

Identification and Evaluation of Novel Drug Targets against the Human Fungal Pathogen *Aspergillus fumigatus* with Elaboration on the Possible Role of RNA-Binding Protein

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ABSTRACT

Background: *Aspergillus fumigatus* is an airborne opportunistic fungal pathogen that can cause fatal infections in immunocompromised patients. Although the current anti-fungal therapies are relatively efficient, some issues such as drug toxicity, drug interactions, and the emergence of drug-resistant fungi have promoted the intense research toward finding the novel drug targets. **Methods:** In search of new antifungal drug targets, we have used a bioinformatics approach to identify novel drug targets. We compared the whole proteome of this organism with yeast *Saccharomyces cerevisiae* to come up with 153 specific proteins. Further screening of these proteins revealed 50 potential molecular targets in *A. fumigatus*. Amongst them, RNA-binding protein (RBP) was selected for further examination. The aspergillus fumigatus RBP (AfuRBP), as a peptidylprolyl isomerase, was evaluated by homology modeling and bioinformatics tools. RBP-deficient mutant strains of *A. fumigatus* were generated and characterized. Furthermore, the susceptibility of these strains to known peptidylprolyl isomerase inhibitors was assessed. **Results:** AfuRBP-deficient mutants demonstrated a normal growth phenotype. MIC assay results using inhibitors of peptidylprolyl isomerase confirmed a higher sensitivity of these mutants compared to the wild type. **Conclusion:** Our bioinformatics approach revealed a number of fungal-specific proteins that may be considered as new targets for drug discovery purposes. Peptidylprolyl isomerase, as a possible drug target, was evaluated against two potential inhibitors, and the promising results were investigated mechanistically. Future studies would confirm the impact of such target on the antifungal discovery investigations. **DOI: 10.18869/acadpub.ibj.21.2.84**

Keywords: *Aspergillus fumigatus*, RNA-binding protein, Peptidylprolyl isomerase, Juglone, RNA interference

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INTRODUCTION

Aspergillus fumigatus is currently the most important airborne fungal pathogen and the leading cause of invasive aspergillosis (~85% of cases)^[1]. Despite the introduction of new effective antifungal drugs, the mortality rate of invasive aspergillosis still exceeds 40%^[2]. Furthermore, the emergence of drug resistance adds more complications

to the treatment process. Hence, new antifungal agents are urgently needed.

While the classical cell-based screening method has been successfully used in drug discovery process^[3], it is an expensive, time-consuming and labor-intensive approach^[4]. Recent advances in application of computation methods in genome, proteome, and metabolome mining coupled with complete genome sequences of pathogen organisms have opened an

alternative path toward identifying new drug targets. For instance, several reports have confirmed the usefulness of comparative genome analysis in identification of pathogen-specific drug and vaccine candidates^[5-7].

The genome of *A. fumigatus* contains ~9900 genes, but few antifungal targets have been developed yet^[8]. In the present study, we compared the whole proteome of *A. fumigatus* with *Saccharomyces cerevisiae*, as a non-pathogen fungal organism, to identify *Aspergillus*-specific proteins. Amongst the proteins, those with close homologues to human were removed, and the final targets were selected. One novel target, the aspergillus fumigatus RNA-binding protein (AfuRBP), was selected and found to be a peptidylprolyl isomerase (PPI). For further functional studies, *AfuRbp* knock out and knock down mutant strains were generated. MIC assay with known inhibitors, phenylglyoxal and juglone, and a synthetic derivative of juglone confirmed the higher sensitivity of the mutants to these compounds when compared to the wild-type strain.

MATERIALS AND METHODS

Comparative analysis of *A. fumigatus* and *S. cerevisiae* proteome

The identification of novel drug targets against *A. fumigatus*, as a human pathogen, was based on alignment of its proteome with the proteome of *S. cerevisiae*. The proteomes of these two fungi were extracted from NCBI (<https://www.ncbi.nlm.nih.gov/genome/browse/>). Three different approaches were used to identify and confirm those sequences that are present in *A. fumigatus* but are absent or significantly diverge from *S. cerevisiae*. LAST (<http://last.cbrc.jp>)^[9] has been used as a fast sequence alignment tool to identify unique sequences for *A. fumigatus*. LAST uses variable-length (spaced) seeds realized by a suffix array^[10]. The expected value of 10 (-e84) was chosen to obtain higher sensitivity (-m1000000), and the other settings and parameters were used as default. In the next step, unique sequences from *A. fumigatus* were compared using suffix tree analysis by Mummer (version 3.0) (<http://mummer.sourceforge.net>)^[11]. Minimum maximal match was seven to restrict output proteins, but other settings were chosen as default. Finally, the proteins that were unique to fungi were identified by BlastP (NCBI Blast v. 2.2.1)^[12]. Modeling was carried out by I-TASSER server (Michigan University, USA)^[13-15]. Docking of the compounds was performed by HEX 6.3 software when required.

Strains, plasmids, and culture conditions

A. fumigatus akuAKU80 and its *pyrG*⁻ derivative (akuAKU80 *pyrG*⁻) with a highly efficient homologous recombination background were used for isolation of *AfuRbp* gene homologue and gene disruption or gene silencing experiments. *E. coli* Top10 (Invitrogen, USA) cells and pGEM-T Easy cloning system (Promega, USA) were used in all DNA recombinant procedures. Plasmid PGEM-GlaA comprising *Aspergillus niger* glucoamylase A (*glaA*) promoter and *glaA* termination signal was used for preparation of RNAi plasmid. All molecular methods including PCR and RT-PCR were performed based on established protocols^[16]. In RT-PCR settings, 1 µg of total RNA was used in cDNA synthesis reactions and the *A. fumigatus* actin gene (AFUA_6G04740) was used as a loading control. Fungal strains were grown and kept on SAB agar or SAB agar medium supplemented with uridine and uracil. Modified Vogel's medium^[17] was used in isolation of fungal transformants.

Construction of *AfuRbp* deletion/silencing cassettes and strains

For construction of the *AfuRbp* deletion cassette, a sequential cloning strategy was applied as described before^[18]. Briefly, 1.5 kb of 5' and 1.2 kb of 3' flanking regions of the gene were amplified separately, using RBP_KO1/RBP_KO2 (containing *SphI/NcoI* restriction sites) and RBP_KO3/RBP_KO4 (containing *EcoRI/SalI* restriction sites) primer sets, respectively (Table 1). These fragments were cloned next together in pGEM-Teasy vector. At the final step, the *A. fumigatus pyrG* gene, as a selection marker, with its own promoter and terminator containing *EcoRI* site at both ends was cloned into *EcoRI* site, between the 5' and 3' flanking regions, resulted in pRBP-KO (Fig. 1A).

To generate the *AfuRbp* silencing cassette, an inducible RNAi transcriptional unit was designed based on a previously described protocol^[19,20]. This unit was driven by the *glaA* promoter and comprises an approximately 500 bp Rbp fragment (sense, nucleotides 342-841), followed by a 100-bp buffer EGFP fragment and the same ~500 bp Rbp fragment in the opposite direction (antisense). Sense and antisense fragments were amplified by PCR using primers RBP_SENSE_F and RBP_SENSE_R carrying appropriate restriction sites (Table 1) and cloned into PGEM-GlaA vector. A 100-bp EGFP fragment was then amplified by PCR using specific primers (Table 1) and cloned between the latter fragments to generate final silencing cassette (pRBP_RNAi) (Fig. 1B).

Table 1. Primers used in this study

| Name | Sequence (5'-3') | Enzymes |
|-------------|--------------------------------|----------------|
| RBP_KO1 | GGCCCCGATGCTCTCTCAGCTCTGTGGTTG | <i>SphI</i> |
| RBP_KO2 | CCATGGCATACTCGAGCACGGTCG | <i>NcoI</i> |
| RBP_KO3 | GAATTC AATGCGTGATGTTG | <i>EcoRI</i> |
| RBP_KO4 | GTCGACGTCAGTTGACAGAGCGAG | <i>SalI</i> |
| RBP_SENSE_F | GAGATCTCCATCCCATTTGTTGTT | <i>BglII</i> |
| RBP_SENSE_R | TGATATCTGCTCCTGCTGTCGGCGT | <i>EcoRV</i> |
| RBP-F | AAGTTCATGGAAAAGTGGGC | ----- |
| RBP-R | AGTCAGTTGACAGAGCGAGG | ----- |
| RBP_rt1 | CCCCTGAGGTTGAGGAGCGG | ----- |
| RBP_rt2 | CGAGGTTCTGCACTTTGGCG | ----- |
| GFP_F | GATATCGTCCAGGAGCGCAC | <i>EcoRV</i> |
| GFP_R | AAGCTTCTCGATGCGGTT | <i>HindIII</i> |
| ACT-F | ATGTCAGTGTGCAGATTGTC | ----- |
| ACT-R | CGTAGAGGGAGAGAACGGCC | ----- |

The prepared constructs were used to transform *A. fumigatus* KU80ΔpyrG strain as described before^[21]. Positive transformants were selected on Vogel's minimal medium lacking uracil/uridine supplements. To find the knock out strains, transformants were screened by PCR using primers RBP-F and RBP-R (Table 1). As the silencing construct, pRBP_RNAi did not contain any fungal selection marker, the pRG3-AMA1 vector containing the pyrG selection marker was used as the second plasmid in co-transformation reaction. RNAi transformants were also selected based on PCR using *gfp*- and *AfuRbp*-specific primers.

Susceptibility testing

MICs were determined based on CLSI Broth Microdilution Method (M38-A2 Document) with some modifications. Briefly, fungal spores (10^4 /well) were inoculated in 96-well microtiter plates containing RPMI 1640 medium enriched by 2% glucose (or maltodextrin), and the susceptibility was assessed at a final compound concentration range of 0-200 μg/ml after 48 h. All MIC assays were performed in triplicates. Based on orthology and EC number (EC: 5.2.1.8) of AFUA_2G07650 (*AfuRBP*), two organic inhibitors of the PPI activity, i.e. phenylglyoxal and juglone (5-hydroxy-1,4-naphthoquinone), were found by searching BRENDA enzyme database (www.brenda-enzymes.info) and tested (Fig. 2). A synthetic derivative of juglone, 5-O-Acetoxy-1,4-naphthoquinone (juglone acetate, JA), was also prepared using a previously published method^[13] and assessed in MIC tests. This derivative was made to make the compound more lipophilic.

Docking and homology modeling

For bioinformatics assessment of the chemical inhibitors used in MIC assays, the 3D structure of *AfuRBP* was predicted by I-TASSER. The predicted binding sites were subsequently used for docking of the inhibitors. HEX 6.3, ArgusLab (v.4.0.1, <http://www.arguslab.com/arguslab.com/Publications.html>), and WebLab Viewer Lite (v. 4.2) were used for docking of the inhibitors and manipulation of the protein.

RESULTS

Identification of target proteins based on *in silico* comparative analyses

Based on the LAST alignment of the *A. fumigatus* proteome (9630 proteins) against *S. cerevisiae*, 474 unique proteins were recognized in *A. fumigatus* as the homologous of these proteins were not present in *S. cerevisiae*. All of the 474 proteins were further examined in KEGG, NCBI, EXPASY, and EBI databases. It was found that the functions of 161 proteins have already been explained, and their accession numbers are available. The remaining 313 proteins were found to be hypothetical (data not shown). Finally, 50 out of 161 protein candidates were chosen as they were fungal-specific but not present in *S. cerevisiae* (Table 2). The RBP, as a potential drug target, was selected for further evaluation. This protein is encoded by an 1158-bp gene located on chromosome 2 and has been annotated as a PPI.

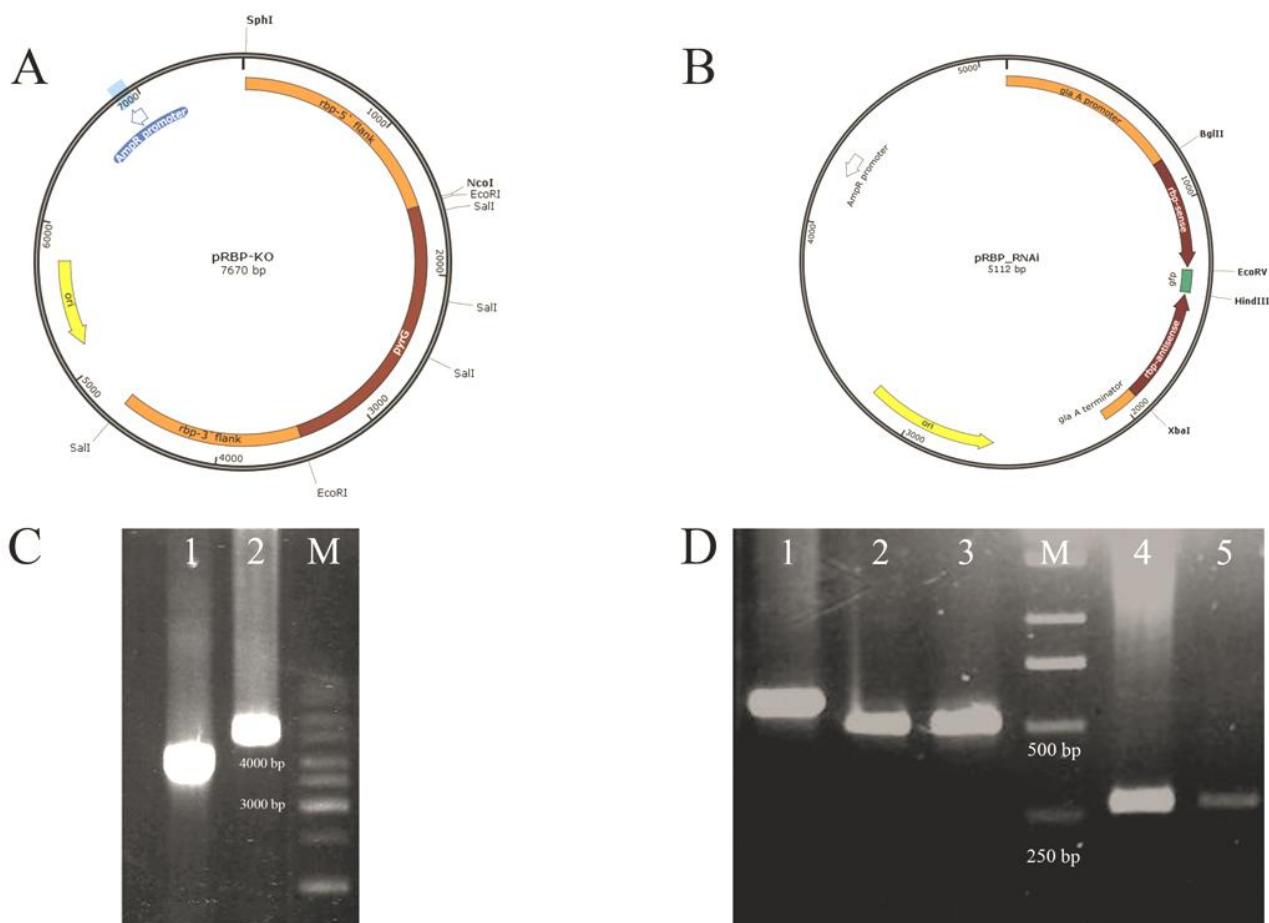


Fig. 1. Gene constructs and molecular validations. Schematic representation of constructed plasmids used in gene deletion (pRBP-KO) (A) and RNAi (pRBP_RNAi) (B) experiments. (C) PCR analysis of wild-type (lane 1) and deletant strain (lane 2) genome using primers RBP-F1 and RBP-R1. The amplification of a 4.6-kb product confirmed the replacement of native *AfuRbp* with the disrupted fragment. (D) RT-PCR analysis of RNAi transformant grown in maltodextrin or glucose medium for 24 h. Lane 1, actin fragment amplified from the genome (560 bp); lane 2, actin fragment amplified from cDNA originated from glucose; lane 3, maltodextrin cultures (485 bp); lane 4, *AfuRbp* expression level in RNAi transformant grown in glucose medium; lane 5, maltodextrin medium. *AfuRBP*-specific RT-PCR primers, RBP_rt1/rt2, amplified a 300-bp product. M, DNA size marker

Disruption of *AfuRbp* in *A. fumigatus*

The *SphI/SalI* pRBP-KO fragment was used for transformation of *A. fumigatus* (akuAKU80 pyrG-) protoplasts. Through the PCR screening of transformants, a deletion strain was isolated and examined further. In this transformant, RBP-F1 and RBP-R1 primers amplified a fragment of ~4.6 kb (KO) instead of a 3.8-kb wild-type fragment, confirming the replacement of native gene with disrupted one (Fig. 1C). RT-PCR analysis of the deletant revealed that *AfuRbp* does not express in this mutants (data not shown). The growth phenotype of this mutant in various media containing different carbon and nitrogen sources was similar to the parental strain, indicating that *AfuRbp* is not essential in *A. fumigatus*. In PCR screening of RNAi transformants, one positive integrant was selected. This transformant was grown in *glaA*-inducing medium containing 1% maltodextrin, as

the sole carbon source. Semi-quantitative RT-PCR analysis using primers RPB-rt1 and RBP-rt2 showed the down-regulation of *AfuRbp* expression in the presence of maltodextrin (Fig. 1D). Phenotypic analysis of this transformant in inducing medium re-confirmed the non-essential role of *AfuRbp* in fungal growth.

Effect of juglone and the synthetic compound on the mutant strains

To test whether the deletion or down-regulation of *AfuRbp* has any effect on drug susceptibility of the fungus, MIC levels were determined for phenylglyoxal, juglone, and JA. Table 3 demonstrates MIC values for these compounds. The results demonstrated a higher sensitivity of all strains to juglone compared to phenylglyoxal and JA. While the phenylglyoxal compound did not appear as a strong

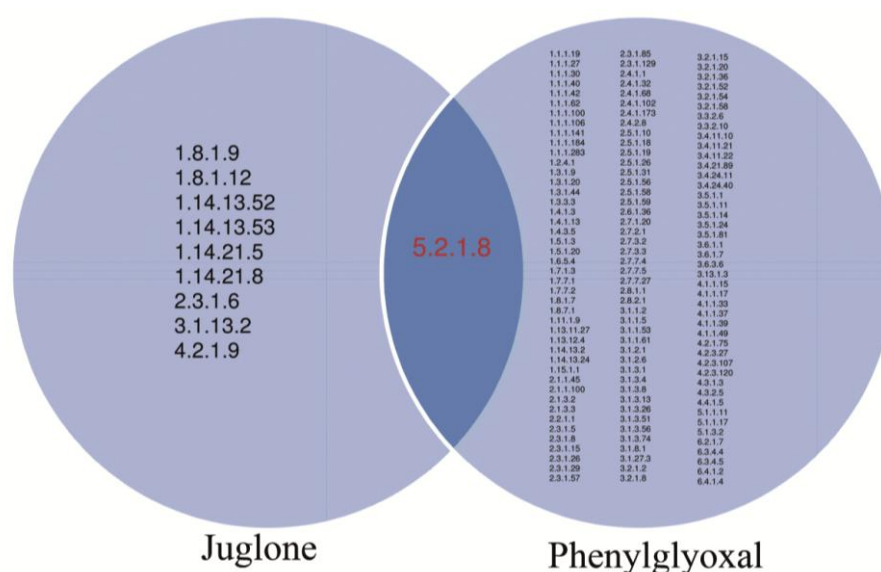


Fig. 2. List of enzymes (EC number) targeted by juglone or phenylglyoxal. The peptidylprolyl isomerase is the only overlap target for both juglone and phenylglyoxal as inhibitors.

antifungal inhibitor, the mutant strains showed more sensitivity to this chemical. MIC assays for RNAi transformant was performed in maltodextrin medium. The higher sensitivity of RNAi transformant in maltodextrin medium compared to the glucose medium indicated that the down-regulation of *AfuRbp* can affect the strain susceptibility to PPI inhibitors.

Outcome of modeling and docking for AfuRBP as a model

As we could not find any template protein having a minimum 35% similarity to our target protein, a threading algorithm for RBP protein modeling was used by I-TASSER server. Five models were generated, and according to the I-TASSER validation criteria, model number one had the least calculation errors and fitted best within the X-ray/NMR 3D population criteria (Fig. 3). In this study, five top models proposed by I-TASSER were used as receptors of the docking experiments, and phenylglyoxal, juglone, and JA were used as ligands. Docking outcome showed that the interaction of model number 4 with JA produced a proper energy (-186.3), which indicates the highest absolute value among the docking results. Other docking outcomes that were sorted by energy are shown in Table 4.

DISCUSSION

Because of the common eukaryotic origin of fungi and mammals, the development of new antifungal drugs has been challenging. This may be the reason for

the introduction of only five classes of antifungal agents, including fluorinated pyrimidine analogs, polyenes, allylamines, azoles, and echinocandins^[22]. Here, we have tried to find some novel drug targets against *A. fumigatus* using bioinformatics. Our alignment has demonstrated a group of proteins/genes (Table 2) that are fungal-specific and may be essential to *A. fumigatus*. Our highlights differ from other studies such as Abadio and colleagues^[23] in comparative alignment as we searched for those novel targets that only exist in *A. fumigatus*. In this study, we found a RNA-binding protein (AfuRBP) that has been annotated as a member of PPI enzyme family. The PPI family contains three members, including cyclophilins, FKBP, and Parvulin^[24]. Cyclophilins are described by an eight-standard β -barrel that forms a hydrophobic pocket. FKBP, instead, contain an amphipatic, five-stranded β -sheet that enfolds around a single, short α -helix^[25,26]. Members of Parvulin family contain a PPI domain consisting of a half β -barrel and its four antiparallel strands surrounded by four α -helices. The results of I-TASSER modeling and docking demonstrated that the AfuRBP is structurally closer to Parvulin family members of PPI enzyme family. To elucidate the function of this protein, *AfuRbp*-deficient strains were generated using gene deletion and RNAi strategies. Normal phenotype of these strains indicated a non-essential role for this protein in growth physiology of *Aspergillus fumigatus*. Some studies have demonstrated that the inactivation of a drug target gene can result in increased sensitivity of the organism to the drug or other compounds that inhibit the same pathway^[27,28]. In this sense, the sensitivity of *AfuRbp*-

Table 2. List of selected proteins specific to fungi other than *S. cerevisiae*

| No. | Accession no. | Description | Taxonomy | Orthology |
|-----|---------------|---|--|---|
| 1 | AFUA_8G06570 | Acetylxylyl esterase ((EC:3.-.-.-)) | Dikarya | Acetylxylyl esterase [EC:3.1.1.72] |
| 2 | AFUA_4G00860 | Cell surface protein | Pezizomycotina | |
| 3 | AFUA_1G13450 | Cell wall proline-rich protein | Pezizomycotina | |
| 4 | AFUA_6G14090 | CFEM domain protein | Leotiomyceta | |
| 5 | AFUA_4G12450 | Conserved lysine-rich protein | Pezizomycotina | |
| 6 | AFUA_4G13230 | Developmental regulatory protein WetA | Saccharomyceta [†] | |
| 7 | AFUA_8G00250 | Dimethylallyl tryptophan synthase (EC:2.5.1.-) | Leotiomyceta | N-dimethylallyltransferase [EC:2.5.1.-] |
| 8 | AFUA_7G01280 | DNA damage response protein RcaA | Metazoa | |
| 9 | AFUA_7G06740 | Endoglucanase (EC:3.2.1.4) | Metazoa | Endoglucanase [EC:3.2.1.4] |
| 10 | AFUA_6G11980 | Exo-beta-1,3-glucanase (EC:3.2.1.58) | Dikarya | glucan 1,3-beta-glucosidase [EC:3.2.1.58] |
| 11 | AFUA_6G14070 | Extracellular glycine/serine-rich protein | <i>Neosartorya fischeri</i> & <i>Aspergillus fumigatus</i> | |
| 12 | AFUA_5G01620 | Extracellular proline-rich protein | Eurotiomycetidae | |
| 13 | AFUA_8G07090 | Extracellular proline-serine-rich protein | Dikarya | |
| 14 | AFUA_7G02060 | Extracellular serine-rich protein | Leotiomyceta | |
| 15 | AFUA_7G02460 | Extracellular serine-rich protein | Trichocomaceae | |
| 16 | AFUA_6G00670 | Extracellular serine-rich protein | Dikarya | |
| 17 | AFUA_7G00320 | Extracellular proline-glycine-rich protein | Leotiomyceta | |
| 18 | AFUA_7G05650 | Glutamine-serine-rich protein MS8 | Leotiomyceta | |
| 19 | AFUA_7G04870 | Glutamine-serine-proline-rich protein | Saccharomyceta [†] | |
| 20 | AFUA_6G10580 | GPI anchored CFEM domain protein | Dikarya | |
| 21 | AFUA_8G04860 | GPI anchored glycoprotein | Leotiomyceta | |
| 22 | AFUA_6G14010 | GPI anchored protein | Leotiomyceta | |
| 23 | AFUA_4G09600 | GPI anchored protein | Metazoa | |
| 24 | AFUA_7G00970 | GPI anchored serine-threonine-rich protein | Leotiomyceta | |
| 25 | AFUA_4G08295 | Histone h1.3. | Leotiomyceta | |
| 26 | AFUA_8G07060 | Hydrophobin | Leotiomyceta | |
| 27 | AFUA_8G01790 | Integral membrane protein | Leotiomyceta | |
| 28 | AFUA_6G04280 | Integral membrane protein | Leotiomyceta | |
| 29 | AFUA_5G00620 | Integral membrane protein | Leotiomyceta | |
| 30 | AFUA_1G11380 | Integral membrane protein | Leotiomyceta | |
| 31 | AFUA_8G01190 | Isoamyl alcohol oxidase | Leotiomyceta | |
| 32 | AFUA_5G03980 | LysM domain protein | Leotiomyceta | |
| 33 | AFUA_6G03050 | Oleate delta-12 desaturase (EC:1.14.99.-) | Dikarya | omega-6 fatty acid desaturase (delta-12 desaturase) |
| 34 | AFUA_8G02230 | Serine-leucine-rich repeat protein | <i>Aspergillus fumigatus</i> | |
| 35 | AFUA_1G13830 | Threonine-rich protein | Eurotiomycetidae | |
| 36 | AFUA_1G02270 | ARS-binding protein Abp2 | Ascomycota | |
| 37 | AFUA_5G05990 | AT DNA-binding protein | Leotiomyceta | |
| 38 | AFUA_3G08110 | Cell wall protein | Ascomycota | |
| 39 | AFUA_5G09580 | Conidial hydrophobin Hyp1/RodA | Leotiomyceta | |
| 40 | AFUA_5G09010 | Conserved glutamic acid-rich protein | Pezizomycotina | |
| 41 | AFUA_3G12790 | Conserved glutamic acid-rich protein | Eurotiomycetidae | |
| 42 | AFUA_1G05290 | Endo-1,3(4)-beta-glucanase (EC:3.2.1.6); K01180 endo-1,3(4)-beta-glucanase [EC:3.2.1.6] | Fungi | endo-1,3(4)-beta-glucanase [EC:3.2.1.6] |
| 43 | AFUA_3G13110 | Extracellular serine-threonine-rich protein | Leotiomyceta | |
| 44 | AFUA_5G14210 | Glucose repressible protein Grg1 | Dikarya | |
| 45 | AFUA_1G11220 | GPI anchored protein | Trichocomaceae | |
| 46 | AFUA_1G03630 | GPI anchored serine-threonine-rich protein (EC:3.1.1.5) lysophospholipase | Fungi | |
| 47 | AFUA_5G13725 | Integral membrane protein | Pezizomycotina | |
| 48 | AFUA_3G08670 | Integral membrane protein | Leotiomyceta | |
| 49 | AFUA_3G00830 | Pfs domain protein | Leotiomyceta | |
| 50 | AFUA_2G07650 | RNA-binding protein (EC:5.2.1.8); K01802 Peptidylprolyl isomerase [EC:5.2.1.8] | Trichocomaceae | peptidylprolyl isomerase [EC:5.2.1.8] |

Orthology is specified where data exist. [†]excluding *S. cerevisiae*

Table 3. *In vitro* activity of compounds against wild-type and *AfuRbp* mutants of *A. fumigatus* by MIC test ($\mu\text{g/ml}$)

| Inhibitor | Wild strain | | KO mutant | | RNA _i mutant | |
|-----------------|------------------|-----------------------|------------------|-----------------------|-------------------------|-----------------------|
| | RPMI/ glucose | RPMI/ maltodextrin | RPMI/ glucose | RPMI/ maltodextrin | RPMI/ glucose | RPMI/ maltodextrin |
| Juglone | 3.12 | 3.12 | 1.56 | 1.56 | 3.12 | 1.56 |
| Phenylglyoxal | 200 | 200 | 100 | 100 | 200 | 100 |
| Juglone acetate | 6.25 | 6.25 | 3.12 | 3.12 | 6.25 | 3.12 |
| Amphotericin B | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 |

disrupted strains to PPI inhibitors was assessed.

Juglone showed the highest inhibitory effect on the mutants and wild strain. In addition, we used JA as an inhibitor to investigate the change in the structure of juglone through modification of its hydroxyl group in order to increase the hydrophobicity. The decision to produce JA was partly due to the predicted activity of this derivative through the docking scores, which had a higher absolute energy of interaction than juglone. Overall, MIC test of JA demonstrated different results for the mutant and wild strains. Although docking showed a high potential for JA, the MIC test indicated slight decrease in activity, which might be due to

lipophilic binding of JA to other cell components.

Considering the specific inhibitory effect of juglone on Parvulin^[29] and the higher sensitivity of *AfuRbp* deficient mutants to this chemical compared to the parental strain, it can be concluded that juglone may act as a specific inhibitor of the AfuRBP. More detailed studies on recombinant AfuRBP may verify this claim.

In conclusion, in this study, the bioinformatics and biological assessments of AfuRBP were carried out, and the presented data can be considered as a start for pin pointing a target towards developing a new drug against *A. fumigatus*.

Table 4. The results of Docking experiments

| Dock name | Produced energy of interaction (kJ/mol) | Position (supercoil) | Position (alphaa helix) | Position (beta sheet) |
|---------------------------|---|---|-------------------------|-----------------------|
| Model4 with JA | -186.3 | 8-9-10-11-243-244-275-317 | 245-246-247 | 276-277 |
| Model3 with JA | -184.7 | 347-348-349-350-366-367-368-369-370-371 | None | 317-318-319-320-321 |
| Model1 with JA | -174.5 | 220-221-222-223-224-225-226-227-228-229-230-231-232-233-234-235-236 | 237-238-239-240 | None |
| Model5 with JA | -174.4 | 109-110-111-112-113-114-115-131-132-133-134-135-136-181-182-183 | None | None |
| Model3 with juglone | -166.4 | 324-325-350-356-357-358-363-364 | None | None |
| Model2 with JA | -162.5 | 80-81-82-83-84-85-126-127-128 | None | 79-86-87 |
| Model4 with juglone | -161.7 | 74-75-156-157-158-351-352 | None | None |
| Model3 with phenylglyoxal | -153.12 | 354-355-356 | None | None |
| Model1 with juglone | -152 | 26-27-28-29-30-31-213-214-215-216 | None | None |
| Model4 with phenylglyoxal | -146.5 | 383-384-385 | None | None |
| Model1 with phenylglyoxal | -146.1 | 243-244-245-246-247 | 279-280 | None |
| Model2 with phenylglyoxal | -143.5 | 143-144-145-157-158 | 229-230-231 | 219-220-221 |
| Model5 with phenylglyoxal | -117.3 | 167-168-169-170-171-172-173-174-175 | None | None |
| Model5 with juglone | -31.9 | 82-83-84-85-86-87 | None | 126-127 |
| Model2 with juglone | 0 | None | None | None |

The synthetic compound (JA), juglone and phenylglyoxal, were used as ligands and the five predicted models (1, 2, 3, 4, and 5) were used as receptors. Position of docked ligand and binding sites for docking results with their energy values are indicated in the Table. Docking was performed by HEX 6.3 software.

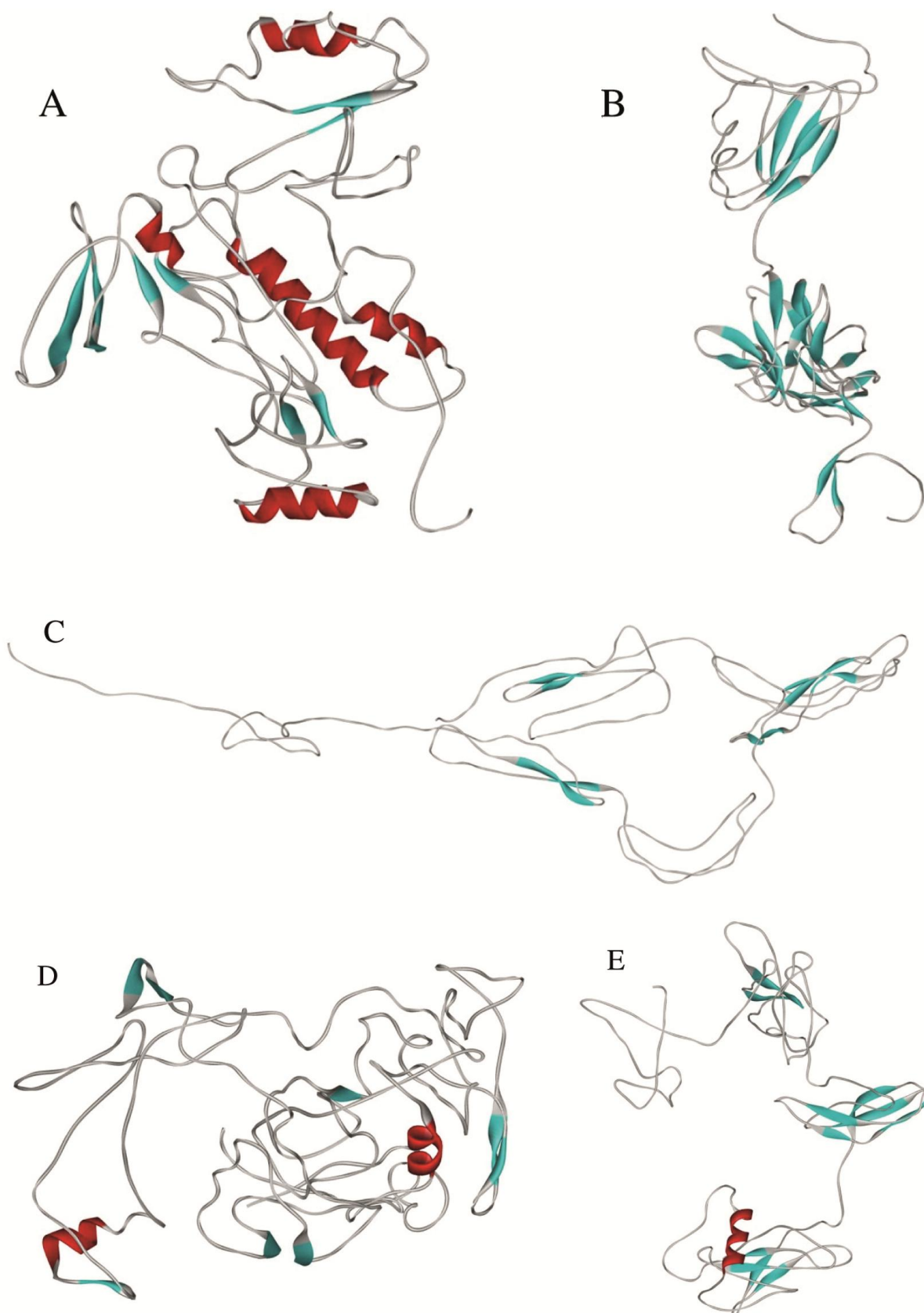


Fig. 3. The five predicted three-dimensional structures of AfuRBP protein obtained by homology modeling with I-TASSER. Model numbers: 1 (A), 2 (B), 3 (C), 4 (D), 5 (E).

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CONFLICT OF INTEREST. None declared.

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