

Original Research Article

Prediction of novel natural inhibitors of avian coccidia (*Eimeria tenella*) through molecular docking

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Abstract

Purpose: To identify some natural molecules (inhibitors) against avian coccidia (*Eimeria tenella*) through molecular docking in order to find the candidate drugs for poultry industry.

Methods: The natural compounds, 6'-de-O-acetylcupacinoside, apigenin, artemisinin, cupacinoside, quercetin and rutin, were screened on the basis of previous research data. These biomolecules were selected as potent inhibitors based on extant literature on calcium-dependent protein kinases (CDPKs) in *Eimeria tenella*.

Results: All the compounds showed good interactions with 6'-de-O-acetylcupacinoside. Based on docking studies, quercetin produced the best interaction, with a binding energy of -7.04 kcal/mol, when compared to the other compounds.

Conclusions: Based on these *in silico* results, the screened compounds have great potential for use against *E. tenella*. In particular, the results suggest that quercetin may be beneficial in the control of avian coccidia through its strong inhibitory potential on CDPKs of *E. tenella*.

Keywords: Apicomplexan, Natural compounds, Calcium-dependent protein kinase (CDPK), *Eimeria*, Docking studies, 6'-Dse-O-acetylcupacinoside, Apigenin, Artemisinin, Cupacinoside, Quercetin, Rutin

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INTRODUCTION

One of the major poultry diseases worldwide is avian coccidiosis which is caused by the parasitic species *Eimeria* [1]. Seven *Eimeria* species which infect chicken have been identified. These are *Eimeria acervulina*, *Eimeria maxima*, *Eimeria praecox*, *Eimeria mitis*, *Eimeria brunetti*, *Eimeria necatrix*, and *Eimeria tenella* [1]. The life cycle of *Eimeria* parasite during its invasion in the intestinal epithelium of chickens is complicated, requiring 4–7 days for completion. The parasite

has 3 different developmental stages in chickens: sporogony, merogony and gametogony [2–4].

The invasion of the parasites in the host begins with its attachment to the host cell. Thus, it is likely that the parasite has a regulatory mechanism for self-invasion and proliferation in the host cells [5]. In order to invade the host cell, the parasites need exocytosis via secretory cellular organelles, rhoptries, micronemes, and dense granules. This mechanism involves some secretory organelles such as rhoptries and micronemes which deliver cargo proteins during

the invasion [6,7]. Calcium is one of the important components needed for cellular invasion of the parasite. The level of cytosolic calcium greatly affects the activities of transporters in cells [8]. The main receptors for Ca^{2+} signals in apicomplexan parasites are calcium-dependent protein kinase (CDPK). These enzymes are characterized as calcium-binding serine/threonine protein kinases [9].

The CDPKs are important proteins containing kinase domains with active sites for serine/threonine protein kinases responsible for phosphorylation of target proteins and another domain i.e., calmodulin-like domain responsible for calcium binding. Apart from the two domains, there is another junction domain that connects calmodulin-like domain and the kinase domain [9-10]. The members of CDPK protein family are not found in animal hosts of these parasites. Therefore, the CDPK protein family may act as potential drug targets for inhibition of parasite invasion in hosts [11].

Recently, it was reported that calmidazolium, trifluoperazine, ophiobolin A and W-7 inhibited calmodulin domain of CDPK protein [11]. Some of these compounds suppress the binding of ATP to the active site of the parasite domain, while some inhibit the binding of the substrate to the kinase, thereby inhibiting the enzyme. In view of these findings, some plant-based compounds were screened for their anti-coccidiosis potential with a view to using them to control *Eimeria* infection in chickens. These phyto-compounds may target CDPKs, and may inhibit the growth of *Eimeria* species at sporogony and merogony stages of their life cycles. Based on this information, 5 natural compounds were screened for their inhibitory effects on *Eimeria* CDPKs [11].

METHODS

Search and retrieval of potential drugs

Based on literature, the following compounds were screened as potential inhibitors of *Eimeria* CDPK: 6'-de-O-acetylcupacinoside, apigenin, artemisinin, cupacinoside, quercetin and rutin. Apigenin is a known kinase inhibitor, while artemisinin is one of the most used and potentially active antimalarial drugs that targets multiple proteins in parasites. One of the targeted proteins is the regulatory subunit of cAMP-dependent protein kinase [12]. Similarly, it has been reported that cupacinoside isolated from *Cupania cinerea* showed antiparasitic activity against *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* [13]. Quercetin inhibits some enzymes such as lipoxigenase

(LOX) and cyclooxygenase (COX). Moreover, it has been reported that quercetin inhibited the phosphorylation of phosphatidylinositol-3-kinase (PI3K)-(p85) tyrosine, while rutin interacted with TGase and TPase domains of glycosyl transferase family protein (PBP2) required for peptidoglycan synthesis in many organisms, thereby inhibiting the enzyme [14].

The structures of these potential drugs were obtained from the structure data file format of chemical database PUBCHEM (<https://pubchem.ncbi.nlm.nih.gov/>).

Furthermore, energy minimization was done for each compound prior to use in docking studies. The structures of the compounds are shown in Figure 1.

Target protein and its active site

Calcium-dependent protein kinase (CDPK) is a vital target in apicomplexan parasite *Eimeria* (www.rcsb.org). The 3-dimensional structure of CDPK from *Eimeria tenella* was obtained from database of Protein Data Bank (www.rcsb.org). The PDB ID of the structure used was 4YSM, and it contained identical chains A and B. Previous studies have shown that the functional domains and important sites associated with *Eimeria* invasion depend on cellular calcium levels, and the targeted site for CDPK inhibition may fall within EF-hands domain. LIGPLOT [<https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/>] was used to identify the target and calcium interactions of bonded contacts at Asp 462, Asp 464, Asp 466, Glu 468 and Glu 473 present in EF-hand domain. Molecular docking was used to identify the best pocket in the protein-containing residues, with bonding contacts predicted by MetaPocket [<https://projects.biotec.tu-dresden.de/metapocket/>].

Molecular docking studies

AutoDock 4.0 was used for molecular docking studies. The software uses Lamarckian Genetic algorithm for docking simulation. Docking was done using 60 conformations/poses, with all other docking parameters set as default. Grid dimensions were set at 60 for x, y and z each, and grid centre was set in line with the active site. Moreover, the protein was set as a rigid molecule, while the ligand was kept flexible. Since the structure contained identical chains, only chain A was kept for docking. Docking analysis was done to find out the best binding conformation/pose of the 6 compounds using Autodock Tools and Discovery studio, and

interaction studies and inspection of the docked complexes were carried out.

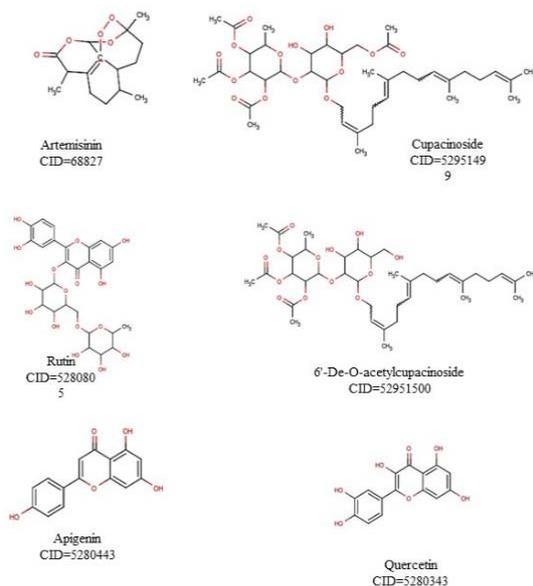


Figure 1: Plant-based natural compounds used as potential drugs against *Eimeria tenella* parasite

RESULTS

Molecular interactions

Eimeria is one of the parasites that affect the chicken industry. In this study, six natural compounds (Figure 1) targeting the CDPK protein were screened. Molecular interactions with 6'-de-O-acetylcupacinoside and cupacinoside showed that the compounds formed hydrogen bonds, but with low binding energies (-0.67 and -1.39 kcal/mol, respectively). Thus, the compounds had weak interactions and were quite unstable. These results are shown in Figure 2 and Table 1. Analysis of the docking of these natural compounds with calcium-dependent protein kinase (CDPK) showed interactions with amino acids via hydrogen bonds, and their respective binding energies were evaluated.

Apigenin, artemisinin, quercetin and rutin formed hydrogen bonds with the target protein, with moderate binding energies. Due to low binding energies, low values of inhibition constant, and H-bond interactions with active site of the protein, apigenin, artemisinin and quercetin had good binding conformations. Apigenin interacted with glutamine (Gln) and asparagine (Asn) with binding energies of -6.44 kcal/mol, whereas artemisinin interacted with valine (Val) with binding energy of -5.83 kcal/mol, indicating good binding stability.

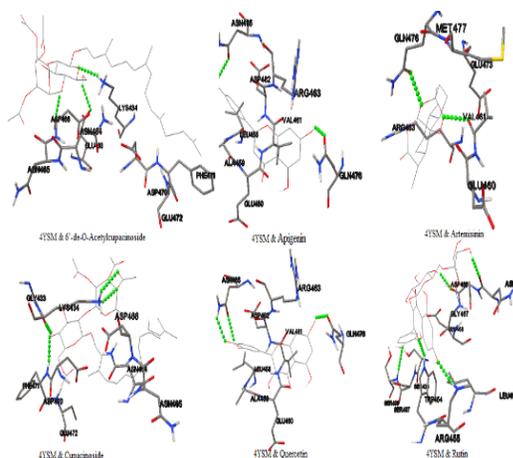


Figure 2: Stick images showing interaction of compounds with Calcium-Dependent Protein Kinase (PDB ID: 4YSM)

The inhibition constants for apigenin and artemisinin were 19.15 and 53.09 μM , respectively. Interactions of quercetin with asparagine (Asn) and glutamine (Gln) generated binding affinity of -7.04 kcal/mol, indicating spontaneous binding. The concentration required to produce half maximum inhibition due to active site pocket interaction with quercetin was 6.94 μM .

The statistical interactions are shown on Table 1. Visual interaction analysis showing the ligand in the protein pocket, pocket residues and formation hydrogen bonds are presented on Figure 2. The present study was aimed at meeting the urgent need for a potent drug against *Eimeria* infections in chicken. Currently, the existing treatments lack cost-effectiveness, safety and efficacy owing to drug resistance.

DISCUSSION

In this study, the plant-based natural compounds 6'-de-O-acetylcupacinoside, apigenin, artemisinin, cupacinoside, quercetin and rutin were selected as inhibitors of avian coccidiosis (*Eimeria*). An earlier report showed that protein kinases from different organisms regulated parasite interaction with host cells through calcium/calmodulin-dependent protein kinases (CDPKs) [11]. These enzymes have also been isolated and characterized from *Eimeria maxima* and *Eimeria tenella* [15]. The therapeutic potential of these compounds have been studied by different investigators. Shukla and Gupta [11] studied the molecular mechanism involved in the antiproliferative and anticancer properties of

Table 1: Docking results for the 6 compounds with CDPK using AutoDock Tools

Compound	Interaction(s)/ bond length(Å)	Binding energy (kcal/mol)	Inhibition constant	Pocket
6'-de-O-Acetylcupacinoside	ASP466:O→ H17/2.048	-0.67	321.46 mM	Yes
	LYS434:HZ3→ O3/2.188			
	GLU468:OE2→ H20/2.15			
Apigenin	GLN476:OE1→ H9/1.841	-6.44	19.15 uM	Yes
	ASN465:HD22→ O5/1.987			
	ASN465:OD1→ H10/2.156			
Artemisinin	VAL461:O→ O4/2.417	-5.83	53.09 uM	Yes
Cupacinoside	GLY433:O→ H17/1.755	-1.39	95.99 mM	No
	LYS434:HZ3→ O13/2.245			
	PHE471:HN→ O9/1.823			
	LYS434:HZ1→ O2/1.998			
Quercetin	ASN465:HD22→O7/2.118	-7.04	6.94 uM	Yes
	GLN476:OE1→H8/1.884			
	ASN465:OD1→H9/2.051			
Rutin	ASP466:O→H167/2.065	-4.24	777.42 uM	Yes
	SER438:HN→O16/2.174			
	ARG455: HE→O14/1.963			
	ASN465:O→H19/1.896			
	TRP:454: HE1→O11/2.108			

apigenin in relation to its modulation of the various tyrosine kinases. It was found that apigenin modulated the MAPK and Akt/PKB pathways through its effects on Rb phosphorylation, CDK, Akt and MAPK pathways, and cell cycle arrest [11]. Salehi *et al* reported that apigenin, the most studied flavonoid inhibited cell cycle, induced apoptosis with lower oxidative stress, and boosted immunity [16]. Other biological roles of apigenin have been reported in cancer cells, hypertension and autoimmune disorder by Zhou *et al* [17]. Wang *et al* determined the mechanism of action of artemisinin and its derivatives at different parasite stages [12]. More recently, it was reported that artemisinin killed parasites by inhibiting various biological processes [12]. The *in vitro* anti-parasitic effect of artemisinin against *Plasmodium* species that cause malaria, as well as its *in vitro* antiviral and antifungal properties have also been reported [12]. Jiao *et al* have reported the inhibitory effects of artemisinin against *E. tenella* infection, and suggested that evasive strategies of intracellular parasites facilitate apoptosis of infected host cells and inhibited their inflammatory responses [18]. In the present study, the docking properties exhibited by artemisinin indicate that it is a promising candidate for treating *Eimeria* infection.

A review of the effects of quercetin on inflammation and immune function by Li *et al* revealed that the mechanism of quercetin action on leukocytes involved targeting of intracellular signaling kinases and phosphatases, as well as enzymes and membrane proteins [14]. The anti-

inflammatory effects of quercetin *in vitro* and in animal studies involved inhibition of activities of PARP-1, cyclooxygenase and lipoxygenase, as well as suppression of arsenite-induced COX-2 expression, thereby blocking the activation of the PI3K signalling pathway [14]. Based on interactions of the 6 compounds with CDPKs, as well as docking analysis, the present study has established that except for 6'-de-O-acetylcupacinoside and cupacinoside, these molecules may be promising inhibitors of *E. tenella*.

CONCLUSION

The data obtained from docking through *in silico* tool AutoDock show that with the exception of 6'-de-O-acetylcupacinoside and cupacinoside, the compounds have good interactions with the target CDPKs. Quercetin has low binding energy and much greater interaction than any of the other compounds. Thus, it may be a potential and promising drug against *Eimeria* infections. This study has revealed that numerous natural molecules or plant-derived compounds may be used to control infections of avian coccidiosis (*Eimeria*) parasite or other parasites at Industrial scale.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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