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Research Article

IN SILICO MODELING AND DOCKING OF Cch1 PROTEIN OF *CANDIDA GLABRATA* WITH FDA-APPROVED DRUGS: A DRUG REPURPOSING APPROACH

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ABSTRACT

Objective: *Candida*-associated mortality rate is increased worldwide in past few years due to increased resistance to available antifungal agents, where *Candida glabrata* has emerged as one of the most upcoming pathogens. To combat the *Candida* infection, new drug molecule is required. Hence, we have studied the antifungal potential of some FDA-approved drug by *in silico* tools against Cch1, membrane Ca^{+2} channel protein of *C. glabrata*.

Methods: The 3D structure of Cch1 was predicted by Swiss modeling tool. Secondary structure was predicted by Sopma software. The docking of FDA-approved drugs with *C. glabrata* Cch1 was done by iGemdock and Hex software separately.

Results: We have tested total nine drugs against Cch1. Amlodipin besylate exhibited best binding energy (–372.16 kcal/mol and –185 kcal/mol for iGemdock and Hex, respectively) followed by Artesunate (–266.97 kcal/mol and –164.6 kcal/mol), Etazolate –244.35 kcal/mol and –163.9 kcal/mol).

Conclusion: Amlodipin besylate has the best antifungal properties and could be used as drug after further *in vitro* and *in vivo* studies. It can be directly come in practice since its toxicological testing has already been done.

Keywords: *Candida glabrata, CCH1*, Calcium channel, Docking, Drug repurposing.

INTRODUCTION

The incidences of candidiasis are increasing alarmingly worldwide due to increasing in the cases of immunocompromised patients and increasing resistance to available antifungals in *Candida* [1]. These infections are associated with high morbidity and mortality. *Candida* is a well-known ubiquitous, commensal, an opportunistic pathogenic fungus which causes infection in immunocompromised patients of intensive care unit [2]. It causes infection ranging from superficial to deep invasive. *Candida albicans* and *Candida glabrata* are the most common causatives of candidiasis. As per the report of CDC, USA 2013 on antibiotic-resistant threats in the United States, *C. glabrata* is considered to be the upcoming threat due to its increasing incidences of resistance to available drugs [3]. The *in vitro* susceptibility of *C. glabrata* is decreased against fluconazole which indicates the high risk associated with patients suffering from invasive candidiasis due to lack of effective drug [4]. The recent national and international reports on the epidemiology of *Candida* suggest that *C. albicans* occurs most frequently in clinical isolates followed by *C. glabrata*, *C. tropicalis,* and *C. parapsilosis* [1,5,6]. In a recent report from a tertiary care hospital of India, *C. albicans*, *C. tropicalis,* and *C. glabrata* were found at the top for biofilm-forming ability [7]. Considering the fact of developing resistance to existing drugs, it seems pertinent to develop new drugs against *Candida*.

Voltage-gated calcium channels (VGCC) are the most potent target for clinically active drugs as they have regulated the functioning of numerous physiological processes [8]. Calcium channel gene, *Cch1* is also found in *C. glabrata.* It has been reported that the mutant having the suppressed or deleted copy of this gene are susceptible to the treatment of azoles and fluconazole work as fungicidal in place of fungi static [9]. Due to the importance of *Cch1* in the survival of *C. glabrata*, we screened few FDA-approved drugs for their binding affinity with Cch1p. The concept of finding new indications of already approved drugs is known as drug repurposing/repositioning [10]. It evaluates the novel therapeutic potentials of an approved drug for exploring the application of it in the treatment of other diseases. Repositioning of the drugs is beneficial because they have been already tested in humans, so detailed information on formulation and toxicity are available hence they take less time to come in the market for treatment of other disease. Initially, we screened the binding affinity of selected drugs to *C. glabrata Cch1* through *in silico* approach, particularly by molecular docking.

Molecular docking is a useful tool for the development of the new drugs. This method helps in the prediction of the binding energy of one molecule with another (receptor and ligand) which is required during their interaction and for the formation of the stable complex [11]. The nature of the interaction (Vander Waals, Hydrogen bonding, electrostatic bonding) between ligand and protein (receptor) may give the direction for the development of new specific and potential drug against any therapeutic target [12].

METHODS

Drugs

We used total nine drugs as ligands given in Table 1, which is known for other indications, e.g., anti-hypertensive, anti-inflammatory, hypolipidemic, anti-malarial, and antifungal too. We included butenafine in our study, which is a well-known antifungal drug but with a different target.

Sequence retrieval

Candida genome database (CGD) is a freely available online database for genomic sequence data retrieval, which contains all information about gene and protein sequence of *C. albicans* and other species together with web-based tools for exploring, visualization, and analysis of these data. The aim of CGD is to help and speed up the research into *Candida* pathogenesis. The resource can be accessible from (http://candidagenome.org/) [23]. The amino acid sequences of *C. glabrata* Cch1 protein was retrieved from this database.

Modeling of *C. glabrata* Cch1p

Modeling is done using Swiss Modeling software. Swiss Modeling is a fully computerized server for automated homology modeling or three

dimensional structure of targeted protein. In Swiss modeling, only the amino acid sequence is submitted to build up the model. The modeling procedure of Swiss model is based on target-template alignment defined by the user. It is freely available at (http://swissmodel.expasy.org/) [24].

SOPMA server

SOPMA is a latest very correct nearest-neighbor method which is used for the prediction of secondary structure of the protein and can be accessible from http://nhjy.hzau.edu.cn/kech/swxxx/jakj/dianzi/Bioinf7/Expasy/Expasy8.htm. It takes only the amino acids sequences of the protein [25].

Parameters used in SOPMA server:

- Window width : 17
- Similarity threshold: 8
- Number of states : 4

SAVE server

SAVE is also a freely available server and used for structural analysis and validation of protein. It runs many programs, i.e., PROCHECK, WHAT_CHECK, VERIFY_ 3D, Ramachandran plot, etc., and can be accessed from http://services.mbi.ucla.edu/SAVES/Ramachandran/[26].

CASTp server

It is a freely available server which can be accessed from http://sts.bioe.uic.edu/castp/calculation.php, and the active site of protein is predicted by uploading the PDB file of the protein model [26].

Ligand preparation

The ligand molecules selected from FDA-approved drugs for the docking process are amlodipine besylate, artesunate, butenafine, diclofenac, carvedilol, lovastatin, mevastatin, simvastatin, and etazolate [27]. The selection was based on the availability of the drugs.

The canonical or isomeric smiles of these drugs was downloaded from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/). PubChem is an open storehouse for biological based information. The task of Pubchem is to deliver free and simple access of all deposited data [28]. The smiles are then converted into PDB format by Open babel software. Open babel is an open chemical toolbox and freely downloaded from (http://openbabel.org/wiki/Category: Installation) which allows anyone to explore, convert, analyze, or store data from molecular modeling, chemistry, biochemistry, or related areas [29].

Molecular docking

The molecular docking of all the ligands with receptor was done by *i*Gemdock software and Hex software.

*i*Gemdock is a graphical-automatic drug designing system that is used for docking, screening, post-analysis, and visualization. It can be freely downloaded software from (http://gemdock.life.nctu.edu.tw/dock/igemdock.php). This software shows the interaction between ligand molecule and receptor, assuming ligand to be flexible [30]. The "PDB" files of both the ligands and receptor were uploaded in the software, which then predict the docking sites by default settings such as population size 200, generation size 70, and a number of solution 2. The *i*Gemdock software consists four major modules, docking/screening module, and post analyzing modules, molecular visualization modules, and parallel processing module based on their protein binding site and ligand interaction.

Hex software is also used for being doubly sure about the results of docking obtained through *i*Gemdock. Hex is interactive molecular graphics software used for calculating and displaying protein-ligand docking. Hex takes the DNA and protein files in PDB format and small molecules in SDB files. This software is only used for rigid docking of protein and ligand where the ligand is supposed to be rigid [31]. Hex is the first protein-ligand docking software accelerates the calculation using modern graphics processor units. The rank list of predicted complexes can be downloaded after the docking process is finished [12].

RESULTS

Sequence of *C. glabrata* Cch1

C. glabarat Cch1 protein (Systematic Name: CAGL0B02211g) is made up of 2124 amino acids. ORF translation (Fig. 2) is retrieved from CGD.

Swiss model

The FASTA format of the amino acid sequence of Cch1 was uploaded to Swiss modeling software, which converted it to three dimensional structure and 3D model of Cch1, is shown in Fig. 1.

Validation of secondary structure

The secondary structure of protein model is predicted by SOPMA server (Table 2). It revealed the presence of 40.3% alpha helices, 20.81% beta sheets, 8.66% beta turns, and 30.23% coils at a different location of Cch1.

Validation of protein model

The structural validation of protein model is done by save server and the rampage server which evaluate the structural and geometrical consistencies of the modeled protein. As shown in Fig. 3, only1.7% of the residue falls under disallowed region in the analysis of Ramachandran Plot, whereas most of the residues falls under most favored regions 84.6% together with allowed regions.

CASTp server

Whole protein was analyzed for active sites through CASTp server. The best pocket site is selected on the basis of area and volume of the active site of the protein model. The area and volume of the best pocket site of this protein 3D model are 1977.4 and 5011.4, respectively.

The best region of pocket sites is show in Fig. 4 laid between 1266 and 1516 amino acids.

Docking results

According to *i*Gemdock analysis, amlodipine besylate has shown the least total energy of binding with Cch1 protein (–372.164 kcal/mol) followed by artesunate (–266.976 kcal/mol), etazolate (–244.357 kcal/mol),

Table 1: Clinically approved drugs used in the study

S. No.	Drugs	Initial indication	Molecular formula	Molecular weight	Reference
1.	Amlodipine besylate	Anti-hypertension and angina	$C_{26}H_{31}ClN_2O_8S$	567.05094	[13]
2.	Diclofenac	Anti-inflammatory drug	$C_{14}H_{11}Cl_2NO_2$	296.14864	[14]
3.	Lovastatin	Hypolipidemic	$C_{24}H_{36}O_5$	404.53964	[15]
4.	Mevastatin	Hypolipidemic	$C_{23}H_{34}O_5$	390.51306	[16]
5.	Simvastatin	Hypolipidemic	$C_{25}H_{38}O_5$	418.56622	[17]
6.	Butenafine	Antifungal	$C_{23}H_{27}N$	317.46718	[18]
7.	Carvedilol	Anti-hypertension	$C_{24}H_{26}N_2O_4$	406.47424	[19]
8.	Artesunate	Anti-malaria	$C_{19}H_{28}O_8$	384.42082	[20]
9.	Etazolate	Neuroprotective drug	$C_{14}H_{19}N_5O_2$	289.33296	[21,22]

butenafine (–238.378 kcal/mol), and others, as shown in Table 3. Lesser the energy better will be the acceptability of the chemical as a drug [12].

Hex docking analysis also exhibited that amlodipin besylate has least total energy of binding with Cch1, i.e., –185.0 kcal/mol followed by others as shown in Table 3.

Fig. 1: 3D model of Cch1 protein downloaded from Swiss model

DISCUSSION

Considering the increase in the resistance to existing drugs and epidemiology shift from *albicans* to non-*albicans Candida*, it has become essential to explore new drugs against *Candid*a pathogens, especially *C. glabrata*. Under the approach of drug repurposing, we selected 9 FDA-approved drugs to analyze their anti-*Candida* effects. In this study, the drugs were screened through *in slico* approaches for their binding with one of the most important putative drug target in *C. glabrata* Cch1. Cch1 is a Ca^{2+} channel in the cell membrane and is essential for the cell viability and fungal pathogenesis [32].

Table 2: Prediction of secondary structure Cch1 protein by SOPMA server

Protein structure, unit	No. of amino acids	% of structural, unit
Alpha helix (Hh)	856	40.30
310 helix (Gg)	0	0.00
Pi helix (Ii)	0	0.00
Beta bridge (Bb)	0	0.00
Extended strand (Ee)	442	0.00
Beta turn (Tt)	184	30.23
Bend region (Ss)	0	20.81
Random coil (Cc)	642	8.66
Ambiguous states	0	0.00
Other states	0	0.00

```
MPMVKRQLTERYEPGNEAQDADDGFVPPFVNIVPPDSDEDKNSSSNSLPIRRIRKRGPYE
DSDEGDYSDNDDDFEERVEYSPRRLEGTFGDDGELDGISEHDHSHRDDMISNTDTAHSDR
VSSLGMSSKAESPSKKWEPRIANLFKSALGNNNAKQKPQLSLRTTSLSTNTQDKARKYSS
SDEPGSPSAKSAVSFHSSGVRSNVLHRSNKSGESSPRRRSISTDGPPRSAKVLSIIDADD
MDEFEDMKRGFQSAIEDKKFAWLPQLQQETESADELEAGDKSKLNIDIKSTLTPRGRSGS
QFRNDSTEDLHFEPGLFSSPSKHSSRRPSIIASSIAHGLGINLEKDKNVDTVSIEVELPD
FDEYENAKKRPPLILHGNSLGYFGPNNPLRYKVAHILLNKYYKIAHILLLTFLTALLAYR
SYEPENFDFLYRFRNWSDYIIFILFILFTSNDIAKIFAFGFWDDSQMFAYHQLEYISLLE
RFGITKLYHLIKKKYGPKIVHFLLPFNVISDESEKETIQRNMKANISAKNDTNSKSLKFN
CPRAFCRSSWNRIDLLSSVCFWLGMFLSINNYDKRVGIRIFKPLAAMRILRLVNTDTGIT
SILRGLKYGMPQLINVGSMLVYFWVFFGILGVQIFKGSFRRQCVWYNPENPNDRYQYQLQ
FCGGYLEPVTKKKMNYIFEDGREGPVSKGFMCPQYSKCVSSSNPFNGRVSFDNIINSMEL
VFIVMSANTFSDIMYYTMNSDEFSACLFFIISIFVLTIWMMNLLIAALVSSFQLAHEEFK
KKKLEESSNESWPIRFALGYWRYFKVKASQTEFPSWAERGLKYYEKIEPVFIILIMFDLI
MRCLIKDTSSQHFVTTLLRIDRGVTIVLFIESIFRLVLHIPNMWKFLTRFNYVYDLFVAI
LTLVITILANAGLLGHGYYWLSFFQITRFYRLVIYIGFTRKLWKEVLGNGIMIWNLTAFY
FFFTFLASIILSLYFEGVIPQDQIEDQLLGMYSLPNSFLSLYSIGSTENWTSILYIIQQY
SPNISSAFFSTVFLIIWFILSNSVILNIFIALISESLEVKEDDKRPMQIKHYLKHIYPQK
IQRFRHASLLARIKKKIFRTDSQEDSRDFKQFLMRGTAIMNIAQNLGDLSKEFNTEQDRD
IVTLLVRLTEYIPILRKFGIYSNNPFYKRNEIMFSETSDLNGRNYMLQLNEFEDEKLDYL
QKHSMFNYSYYLFSPRHRFRKFCQKLVPPSYGKRTDNYKFYEDDTDTYSRKTYFNHIWRD
LFVVFYAAATVLLIVFSCYVTPIYRMKRNMSSKNWVTYVDAAFLAIFCVEFIIKTVADGF
IYTPNAYARNPWNLIDFFVLISMWINLIAFLRNDGNLSRVFKGLTALRALRCLTISNTAR
ETFKVVLFNGIGKILEAGLLSLTLLFPFTVWGLNLFRGRLGVCNDGDLGRDECFNEFSNE
VFQWNVMMPRVYDNPELYLDSFTSAFNSLFQIISLEGWTDLLGNLMNSTGVGKPASLLAS
TGNAVFIIAFNFLSIVFILNLFVSFIVNNQAKSTGSAYFTSEEKAWLESQKLLSQAKPIA
IPTFHDISRVRIFLYNIAVEKNNIYYAIFLQTVTYIHIIMLLSLTYKDHGSGLLYSQVYF
MFSTTVFLLQELFHVYGAGLRIYKMNKWNLIRIQILIVSFLLTLISFNVDRAYIWYHNVN
GFFHLVIFLFIIPQNDTLSELIETAMASLPPILSLTYTWFILFLVYAIALNQIFGLTRLG
PNTTDNINFRTVLKALIVLFRCSFGEGWNYIMDDLKVKEPYCSQRVKGNYSDCGSQTYAY
ILLISWNILSMYIFLNMFISLIIGNFSYVYRKGGTKSEVNRGEIRKYVETWARFDPDGTG
KLDFEYLPKIMHSFNGPLSFKIWEGKLTVKNLVKNYMEVNPNDPYDVKVDLEGLNKELDS
INMESIMNRRLQYRRFVQEVYYTQAYMGYMKFSTLLDLVPLYTTYDPRECLGIDQYVRHL
YIMGKVDRYLDNERNVDVLDMVVTRWKHHLKRKYGPNYKLDQDSIKKDLNIKVPEIKTWN
ESVESVTTPRVSYGVNDFIWSPRNNSNSSAYSKPLPQSHARPIPPHIQISNSRVMEYTNR
  DSLSSLENIHNQSDEELKDPFRDI
```

Fig. 2: Amino acid sequence of *Candida glabrata* Cch1

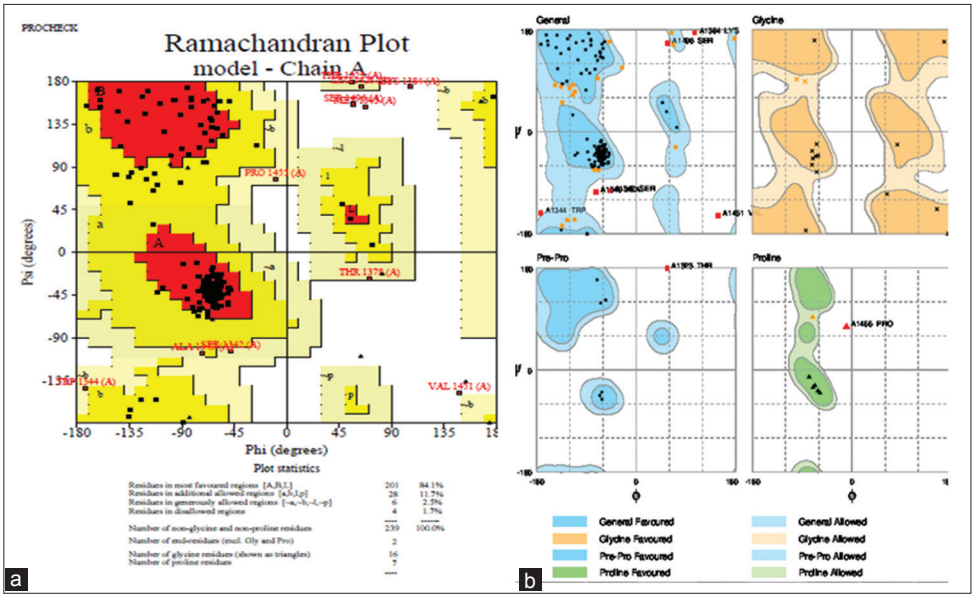

Fig. 3: Ramachandran plot of Cch1 protein obtained from (a) Save server (b) Rampage Server

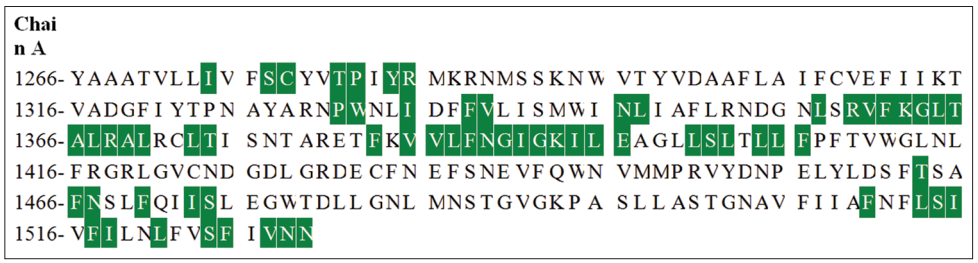

Fig. 4: Active site prediction of protein model by CASTp server

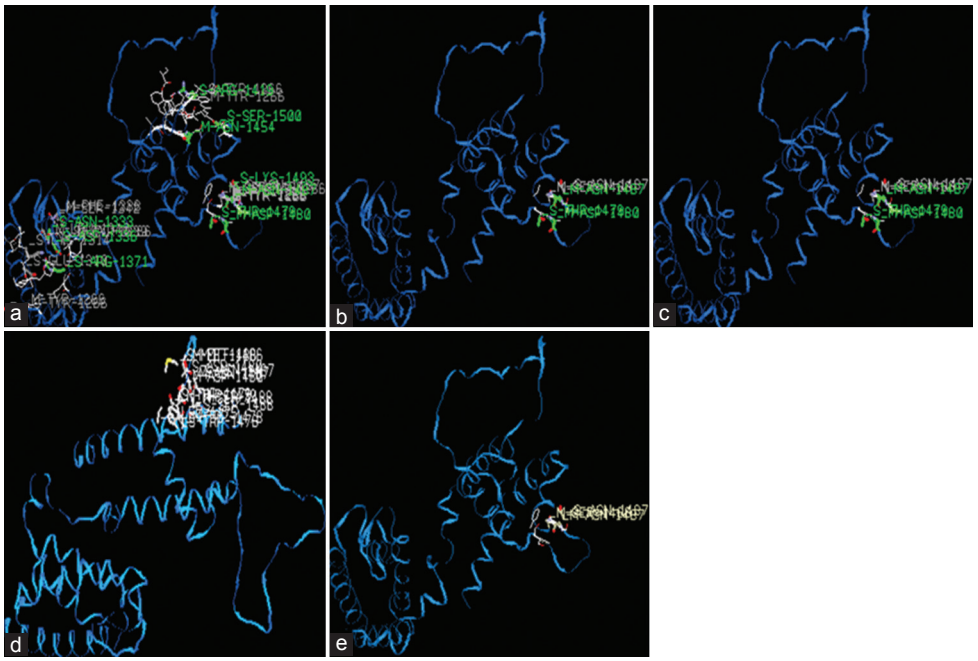

Fig. 5: Docking result from *i*Gemdock: (a) With all the ligands, (b) amlodipine besylate, (c) artesunate, (d) etazolate, and (e) butenafine

Modeled Cch1 exhibited the majority of favored region in Ramachandran plot is 84.6% (Fig. 3) and several active sites in the middle of the protein (Fig. 4). All the ligands showed a promising binding affinity for *C. glabrata* Cch1. From both the docking softwares, it can be inferred that amlodipine besylate is the best ligand for the receptor Cch1. The docking result of *i*Gemdock and Hex have shown different total energies of binding because both the software is based on different algorithm, hex is for rigid docking that cannot change their spatial shape on the

Table 3: Results of molecular docking by using

S. No.	Ligands	Total energy by *i*Gemdock (kcal/mol)	Total energy by Hex (kcal/mol)
1.	AB	−372.164	−185.0
2.	Artesunate	−266.976	−164.6
3.	Etazolate	−244.357	−163.9
4.	Butenafine	−238.378	−164.1
5.	Diclofenac	−184.298	−164.1
6.	Carvedilol	−153.089	−169.7
7.	Simvastatin	−103.718	−170.0
8.	Mevastatn	−95.3192	−144.7
9.	Lovastatin	−85.1319	−157.6

AB: Amlodipine besilate

time of docking process [33], whereas *i*Gemdock is used for flexible docking so that the ligand can be bind after changing the shape [34]. *i*Gemdock docking analysis has shown that most of the ligands are binding in the region of best pocket site/active sites, as predicted by CASTp server (Figs. 4 and 5).

Amlodipine besylate is already an approved anti-hypertension drug, that targets mammalian VGCC [35]. Similarly, we found that amlodipine has a maximum affinity for the *C. glabrata* Cch1. Amlodipine besylate has also been proven to possess antibacterial activity [36]. Therefore, it seems pertinent to analyze the anti-*Candida* properties of amlodipine besilate to end up with an effective anti-*Candida* drug.

CONCLUSION

C. glabrata Cch1 protein model has several active sites for the binding of ligands. All the ligands have exhibited the binding affinity with *C. glabrata* Cch1. Among all, amlodipine besylate has shown the minimum total energy of binding with *C. glabrata* Cch1. This study suggests that these drugs (especially amlodipine besylate) could be screened for their anti-*C. glabrata* activities through *in vitro* and *in vivo* models.

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