PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.2972

HORIZON
e-Publishing Group

RESEARCH ARTICLE

Evaluation of the therapeutic potentials of natural compounds against *Eimeria tenella* hexokinase for poultry coccidiosis: bioinformatics and *in-silico* study

Felix Oladele Okunlola[1*], Success Joanna Mishael[1], Okon-Imeh Divine Uwanna[1], Kehinde Foluke Paul-Odeniran[1], Peace Abiodun Olajide[1], Babatunde Oluwafemi Adetuyi[1], Darasimi Deborah Okunlola[1], Ransford Oduro Kumi[3], Philip Obiri Ankomah[3], Captain Cyrille Kablan Nouoma[4] & Mahmoud E S Soliman[2]

[1]Department of Natural Sciences (Biochemistry Option), Faculty of Pure and Applied Sciences, Precious Cornerstone University, Ibadan, Nigeria

[2]Molecular Bio-computation and Drug Design Laboratory, School of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban 4001, South Africa

[3]Faculty of Health and Allied Sciences, Takoradi Technical University, Takoradi, Ghana

[4]Molecular, Forensic and Research Laboratory, Pathology Division, 37 Military Hospital, Ghana Armed Forces

*Email: felixokunlola@pcu.edu.ng

OPEN ACCESS

ARTICLE HISTORY
Received: 22 September 2023
Accepted: 12 February 2024
Available online
Version 1.0 : 01 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

CITE THIS ARTICLE
Okunlola FO, Mishael SJ, Uwanna OD, Paul-Odeniran KF, Adetuyi BO, Okunlola AR. Evaluation of the therapeutic potentials of natural compounds against *Eimeria tenella* hexokinase for poultry coccidiosis: bioinformatics and *in-silico* study. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.2972

Abstract

Eimeria tenella, the causative agent of coccidiosis infection, is primarily found in poultry intestines and is recognized by the formation of clotted, red droppings. It has been found that because chickens have developed a resistance to anticoccidial medications and vaccines, their use alone is no longer as effective. But as a result, researchers have been looking for different treatment approaches to manage this illness and natural products have emerged as interesting possibilities. We used binding energy studies and molecular dynamics modeling to determine the mechanistic inhibitory capability of 5 natural substances against hexokinase (HK). Comparing CPD4 (Zinc 000002111835) to other compounds, the results showed that it had the highest binding activity, with a total binding energy of -32 kcal/mol. The PRED method discovered key CPD4 moieties as well as a number of chemical interactions, including hydrogen bonds, pi-alkyl bonds and pi-anion bonds, that are important to its binding ability. As demonstrated by their consistent complementary interactions over the course of the simulation, PRO160, PHE159, SER158 and ILE240 were important contributors to CPD4's effective binding activity. We suggest CPD4 as a possible lead molecule based on this study in order to address the shortcomings of the available treatment choices and encourage more experimental research towards the development of anticoccidial medications.

Keywords

Coccidiosis; hexokinase; natural compounds; binding residues; MM/ PBSA; PRED

Introduction

Globally, coccidiosis infections in poultry are caused by *Eimeria tenella*, a form of eimeria that causes clotted, bloody droppings to accumulate in the intestines of the birds (1-4). An Eimeria parasite's life cycle is complex and takes 4 to 7 days to finish when it infiltrates a chicken's intestinal epithelium. The parasite develops in hens in three stages: sporogony, merogony and gametogony (4, 5). The start of the parasite's invasion of the host is indicated by its attachment to the host cell. For self-invasion and proliferation in the host cells, the parasite most likely possesses a control system (6). The presence of oocysts in feces is necessary for diagnosis. Prophylactics, anticoccidial medications, and immunization of young birds can all help prevent the spread of infection even though a viable cure has

not yet been found (4). Nowadays, chemoprophylaxis and anticoccidial feed additives are the most used methods of coccidiosis prevention and therapy. Since humans are the ultimate consumers, there is strong opposition to the use of commercial anticoccidial medications and vaccines on animals, even though they are readily available. Although there isn't yet a solution that can permanently guard against coccidiosis, researchers are still looking for other ways to treat the illness and natural remedies have showed a lot of promise in treating chicken coccidiosis (7).

Eimeria tenella's phosphorylation of glucose is catalyzed by the important enzyme hexokinase (HK), whose biochemical activities make it a promising candidate for investigation as a potential coccidiosis therapeutic target (8). It has been discovered that depending solely on vaccinations and anticoccidial drugs is no longer effective because hens have evolved a strong tolerance to them.

Plant extracts, veggies, fruits, herbs, insects and animals all contain natural substances that have historically been utilized to cure a variety of illnesses. These are substances that possess biological properties that can be investigated in the process of finding and creating new drugs (9, 10). Natural chemicals or their derivatives make up most of the best-selling medications on the market. Natural substances are still a significant and vital source of medications for the treatment of illnesses in humans (11). Natural and phytochemical-based chemoprevention has gained a lot of interest lately. It is regarded as a realistic, doable, acceptable and approachable method for managing, reversing, and controlling a variety of illnesses (11, 12). Preclinical and clinical experiments are still being conducted on a number of natural compounds to cure infections (13). The hunt for an alternative to the present anticoccidial medications and vaccines for the treatment of coccidiosis led to the necessity for this investigation. The current treatments are not safe, effective, or cost-effective because of medication resistance. For treating poultry coccidiosis, the current study employed molecular docking, molecular dynamics simulation, and binding energy assesses at the atomistic level to clarify the underlying mechanisms of bonding and different interactions and pharmacophoric moieties involved in the binding procedures of the selected natural compounds regarding the inhibition of HK. The purpose of this study is to illustrate the value of natural chemicals as a substitute treatment for chicken coccidiosis. The findings of this study could provide clinical trial teams with a feasible pharmacological lead, which would likely address the shortfall of current drugs for the treatment of coccidiosis.

Computational Methods

Data Collection, Molecular Docking and Molecular Dynamics (MD) Simulation

With PDB code 6KSR, the hexokinase complex's crystalline x -ray structure was made available by the Protein Data Bank Repository (RCSB PDB). After that, the ZINC database yielded 10000 natural chemicals (14). The protein as well as the ligand files were created and adjusted to help in molecular docking. Then, GAFF was used to optimize the ligand structures in order to reduce them. The AutoDock suite's integrated program Raccoon utilized Maestro software to convert the ligand SDF files to the mol2 format, which was then converted to the pdbqt format (15). The Autodock Vina provided the binding site's required grid box scale and positions prior to docking [center (X = 21.22, Y = 58.20, Z = 73.53) and size (X = 12.26, Y = 14.13, Z = 12.37)]. For the corresponding ligand docking process and calculations, the Autodock Vina was utilized, producing up to eight docked conformations per molecule (16, 17). The compounds with the highest binding affinities to hexokinase after docking were chosen for this investigation. Each of the five compounds' docked conformation was displayed using Chimera's ViewDock plugin (17). Five systems, Hk-cpd-1, Hk-cpd-2, Hk-cpd-3, Hk-cpd-4, Hk-cpd-5, were set up for a 50ns MD run via the graphic processor unit (GPU) in AMBER 18, comprising of LEAP and PMEMD (18). The five selected ligands were subjected to varying degrees of fractional charge using the GAFF charge feature of the ANTECHAMBER program. The AMBER ff14SB was used to parameterize the components of the 5 systems (19). To prepare the PDB file for the LEaP module, we used pdb4amber at a constant pH. In order to neutralize the systems, introducing ions was one of the LEAP module's components (20). Furthermore, TIP3P fluids were added to a 10Å-cubic box for solvating each system (21). Ultimately, prior to the MD simulation, the coordinates and topology of each system were created. Prior to the MD run, we carried out full minimization (1000 steps) without any energy constraint and partial minimization (2500 steps) with a 500 kcal/mol energy constraint potential. Each system was progressively heated from 0 to 300k for 50ps using the NVT canonical ensemble, which included a Langevin thermostat with a harmonic restraint of 5 kcal/mol^2. System equilibration was performed at 1000 ps at 300k and 1 bar of pressure using a Berendsen barostat (22), followed by the 50ns MD run. For subsequent post-MD assessments, the coordinates and trajectory data for each system were generated and saved at intervals of one perisecond using the AMBER18 PTRAJ and CPPTRAJ modules (23). Microcal Origin was responsible for implementing data visualizations and 3D representations (24) and UCSF Chimera respectively (17).

Thermodynamics calculation (MMPBSA.)

The degree of ligand interaction in the binding pocket of a target protein as indicated by binding energy (BE) (25, 26). We estimated the BE of the corresponding bound chemicals in our investigation using Molecular Mechanics Generalized Poisson Boltzmann Surface Area (MM/PBSA) (26,27). For this investigation, the MM/PBSA.py Python script was used in conjunction with AmberTools18, utilizing a continuous solvent model (15). It is an extremely effective, eye-catching and trustworthy analytical instrument (28, 29).

Mathematically,

$$\Delta G_{bind} = G_{complex} - (G_{lig} + G_{rec})$$

$$E_{gas} = E_{ele} + E_{vdw} + E_{int}$$

The components of E_{int} (internal energy), E_{vdw} (van der Waals), and E_{ele} (electrostatic) add up to E_{gas} (gas-phase energy). The product of ΔE_{angle}, ΔE_{bond} and $\Delta E_{torsion}$ yields ΔE_{int}.

$$G_{sol} = G_{PB} + G_{SA}$$

The contributions of GSA (non-polar solvation) and GPB (polar solvation) make up G_{sol} (solvation energy).

$$G_{SA} = \gamma SASA$$

Solvent-accessible surface area, or SASA, is used to measure GSA using a 1.4 Å water probe radius.

Using Molecular Mechanics Generalized Poisson Boltzmann Surface Area (MM/PBSA), we calculated the BE of the corresponding bound compounds in our study.

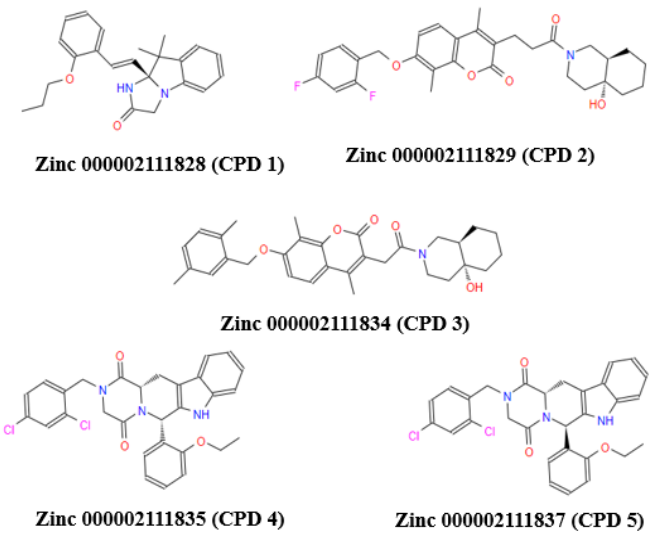

Fig. 1. 2D representations of the five selected natural compounds.

Results and Discussion

Binding energy profiles of selected compounds

Calculating binding energy is a dynamic, economical, and popular method for determining how strongly a pharmaceutical molecule binds to its target. It emphasizes how every binding residue has a role in deciding how stable a ligand is at the binding site. The binding free energies of the chosen compounds were calculated via MD simulation. Since the conformational entropy impact is difficult to estimate using the conventional method (20), and its impact on binding energy is unknown, we did not take it into consideration (28,30,31). Snapshots from 10 to 40 ns showed relative stabilities and were sampled for the energy estimation to minimize conformational entropy. All the compounds had moderately favorable binding energies, as shown in Table 1. But the highest binding energy (ΔGbind) was found for CPD4 (-32.43kcal/mol), revealing its affinity and potential for binding to HK. With values of -23.35 and -23.30 kcal/mol, respectively, CPD2 and CPD5 were the next in line. The two with the lowest binding energies were CPD1 (-17.92 kcal/mol) and CPD3 (-19.97 kcal/mol). According to their ΔGbind, CPD2 and CPD5 showed comparable binding activities in our analysis; however, CPD1 showed the least binding activity. Table 1 illustrates that the highest binding energy for CPD4 was a result of the high van der Waals effect (-44.02 kcal/mol), which was a major factor in CPD4 binding. Additionally, we found that CPD4 had the highest ΔGgas, which represents the combined effects of van der Waals and electrostatic forces that favor CPD4's binding over other substances. Fig. 2 clearly illustrates the important residues that add to the HK-CPD4 complex's binding energy.

PRED Analysis upon ligand's binding

PRED was constructed using MM/PBSA in order to evaluate the energy contribution of crucial site residues implicated in the heterogeneous binding of the selected drugs against HK. As illustrated in Fig. 2, the energy that crucial residues contributed to the stability of the protein-ligand complex was calculated by breaking down the total binding free energy per residue (32). We hypothesised those critical site residues with total binding energies equal to or greater than -0.5 mostly affected CPD4's optimal binding activity. The effectiveness of HK's differential interaction with the chosen ligands was thought to be largely dependent on these residues: ILE 240 (-1.10 kcal/mol), ASN 246 (-0.58 kcal/mol), PHE 159 (-2.14 kcal/mol), PRO 160 (-3.58 kcal/mol), CYS 161 (-0.63 kcal/mol), THR 217 (-0.75 kcal/mol), ILE 240 (-1.10 kcal/mol), SER 158 (-1 kcal/mol), ARG 56 (-0.69 kcal/mol) and CYS 161 (-0.63 kcal/mol). As evidenced by their individual total binding energy contributions, these residues positively impacted the complimentary interactions between HK and CPD4. The relationship between these important residues and HK is shown in Fig. 2. The ligand moieties created various interactions with the residues of the crucial sites, supplying this energy. The chemical architecture of these compounds was the cause of the binding ability and prospective pharmacological benefit of coccidiosis.

Exploring the interaction dynamics of HK with CPD4

To gain further insight into the beneficial interactions of CPD4 in the binding pocket of HK, we investigated the dynamics of the molecular interaction that took place at the different MD time points (snapshots at 10 ns, 20 ns, 30 ns and 40 ns were chosen for this investigation). A clear representation of CPD4 dynamics can be found in Fig. 3. SER 158 consistently produced a typical hydrogen bond at 10, 20 and 40 ns. During the simulation period, GLU267, ASP216 and LYS176 were creating Pi-Anion interaction inconsistently (Fig. 3). Nevertheless, as Fig. 2 illustrates, GLU267's interaction is

Table 1. Binding energy profiles of screened natural compounds against Hexokinase (HK.)

Complexes	Binding Energy Components (kcal/mol)				
	ΔE_{vdw}	ΔE_{ele}	ΔG_{gas}	ΔG_{sol}	ΔG_{bind}
HK-CPD1	-23.50± 0.35	-10.17 ± 0.22	-33.67 ± 0.53	15.74 ± 0.26	-17.92 ± 0.28
HK-CPD2	-34.59 ±0.17	-14.74 ± 0.18	-49.33 ± 0.30	25.98 ± 0.14	-23.35 ± 0.20
HK-CPD3	-30.34 ± 0.15	-17.8 ± 0.16	-48.17 ± 0.25	28.20 ± 0.16	-19.97 ± 0.11
HK-CPD4	-44.02 ± 0.28	-10.56 ± 0.17	-54.59 ± 0.38	22.16 ± 0.19	-32.43 ± 0.21
HKCPD5	-32.34 ± 0.56	-11.13 ± 0.24	-43.48 ± 0.77	20.17 ± 0.38	-23.30 ± 0.41

detrimental to CPD4's ability to bind. In addition to LEU58 and LYS176, which showed somewhat comparable interactions, PRO160, PHE159 and ILE240 also showed several interactions including Pi-Alkyl and Amide-Pi Stacked interactions. Important contributions in the binding activity of CPD4 have been shown for PRO160, PHE159, SER158 and ILE240, as evidenced by their high affinity and persistent complementary multiple interactions to the compound across the simulation time. We discovered that the ligand maintained consistent connections with several of these key residues (Fig. 3), even though the protein structure was always moving. This information was obtained from the interactions revealed by CPD4. These interactions support CPD4's binding capability as well as the significance of natural substances as possible HK inhibitors as a different approach to treating coccidiosis. Natural chemicals' potential for medicinal use has already been investigated via an in silico method (10). The several interactions that CPD 4 elicited with crucial site residues suggest that it may be a blocking agent of *Eimeria tenella* hexokinase, according to the work's findings.

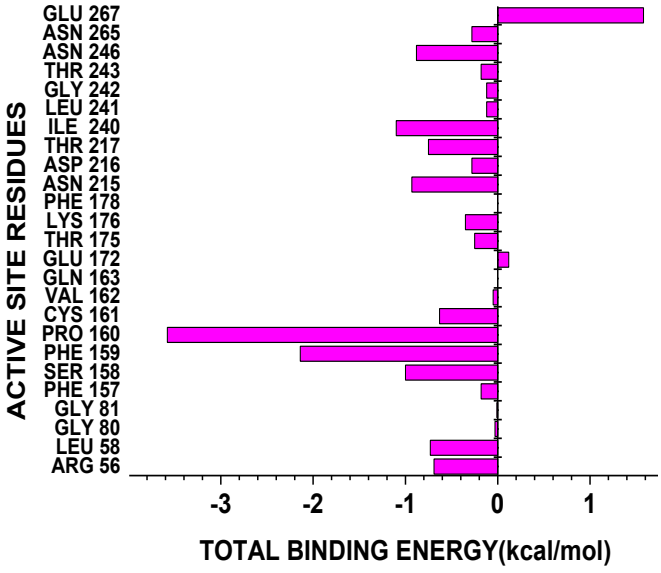

Fig. 2. Plot of energy per-residue decomposition showing key site residues with their respective total energy contributions to the binding activity of CPD4.

Conclusion

Worldwide, coccidiosis in chicken is caused by the eimeria species *Eimeria tenella*. The collection of clotted, bloody droppings is a telltale sign of a chicken intestine coccidiosis infection. Significant damage to the intestinal mucosa is the disease's defining feature and it spreads quickly over 4 to 7 days. Nowadays, the most popular prophylactic and curative approaches are chemoprophylaxis and anticoccidial feed additives. Given that it is a crucial enzyme in the glycolytic pathway that stimulates the body's phosphorylation of glucose, *Eimeria tenella* Hexokinase (HK) is being studied as a potential therapeutic target for coccidiosis. However, no product has been made available for permanent protection against coccidiosis. It has been found that merely using anticoccidial drugs and vaccines is no longer as effective because poultry has developed a resistance to them. Natural products have showed potential in the fight against chicken coccidiosis, but this has led to the quest for other therapeutic approaches to control this disease. To clarify the fundamental binding mechanisms, numerous interactions and various pharmacophoric moieties connected to the binding activities of the selected natural compounds toward the blocking action of HK for the curative purpose of coccidiosis in poultry, the current study used in silico techniques. The purpose of this study is to illustrate the value of natural chemicals as a substitute treatment for chicken coccidiosis. With a binding energy of -32 kcal/mol, CPD4 had the highest binding activity among the 5 compounds that were chosen. The simulation period has demonstrated that PRO160, PHE159, SER158 and ILE240 exhibit continuous complimentary interactions, indicating their significant roles in the successful binding of CPD4. The binding capability of CPD4 and the significance of natural chemicals as possible HK inhibitors as a substitute therapeutic option for coccidiosis are further supported by these interactions. The study's conclusions suggested that natural substances might be good alternatives to *Eimeria tenella* hexokinase inhibitors in the treatment of poultry coccidiosis. These findings could also help develop new inhibitors and medications for HK that have better therapeutic qualities for coccidiosis.

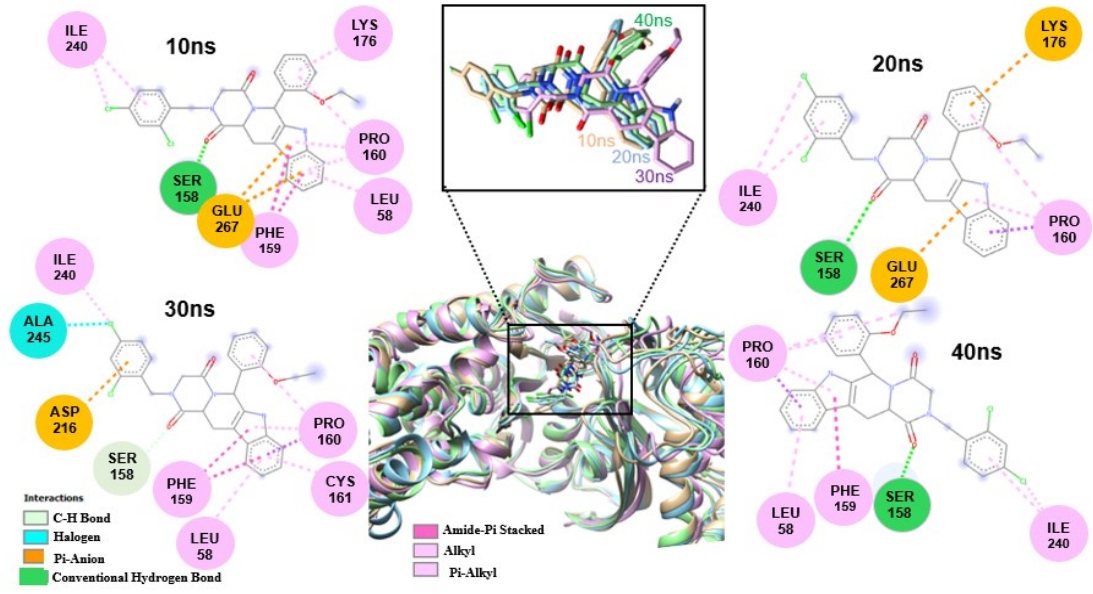

Fig. 3. Molecular interaction dynamics of CPD 4 with the key binding site residues of HK at different nanoseconds.

Acknowledgements

The authors appreciate the Centre for High Performance Computing (CHPC), Cape Town, South Africa, for making computational resources available.

Authors' contributions

FOO, ODU, KFP, SJM participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. FOO, ODU, KFP, SJM, BOA, ARO participated in the sequence alignment. FOO, KFP, BOA, ARO participated in the design of the study and performed the statistical analysis. FOO, KFP conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Chapman HD, Barta JR, Blake D, Gruber A, Jenkins M, Smith NC, et al. A selective review of advances in coccidiosis research. Advances in Parasitology. 2013;83:93-171. https://doi.org/10.1016/B978-0-12-407705-8.00002-1
2. Dalloul RA, Lillehoj HS. Poultry coccidiosis: Recent advancements in control measures and vaccine development. Expert Rev Vaccines. 2006;5(1):143-63. https://doi.org/10.1586/14760584.5.1.143
3. Allen PC, Fetterer RH. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin Microbiol Rev. 2002;15(1):58-65. https://doi.org/10.1128/CMR.15.1.58-65.2002
4. Noack S, Chapman HD, Selzer PM. Anticoccidial drugs of the livestock industry. Parasitol Res. 2019;118(7):2009-26. https://doi.org/10.1007/s00436-019-06343-5
5. Muthamilselvan T, Kuo TF, Wu YC, Yang WC. Herbal remedies for coccidiosis control: A review of plants, compounds and anticoccidial actions. Evidence-based Complement Altern Med. 2016;2016. https://doi.org/10.1155/2016/2657981
6. Schmid-Hempel P. Immune defence, parasite evasion strategies and their relevance for “macroscopic phenomena” such as virulence. Philos Trans R Soc B Biol Sci. 2009;364(1513):85-98. https://doi.org/10.1098/rstb.2008.0157
7. Cobaxin-Cárdenas ME. Natural compounds as an alternative to control farm diseases: *Avian coccidiosis*. Farm Anim Dis Recent Omi Trends New Strateg Treat. 2018; https://doi.org/10.5772/intechopen.72638
8. Sun M, Liao S, Zhang L, Wu C, Qi N, Lv M, et al. Molecular and biochemical characterization of *Eimeria tenella* hexokinase. Parasitol Res [Internet]. 2016;115(9):3425-33. https://doi.org/10.1007/s00436-016-5104-4
9. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv [Internet]. 2015;33(8):1582-614. https://doi.org/10.1016/j.biotechadv.2015.08.001
10. Aljedaie MM, Alam P, Al-Malki ES. Prediction of novel natural inhibitors of avian coccidia (*Eimeria tenella*) through molecular docking. Trop J Pharm Res. 2021;20(7):1441-45. https://doi.org/10.4314/tjpr.v20i7.17
11. Enrico C. Nanotechnology-based drug delivery of natural compounds and phytochemicals for the treatment of cancer and other diseases. Studies in natural products chemistry. 2019;62:91-123. https://doi.org/10.1016/B978-0-444-64185-4.00003-4
12. Forni C, Facchiano F, Bartoli M, Pieretti S, Facchiano A, et al. Beneficial role of phytochemicals on oxidative stress and age-related diseases. BioMed research international.2019;2019(1). https://doi.org/10.1155/2019/8748253
13. Maruca A, Catalano R, Bagetta D, Mesiti F, Ambrosio FA, Romeo I, et al. The Mediterranean Diet as source of bioactive compounds with multi-targeting anti-cancer profile. European Journal of Medicinal Chemistry. 2019;181. https://doi.org/10.1016/j.ejmech.2019.111579 .
14. Irwin JJ, Shoichet BK. for Virtual Screening. 2006;45(1):177-82. https://doi.org/10.1021/ci049714+
15. Omolabi KF, Iwuchukwu EA, Agoni C, Olotu FA, Soliman ME. A probable means to an end: exploring P131 pharmacophoric scaffold to identify potential inhibitors of *Cryptosporidium parvum* inosine monophosphate dehydrogenase. Journal of Molecular Modeling. 2021;27:1-4. https://doi.org/10.1007/s00894-020-04663-3
16. Allouche A. Software News and Updates Gabedit - A graphical user interface for computational chemistry softwares. J Comput Chem. 2012;32:174-82. https://doi.org/10.1002/jcc.21600
17. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera - A visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-12. https://doi.org/10.1002/jcc.20084
18. Case DA, Walker RC, Cheatham TE, Simmerling C, Roitberg A, Merz KM, et al. Amber 18. Univ California, San Fr [Internet]. 2018.
19. Maier JA, Martinez C, Kasavajhala K, Wickstrom L, et al. ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from ff99SB. J Chem Theory Comput. 2015;11(8):3696-713. https://doi.org/10.1021/acs.jctc.5b00255
20. Case DA, Cheatham TE, Darden T, Gohlke H, Luo R, Merz KM, et al. The Amber biomolecular simulation programs. J Comput Chem. 2005;26(16):1668-88. https://doi.org/10.1002/jcc.20290
21. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. J Chem Phys. 1983;79(2):926-35. https://doi.org/10.1063/1.445869
22. Berendsen HJC, Postma JPM, Van Gunsteren WF, Dinola A, Haak JR. Molecular dynamics with coupling to an external bath. J Chem Phys. 1984;81(8):3684-90. https://doi.org/10.1063/1.448118
23. Roe DR, Cheatham TE. PTRAJ and CPPTRAJ: Software for processing and analysis of molecular dynamics trajectory data. J Chem Theory Comput. 2013;9(7):3084-95. https://doi.org/10.1021/ct400341p
24. Seifert E. OriginPro 9.1: Scientific data analysis and graphing software - Software review. J Chem Inf Model. 2014;54(5):1552. https://doi.org/10.1021/ci500161d
25. Kollman PA, Massova I, Reyes C, Kuhn B, Huo S, Chong L, et al. Calculating structures and free energies of complex molecules: Combining molecular mechanics and continuum models. Acc Chem Res. 2000;33(12):889-97. https://doi.org/10.1021/ar000033j
26. Hou T, Wang J, Li Y, Wang W. Assessing the Performance of the MM_PBSA and MM_GBSA Methods. 1. The Accuracy.pdf. J Chem

Inf Model. 2011;51:69-82. https://doi.org/10.1021/ci100275a

27. Genheden S, Ryde U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Expert Opin Drug Discov. 2015;10(5):449-61. https://doi.org/10.1517/17460441.2015.1032936

28. Chaudhary N, Aparoy P. Deciphering the mechanism behind the varied binding activities of COXIBs through Molecular Dynamic Simulations, MM-PBSA binding energy calculations and per-residue energy decomposition studies. J Biomol Struct Dyn. 2017;35(4):868-82. https://doi.org/10.1080/07391102.2016.1165736

29. Gupta A, Chaudhary N, Aparoy P. MM-PBSA and per-residue decomposition energy studies on 7-Phenyl-imidazoquinolin-4 (5H)-one derivatives: Identification of crucial site points at microsomal prostaglandin E synthase-1 (mPGES-1) active site. Int J Biol Macromol [Internet]. 2018;119:352-9. Available from: https://doi.org/10.1016/j.ijbiomac.2018.07.050

30. Yang T, Wu JC, Yan C, Wang Y, Luo R, Gonzales MB, et al. Virtual screening using molecular simulations. Proteins Struct Funct Bioinforma. 2011;79(6):1940–51. https://doi.org/10.1002/prot.23018

31. Weis A, Katebzadeh K, Söderhjelm P, Nilsson I, Ryde U. Ligand affinities predicted with the MM/PBSA method: Dependence on the simulation method and the force field. J Med Chem. 2006;49 (22):6596-606. https://doi.org/10.1021/jm0608210

32. Woods CJ, Malaisree M, Michel J, Long B, et al. Rapid decomposition and visualisation of protein-ligand binding free energies by residue and by water. Faraday Discuss [Internet]. 2014;169:477-99. Available from: https://doi.org/10.1039/C3FD00125C