Original Article

Antifungal Indole and Pyrrolidine-2,4-Dione Derivative Peptidomimetic Lead Design Based on *In Silico* Study of Bioactive Peptide Families

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Abstract

Background: The rise of opportunistic fungal infections highlights the need for development of new antimicrobial agents. Antimicrobial Peptides (AMPs) and Antifungal Peptides (AFPs) are among the agents with minimal resistance being developed against them, therefore they can be used as structural templates for design of new antimicrobial agents.

Methods: In the present study four antifungal peptidomimetic structures named C_1 to C_4 were designed based on plant defensin of *Pisum sativum*. Minimum inhibitory concentrations (MICs) for these structures were determined against *Aspergillus niger* N402, *Candida albicans* ATCC 10231, and *Saccharomyces cerevisiae* PTCC 5052.

Results: C_1 and C_2 showed more potent antifungal activity against these fungal strains compared to C_3 and C_4. The structure C_2 demonstrated a potent antifungal activity among them and could be used as a template for future study on antifungal peptidomemetics design. Sequences alignments led to identifying antifungal decapeptide (KTCENLADTY) named KTC-Y, which its MIC was determined on fungal protoplast showing 25 (*μg/ml*) against *Aspergillus fumigatus* Af293.

Conclusion: The present approach to reach the antifungal molecules seems to be a powerful approach in design of bioactive agents based on AMP mimetic identification

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Keywords: Antifungal agents, Defensins, Drug design, Peptidomimetics, Protoplasts

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Introduction

In the past 10 years, the incidence of fungal infections has increased obviously [1]. The emerging multiple-drug resistance has led to induce research for the detection of novel drugs through various mechanisms. AMPs and AFPs are evolutionarily preserved modules of the innate immune response and are established along the entire classes of life [2]. These peptides are effective, broad spectrum antibiotics which reveal potential as new therapeutic agents [3]. These AMPs are distributed in various sources ranging from primary eukaryotes to mammalians containing normally less than 60 amino acids long, having cationic amino acid residues and an amphiphilic structure bound to the membrane (α-helical and/or β-sheet) [4,5]. The application of these antifungal peptides as drugs is not easy due to their poor oral and tissue absorption, rapid proteolytic cleavage and weak

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half-life or stability. Since the majority of proteins and small peptides are simply proteolyzed, quickly excreted and poorly bioavailable, attempts have been made to determine the ways of replacing peptide portions. These structures are termed as peptidomimetics which are to mimic peptides in the anticipation of attaining more bioavailable units [6]. Their artificial backbone is resistant to proteases as well. Peptidomimetics are one set of probes utilized in the shift pathway of tiny molecule drug design.

Recently, significant progress has been made in the use of Computer-Aided Molecular Design (CAMD) of novel molecules with desired properties. These methods typically rely on two stages: the first stage is forward modelling [7], through which Quantitative Structure Activity Relationship (QSAR) process is accomplished by application of non-linear modelling procedures such as Artificial Neural Networks (ANN). As example of this strategy, in our group [8], over 100 antifungal peptides were analyzed by artificial neural networks and observed that most important physicochemical parameters affecting bioactivity of antifungal peptides are Log P and relative amphipaticity.

In the present study, 4 antifungal peptidomimetics structures were designed based on antifungal peptide structures, using bioinformatics and peptidomimetics strategy. The designed compounds were prepared and tested for antimicrobial activity.

Materials and Methods

Data collection

For this study, more than 60 antifungal peptides were selected. Their amino acids sequences were used as input data for sequences alignments in order to find out the shared amino acids sequences among them.

Sequences alignments

T-Coffee v5.13, 2007, and ClustalW (1.82) multiple sequence alignment European Bioinformatics Institute [53] were used for sequences alignments of antifungal peptides. T-Coffee is a multiple sequence alignment package [54]. Given a set of sequences (Proteins or DNA), T-Coffee generates a multiple sequence alignment. After sequence alignment, some correlations between certain sequences were found, which have been repeated in all of antifungal peptides in each group. AFPs with more sequence homology were selected to extract final pattern among them using CLASTALW alignment. Peptidomimetic structures were designed based on the final pattern obtained from final sequence alignment using T-coffee in which scores are based on colours; positions that have no consistency with the in-house peptide library are in blue, a little in green, better positions in yellow, then orange, and finally red. Yellow to red positions are expected to be entirely correct.

Similarity search was done to find close amino acid sequences to final pattern from sequences alignments in order to find crystallography structures. In the case of our study, T-coffee sequence alignments showed scores between average and good by having dark orange to red colours. The Basic Local Alignment Search Tool (BLAST) [55], and RCSB Protein Data Bank [56] were used to achieve this goal.

Mimetic design

In order to design mimetic counterparts, SuperMimic software [57,58] was used. This tool is being used for finding potential non-peptidic building blocks that can replace or mimic parts of a protein, or peptide and conversely for identifying locations within a protein where such building blocks can be inserted. It identifies compounds that mimic parts of a protein, or positions in proteins that are suitable for inserting mimetics. SuperMimic provides a library of 126 peptidomimetic structures which have been arranged in sublibraries such as beta-turn- or gamma-turn-mimetics [59]. SuperMimic program ©2006 was used to replace KTC-Y with peptidomimetics structures which are in SuperMimic library.

Peptide and mimetic preparation

The decapeptide KTC-Y (KTCENLADTY) was synthesized and purchased from SBS biocompany, Beijing, China. Compounds C_1

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and C_2 were purchased from Sigma Aldrich, Eteinhim, Germany, and C_3 and C_4 purchased from Enamine chemical supplier company.

Protoplast preparation

Protoplasts were prepared according to the protocol described by Osherov and Romano [57] with some modifications. Briefly, 20 *ml* of Sabouraud dextrose broth medium was inoculated with 10^9/*ml* fresh spores of *Aspergillus niger* (*A. niger*) N402 or *Aspergillus fumigatus* (*A. fumigatus*) Af293 and incubated for 6-7 *hr* in a shaker incubator, 32^oC, 250 *rpm* until more than 70% of conidia formed germ tube. Germinated spores were then harvested and resuspended in a lytic enzyme mix (5% Glucanex in 0.6 *M* KCl, 0.05 Citric acid pH=5.8, 1% glucose) and incubated for 2 *hr* at 30^oC, 100 *rpm*. Resulting protoplasts were harvested by centrifugation for 5 *min* at 2000 *rpm* in a bench top centrifuge. Protoplasts were washed twice in 20 *ml* 0.6 *M* KCl and resuspended in 1 *ml* of RPMI 1640 medium, prepared without bicarbonate, buffered with 0.165 *M* 3-(N-morpholino) propanesulfonic acid (MOPS), and adjusted to pH=7.0 with 10 *M* NaOH. This medium also contained 2% glucose and 1.2 *M* sorbitol as an osmotic stabilizer. Antifungal peptide was obtained in powder form and a stock solution of 5 *mg/ml* in water was prepared. MIC level was determined in 96-well microtiter plates through preparation of twofold serial dilutions across the concentration range (0-200 *μg/ml*) of antifungal peptide in the above medium. Total volume of each well was 100 *μl* containing 10^4 fungal cells. The plate was covered and incubated at 37°*C* for 24-48 *hr*. The MIC values were obtained by reading the concentration of the well with no growth.

Antifungal screening

A. niger N402, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* PTCC 5052 were used as test strains. The compounds were dissolved in Dimethyl Sulfoxide (DMSO) to reach a concentration of 10 *mg/ml*. The broth microdilution method was performed for antifungal activity tests. The absorbance was read at 530 *nm* for fungi inoculums to reach the suitable density of microorganisms, equal to 10^3 fungi in each well of 96-well microplate after the final dilutions. Working fungal culture was prepared from the stock fungal culture, a 1:1000 dilution with broth (*e.g.*10 *μl* stock fungal culture: 10 *ml* broth). Sabouraud Maltose Broth (SMB) was used as medium.

Modified antimicrobial susceptibility testing based on NCCLS M27-A method was performed [60,61]. Broth (100 *μl*) was added to each well of a 96-well plate and then 40 *μl* of compounds and 60 *μl* broth were added to well (A), then a solution (100 *μl*) serially diluted from well (A) by taking 100 *μl* into (B) was obtained. This two-fold dilution was continued down the plate and 100 *μl* from the last well (H) was discarded. Then all the wells were filled with 100 *μl* of working yeast culture. Itraconazole was used as a reference in fungi test. For this experiment the following controls were prepared: wells containing serial dilution of DMSO and itraconazole only. The plate was covered and incubated at 37°*C* for 24-48 *hr*. The MIC values were obtained by reading the concentration of the well with no growth.

Results

Sequences alignments

Various sets of sequence alignments were carried out in order to find better homology among antifungal peptide sequences (Table 1). The final peptide pattern which was designed in our study and led to KTC-Y was obtained from 10 antifungal peptides that showed more sequence homology to each other with high score -using T-COFFEE- shown in figure 1. The final sequence alignments pattern was K-CENLA-DTY in which "-" could be any amino acid. Source of the all 10 AFPs which were used for the final sequence alignments and the final pattern obtained from them was originally from plants. Hence, we obtained a pattern close to defensin sequence which is AFP from plant kingdom. This pattern was used in similarity search to find crystallography structures close

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Table 1. Antifungal peptides, their amino acids sequences, molecular weight and their sources

Peptide	Molecular weight	Sequence	Source	References
Pleurostrin	6500	VRPYLVAF	Oyster mushroom	(9)
Vulgarinin	7000	KTCENLADTYKGPCFTSGGD	Haricot beans (*Phaseolus vulgaris*)	(10)
Cicerin	8200	ARCENFADSYRQPPISSSQT	Seeds of the chickpea	(11)
Arietin	5600	GVGYKVVVTTTAAADDDDVV	Seeds of the chickpea	(11)
P1	7800	RGPQAFYEERLM	Seeds of a *Brassica species*	(12)
Fa-AMP1	3879	AQCGAQGGGATCPGGLCCSQWGWCGSTPKYCGAGCQSNCK	*Fagopyrum esculentum* Moench	(13)
Fa-AMP2	3906	AQCGAQGGGATCPGGLCCSQWGWCGSTPKYCGAGCQSNCR	*Fagopyrum esculentum* Moench	(13)
Lunatusin	7200	KTCENLADTFRGPCFATSNC	Lima beans (*Phaseolus lunatus* L.)	(14)
Dolabellanin B2	3873	SHQDCYEALHKCMASHSKPFSCSMKFHMCLQQQ	The sea hare *Dolabella auricularia*	(15)
Pleurotus AFP	1600	DNGEAGRAAR	Mushroom *Pleurotus erygii*	(16)
Tu-AMP1	4988	KSCCRNTVARNCYNVCRIPGTPRPVCAATCDCKLITGTKCPPGYEK	*Tulipa gesneriana1*	(17)
Tu-AMP2	5006	KSCCRNTTARNCYNVCRIPGTPRPVCAATCDCKIITGTKCPPGYEK	*Tulipa gesneriana2*	(17)
Ib-AMP1	2200	EWGRRCCGWGPGRRYCVRWC	*Impatin balsamina*	(18)
Berevin -2	3630	GLLDSLKFAATAGKGVLQSLLSTASCKLAKTC	Skin of frog	(8)
Ib-AMP4	2350	EWGRRCCGWGPGRRYCRRWC	*Impatin balsamina*	(18)
Sesquin	7000	KTCENLADTY	Ground beans	(19)
EAFP1	4212	QTCASRCPRPCNAGLCCSIYGYCGSGNAYCGAGNCRCQCRG	Olive	(20)
EAFP2	4169	QTCASRCPRPCNAGLCCSIYGYCGSGAAYCGAGNCRCQCRG	*Eucommia ulmoides* Oliv	(20)
Cucurmoschin	8000	PQRGEGGRAGNLLREEQEI	Black pumpkin seeds	(21)
CW-3	7150	PEDPQRRYQEEQRRE	*Malva parviflora* seeds	(22)
CW-4	5000	DRQIDMEEQQLEKLNKQDRPGLRYAAKQQMTRMG	*Malva parviflora* seeds	(22)
CW-5	7300	ITCGQVTSQVAGCLSYLQRGGAPAPGIRNLMA	*Malva parviflora* seeds	(22)
Coccinin	6700	KQTENLADTY	Runner beans	(23)
Agrocybin	9000	ANDPQCLYGNVAAKF	Mushroom *Agrocybe cylindracea*	(24)
AC-AMP	3520	GECVRGRCPSGMCCSQFGYCGKCGKPKYCGR	*Aarnthus caudatus*	(8)
Pn-AMP1	43000	QQCGRQASGRLCGNRLCCSQWGYCGSTASYCGAGCQSQCRS	Seed of *Pharbitis nil* L.	(25)
Pn-AMP2	43570	QQCGRQASGRLCGNRLCCSQWGYCGSTASYCGAGCQSQCR	Seed of *Pharbitis nil* L.	(25)
Lentil AFP	11000	TETNSFSITKFSPDGNKLIFQGDGYTTKGK	Red lentil seeds	(26)
Alveolarin	2800	GVCDMADLA	Mushroom *Polyporus alveolaris*	(27)
Lentin	2750	CQRAFNNPRDDAIRW	Shitake mushroom	(28)
Lyophyllin	8950	ITFQGASPARQTVITNAITRARADVRAAVSALPTKAPVST	*Lyophyllum shimeji*	(29)
IWF6	4070	RPRPRPCIRAGGYCNILNVCCAGLTCKKHDIQDAACV	Sugar beet leaves	(8)
LAP	7600	AGTEIVTCYNAGTKVPRGPSAGGAIDFFN	Fruiting bodies of *L. shimeji*	(29)
Psc-AFP	6700	EPILDVNGGSSYYMVPRIWA	*Psoralea corylifolia* seeds	(30)
Red bean	5700	RTCENLANTYRGPCI	Seeds of the red bean	(31)
Pinto bean	5600	KTCENLADTYKGPCFT	Seeds of the pinto bean	(31)
α-Basrubrin	4300	GADFQECMKEHSQKQHQHQG	Ceylon spinach seeds	(32)
PAFP-s	3929	AGCIKNGGRCNASAGPPYCCSSYCFQIAGQSYGVCKNR	Seeds of *Phytolacca americana*	(33)
SMAP-29	3198	RGLRRLGRKIAHGVKKYGPTVLRIIRIA	Sheep leukocytes	(34)
Cicadin	16500	NEYHGFVDKANNENKRKKQQGRDDFVVKPNNFANRRRKDDYNENY-YDDVDAADVV	Dried Juvenile cicadas	(35)
Pe-AFP2	2750	QSERFEQQMQGQDFSHDERFLSQAA	Passion fruit *Passiflora edulis*	(17)
Eryngin	10500	ATRVVYCNRRSGSVVGGDDTVYYEG	Mushroom *Pleurotus eryngii*	(36)
β-Basrubrin	5900	KIMAKPSKFYEQLRGR	Ceylon spinach seeds	(32)
Psalmotoxin	3300	ACGILHDNCVYVPAQNPCCRGLQLQRYGKCLVQV	Venom of tarantula	(37)
Pe-AFP1	5600	QSERFEQQMQGQDFSHDERFLSQAA	*Passiflora edulis* seeds	(17)
Drosomycin	4840	DCLSGRYKGPCAVWDNETCRRVCKEEGRSSGHCSPSLKCWCEGC	Drosophila	(38)
Histatin 3	3410	DSHAKRHHGYKRKFHEKHHSHRGKRSNKLYDN	Saliva of humans	(39)

to it. WU-BLAST2 - Protein Database Query, NCBI-BLAST2 - Protein Database Query, FASTA and SSEARCH - Protein Similarity Search, and RCSB Protein Data Bank were used to discover the structures. Interestingly, a structure with similarity of 90.909% to our final pattern was found which its PDB ID is 1JKZ.

Information about this protein and its structure as indicated in Protein Data Bank (PDB)

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Table 1. Antifungal peptides, their amino acids sequences, molecular weight and their sources (Continued)

Peptide	Molecular weight	Sequence	Source	References
GAFP	4180	DPTCSVLGDFKCNPGRCCSKINYCGACYQWRFGLTPAR	Leaves of *Ginkgo biloba*	(40)
Tenecin	4730	VTCDILSVEAKGVKLNDAACAAHCLFRGRSGGYCNGKRVCVCRr	Insect defencin peptides	(41)
Triptecin	1430	VRRFPWWWPFLRR	Synthetic	(8)
Esculentin-1	5170	GIFSKLGRKKIKNLLISGLKNVGKEVGMDVVRTGIDIAGCKIKGEC	Skins of frogs	(42)
Melitin	2750	GIGAVLKVLTTGLPALISWIKRKRQQ	Apis mellifera	(43)
MP1	1210	KKVVFKVKFKK	Combinatorial chemistry	(44)
MP6	1320	CNHKVVFKVKFK	Synthetic from MP	(44)
DSP	4510	AVRIGPCDQVCPRIVPERHECCRAHGRSGYAYCSGGGMYCN	Leaf beetle	(45)
Gymnin	6500	KTCENLADDY	Yunnan bean	(46)
Halocidin	2530	RIIDLLWRVRRPQKPKFVTVWVR	Hemocytes of the tunicate	(47)
Ascalin	9500	YQCGQGG	Shallot bulbs	(48)
Pisum sativum	1500	AKKKKTEEN	*Pisum sativum* var. arvense Poir	(49)
Cicerarin	8000	VKSTGRADDDLAVKTKYLPP	Green chickpea	(50)
Ar-AMP	3156	AGECVQGRCPSGMCCSQFGYCGRGPKYCGR	*Amaranthus retroflexus* L. seeds	(51)
Actinchinin	2500	GAKRAYDEVEAQN	The gold kiwi fruit	(52)

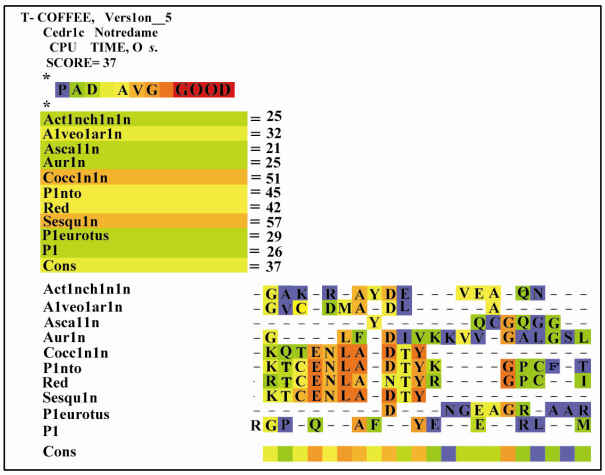

Figure 1. Final sequences alignment among AFPs that led to the selected pattern

included, molecular description DEFENSE-RELATED PEPTIDE 1; classification: antifungal protein; source scientific name: *Pisum sativum*; family: plant defensins. The final peptide pattern obtained from multiple alignment led to the structure that its source was from plant, and belonged to defensins family. Having high sequence identity to 1JKZ, starts from N-terminal part of peptide which is important part of peptide for its antifungal activity.

In this study, it was necessary to input just N-terminal part of crystallized structure of 1JKZ (which contains 10 amino acid sequences with high similarity to final pattern) into SuperMimic program and the other parts of this structure were not needed. Therefore, it was needed to separate the mentioned area (N-terminal) from the other parts of its structure. Swiss-pdb Viewer [62] was used to cut N-terminal sequences of our candidate in order to obtain the 3D structure of related segment. This structure named KTC-Y and was used as an input structure in SuperMimic program in order to obtain peptidomimetic structures.

Mimetic design

About 114 peptidomimetics structures were matched to place in KTC-Y using SuperMimic program. Peptidomimetics with lower than 9×10^{-3} *Å* Root Mean Square Deviation (RMSD) and more similarity to KTC-Y back bone and its side chains were selected. KTC-Y was divided into 2 parts to study and determine potent antifungal region of its structure between its N-terminal and C-terminal. Four peptidomimetics structures were obtained; two structures were designed for its N-terminal part and two structures for its C-terminal part (Figure 2). Two peptidomimetic structures were generated by replacing of the back bone and side chains of N-termini domain of KTC-Y, named A1 and A2 with RMSD equal to 0.009 and 0.007 *Å*, respectively. Molecules B_1 and B_2 represent peptidomimetics structures from C-terminal part of KTC-Y that contains amino acids of 5 to 10 and resulting RMSD equal to 0.008 and 0.007 *Å*,

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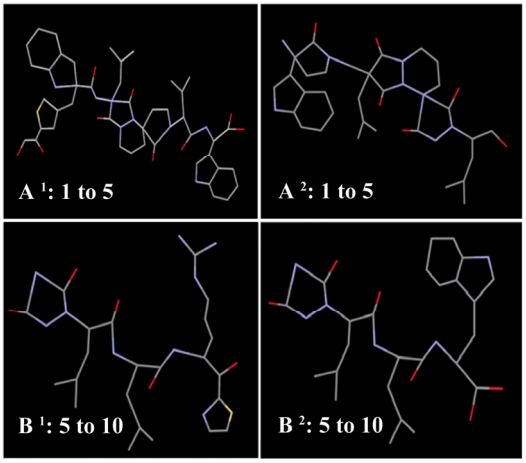

Figure 2. Peptidomimetic structures that designed by Super-Mimic Program

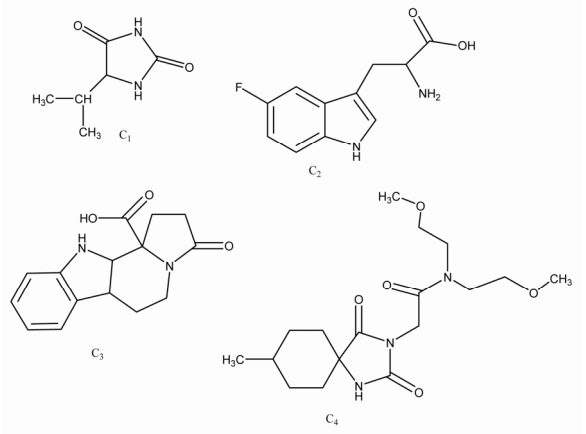

Figure 3. Final compounds for antifungal screening, named C_1, C_2, C_3 and C_4

respectively. These structures are not small enough to possibly pass through the fungal membrane.

Consequently, similarity search was done to find smaller structures with high similarity to the four peptidomimetics. Using Enhanced NCI Database Browser [63], this database also shows summary of data about structures and demonstrates that whether the selected structure has been studied previously or not. This database can also predict activity of structures and can help to estimate the activity of structure against microorganisms. Among all structures that were found, structures that had not been studied before for antifungal activity were selected. ZINC [64] as a database of commercially-available compounds for virtual screening was applied to uncover these compounds availability through companies for purchase. Finally, four structures were chosen among the resulting molecules for antifungal bioactivity screening (Figure 3).

Protoplast and antifungal screening

Four compounds that were obtained through similarity search (Figure 3) were screened for their protoplast and antifungal properties. These compounds were tested to identify the level of inhibition and the results are shown in table 2. The general ability of the test was affirmed by including a positive control agent. There was no inhibition by DMSO solvent as a negative control. Among the compounds tested, C_3 and C_4 did not show acceptable antifungal activities. C_1 and C_2 had more antifungal activities against *A. niger* and *Saccharomyces cerevisiae* (*S. cerevisiae*). Compound C_2 represents the least MIC among the compounds against *A. niger* and *S. cerevisiae*. Compound C_2 is more effective against *A. niger* than *Candida albicans* (*C. albicans*) and shows more antifungal activity on the tested fungal strains than C_1, C_3 and C_4. Due to these results C_2 and C_1 could be an option for future studies and developmental research.

The decapeptide KTC-Y was tested in antifungal bioassay and showed no activity up to 2000 *µg/ml* in neither of fungal strains assayed; it means that higher concentrations of KTC-Y should be used in order to detect its fungal inhibitory effect or the peptide cannot pass through the cell membrane. To test the second possibility, when KTC-Y was tested against *A. fumigatus* in the protoplast assay, it showed a potent activity, MIC=25 *µg/ml* (Table 2). The above-mentioned test provides further support for the possibility that KTC-Y peptide is active and it might be the cell wall of *A. fumigatus* that does not allow KTC-Y penetration into its active site on the cell membrane or in the cell itself.

Discussion

An alarming rise in life-threatening systemic fungal infections of varying types and frequencies is seen in immunocompromised patients such as AIDS, cancer and transplant

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Table 2. Protoplast assay result of KTC-Y against *A. fumigatus* Af293, and antifungal effect of four mimetic compounds against *A. niger*, *C. albicans*, and *S. cerevisiae*, as obtained by the broth dilution method; MIC values are expressed in *μg/ml*

Microorganisms	*C. albicans*		*S. cerevisiae*		*A. niger*		Protoplast assay**
Compound/Time	24 *hr*	48 *hr*	24 *hr*	48 *hr*	24 *hr*	48 *hr*	
KTC-Y	-*	-	-	-	>2000	>2000	25
C1	-	1000	-	500	-	250	nt***
C2	250	500	-	125	-	125	nt
C3	-	1000	-	1000	-	1000	nt
C4	-	1000	-	>1000	-	1000	nt
Itraconazole	0.12	2	-	1	-	8	<0.1

*"-" means no readable growth; ** The test was performed on *A. fumigatus*; *** "nt" means not tested

patients. Invasive medical procedures such as the use of vascular catheters, peritoneal dialysis, haemodialysis and parenteral nutrition have also contributed to the rise in fungal infections. The problem is made worse by the emergence of fungal strains that are resistant to currently used antifungal agents. Therefore, the design of new drugs and further improvement of currently used drugs are essential. The searches for appropriate cellular targets as well as the complete biophysical characterisation of these molecules have begun.

Recent advances in cell and molecular biology, microbiology, biochemistry and recombinant DNA technology have greatly contributed to the characterisation of molecular targets. The modern approach to drug discovery focuses on identifying and describing biochemical targets - mainly proteins - and subsequently matching those targets with small molecules that have the properties needed to inactivate them, *i.e.* structure-based drug design.

Three-dimensional characterisation of antifungal drug targets and understanding the mode of action of antifungal agents are indispensable tools in modern drug research. The success of structure-based drug design demands an iterative procedure [65]. The structure of the protein analyzed in detail allows the design of a ligand that must then be synthesised and tested. Biological data and new protein-ligand complex determination help to refine working hypotheses about complementarity. Repeating these steps leads to incremental improvement in the initial compound.

Proteins achieve their functions by folding into compact, well-ordered structures [66]. Multiple alignments of protein sequences are important in many applications, including phylogenetic tree estimation, secondary structure prediction and critical residue identification [67]. Sequence alignment is a common tool in bioinformatics and comparative genomics [68]. In the current study, investigation for extracting pattern with homology among all AFPs amino acids sequences using ClustalW, BLAST, and T-Coffee led to final pattern with 10 amino acids sequences. AFPs that showed more homology to this pattern belonged to plants. Crystallized structure that belonged to defensins group was obtained from this pattern. Defensins are major AFPs from plant [69].

Combining these results and information together, and with the help of sequence alignments, we could realize that final pattern are from antifungal peptides originating in plants, and therefore, the crystallized structure extracted similar to KTC-Y structure, belongs to defensins that are most important AFPs in plants. Pleurocidin is a 25-residue peptide which has been studied for its interaction with membrane of human pathogenic fungus *C. albicans*. It was shown that this peptide exerted fungicidal activity against *C. albicans* with the disruption of plasma membrane [70]. Piscidin is another example of cationic peptide which has been investigated for its antifungal activity, being able to permeabilize the model phospholipids membranes [71].

Design of mimetic molecules has been reported previously. For example, Fullbeck *et al*

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[59] used SuperMimic software in order to be able to present folding of the 35-residue subdomain (H35) at its C-terminus of the villin headpiece by irradiation, to replace parts of its main chain without changing the overall structure of the subdomain. Our efforts to design peptidomimetics structures led to finding four compounds through *in silico* analysis. Antifungal assay results indicated that C_2 posses more antifungal activity against selected microorganisms *C. albicans* and *S. cerevisiae*, and *A. niger* rather than the others compounds. The question that we faced was that why this compound is more potent for its antifungal activity or whether this compound acts on lipid bilayer membrane of tested microorganisms.

Looking into this compound's structure, it is realized that this structure consists of tryptophan which could affect its antifungal activity. Therefore, a literature survey was carried out to discover influence of tryptophan on antifungal activity. Tryptophan is generally not an abundant amino acid residue in peptides or proteins. The effect of tryptophan derivative here might be corresponding to its behaviour in AMP in which the partitioning of peptides into membranes is imposed by its propensity to position itself near the membrane/water interface. Examples of antimicrobial peptides exist that are rich in tryptophan include tritripticin (VRRFPWWWPFLRR) and indolicidin (ILPWKWPWWPWRR-amide) [72]. Indolicidin exhibits potent antifungal and antiviral activities that are rich in tryptophan residues [73]. Lawyer and his colleagues [74], found that a 13 amino acid tryptophan-rich region with the sequence of VRRFPWWWPFLRR had strong antimicrobial activity with a wide spectrum.

Tryptophan residues play a crucial role in membrane spanning proteins as well, has a strong preference for the interfacial regions of lipid bilayers. In addition, tryptophan residues are involved in protein folding, forming both native and non-native hydrophobic contacts even in denatured proteins to ensure their proper folding. Further testament to the significance of tryptophan is the fact that upon screening of a complete combinatorial library of hexapeptides, peptides rich in these two amino acids (W and R) were found to possess the highest antimicrobial activities [75].

Ramamoorthy *et al* [76], investigated the correlation between cell selectivity and membrane interactions of G15, which is antimicrobial peptide granulysin, and discovered the role of interaction of tryptophan residues and possibly the hydrophobic sidechains with the phosphate head groups for a tight binding of the G15 to the lipid bilayers. They suggested that the tryptophan residue is located at the bilayer interface and not deep inside the hydrophobic region of the membrane. Andreu and colleagues [31], during study on cecropins suggested that AMPs containing more tryptophan are being active against microorganisms.

The mimetic design has many applications in biochemical studies for development of new therapies and also pest control [78,79]. Recently, some halide substituted indole dihydropyrimidines were synthesized and their antifungal activities have been reported [80]. Singh and colleague [81] have isolated an indole alkaloid *Alstonia venenata* named venenatine, which shows antifungal activity against some plant pathogenic and saprophytic fungi.

Conclusion

In this study, we investigated a group of antifungal peptides through bioinformatics investigation that could extract a general pattern to be in common in the N-terminal region. After further analysis, the KTC-Y peptide was chosen to be synthesized. In our preliminary studies, the mentioned decapeptide showed to be active only in protoplast cells, which is an indicator of deep target site for such molecule and role of cell wall as a penetration barrier for this peptide. By computational tools and design of mimetics of and generation similar to mimetics, we were able to reach a group of molecules that are active and can be used for further studies in antifungal drug discovery and design processes. Further experiments on such compounds to determine their mode of

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action and synthesis of their derivatives are currently in place in our group.

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