RESEARCH ARTICLE

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Theoretical studies of phytochemicals with feline infectious peritonitis virus proteins: a search for novel antivirals

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Abstract

Feline Infectious Peritonitis Virus (FIPV) is a highly lethal pathogen affecting cats worldwide. Developing effective antiviral treatments is crucial for managing this disease. This study investigates the potential of flavonoids to act as antiviral agents and allosteric modulators against the FIPV spike protein using molecular docking simulations. Thirteen flavonoids were docked against the FIPV spike protein (PDB ID: 6JX7) in both ligand-free (cleaned) and ligand-bound (uncleaned) states to assess their binding affinities and potential allosteric effects. The docking results revealed that all tested flavonoids exhibited strong binding affinities, with docking scores ranging from -7.9 to -9.6 kcal/mol in the cleaned receptor state. Notably, Hesperidin, Morin, Hesperetin, and Quercetin maintained or even improved their binding affinities in the presence of native ligands, suggesting their potential as allosteric modulators. Comparative analysis of the binding modes in the cleaned and uncleaned receptor states further supports the allosteric modulator potential of Morin, Hesperetin, and Hesperidin. These findings highlight the promising role of flavonoids as antiviral agents and allosteric modulators targeting the FIPV spike protein. Further experimental validation and optimization of these compounds could lead to the development of effective treatments for feline infectious peritonitis. This study provides valuable insights into the application of flavonoids in the management of viral diseases and contributes to the ongoing efforts in antiviral drug discovery.

Keywords: FIPV, Flavonoid, Docking, Allosteric Modulators

Type: Research Article

Subject: Biochemistry and Cell Biology

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INTRODUCTION

Feline Infectious Peritonitis Virus (FIPV) is an important viral pathogen affecting cats worldwide and causes a complex and often fatal disease characterized by systemic inflammation and peritonitis. Recent advancements in understanding the molecular biology of FIPV and therapeutic interventions have provided new insights into the management and potential eradication of the disease. A recent study has highlighted the antiviral activity of an extract from the d3 Chinese herb against FIPV, suggesting a promising avenue for natural component-based therapies (Nishijıma et al., 2023). Furthermore, flavonoids such as isoginkgetin and luteolin have been found to inhibit FIPV replication and exhibit virucidal effects, providing a foundation for the development of flavonoid-based antiviral drugs (Triratapiban et al., 2023).

The strategic application of immunoinformatic approaches to develop FIPV vaccines is also gaining importance. The strategic application of immunoinformatic approaches to develop FIPV vaccines is also gaining importance. A recent study

by (Chawla et al., 2023) has used immunoinformatic techniques to predict and evaluate T-cell epitopes in the spike protein of the virus, paving the way for more targeted vaccine strategies. Another promising research direction is the identification of potential epitopes for FIPV using phylogenetic analysis, which can aid in the design of effective preventive measures (Aksono et al., 2023; Meshram & Gacche, 2015).

Antiviral drugs like GS-441524 and remdesivir have been investigated for their efficacy against FIPV, and GS-441524 has shown promising results in reducing viral replication (Barua et al., 2023). This highlights the potential for re-evaluating current antiviral agents for FIPV management. Additionally, understanding the role of specific viral proteins and genetic factors in FIPV pathogenesis can contribute to the development of targeted therapies. For instance, the ORF7a protein has been found to increase the inflammatory pathology in infected cats, pointing to a critical target for therapeutic intervention (Jiao et al., 2024).

Another study by Zeedan et al. has highlighted the antiviral effects of plant extracts on significant animal viruses, including FIPV. The study suggests that certain plant extracts demonstrate therapeutic potential by inhibiting viral replication and early stages of viral adsorption (Zeedan & Abdalhamed, 2021).

In summary, the research landscape for Feline Infectious Peritonitis Virus is rapidly evolving, with significant contributions from antiviral compounds, immunological approaches, and molecular diagnostic studies. These advancements hold the potential to not only improve the management of FIPV but also provide new insights into coronavirus biology that may be applicable to other species, including humans.

In this study, 13 flavonoids that have shown viral activity in previous studies were selected (Davies & Yáñez, 2012; Kasprzak et al., 2015; Rizk et al., 2018; Paredes et al., 2003), and their docking analyses were performed. The analyses were conducted against the FIPV virus spike protein, both with the ligands removed and in the presence of natural ligands. This approach allowed for the discussion of the competitive advantages of the flavonoid drug candidates against other ligands and the examination of their allosteric modulator potential. The results indicated that the selected flavonoids are both promising drug candidates and potential allosteric modulators.

MATERIALS AND METHODS

A total of 13 flavonoids, which have demonstrated antiviral properties in prior research, were chosen. (Davies & Yáñez, 2012; Kasprzak et al., 2015; Rizk et al., 2018; Paredes et al., 2003). Then, all ligands were obtained in 3D mol2 format from the databases provided in the reference (Pence & Williams, 2010; Groom et al., 2016; Irwin et al., 2012) . Then, using the OPENMM 7 program (Eastman et al., 2017) on Google Colab with Nvidia Tesla A100 80 GB GPUs hardware and gaff parameters (Wang et al., 2004), all ligands were optimized. As the receptor, the structure of the spike protein of feline infectious peritonitis virus strain UU4 obtained by X-ray diffraction was retrieved from the Protein Data Bank (PDB ID: 6JX7) from https://www.rcsb.org/ (Berman, 2000). Next, prepare_receptor4.py and prepare_ligan4.py scripts in MGL TOOLS (Eberhardt et al., 2021; Morris et al., 2009) were used to prepare the molecules for docking. These scripts were used to remove water molecules from the receptor, add polar hydrogens, calculate Kollman charges, and convert the files to pdbqt format. Box dimensions were calculated python script program that I developed, which uses the geometric center of the receptor as a reference. Then, virtual screening was started and docking of all ligands to the receptor was performed using vina.exe, and the results generated by vina were parsed using the vina_split.exe program. The "grep" command in Linux was used to quickly tabulate the results. Finally, the results were visualized using the Discovery Studio Visualizer program (BIOVIA, 2019).

RESULTS AND DISCUSSION

The results of the mini virtual-screening analysis are shown in the second column of Table 1. According to these results, it was concluded that all of the phytochemicals used in this study, which belong to the flavonoid class, are effective against the FIPV. All 13 compounds studied showed a docking score of -7.9 or better. This indicates that they are very potent drug candidates and is an important result of the study in terms of the binding of flavonoids to the FIPV spike protein (Xue et al., 2022).

The unexpectedly high efficacy of the ligands may necessitate a more challenging test. Typically, in the docking process, the water and ligands within the protein designated as the receptor are removed, and then water and ions are added to these "clean" receptors in molecular dynamics simulations. In most cases, the molecular dynamics results indeed support the docking results. Therefore, to address doubts about whether the ligands tested here are truly drug candidates, it would be more appropriate to investigate the allosteric effect rather than proceeding with molecular dynamics simulations using the same ligand and receptor, which are already known in the literature to mostly support

the docking process results. Consequently, in the second stage of testing, the drug candidate ligands were subjected to the docking process with the receptor whose ligands were not removed (uncleaned receptor). This way, the drug candidate ligands will compete with the naturally placed ligands inside the receptor, and the allosteric effect will also be examined. If our drug candidate ligands still show above-average efficacy in this scenario, we can confidently state that these candidates are indeed potent.

The docking results performed with the uncleaned receptor, without removing the natural ligands, are also presented in the third column of the same table (Table 1). When comparing the effects of the ligands in the clean and uncleaned receptors, it is observed that the activities of Morin, Hesperetin, Quercetin, and Hesperidin increase even in the presence of other ligands. Among these, Hesperidin exhibits the best binding score in both scenarios, leading us to conclude that it is the most promising drug candidate in this study. Luteolin and Isoquercitrin showed the same binding score despite the allosteric effect and appear unaffected by the situation, suggesting that they are more stable and predictable drug candidates. By solely examining the docking scores without conducting molecular dynamics simulations, we can also infer that these two ligands are not affected by competition with other ligands, and their drug activities within the cell will not decrease, at least in terms of competition with other ligands. It can be observed that Scutellarin, Taxifolin, Biochanin A, and Naringenin are slightly negatively affected by the allosteric effect. Catechin, Kaempferol, and Fisetin appear unable to compete with other ligands, with Fisetin showing the worst binding energy in both scenarios. However, it is still possible to say that even a -7.40 kcal/mol docking score is sufficient to keep it on the list of drug candidates.

The ability of the flavonoid drug candidates to bind to the protein even in the presence of other ligands suggested that they may act as allosteric modulators. As we will provide more detailed information in the conclusion section, allosteric modulators are highly important in drug targeting. We can determine whether a drug candidate possesses allosteric modulator properties by examining where it binds in the absence and presence of allosteric effects.

In the figures below, Figure 1 and Figure 5 show the binding modes of Morin to the cleaned and uncleaned receptors, respectively, and they are quite different from each other. In the clean receptor, the binding modes involve ASN C:3266, PHE C:3267, LYS C:3265, ASP C:3471, and GLN C:3685, whereas in the uncleaned receptor, these have changed to ASN A:776, GLN A:1195, and GLN A:979. Similarly, Figure 2 and Figure 6 for Hesperetin, and Figure 4 and Figure 8 for Hesperidin, show that they bind to very distant regions in the two different versions of the same receptor. This suggests that Morin, Hesperetin, and Hesperidin could be allosteric modulators. In the binding modes of Quercetin in Figure 3 and Figure 7, there is a situation where in Figure 3, Quercetin binds to PHE A:777, ASN A:776, LEU A:983, GLN A:979, GLN A:1195, and PHE A:1194, while in Figure 7, it binds to PHE A:777, LEU A:990, GLN A:979, and ASP A:981. Although it binds to different amino acids in both cases, its affinity for the PHE A:777 amino acid is high. However, it still has the potential to be an allosteric modulator, and this can be clarified with further studies.

Table 1. Docking Score of flavonoids with a ligand-free (clean) and uncleaned receptor (PDB ID: 6JX7)

Ligands	**Docking Score with Cleaned Receptor(S2) (kcal/mol)**	**Docking Score with Uncleaned Receptor(S1) (kcal/mol)**	**Difference (S2-S1)**
Hesperidin	-9.6	-9.7	0.1
Scutellarin	-9.2	-9.1	-0.1
Catechin	-8.5	-8	-0.5
Isoquercitrin	-8.5	-8.5	0
Kaempferol	-8.5	-8	-0.5
Taxifolin	-8.5	-8.4	-0.1
Biochanin A	-8.2	-8.1	-0.1
Morin	-8.2	-8.6	0.4
Quercetin	-8.2	-8.3	0.1
Hesperetin	-8	-8.4	0.4
Luteolin	-8	-8	0
Naringenin	-8	-7.8	-0.2
Fisetin	-7.9	-7.4	-0.5

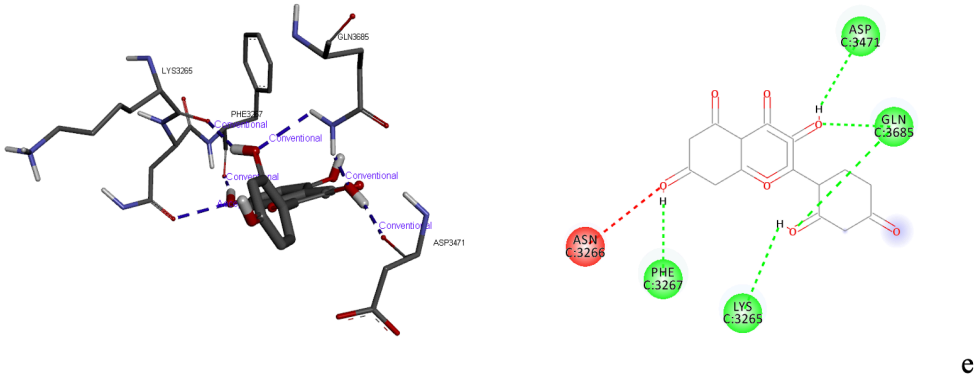

Figure 1. Morin's binding sites with clean receptor

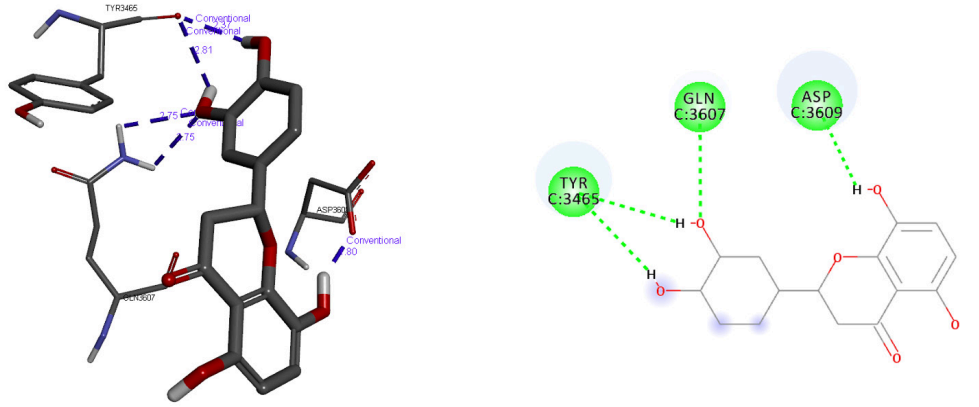

Figure 2. Hesperetin's binding sites with clean receptor

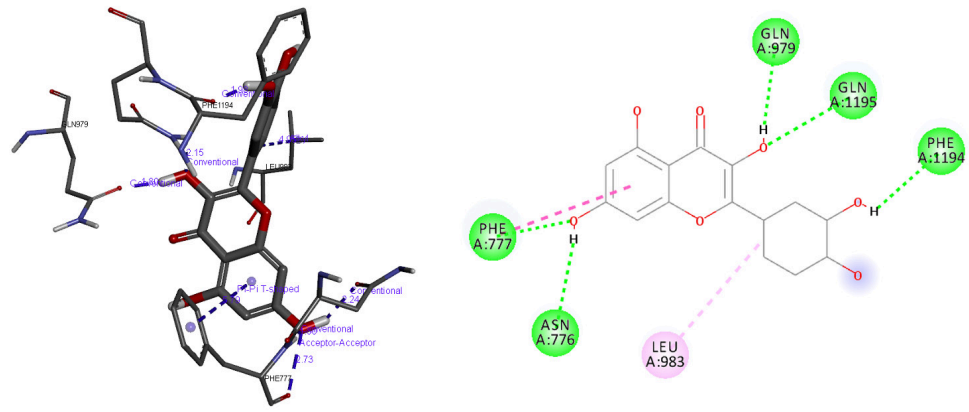

Figure 3. Quercetin's binding sites with clean receptor

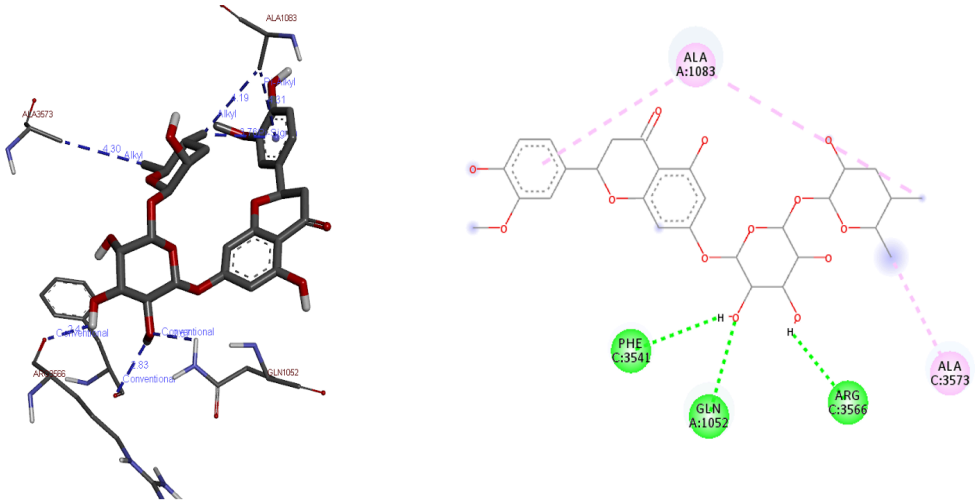

Figure 4. Hesperidin's binding sites with clean receptor

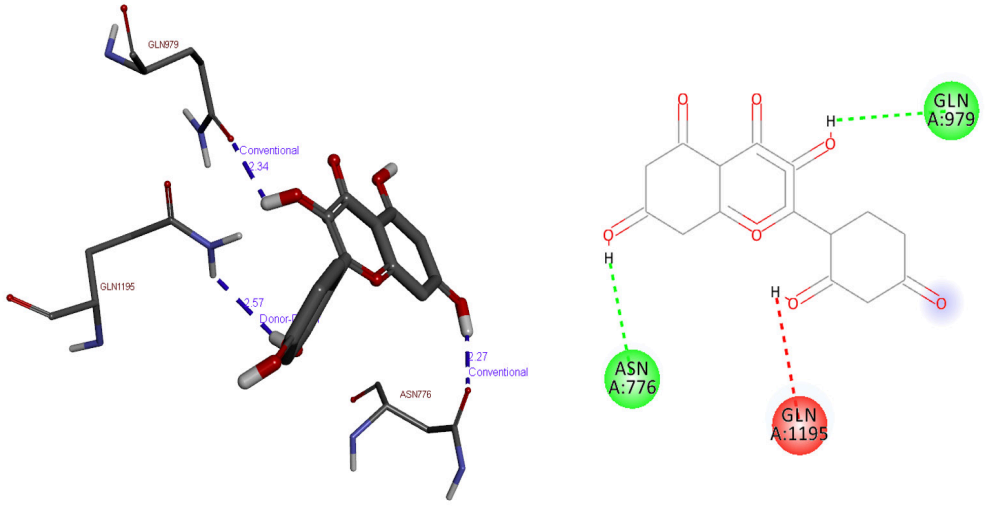

Figure 5. Morin's binding sites with uncleaned receptor

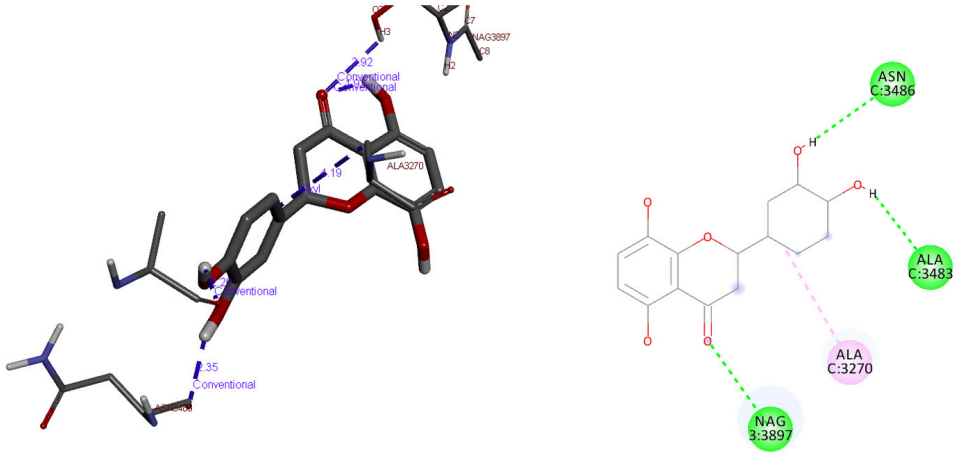

Figure 6. Hesperetin's binding sites with uncleaned receptor

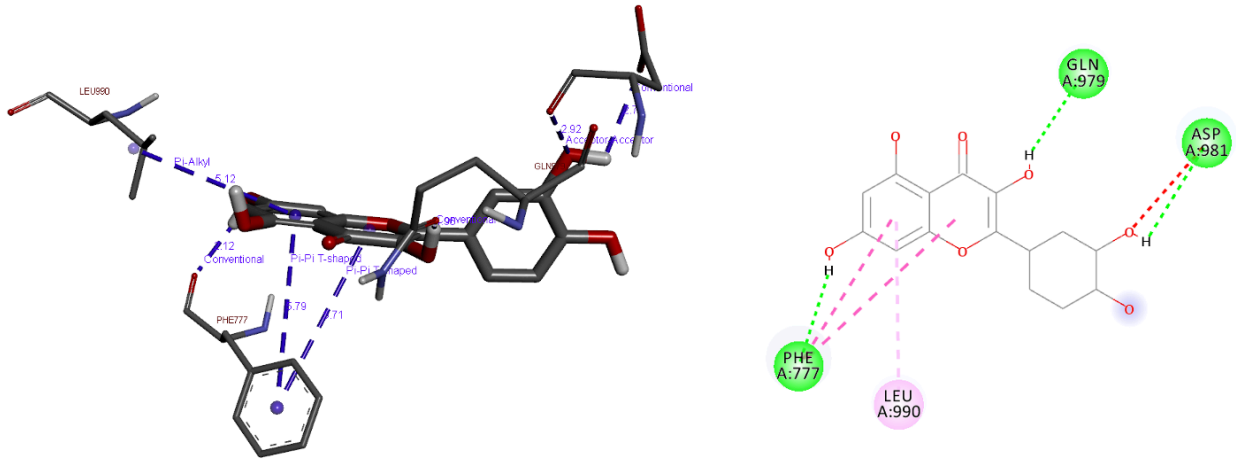

Figure 7. Quercetin's binding sites with uncleaned receptor

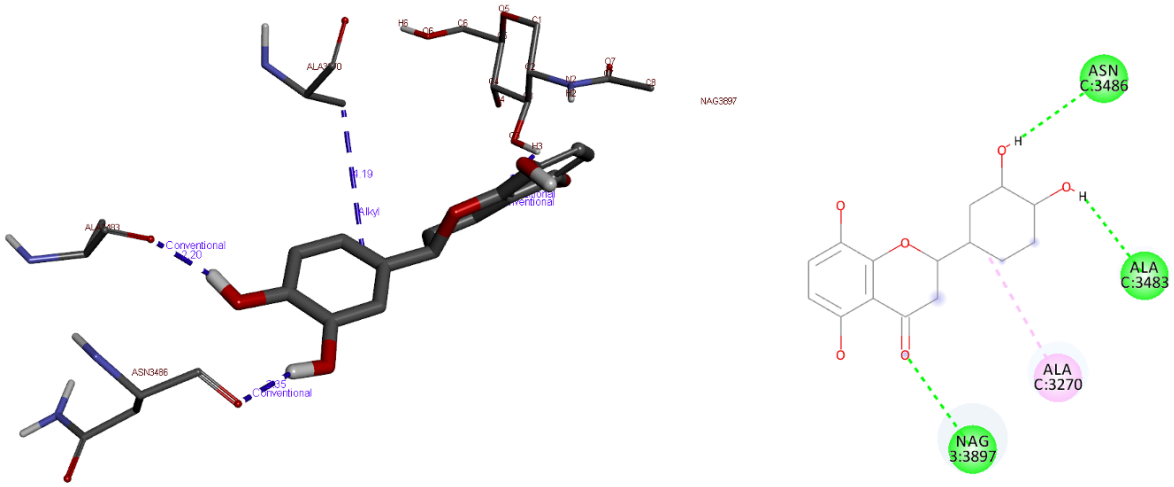

Figure 8. Hesperidin's binding sites with uncleaned receptor

CONCLUSION

Feline Infectious Peritonitis Virus is a highly lethal virus for cats, and efforts to develop drugs for its rapid, inexpensive, and effective treatment are ongoing. In this study, the potential outcomes of applying the general binding affinities exhibited by flavonoids against viral spike proteins to this virus were discussed, and the situation was elucidated through docking studies. Initially, a standard docking process was performed, where the virus spike protein was cleaned of its own ligands and water, and docking studies were conducted with 13 selected flavonoids, yielding surprisingly good results. Subsequently, the effect of these flavonoids on the viral protein in the presence of other ligands was investigated, and a second docking study was performed without removing the ligands from the protein. The results of both studies are presented in Table 1, indicating that flavonoids achieve a considerable binding score to the viral spike protein in both scenarios. At this stage, the study was taken a step further to investigate whether the flavonoid drug candidates act as allosteric modulators.

Allosteric modulators bind to the allosteric regions of proteins, altering their shape and function. This property is utilized to modulate the biological activity of proteins and offers great potential, particularly in drug discovery. They can induce conformational changes in the viral membrane spike protein, which may affect interactions with cell surface receptors (Markwell et al., 1985). These changes can influence the virus's ability to bind to and fuse with cells, thereby affecting infectivity (Clapham & McKnight, 2002). Furthermore, these modulators can weaken the interfaces

of the viral spike protein by targeting specific regions that directly interact with the body (such as ACE2 for COVID-19), thereby reducing viral infectivity. This targeting can provide a strategic advantage in antiviral defenses (Olotu et al., 2020).

In summary, allosteric modulators can provide higher target selectivity compared to traditional orthostatic ligands, helping to reduce side effects and increase drug efficacy. In this study, the allosteric modulator status of flavonoids that successfully competed for binding with other ligands under allosteric effects was examined, and positive results were obtained. In particular, it was found that Morin, Hesperetin, and Hesperidin could not only be good drug candidates against the FIPV spike protein but also potential allosteric modulator molecules.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

No conflict of interest is declared by the author.

Author contribution

Bariş KURT designed the study, conducted the research, analyzed the data, and wrote the manuscript.

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Data availability

All data are provided in the manuscript.

Consent to participate

Not applicable

Consent for publication

Not applicable

REFERENCES

Aksono, E. B., Iradatya, K. R., Sucipto, T. H., Fajar, N. S. & Yuniarti, W. M. (2023). Phylogenetic analysis of feline infectious peritonitis virus, feline enteric coronavirus, and severe acute respiratory syndrome coronavirus 2 of cats in Surabaya, Indonesia. Veterinary World, 76–81. https://doi.org/10.14202/vetworld.2023.76-81

Barua, S., Kaltenboeck, B., Juan, Y.-C., Bird, R. C. & Wang, C. (2023). Comparative Evaluation of GS-441524, Teriflunomide, Ruxolitinib, Molnupiravir, Ritonavir, and Nirmatrelvir for In Vitro Antiviral Activity against Feline Infectious Peritonitis Virus. Veterinary Sciences, 10(8), 513. https://doi.org/10.3390/vetsci10080513

Berman, H. M. (2000). The Protein Data Bank. Nucleic Acids Research, 28(1), 235–242. https://doi.org/10.1093/nar/28.1.235

BIOVIA. (2019). Discovery Studio Visualizer. Dassault Systèmes.

Chawla, M., Cuspoca, A. F., Akthar, N., Magdaleno, J. S. L., Rattanabunyong, S., Suwattanasophon, C., Jongkon, N., Choowongkomon, K., Shaikh, A. R., Malik, T. & Cavallo, L. (2023). Immunoinformatics-aided rational design of a multi-epitope vaccine targeting feline infectious peritonitis virus. Frontiers in Veterinary Science, 10. https://doi.org/10.3389/fvets.2023.1280273

Clapham, P. R. & McKnight, Á. (2002). Cell surface receptors, virus entry and tropism of primate lentiviruses. Journal of General Virology, 83(8), 1809–1829. https://doi.org/10.1099/0022-1317-83-8-1809

Davies,N.M.&Yáñez,J.A.(Eds.).(2012).FLAVONOIDPHARMACOKINETICS.Wiley.https://doi.org/10.1002/9781118468524

Eastman, P., Swails, J., Chodera, J. D., McGibbon, R. T., Zhao, Y., Beauchamp, K. A., Wang, L.-P., Simmonett, A. C., Harrigan, M. P., Stern, C. D., Wiewiora, R. P., Brooks, B. R. & Pande, V. S. (2017). OpenMM 7: Rapid development of high performance algorithms for molecular dynamics. PLOS Computational Biology, 13(7), e1005659. https://doi.org/10.1371/journal.pcbi.1005659

Eberhardt, J., Santos-Martins, D., Tillack, A. F. & Forli, S. (2021). AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. Journal of Chemical Information and Modeling, 61(8), 3891–3898. https://doi.org/10.1021/acs.jcim.1c00203

Gamil Zeedan, G. S. & Abdalhamed, A. M. (2021). Antiviral Effects of Plant Extracts Used in the Treatment of Important Animal Viral Diseases. World's Veterinary Journal, 11(4), 521–533. https://doi.org/10.54203/scil.2021.wvj67

Groom, C. R., Bruno, I. J., Lightfoot, M. P. & Ward, S. C. (2016). The Cambridge Structural Database. Acta Crystallographica Section B Structural Science, Crystal Engineering and Materials, 72(2), 171–179. https://doi.org/10.1107/S2052520616003954

Irwin, J. J., Sterling, T., Mysinger, M. M., Bolstad, E. S. & Coleman, R. G. (2012). ZINC: A Free Tool to Discover Chemistry for Biology. Journal of Chemical Information and Modeling, 52(7), 1757–1768. https://doi.org/10.1021/ci3001277

Jiao, Z., Wang, P., Hu, X., Chen, Y., Xu, J., Zhang, J., Wu, B., Luo, R., Shi, Y. & Peng, G. (2024). Feline infectious peritonitis

virus ORF7a is a virulence factor involved in inflammatory pathology in cats. Antiviral Research, 222, 105794. https://doi.org/10.1016/j.antiviral.2024.105794

Kasprzak, M. M., Erxleben, A. & Ochocki, J. (2015). Properties and applications of flavonoid metal complexes. RSC Advances, 5(57), 45853–45877. https://doi.org/10.1039/C5RA05069C

Markwell, M. A., Portner, A. & Schwartz, A. L. (1985). An alternative route of infection for viruses: entry by means of the asialoglycoprotein receptor of a Sendai virus mutant lacking its attachment protein. Proceedings of the National Academy of Sciences, 82(4), 978–982. https://doi.org/10.1073/pnas.82.4.978

Meshram, R. J. & Gacche, R. N. (2015). Effective epitope identification employing phylogenetic, mutational variability, sequence entropy, and correlated mutation analysis targeting NS5B protein of hepatitis C virus: from bioinformatics to therapeutics. Journal of Molecular Recognition, 28(8), 492–505. https://doi.org/10.1002/jmr.2466

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry, 30(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Nishijıma, R., Endo, T., Gankhuyag, E., Khın, S. T. M. M., Jafar, S. M., Shinohara, Y., Tanaka, Y., Sawakamı, K., Yohda, M. & Furuya, T. (2023). Detection of anti-feline infectious peritonitis virus activity of a Chinese herb extract using geneLEAD VIII, a fully automated nucleic acid extraction/quantitative PCR testing system. Journal of Veterinary Medical Science, 85(4), 22–0185. https://doi.org/10.1292/jvms.22-0185

Olotu, F. A., Omolabi, K. F. & Soliman, M. E. S. (2020). Leaving no stone unturned: Allosteric targeting of SARS-CoV-2 spike protein at putative druggable sites disrupts human angiotensin-converting enzyme interactions at the receptor binding domain. Informatics in Medicine Unlocked, 21, 100451. https://doi.org/10.1016/j.imu.2020.100451

Paredes, A., Alzuru, M., Mendez, J. & Rodríguez-Ortega, M. (2003). Anti-Sindbis Activity of Flavanones Hesperetin and Naringenin. Biological and Pharmaceutical Bulletin, 26(1), 108–109. https://doi.org/10.1248/bpb.26.108

Pence, H. E. & Williams, A. (2010). ChemSpider: An Online Chemical Information Resource. Journal of Chemical Education, 87(11), 1123–1124. https://doi.org/10.1021/ed100697w

Sameh A Rizk1, MohyEldin A Abdel-Atti, A. E. A.-R. (2018). Regio-Selective Reaction, Spectroscopic Characterization and Computational Chemical Study of (Hesperidin) Hesperetin-7-O-Rutinoside Analogs as Antimicrobial Agents. Journal of Chemistry: Education Research and Practice, 2(1). https://doi.org/10.33140/JCERP.02.01.13

Triratapiban, C., Lueangaramkul, V., Phecharat, N., Pantanam, A., Lekcharoensuk, P. & Theerawatanasirikul, S. (2023). First study on in vitro antiviral and virucidal effects of flavonoids against feline infectious peritonitis virus at the early stage of infection. Veterinary World, 618–630. https://doi.org/10.14202/vetworld.2023.618-630

Wang, J., Wolf, R. M., Caldwell, J. W., Kollman, P. A. & Case, D. A. (2004). Development and testing of a general amber force field. Journal of Computational Chemistry, 25(9), 1157–1174. https://doi.org/10.1002/jcc.20035

Xue, Q., Liu, X., Russell, P., Li, J., Pan, W., Fu, J., Zhang, A., 2022. Evaluation of the binding performance of flavonoids to estrogen receptor alpha by Autodock, Autodock Vina and Surflex-Dock. Ecotoxicol. Environ. Saf. 233, 113323. https://doi.org/10.1016/j.ecoenv.2022.113323