natural/semisynthetic compounds. The 3 new hits, on the right of the figure, are rendered as stick with the carbon color indicated in brackets, ZINC **72325331** (green), **72325699** (violet), **72325788** (grey). They are in the best alignment solution, mapped to the different chemical features of the pharmacophore hypothesis described in the previous figure. All non-carbon atoms are colored according to atom types. (For the colored version of this Figure, please refer to the online article.).

chemico-physical properties prediction was carried out by Canvas and reported in Table S2, concluding that all compounds have a drug-like profile[19–21].

Antifungal activity

The antifungal activity of the three coumarins, purchased by MolPort with purity of 92–95%, was evaluated against six clinical fungal isolates of *Candida albicans* and compared with topical and systemic reference drug fluconazole (Table 1) by using the same experimental protocols carried out for our synthetic library of (4-aryl-thiazol-2-yl)hydrazine derivatives. The used clinical isolates were recently collected from specimens of patients at the ''Azienda Policlinico Umberto I'' (Sapienza University of Rome) and were obtained from haematology/oncology and surgery departments, which also included an intensive care unit. In particular, the samples were isolated from the upper and lower respiratory tract, blood and indwelling venous catheters; the isolates were identified by conventional methodologies. Prior to testing, each isolate was subcultured on a qualified medium to ensure purity and optimal growth. All derivatives were dissolved in DMSO. The *in vitro* antifungal activities were determined by the broth microdilution method with Sabouraud dextrose broth (BBL Microbiology Systems, Cockeysville, MD) as recommended by the NCCLS. Briefly, microtiter plates containing serial dilutions of each compound were inoculated with each organism to yield the appropriate density (10^3/mL) in a 100 μL final volume; each plate included positive controls (fungi without a compound) and a negative control (medium only). The plates were incubated for 24 h at 37 °C. The MIC for all isolates was defined as the lowest concentration of antifungal agents that completely inhibited the growth of the organism, as detected by unaided eye[22,23]. From these data it was possible to extrapolate that these new compounds are active antifungal agents endowed with the same anti-*Candida* efficacy of Fluconazole *in vitro*.

Results and discussion

The selected coumarin scaffold displayed good biological activity confirming that the aliphatic chain (methyl, isopropyl and isobutyl) at the lateral amidic portion was not crucial for the *in vitro* anti-*Candida* efficacy. Actually, in literature, several works demonstrate the anti-fungal activity of coumarin derivatives[24–29]. In particular, for the methyl and isopropyl ZINC compounds, we found a reference that previously reported the antimicrobial activity[30], confirming the good ability of the pharmacophore hypothesis to early retrieve anti-*Candida* compounds. In fact, our computational studies permitted to identify *in silico*, with reduced time and costs, the most promising candidates to focus the

Table 1. Minimal inhibitory concentration (MIC) of the coumarin derivatives, displayed as ZINC codes, and reference compound (fluconazole) against six clinical strains of *Candida albicans*.

Compound	*C. albicans* (MIC μg/mL)
72325331	2-8
72325699	2-4
72325788	2-4
Fluconazole	2-4

experimental efforts on, starting from a large database of natural and semisynthetic ligands. In particular, by a comparison between the ''Training Set'' molecules, used to generate the ligand-based pharmacophore model, and the three coumarin hit compounds identified by our LBVS study, we observed the importance of a shared heterocycle with a HBA (e.g. thiazole and pyridine) as central feature linked to HBD/acceptor sites (e.g. hydrazone and carbonylic moiety) and to aromatic/hydrophobic features (e.g. phenyl and coumarin portions). In conclusion, our study represents the first example of a successful application of a LBVS approach to identify promising anti-*Candida* compounds. The ligand-based pharmacophore hypothesis generated *in silico*, and validated by the experimental results, may be used for future virtual screening studies starting from *in house* libraries of natural, semisynthetic and synthetic compounds. Further investigations will also focus on the identification of the macromolecular target and more potent anti-*Candida* agents.

Declaration of interest

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Supplementary material available online