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ORIGINAL ARTICLE

Docking analysis of hexanoic acid and quercetin with seven domains of polyketide synthase A provided insight into quercetin-mediated aflatoxin biosynthesis inhibition in *Aspergillus flavus*

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Abstract

Studies on phytochemicals as anti-aflatoxigenic agents have gained importance including quercetin. Thus, to understand the molecular mechanism behind inhibition of aflatoxin biosynthesis by quercetin, interaction study with polyketide synthase A (PksA) of *Aspergillus flavus* was undertaken. The 3D structure of seven domains of PksA was modeled using SWISS-MODEL server and docking studies were performed by Autodock tools-1.5.6. Docking energies of both the ligands (quercetin and hexanoic acid) were compared with each of the domains of PksA enzyme. Binding energy for quercetin was lesser that ranged from −7.1 to −5.25 kcal/mol in comparison to hexanoic acid (−4.74 to −3.54 kcal/mol). LigPlot analysis showed the formation of 12 H bonds in case of quercetin and 8 H bonds in hexanoic acid. During an interaction with acyltransferase domain, both ligands showed H bond formation at Arg63 position. Also, in product template domain, quercetin creates four H bonds in comparison to one in hexanoic acid. Our quantitative RT-PCR analysis of genes from aflatoxin biosynthesis showed downregulation of *pksA, aflD, aflR, aflP* and *aflS* at 24 h time point in comparison to 7 h in quercetin-treated *A. flavus*. Overall results revealed that quercetin exhibited the highest level of binding potential (more number of H bonds) with PksA domain in comparison to hexanoic acid; thus, quercetin possibly inhibits via competitively binding to the domains of polyketide synthase, a key enzyme of aflatoxin biosynthetic pathway. Further, we propose that key enzymes from aflatoxin biosynthetic pathway in aflatoxin-producing *Aspergilli* could be explored further using other phytochemicals as inhibitors.

Keywords *Aspergillus flavus* · Docking · Polyketide synthase A · Aflatoxin biosynthesis · Quercetin · Hexanoic acid

Abbreviations

qRT-PCR	Quantitative real-time PCR
PksA	Polyketide synthase A
AF	Aflatoxin
KS	Ketoacyl synthase
AT	Acyltransferase
ACP	Acyl carrier proteins
KR	Ketoreductase
DH	Dehydratase
ER	Enol reductase
CYC	Cyclase
MT	Methyltransferase

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Introduction

Aspergillus flavus is one of the potent contaminants of raw food commodities during pre- and post-harvest crops by producing aflatoxin (AFB1 and AFB2) (Zain 2011). These toxins have carcinogenic effect and are grouped into type I toxin by International Agency for Research on Cancer (http://monographs.iarc.fr/). The exposure level of toxin is regulated by various safety administrations to prevent its severe effect on humans and animals (Unnevehr and Grace 2013). Additionally, *A. flavus* is one of the leading cause of aspergillosis in immunocompromised patients (Thakur et al. 2015).

Aflatoxin biosynthesis in *A. flavus* is a sequential event and is produced via polyketide biosynthetic pathway (Zain 2011; Roze et al. 2013). Aflatoxin production is influenced

by temperature, pH (Patel et al. 2014), carbon source (Tiwari et al. 2016) and the developmental stages of *A. flavus* (Shankar et al. 2018). One of the key enzymes of polyketide biosynthetic pathway is polyketide synthase (PKS) which comprises of seven domains. PKS, a multidomain protein, involves utilization of acyl units to produce complex natural products (Crawford and Townsend 2010). Fungi belonging to type I polyketide synthase consists of very large multifunctional protein (180–250 kDa) with multiple domians. Ketoacyl synthase (KS), acyl transferase (AT) and acyl carrier proteins (ACP) are the major domain categories. Other domains are ketoreductase (KR), dehydratase (DH), enol reductase (ER), cyclase (CYC), and methyl transferase (MT) domains (Cox and Simpson 2009; Liu et al. 2015; Fujii et al. 2001; Kroken et al. 2003; Sarma et al. 2017). There are approximately 30 genes and a major regulatory gene (*aflR*) in *A. flavus* involved in aflatoxin biosynthesis including fatty acid synthases (Yu 2012). Study on *Aspergillus nidulans* revealed that mutant of fatty acid synthase when provided with hexanoic acid the production of secondary metabolite has been restored in mutants (Brown et al. 1996; Watanabe and Townsend 2002; Schümann and Hertweck 2006). Newman et al. (2012) have suggested that polyketides are the frontline for the development of therapeutics against aflatoxin contamination in various agricultural crops (Newman et al. 2012).

Application of biological agent to out-compete toxic effects of aflatoxins in pre- and post-harvested food crops has proven to be effective in reducing aflatoxin contamination; however, certain limitations exist (Yin et al. 2008). Application of phytochemicals extracted from various plant sources could be an alternative approach against fungal contamination (Pooja et al. 2012). Quercetin is a plant polyphenol that belongs to flavonoids, commonly found in fruit, vegetables, seeds, tea, wine, flower, nuts, propolis and honey (Kressler et al. 2011). Zhou et al. (2015) studied on biologically derived polyphenols such as gallic acid, catechin, epigallocatechin, and quercetin, among which quercetin showed maximum inhibition towards AFB1 production in *A. flavus*. Quercetin has been found to be an anticancerous through mitochondrial apoptosis-mediated tumor regression (Srivastava et al. 2016). Also, using HPLC and proteomic analysis, quercetin showed inhibition of aflatoxin biosynthesis when compared with control at MIC_{50} (113 µg/mL) and enzymes from the pathway (Tiwari and Shankar 2018). However, it is not clear how quercetin inhibits aflatoxin biosynthesis in *A. flavus*, thus computational approach could be an important asset to understand the mechanism of aflatoxin inhibition. Translational processes depend on both transcriptome and proteome relationships that may lead to alter dynamic transitions of biological processes under certain stress conditions. Hence, development of a novel target requires the understanding at both mRNA and protein expression systems of host in response to stress (Liu et al. 2016). Thus, to understand the quercetin and PksA interaction, protein sequence of PksA from *A. flavus* was obtained from UniProt database to perform homology modeling. Further, domains and molecular docking studies were performed using hexanoic acid and quercetin as ligands. Also, qRT-PCR analysis of selected genes of *A. flavus* involved in aflatoxin biosynthetic pathway was performed to understand the expression of genes in response to quercetin-mediated stress. The overall results of molecular docking and LigPlot analysis including binding energy, electrostatic energy, H bonding, bond length and hydrophobic interaction revealed that quercetin exhibited the highest level of binding potential with PksA domains in comparison to hexanoic acid.

Materials and methods

Selection of biological data, sequence retrieval and phylogenetic analysis

The complete amino acid sequence of *A. flavus* polyketide synthase (strain ATCC 200026/FGSC A1120/NRRL 3357/JCM 12722/SRRC 167) was acquired from nucleotide database of NCBI (http://www.ncbi.nlm.nih.gov/) with the NCBI Gene ID of 7914331, gene symbol: AFLA_139410, gene description: aflC/PksA/pksL1/polyketide synthase and UniProt accession number B8NI04_ASPFN. The amino acid FASTA sequence was also used as a query to search for a homologous sequence using pBLAST in NCBI (http://www.ncbi.nlm.nih.gov/Blast.cgi). The homologous sequences of more than 90% identity were retrieved and aligned using MEGA 6.06 (http://www.megasoftware.net/) with Muscle analysis and converted into MEGA format (Tamura et al. 2013; Thakur and Shankar 2016). Phylogenic tree was constructed using the maximum likelihood method (Jones et al. 1992).

Ligand preparation

The 3D structure of quercetin and hexanoic acid was obtained from PubChem structure search (https://pubchem.ncbi.nlm.nih.gov/), modified in PyMOL molecular graphic system 1.3 (http://www.pymol.org/funding.html) and validated by Lipinski's rule of five (http://www.scfbio-iitd.res.in/). The parameters were input ligand file; PDB format; pH 7.

Domain investigation, homology modeling and validation

Aspergillus flavus target protein AflC/PksA/pksL1/polyketide synthase (UniProt accession; B8NI04_ASPFN) was

subjected to NCBI database and seven different domains of PksA were retrieved from NCBI database (**Fig. S1**). The conserved domain locations of nucleotide sequences were inserted into the DNA to protein online conversion tool (http://insilico.ehu.es/translate/), translated to amino acid sequence and subjected to homology modeling using SWISS-MODEL server (http://swissmodel.expasy.org/) (Kiefer et al. 2009). The generated models were ordered by sequence identity. Two domains out of seven, i.e., ACP transacylase domain and ketoacyl synthase C-terminal domain, have shown identities between 18 and 19%. These protein structures were saved in PDB format for further analysis and validated by PROCHECK (http://www.ebi.ac.uk/thornton-srv/). For each modeled protein structure, Ramachandran plot analysis showed that majority of the residues of the protein are in the favorable regions.

Molecular docking studies

Autodock tools-1.5.6 was used to calculate the docking scores. Kollmann charges were incorporated into the proteins. All H atoms were merged and atoms were assigned to AD4, removed water molecules and saved in PDBQT format. Ligand was prepared by adding Gasteiger charges, polar H bonds, and all the rotatable bonds were defined. Random setting was used for initial position, orientation, and torsions of the ligand molecules. During docking analysis, rotatable torsions were released. Search parameters were Lamarckian genetic algorithm followed by 50 runs. Autogrid program was used to generate affinity (grid) maps of $90 \times 90 \times 90$ xyz Å grid points and 0.375 Å spacing. Autodock parameter with distance-dependent dielectric functions was selected to calculate the van der Waals and the electrostatic terms. Data from each docking experiment were derived from 50 different runs that terminate after 25 k energy evaluations. Other docking parameters were used as described by Zhou et al. (2016). During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied (Lin and Lapointe 2013; Xiao et al. 2009). The results were binding free energy, electrostatic energy, inhibition constant and final intermolecular energy, which include van-der Waals, H bond, and distortion energy. The best result was obtained from 50 runs and compared. The hydrophobic and H bond interactions between the ligands and receptor of the docked complex were analyzed using LigPlot program, which were further visualized in PyMOL in three dimensions. Interactions of both the ligands were compared with each domain to determine binding efficiency.

Quantitative real-time PCR (qRT-PCR)

Total RNA from *A. flavus* treated with and without quercetin treatment at 7-h (germinating conidia stage) and 24-h time points was extracted from two biological replicates using TRIzol reagent as per the manufacturer's instruction (Invitrogen, USA) (Shankar et al. 2011). Spectrophotometer (Thermo Scientific, USA) was used at A260/A280 nm to verify the quality and quantity and 1.2% agarose gel electrophoresis was performed to check the integrity of the extracted RNA. Total RNA (1 µg) was used for cDNA synthesis using verso-cDNA synthesis kit (Thermo Scientific, USA) as provided with the manufacturer's instructions. Primers for selected *A. flavus* genes of aflatoxin biosynthetic pathway were designed using Primer-BLAST tool (NCBI) (Ye et al. 2012) for expression study (Table S1). Further, qRT-PCR was performed for selected genes including tubulin (house keeping gene) in Bio-Rad machine CFX96, Bio-Rad, USA. qRT-PCR from two biological replicates containing three technical replicates from each were performed using SYBER-Green master-mix (Bio-Rad) according to the manufacturer's instructions (Thakur and Shankar 2017). The thermal cycling conditions for qRT-PCR were initial denaturation at 95 °C for 3 min, 39 cycles of 95 °C for 10 s, 49 °C for 45 s and 72 °C for 30 s. A melting curve was plotted to check the specificity of primers (Monteiro et al. 2009). We used 'ΔΔCt' method to quantify the expression of selected genes involved in aflatoxin biosynthesis in *A. flavus* (Gautam et al. 2008).

Results

Sequence alignment, phylogenetic analysis and homology modeling

pBLAST results revealed 12 homologues for protein sequence (PksA) having similarity score more than 90%, which were further analyzed for the phylogenetic relationship of *A. flavus* PksA. The evolutionary tree results shown in Fig. 1 suggest that *A. flavus* shares PksA sequence similarity with *Aspergillus* species, majorly with *A. oryzae, A. sojae, A. nomius* and *A. bombycis*. Three-dimensional structure of two ligands, viz. quercetin and hexanoic acid was obtained from PyMol (Fig. 2) and validated using Lipinski's rule of five, represented in Table 1. The result suggested drug-like properties for these two ligands including 3D structure for molecular docking studies. Seven different domains of PksA from *A. flavus* were selected from NCBI conserved domain sequence database (Fig. S1). All the seven domains of PksA were modeled in SWISS-MODEL server and the best possible model was selected based on the sequence identified for homology modeling (Fig. 3). The SWISS-MODEL Template Library uses both BLAST and HHblits search engines to identify templates and to obtain target–template alignments. These approaches guarantee good alignments at all the sequence identity levels and result

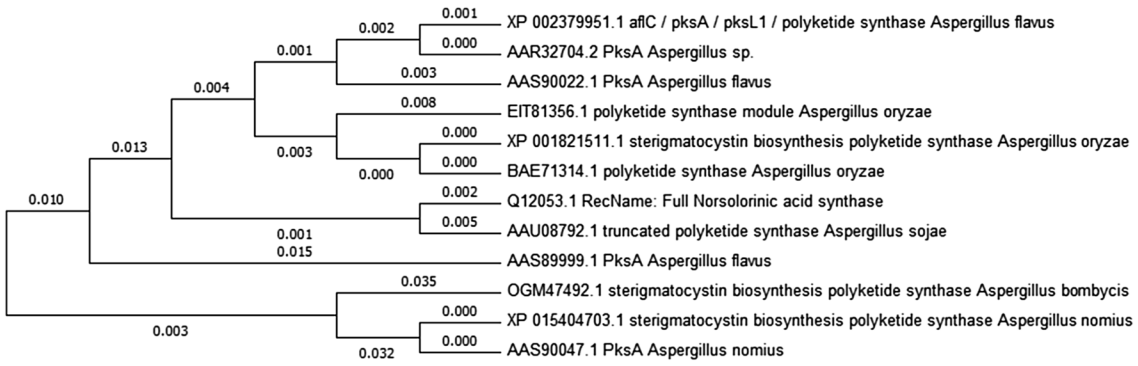


Fig. 1 Phylogenetic analysis of polyketide synthase A gene of *Aspergillus flavus* by maximum likelihood method based on the JTT matrix-based model using MEGA 6.06 software

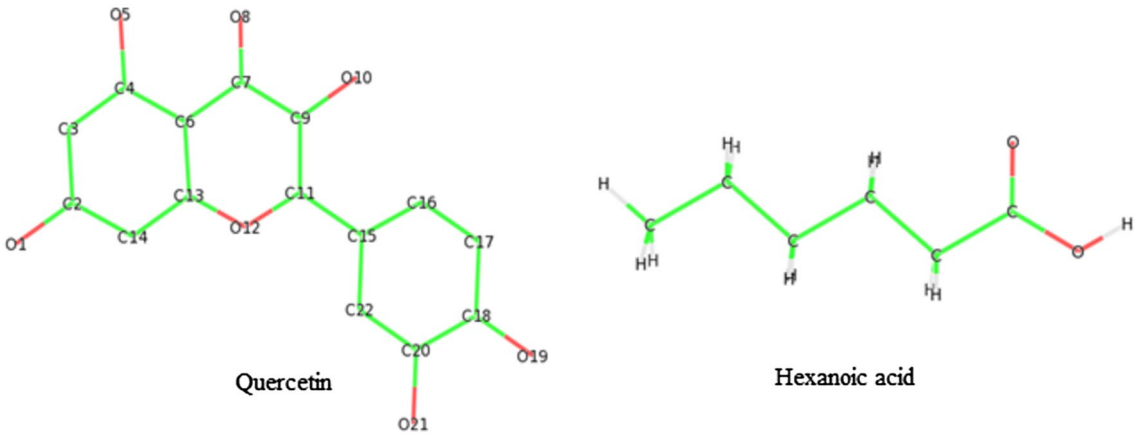


Fig. 2 Three-dimensional structure of quercetin (Id: 5280343) and hexanoic acid (Id: 8892) were obtained from Pubchem

Table 1 Molecular properties of ligands on the basis of Lipinski's rule of 5

Compound	Molecular weight (g/mol)	H-bond donor	H-bond acceptor	Log P	Molar refractivity	Pubchem CId	Molecular formula
Quercetin	302	5	7	2.010899	74.050484	5280343	$C_5H_{10}O_7$
Hexanoic acid	116.16	1	2	0.316600	29.148993	8892	$C_6H_{12}O_2$

in experimental approach, resolution (if applicable), quaternary state assignment, sequence identity to the target, coverage, etc., which suggest the best available template for our structure. The sequence of aligned and selected template is given in Table S2 (A). As indicated by Ramachandran plot (Table 2), 81.1–89.6% of all the domains were ranged in the most favored region, 6–13.5% in the additionally allowed region, 1.4–6.1% in the generously allowed region and 0–1.5% in the disallowed region. The overall goodness factor (G factor) lay within 0.11–0.36, distribution of main chain bond length of domains ranged between 96.5 and 100% and the range of covalent bond angle lay between 89.9 and 92%. Based on these validations, homology models were further used in molecular docking studies.

Docking scores

Quercetin was docked with different domains of PksA of *A. flavus* and compared with the substrate, viz. hexanoic acid known to be involved in PksA activation and

regulation of aflatoxin biosynthesis. Different scores such as binding free energy, intermolecular energy, electrostatic energy and inhibition constant values are listed in Table 3. The results showed that the binding energy of quercetin with all the respective domains was found to be less than that of hexanoic acid. The binding energy of quercetin ranged between − 7.1 and − 5.25 kcal/mol and that of hexanoic acid between − 4.74 and − 3.54 kcal/mol. Comparison between electrostatic energy of quercetin (− 0.29 to − 0.1 kcal/mol) and hexanoic acid (− 2.93 to − 1.05 kcal/mol) with that of domains also coincides with the binding energy. Intermolecular energy is directly proportional to binding energy, which was in the range between − 6.62 and − 5.55 kcal/mol and that of hexanoic acid − 6.25 kcal/mol and − 5.03 kcal/mol. A decrease in intermolecular energy was observed, which suggested quercetin is an efficient binder with these domains in comparison with hexanoic acid.

To locate and compare the site of H bonding and hydrophobic interaction of quercetin and hexanoic acid with seven different domains of PksA, LigPlot software was used. Quercetin molecule creates a total of 12 H bonds in all the domains whereas hydroxylic acid creates a total of 8 H bonds in all domains of PksA. The total number of hydrophobic interacting molecules in quercetin interaction is 46, whereas in the case of hexanoic acid it is 22. This comparative analysis for quercetin and hexanoic acid interaction with seven different domains of PksA is listed in Table 4 and represented in Fig. 4. On interaction of acyltransferase and domain of PksA, both the ligands were found to form H bond at Arg63 position but the bond length in case of quercetin was found to be 2.82 Å, whereas in case of hexanoic acid it was found to be 2.88 Å. In the product template domain, quercetin creates four H bonds whereas hexanoic creates only one H bond. In phosphopantetheine attachment site, again quercetin was found to create two H bonds whereas hexanoic acid created a single H bond. The active amino acid residues of each domain are available in Table S2 (B). The overall results of molecular docking and LigPlot analysis including binding energy, electrostatic energy, H bonding, bond length and hydrophobic interaction revealed that quercetin exhibited the highest level of binding potential with PksA domain in comparison to hexanoic acid.

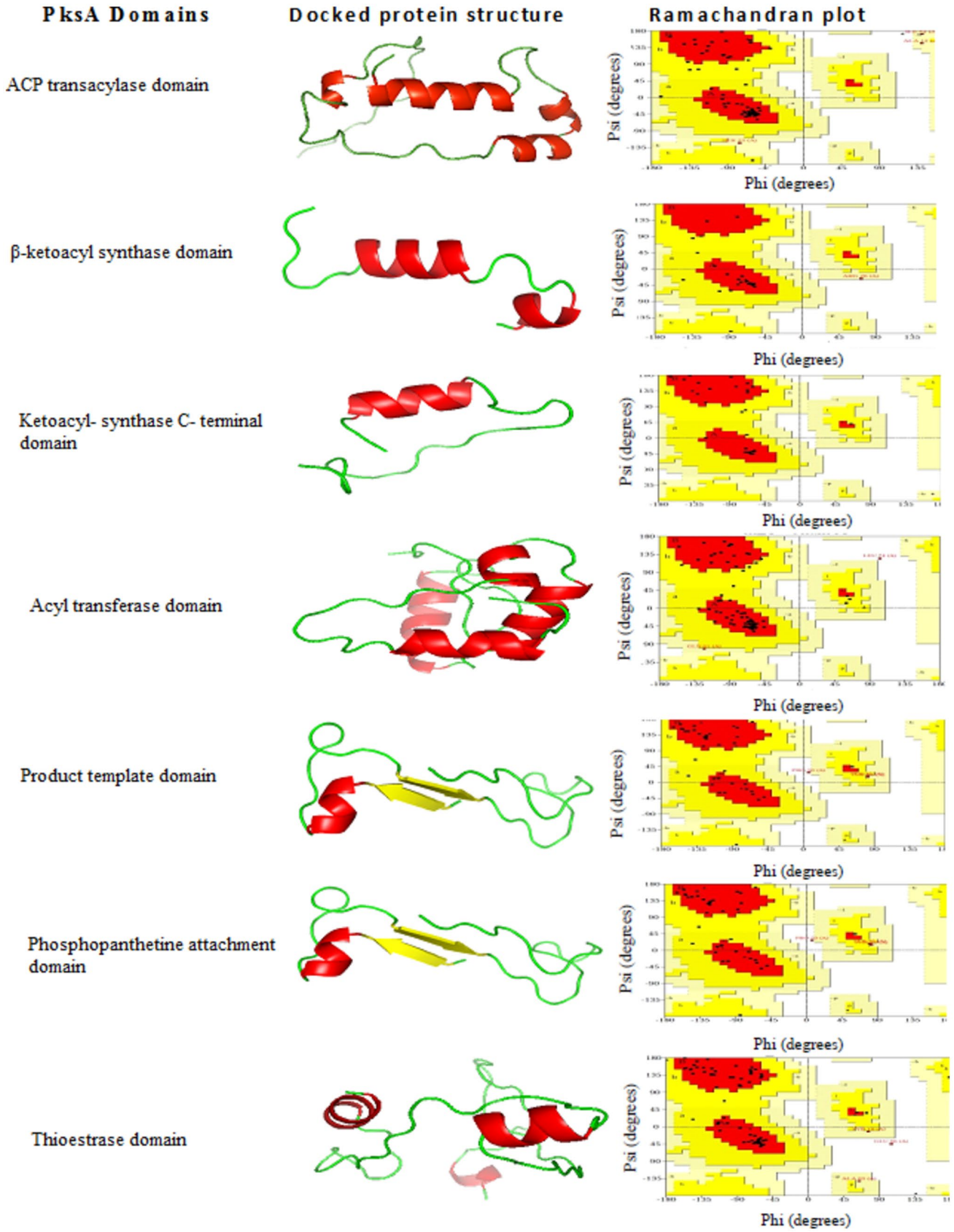
Expression of mRNA of selected genes of *A. flavus* involved in aflatoxin biosynthetic pathway in response to quercetin

To quantify the expression of selected transcripts in response to quercetin, the following genes *aflR, aflS, aflP, aflD* and *pksA* were considered for qRT-PCR analysis at 7 and 24-h time points. We observed downregulation of these genes at 24-h time point in comparison to 7-h in quercetin-treated *A. flavus* (Table 5).

Discussion

Prevention is the best approach to contain the limit of aflatoxin exposure in agricultural crops. Experimental data showed quercetin exhibits anti-aflatoxigenic properties and antiaspergillus properties (Choi et al. 2010; Zhou et al. 2015). Quercetin-mediated aflatoxin inhibition in *A. flavus* was observed in the proteomic and HPLC studies (Tiwari and Shankar 2018). PksA contains an active binding site for hexanoyl CoA as a precursor unit in aflatoxin biosynthetic pathway (Yu 2012). Efforts toward the identification of novel compound that may lead to inhibition of aflatoxin biosynthesis in *A. flavus* require a systematic, multi-pronged approach.

Acyltransferase, domain of PksA, is used in the translocation of substrate from coenzyme A to SAT (Bunkoczi et al. 2009). In our analysis, quercetin and hexanoic acid showed the same binding patterns forming a single H bond at Arg63. It also suggests quercetin affinity to bind stronger in comparison to hexanoic acid; the binding energy of quercetin was − 6.15 kcal/mol in comparison to hexanoic acid (− 3.79 kcal/mol). Also, hydrophobic interactions showed the involvement of nine amino acids for quercetin and three amino acids for hexanoic acid. Fungal iterative type I Pks contains ACP transacylase domain which accepts the end product of fatty acid biosynthetic pathway, often a six-carbon acyl group (Crawford et al. 2006). Previous studies have shown that all the hexanoyl acceptors contain a conserved alanine group (Vagstad et al. 2012). Our data also showed Ala32 in the hydrophobic region in the interaction with hexanoic acid, whereas absent in interaction of quercetin with ACP transacylase domain. This suggests that both quercetin and hexanoic acid have different binding sites in ACP transacylase domain of PksA. However, the binding specificity of quercetin with ACP transacylase domain was observed to be stronger than hexanoic acid in terms of binding energy (− 6.33 kcal/mol/− 4.74 kcal/mol) and hydrophobic interactions (eight for quercetin and five for hexanoic acid). β-Ketoacyl synthase domain catalyzes extension of enzyme-bound substrate followed by another domain of ketoacyl synthetase that extended to C-terminus (Crawford et al. 2008). In our results, the binding affinity of quercetin in these two domains was found to be more stronger in quercetin than hexanoic acid, as quercetin required less binding energy for β-ketoacyl synthase domain (− 5.6 kcal/mol) as well as ketoacyl synthetase C-terminal (− 7.1 kcal/mol) whereas


PksA Domains
Docked protein structure
Ramachandran plot
ACP transacylase domain
β-ketoacyl synthase domain
Ketoacyl- synthase C- terminal domain
Acyl transferase domain
Product template domain
Phosphopanthetine attachment domain
Thioestrase domain
Psi (degrees)
Phi (degrees)

◂**Fig. 3** Docking of different domains of polyketide synthase A of *Aspergillus flavus* using Autodock software. The red color shows the α-helix and the yellow color shows the β-sheets. Structure stability is validated by the Ramachandran plot using Procheck software. Red color region denotes residues of the respective domains in the most favorable region, yellow color denotes residues in the additionally allowed region, and light shade indicates residues in the generously allowed region

hexanoic acid required − 4.41 kcal/mol and − 3.54 kcal/mol, respectively.

Product template domain is present adjacent to ACP domain and also stabilizes poly-β-keto intermediates by catalysing its stepwise cyclization and aromatization (Udwary et al. 2002; Crawford et al. 2009). Phosphopantetheine localization channel is present in the substrate-binding regions of product template domain that binds to the extended phosphopantetheine arm of the ACP domain helping in substrate transport; the central cyclization cyclizes and accommodates the delivered substrate; and the hexyl-binding region binds to fatty acid starter unit (Liu et al. 2015). Until now, only *Aspergillus parasiticus* product template domain crystal structure has been studied, which is involved in aflatoxin B1 biosynthesis (Crawford et al. 2009). Our in silico analysis showed the active binding of quercetin with product template domain in comparison to hexanoic acid, both forms H bond at Arg15 position, but the stability of quercetin was more due to the formation three extra H bonds at Leu41 and two at Ser37 residues. In addition, quercetin showed stronger hydrophobic interactions in comparison with hexanoic acid due to lesser binding energy in case of quercetin–product template domain complex. Also, phosphopantetheine attachment domain of PksA showed more specific binding

Table 2 Parameters used for protein structural assessment by PROCHECK analysis

Domains	Ramachandran plot (%)	*G* factor	MCBL (%)	CBA (%)	Residues
ACP transacylase domain	81.8[a]; 12.1[b]; 6.1[c]; 0.0[d]	0.21[e]; 0.50[f]; 0.29[g]	96.6	86.3	72
β-Ketoacyl synthase domain	70.8[a]; 25.0[b]; 4.2[c]; 0.0[d]	0.25[e]; 0.62[f]; 0.36[g]	97.8	85.9	28
Ketoacyl synthetase C-terminal domain	88.5[a]; 11.5[b]; 0.0[c]; 0.0[d]	0.16[e]; 0.29[f]; 0.19[g]	96.5	90.6	33
Acyl transferase domain	77.0[a]; 9.6[b]; 1.4[c]; 1.4[d]	0.24[e]; 0.31[f]; 0.24[g]	97.8	89.9	83
PT domain	81.1[a]; 13.5[b]; 5.4[c]; 0.0[d]	0.26[e]; 0.08[f]; 0.17[g]	100	92.0	48
Phosphopantetheine attachment site	81.1[a]; 13.5[b]; 5.4[c]; 0.0[d]	0.33[e]; 0.08[f]; 0.21[g]	100	92.3	48
TE domain	89.6[a]; 6.0[b]; 3.0[c]; 1.5[d]	0.11[e]; 0.13[f]; 0.11[g]	98.7	94.6	79

MCBL distribution of main chain bond length, *CBA* distribution of covalent bond angle

[a]Residue in favored regions

[b]Residue in allowed regions

[c]Residue in generously allowed regions

[d]Residue in disallowed regions

[e]*G* factor score of dihedral bond

[f]G factor score of covalent bond

[g]Overall *G* factor score

Table 3 Comparative analysis of known protein domains and ligands (quercetin and hexanoic acid) on target molecule domains

Ligands and Domains	Binding energy	Inhibition constant (nM)	Intermolecular energy	Electrostatic energy	Total internal energy
Quercetin vs hexanoic acid					
ACP transacylase domain	− 6.33/− 4.74	23.08/327.21	− 6.62/− 6.25	− 0.14/− 2.62	− 0.39/− 0.09
β-Ketoacyl synthase domain	− 5.6/− 4.41	78.19/585.52	− 5.9/− 5.9	− 0.24/− 2.75	− 0.26/− 0.07
Ketoacyl synthetase C-terminal domain	− 7.1/− 3.54	6.3/2.55	− 7.39/− 5.03	− 0.29/− 1.05	− 0.39/− 0.11
Acyl transferase domain	− 6.15/− 3.79	31.17/1.67	− 6.45/− 5.28	− 0.1/− 2.02	− 0.35/− 0.05
Product template domain	− 5.78/− 4.63	58.45/406.11	− 6.07/− 6.12	− 0.18/− 2.38	− 0.37/− 0.08
Phosphopantetheine attachment site	− 5.8/− 4.57	56.08/446.44	− 6.1/− 6.06	− 0.19/− 2.54	− 0.37/− 0.05
Thioesterase domain	− 5.25/− 3.86	141.61/1.49	− 5.55/− 5.35	− 0.11/− 2.93	− 0.38/− 0.07

Table 4 Comparative analysis on the basis of hydrophobic and hydrogen bonding of quercetin and hexanoic acid with different PksA domains of *A. flavus*

Domains (interacting amino acids)	No. of H bonds	H bonding	Bond length (Å)	Hydrophobic bonding
Hexanoic acid vs quercetin				
ACP transacylase domain	1	Lys68	2.82	Leu18, Pro16, Lys17, Arg19, Val15
	1	Leu34	2.86	Met65, Val70, Tyr69, Val66, Leu30, Ala32, Phe33, Leu34
β-Ketoacyl synthase domain	1	Arg21	2.66	Ile19, Ser20
	1	Ile19	2.85	Val33, Ile37, Cys34, Ile30, Ala29, Ala22
Ketoacyl synthetase C-terminal domain	0	-	-	Leu28, pro29, Ser1,Leu7, Ile26
	1	Ile24	2.94	Thr21, Ser1, Ala22, Leu7, Pro29, Leu28, Leu23, Ile26
Acyl transferase domain	1	Arg63	2.88	Lys66, Tyr17, Pro16
	1	Arg63	2.82	Pro16, Lys66, Tyr17, Val71, Ile70, Pro72, Met81, Val52, Leu56
Product template domain	1	Arg15	2.72	Ala39, Leu41
	4	Arg15, Leu41, Ser37	2.63 2.83 2.49, 2.49	Ala39, Ser40, Met16, Ile18, Gln38
Phosphopantetheine attachment site	1	Arg15	2.63	Ala39, Ser40, Leu41
	2	Trp35, Lys22	2.86, 2.78	Arg15, Trp17, Pro19, Met16
Thioesterase domain	3	Asp5, Leu4, Gly3	2.90, 2.89, 2.69	Arg28, Leu10
	2	Lys17, Gly25	3.10, 2.85	Cys16, Pro18, Tyr26, Phe9, Asp24, Ala23

for quercetin in comparison to hexanoic acid in terms of binding energy, H binding and hydrophobic interactions.

However, thioesterase domain with the help of product template domain is involved in the release of product, that is, first aflatoxin biosynthesis intermediate, norsolorinic acid, by catalysing nucleophilic attack on starter ACP domain (Korman et al. 2010; Xu et al. 2013). Sometimes product template domain in collaboration with ketoacyl synthase and thioesterase domain is involved in forming building blocks to hexanoyl starter unit (Du and Lou 2010). In silico approaches on the interaction of quercetin with thioesterase domain of *A. flavus*, PksA, in comparison to hexanoic acid showed more stronger binding of quercetin than hexanoic acid in terms of binding energy which was − 5.25 kcal/mol and − 3.86 kcal/mol, respectively, suggesting quercetin may be involved in the inhibition of norsolorinic acid, hence, inhibiting aflatoxin biosynthesis in *A. flavus*.

Aflatoxin biosynthetic pathway consists of consensus gene cluster which includes 30 genes (Yu 2012). Aflatoxin pathway of *A. flavus* is regulated by *aflR* and *aflS*, which mediate transcriptional activation (Yu et al. 2011). Kim et al. showed the inhibition of *aflR* by gallic acid in *A. flavus* (Kim et al. 2006). Additionally, 4-hydroxy-7-methyl-3-phenyl coumarin and 2,3-dihydrobenzofuran resulted in downregulation of *aflR* and *aflS* in *A. flavus*, respectively (Moon et al. 2017). *aflD* and *aflP* are the clustral genes which encode ketoreductase and methyltransferase activities, respectively (Yu 2012). aflD was also one of the targets of 4-hydroxy-7-methyl-3-phenyl coumarin (Moon et al. 2017). Also, *aflD* and *aflP* transcripts of aflatoxin biosynthesis gene cluster are used to distinguish between toxigenic and non-toxigenic strains of *A. flavus* (Scherm et al. 2005). Hence, reviewing the above literature, we have designed primers of five key genes (*aflR, aflS, aflP, aflD* and *pksA*) of *A. flavus* involved in aflatoxin biosynthetic pathway. Using qRT-PCR, it has been observed that genes encoding for *aflR, aflS, aflP, aflD* and *pksA* were downregulated at 24-h time point in comparison to 7 h in quercetin-treated *A. flavus*. In our previous study, PksA was observed during germination of *A. flavus* conidia that may contribute to aflatoxin biosynthesis. However, polyketide synthase and subsequent enzymes from aflatoxin pathway were found to be inhibited in quercetin-treated *A. flavus* (Tiwari and Shankar 2018). Also, our present data on qRT-PCR showed downregulation of *pksA*, which may suggest that quercetin may be a potent inhibitor of polyketide synthase enzyme. Additionally, in silico data using 3D

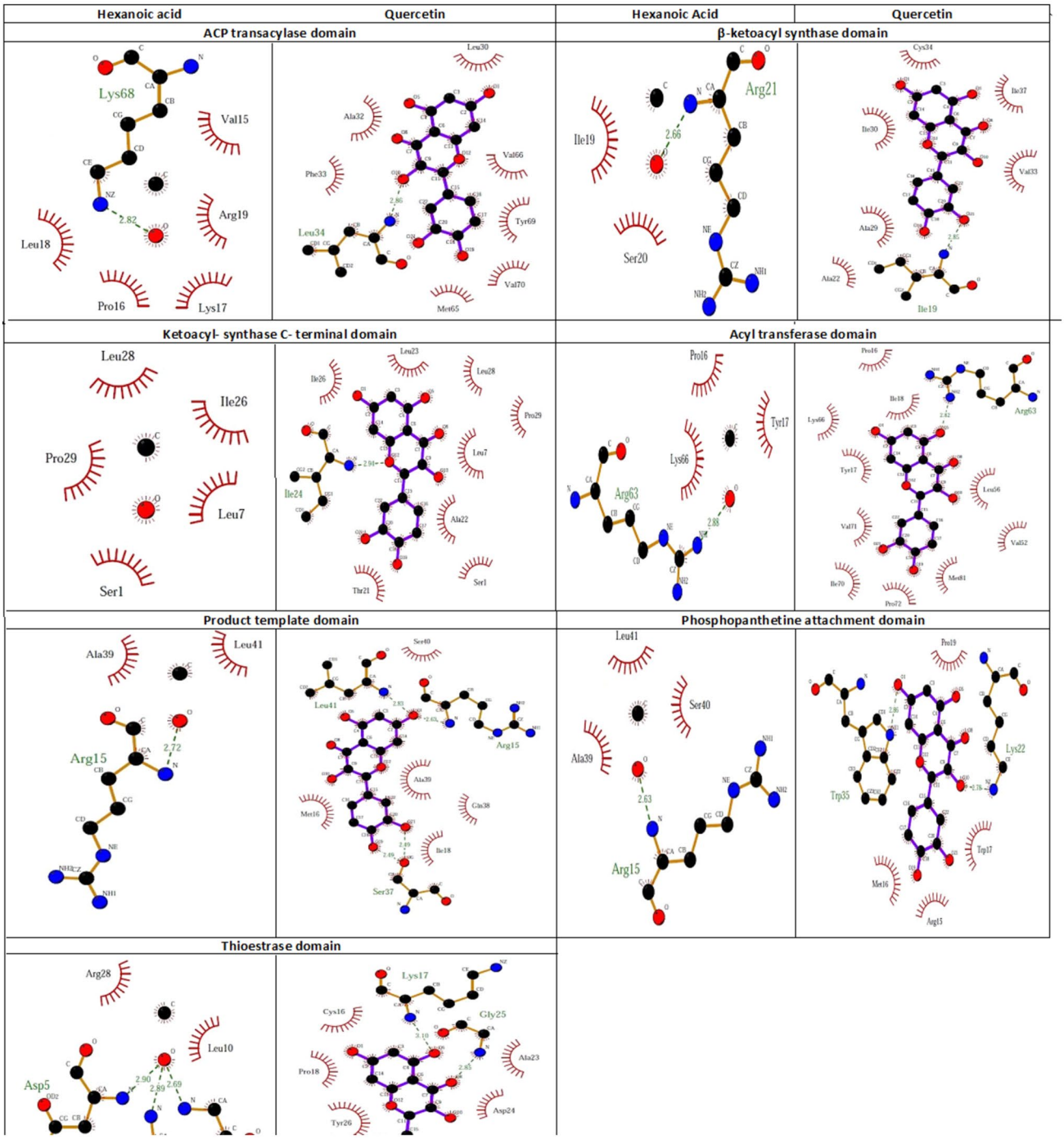


Fig. 4 Postdocking interactions between active residues of seven domains of PksA of *Aspergillus flavus* with two different ligands, quercetin and hexanoic acid showing a schematic drawing of types of interaction of ligands generated using LigPlot

structures of seven domains of PksA enzyme of *A. flavus* on binding energy, electrostatic energy, H bonding, bond length and hydrophobic interaction, the molecular docking of quercetin or hexanoic acid with each domain of PksA showed quercetin as potent candidate for polyketide synthase inhibitor. Thus, computational and selected transcript

Table 5 qRT-PCR analysis of quercetin-treated *Aspergillus flavus*/control at 7 and 24-h time points

S. no.	Gene name	7-h (quercetin-treated *A. flavus*/control) $\log_2$ ratio	24-h (quercetin-treated *A. flavus*/control) $\log_2$ ratio
1	*aflS* (transcriptional factor)	0.22	−2.01
2	*aflP* (O-methyltransferase A)	5.14	−2.6
3	*aflR* (transcriptional factor)	4.5	−5.03
4	*aflD* (reductase)	3.95	−2.39
5	*pksA* (polyketide synthase)	0.36	−1.48

analysis added insight into the quercetin-based inhibition of aflatoxin biosynthesis in *A. flavus* and could serve as a resource for experimental studies.

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Author contributions ST and JS conceived and designed the experiments. ST and SKS performed the experiments. ST, SKS and JS analyzed the data. JS contributed reagents/materials/analysis tools. ST and JS contributed to writing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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