Molecular modeling of inhibitors against fructose bisphosphate aldolase from *Candida albicans*

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Abstract

Candida albicans is an opportunistic pathogen that causes from vulvovaginal and oropharyngeal candidiasis to systemic infections. The enzyme 1,6-fructose bisphosphate aldolase class II (FBA II), is a macromolecule existing only in lower organisms, being essential for the survival of the pathogen due to its function of maintaining the glycolysis process. The aim of this paper was to evaluate the inhibitors of FBA II regarding their physicochemical, pharmacokinetic and toxicological properties and apply concepts of rational drug development to propose new compounds for the treatment of fungal infections of *C. albicans*. Physicochemical (HyperChem software and the webserver cactus) and ADME/Tox (PreADMET webserver) properties were calculated to four inhibitors described in the literature and three analogues. None of the compounds presented in this study violated RO5, however all inhibitors demonstrated low or moderate human intestinal absorption (HIA), as well as low or moderate permeability in Caco-2 and MDCK, poor plasma proteins binding (PPB) and low permeability of the blood–brain barrier (BBB); however, Compound 4 is the exception for BBB permeability, being also the only non-mutagenic compound, and therefore, used as a lead compound. Analogues B and C presented high HIA, weak PPB and low BBB permeability, as well as a positive prediction for carcinogenicity in rats and mouse and non-mutagenicity in the Ames test. Through the evaluations carried out, it was concluded that the analogues B and C have proved to be promising candidates for oral administration drugs in the treatment of fungal infections of the genus *Candida*.

Keywords *Candida albicans* · Fructose bisphosphate · Aldolase · Molecular modeling · Treatment

Introduction

The fungi are eukaryotic organisms and saprobes, they can be free living or associated with other organisms through symbiosis, widely distributed throughout the body surface and capable of moving from the condition of commensal to pathogen, when there are favorable conditions in the host. Morphologically distinguish themselves in filamentous and yeast forms (Tortora et al. 2012; Cardoso 2013; Macêdo et al. 2009; Cavalheiro 2003). *Candida albicans* yeast is an

opportunistic pathogen normally present in the skin, mucosa, gastrointestinal and genitourinary tract (Cavalheiro 2003).

Any variable that causes microbiota imbalance or lesion of the gastrointestinal mucosa may be a facilitating agent for the translocation of *Candida* spp. to the mesenteric capillaries, thus leading to infections, the most common being vulvovaginal candidiasis (Thrush, VVC), oropharyngeal candidiasis (Oral thrush, OPC) and, in severe cases, systemic infections (Tortora et al. 2012; Serracarbassa and Dotto 2003; Cardoso 2013; Macêdo et al. 2009; Cavalheiro 2003; Favalessa et al. 2010; Kabir et al. 2012; Etgeton et al. 2011; Castro et al. 2006).

The VVC affects millions of women causing discomfort and interfering in sexual relations and is considered a major public health problem worldwide. In Europe, it is the first cause of vulvovaginitis and the second cause in the US and Brazil. It is responsible for about 20–25% of infectious vaginal discharge and it is estimated that approximately 75% of adult women have at least one episode of VVC in their

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lifetime. The prevalence of candidiasis in pregnant women is high, even when it is asymptomatic, ranging from 12.5 to 33% (Favalessa et al. 2010; Kabir et al. 2012; Souza et al. 2012; Barbosa 2015; Santos 2010).

Intrinsic properties of *Candida* spp. cells are described, referred as virulence factors, which give the microorganism the ability to cause disease. Since the initial virulence process of *C. albicans* is its adhesion to host cells, it is necessary for the survival of the fungus that it is adhered or internalized in the epithelial cells. Adhesion occurs through adhesins which are Agglutinin-Like Sequence (ALS) (Zhao et al. 2004) proteins, which has expression regulated by environmental or physiological conditions found in the host (Álvares et al. 2007).

Moreover, as an essential factor for the maintenance of the glycolysis process of these microorganisms is the presence of the enzyme 1,6-fructose biphosphate aldolase class II (FBA-II). It is responsible for catalyzing a reversible cleavage reaction of fructose-1,6-bisphosphate (FBP) in dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (G3P) (Fig. 1).

These enzymes are only found in lower organisms, presenting in the homodimeric form and requiring a divalent ion, generally Zn^{2+} , in its structure to stabilize the intermediate metabolites during their catalytic activity (Barbosa 2015; Fonvielle et al. 2004; Han et al. 2015; Labbé et al. 2012).

Currently, the treatment of *Candida* spp. infections is carried out with antifungal drugs of the polyene and azole classes in pharmaceutical presentations ranging from those intended for oral use, such as nystatin, as well as those for intravaginal use. Amphotericin B is a drug of the polygenic class, being highly effective but barely used, due to its serious toxic effects, especially on the liver. Among the azolic drugs, there are several alternatives available for topical use, in the form of creams or ova for vaginal application such as clotrimazole, miconazole, isoconazole, as well as for oral

systemic use, such as fluconazole, ketoconazole and Itraconazole (Barbosa 2015).

Since FBA-II is absent in humans, occurs in microorganisms and is still essential for pathogen survival, these enzymes become promising targets for the development of new drug candidates that specifically inhibit class II aldolase. Thus, using chemical-computational methods, this study aimed to evaluate the physicochemical, pharmacokinetic and toxicological properties of FBA-II inhibitors and to apply rational drug development concepts to propose new compounds for the treatment of fungal infections belonging to species *C. albicans*.

Materials and methods

FBA-II inhibitors

In a study performed by Daher et al. (2010), four inhibitors of the FBA-II enzyme were described, and these inhibitors were selected and subsequently designed with the ChemBioDraw software v.14.0.0.11719 (ChemBioOffice Ultra for Windows 2014) and structurally optimized through energy minimization and geometry optimization by the semi-empirical RM1 method in the HyperChem v.80.6 software (2000).

Physical and chemical properties

These properties are used to predict the oral bioavailability of drugs (Chemplus 2000), considering the theoretical approach of pharmacokinetic parameters according to Lipinski (1997). In order to calculate the oil/water partition coefficient (LogP) and molecular mass (MM), it was used the HyperChem v.80.6 software (2000) and for number of donors and acceptors of hydrogen bonds (nHBD and nHBA, respectively), the cactus webserver (<http://cactus.nci.nih.gov/chemical/structure>) was used.

Pharmacokinetic and toxicological properties (ADME/Tox)

The prediction of pharmacokinetic properties: absorption, distribution, metabolism and excretion (ADME) and toxicological properties (mutagenicity and carcinogenicity) were performed using the PreADMET webserver (<https://preadmet.bmdrc.kr/>). The ADME properties were evaluated through human intestinal absorption index (HIA), permeability in human adenocarcinoma cells (pCaco-2), permeability in canine kidney derived cells (pMDCK), plasma protein binding (PPB) and permeability of blood–brain barrier (BBB).

Fig. 1 FBA-II catalyzed reactions, where (1) fructose-1,6-bisphosphate, (2) dihydroxyacetone phosphate and (3) D-glyceraldehyde-3-phosphate

Pharmacophore derivation

Pharmacophore derivation is one of the primary steps in drug design as it evidences the potential subunit(s) responsible for the primary molecular recognition of drug candidates by the site of the therapeutic target. For generate of the pharmacophore hypothesis, the four inhibitors previously described were submitted to PharmaGist webserver (<http://bioinfo3d.cs.tau.ac.il/PharmaGist/>), which aligned these inhibitors (Dror et al. 2004; Schneidman-Duhovny et al. 2008; Rodrigues 2009).

Designing analogues

Once the pharmacophore hypothesis for FBA-II enzyme inhibitors was calculated by considering their electronic characteristics and analyzing physicochemical, pharmacokinetic and toxicological properties, three novel inhibitor analogues were designed based on compound that presented the best results.

Results and discussion

For reasons such as convenience, safety and patient acceptability of treatment, drugs are preferably intended for oral administration (Rodrigues 2009). Thus, some in silico assays, such as those carried out in this study, are used in research and development (R&D) strategies to predict the probability of certain compounds be good candidates by the path chosen and enabling rational planning using molecular modeling techniques (Moda 2007).

FBA-II inhibitors

The selected inhibitors (Fig. 2) are monophosphorylated derivatives of *N*-(3-hydroxypropyl)-phosphoglycohydroxamic acid bisphosphate, described, synthesized and tested by Daher et al. (2010). In order to explore the use of compounds that are intermediate analogues of the transition state, the authors have designed four inhibitors structurally similar with the natural substrate (FBP) based on *N*-substituted hydroxamic acid that has the function of mimicking the electronic delocalization that occurs during cleavage of the FBP (Daher et al. 2010).

These compounds were tested in FBA-II of four microorganisms (*C. albicans*, *Helicobacter pylori*, *Mycobacterium tuberculosis* and *Yersinia pestis*) and in a FBA I of mammal, the results being compared later. All inhibitors showed inhibitory activity and highly selectivity for FBA-II, with the best inhibition values obtained for the *C. albicans* microorganism (Daher et al. 2010).

Physicochemical properties

The physicochemical properties of the four inhibitor compounds (Fig. 2) were analyzed according to the Rule of Five or RO5 described by Lipinski et al (1997), where substances that obey three or more parameters are more likely to be good candidates for oral drugs. Four physical and chemical parameters related to absorption and permeability are evaluated, thus good candidates must present: (a) molecular mass (MM) ≤ 500 Da, (b) Oil/water partition coefficient (LogP) ≤ 5 , (c) Number of Hydrogen Bond Acceptors (nHBA) ≤ 10 e (d) Number of Hydrogen Bond Donor (nHBD) ≤ 5 (Abreu 2011; Afonso 2008; Buzzi 2007; Silva 2008). The results

Fig. 2 Monophosphorylated derivatives of *N*-(3-hydroxypropyl) phosphoglycohydroxamic acid bisphosphate, hereinafter called, from the right to the left, compounds 1 (C1), 2 (C2), 3 (C3) e 4 (C4)

Table 1 Physicochemical properties of the studied compounds—rule of 5

	Compounds			
	C1	C2	C3	C4
MM	243.15	229.13	327.31	427.48
Log P	− 0.04	− 0.49	1.47	4.42
nHBA	7	7	7	8
nHBD	4	4	4	4
Violations	0	0	0	0

showed that all compounds fulfill this rule by displaying values in the following ranges: MM = 229.13–427.48 g/mol, LogP = −0.04–4.42, nHBA = 7–8, e nHBD = 4 (later one is common in all compounds), being characterized as promising candidates for future drugs with oral administration (Table 1).

Pharmacokinetic and toxicological properties (ADME/Tox)

In silico prediction of pharmacokinetic properties—absorption, distribution, metabolism and excretion—and toxicological properties (ADME/Tox) turns out to be very important during the preliminary stages of drug planning and development (Yamashita and Hashida 2004). As it is possible to eliminate inappropriate candidates that could be discarded in the clinical phase in the future and reduce both costs and time, besides reducing the number of animals used for toxicity testing (Moda 2007; Abreu 2011; Silva 2008; Buzzi 2007; Afonso 2008; Magalhães 2009; Galves 2011).

Table 2 Pharmacokinetic properties of the studied compounds

	Compounds			
	C1	C2	C3	C4
HIA (%)	11.512	10.409	25.561	47.841
pCaco-2 (nm/s)	0.417	4.965	1.112	1.466
pMDCK (nm/s)	0.584	0.531	1.264	47.204
PPB (%)	20.162	14.636	43.236	84.547
BBB (Cbrain/Cblood)	0.058	0.052	0.078	0.288

Table 3 Toxicological properties of the studied compounds

	Compounds			
	C1	C2	C3	C4
Ames test	Mutagenic	Mutagenic	Mutagenic	Non-mutagenic
Carcinogenicity (rats)	Positive	Positive	Positive	Positive
Carcinogenicity (mouse)	Negative	Negative	Negative	Negative

Among the characteristics evaluated by the PreADMET webserver, regarding pharmacokinetics, are: human intestinal absorption (HIA), Cell permeability in human colon adenocarcinoma cells (pCaco-2), Permeability to cells derived from canine kidney (Madin–Darby canine kidney—pMDCK), plasma protein binding (PPB) and permeability of blood–brain barrier (BBB). The results are shown in Table 2.

Both Madin–Darby canine kidney cells (MDCK) and human colon adenocarcinoma cells (Caco-2) are tools used to analyze the metabolism and transport of drugs during their development, including qualitative prediction and absorption classification (Irvine et al. 1999; Volpe 2011).

In silico studies demonstrated that 75% of the compounds have low permeability in Caco-2 cells, with values of 0.417 for C1, 1.112 for C3 and 1.466 for C4, and the compound C2 average permeability with value of 4965. As for permeability in MDCK cells, C1 to C3 were shown to have low permeability (values smaller than 25), while the compound 4 showed average permeability (values between 25 and 500).

Still, all of them bind poorly to plasma proteins < 90% and compounds C1, C2 and C3 have low BBB permeability as represented by values < 0.1 and only C4, presents average permeability (values between 0.1 and 2.0).

In the predictions of toxicity, the PreADMET webserver showed that of the four compounds, only C4 did not show mutagenicity in the Ames test, and none showed clear evidence of carcinogenicity to rats (positive prediction) (Table 3).

Pharmacophore derivation

Pharmacophore is the spatial arrangement of molecular characteristics that allow the supramolecular interaction between a molecule and the target receptor to stimulate or inhibit its biological response, and it is possible to establish the types of interactions the ligands in common make with the receptor site (Dror et al. 2004; Schneidman-Duhovny et al. 2008; Rodrigues 2009).

There are two approaches to the development of pharmacophore models, in that case: (1) based on the structure of the protein–ligand interaction or protein alone; and (2) ligand-based. The PharmaGist webserver is used to elucidate the pharmacophores that are known to bind to the biological target, in a way that the molecules undergo multiple flexible alignments and are organized according to the scores (Dror

Fig. 3 Alignment of all molecules of fructose biphosphate aldolase class II inhibitors—hydrogen bonds acceptor regions represented in yellow color and negative region represented in red color

et al. 2004; Schneidman-Duhovny et al. 2008; Rodrigues 2009).

The webserver provided 03 alignments, of which the best score obtained was 18.520 in the alignment of all compounds revealing five essential characteristics for biological activity, being: four hydrogen bonds acceptors regions and one negative region (Fig. 3).

Designing analogues

Since all inhibitors, according to RO5, were presented as good candidates for drugs for oral administration, choosing the lead compound took into account the pharmacophore hypothesis and the ADME/Tox properties, since they all have an average or low permeability in pCaco-2 and

pMDCK cells, these parameters were not considered as selection criteria.

Therefore, the parameters evaluated were HIA, binding to PPB, permeability of BBB and mutagenicity for the following reasons:

- After administration, the drug must be dissolved and solubilized in the gastrointestinal tract (Moda 2007);
- PPB significantly affects the distribution, excretion, and both pharmacological and toxicological effects (Moda 2007);
- BBB permeability, since the target macromolecule belongs to a microorganism and not to the human (Abreu 2011); and

Fig. 4 Lead compound (compound 4) and proposed modifications

Table 4 Physicochemical properties of analogues—rule of 5 (Lipinski et al. 1997)

	Compounds			
	C4	Analogue A	Analogue B	Analogue C
MM	427.48	272.19	415.38	417.4
Log P	4.42	− 0.18	2.4	3.15
nHBA	8	8	8	8
nHBD	4	4	4	4
Violations	0	0	0	0

Table 5 Pharmacokinetic properties (ADME) of the analogues

	C4	Compounds		
		Analogue A	Analogue B	Analogue C
HIA (%)	47.841	7.407	72.675	72.587
pCaco-2 (nm/s)	1.466	0.751	2.201	1.465
pMDCK (nm/s)	47.204	0.515	1.533	1.144
PPB (%)	84.547	0.000	75.352	72.576
BBB (Cbrain/Cblood)	0.288	0.041	0.089	0.098

- (d) Mutagenicity, since it is known that currently a high number of drugs have been disapproved in clinical trials for this reason (Abreu 2011).

It was observed that only C3 and C4 were moderately absorbed, being C4 the only non-mutagenic, hence, chosen as the lead compound (Fig. 4). Although all the compounds have weak PPB and the selected molecule is close to 90% and has an average BBB permeability (which is not desired in this case), these two parameters can be modified through the use of molecular modeling strategies.

Molecular mass, lipophilicity, rotational points and formation of covalent bonds are important characteristics in

the aspects of absorption and toxicity (Volpe 2011). From the template compound, three modifications were proposed aiming at the reduction of these characteristics and also the improvement of the properties of HIA, pCaco-2 and pMDCK cells (as these allow to evaluate the potential HIA for different routes and renal absorption, respectively), PPB and Carcinogenicity to mouse.

In order to confer hydrosolubility to the structure, since this characteristic is essential because lipophilic substances have high permeability and low solubility, implying low HIA and pCaco-2 (Moda 2007; Abreu 2011; Afonso 2008; Magalhães 2009; Galves 2011; Korolkovas and Burckhalter 1998), in analogue A (Fig. 4) it was replaced the carbon chain from the carbon 18 by a group NH₂.

It is believed that the formation of covalent bonds between a chemical compound and DNA is primordial in the process of carcinogenicity (Moda 2007). In addition, the rigidification of the structure is a widely used strategy offering stability and reduction of rotational points (Volpe 2011; Junior 2013; Patrick 2009). In analogues B and C (Fig. 4) we used two special strategies of molecular modification (Korolkovas and Burckhalter 1998), respectively, closing a ring from carbon 22 and introducing double bonds between the carbons 17–18, 19–20, 21–22, 23–24 and 25–26.

After modifications, it can be seen that the molecular weight of the compounds was reduced successfully, from 427.4 to (A) 272.19, (B) 415.38 and (C) 417.4. Furthermore, there were no changes regarding the donor and acceptor properties of hydrogen bonds (Table 4).

In the calculations of the ADME properties, it can be observed that there was a considerable improvement in the HIA in analogues B (72.675) and C (72.587). Besides, it was observed that all of them reduced both the percentage of PPB (thus implying a greater amount of bioavailable drug) and the low BBB permeability (Table 5).

For toxicological properties, analogues B and C were non-mutagenic and had no clear evidence of carcinogenicity for rats and mouse (positive prediction) (Table 6).

Table 6 Toxicological properties of analogues

	Compounds			
	C4	Analogue A	Analogue B	Analogue C
Ames test	Non-mutagenic	Mutagenic	Non-mutagenic	Non-mutagenic
Carcinogenicity (rats)	Positive	Positive	Positive	Positive
Carcinogenicity (mouse)	Negative	Negative	Positive	Positive

Conclusion

Based on the results of this in silico study, it can be observed that analogues B and C for new inhibitors of the enzyme FBA-II have been demonstrated as potential drug candidates. The pharmacophore characteristics were maintained and no physicochemical parameters were violated, which, associated with high intestinal absorption revealed by the evaluation of pharmacokinetic properties, characterizes them as promising candidates for oral administration.

Although pCaco-2 and pMDCK cells presented average and low results, there are now methodologies within pharmaceutical technology that are capable of improving the absorption, release and excretion of drugs, thus opening doors for further studies.

Further studies of the structure–activity relationship are required yet to investigate the use of these compounds in the treatment of fungal infections of the genus *Candida*.

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