Depicting Molecular Interactions of Luteolin with Human Cytomegalovirus Envelope Glycoprotein N and Glycoprotein H: An *In Silico* Approach

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Abstract

Human cytomegalovirus (HCMV) poses a significant threat causing congenital viral infection in new-borns and in individuals with weakened immune system. The rise of drug resistance in one or more approved antiviral medications, has created an urgent need for the development of the new antiviral medications. This study explored the role of a plant-derived flavonoid luteolin, as a potential antiviral agent targeting human cytomegalovirus glycoproteins N and H by molecular docking. Using computational methods, we investigated luteolin's interaction with two key HCMV envelope glycoprotein N (UniProt ID:F5HHQ0) and envelope glycoprotein H (UniProt ID:Q6SW67). Molecular docking performed with AutoDock Vina 4.2 predicted binding affinities and assessed luteolin's drug-likeness using Lipinski's Rule of Five via SwissADME. Luteolin demonstrated higher binding affinities of -6.4 kcal/mol binding energy with Envelope glycoprotein N, forming hydrogen bonds with Thr52(A), Thr135(A) and Cys116(A).

Against Envelope glycoprotein H, luteolin has a stronger binding affinity of -7.1 kcal/mol, interacting with Ser577(A) (two bonds) and Ile666(A). Notably, luteolin met Lipinski's criteria for drug-likeness. This study highlights luteolin as a promising novel antiviral against human cytomegalovirus, exhibiting higher binding affinities to envelope glycoproteins. This warrants further experimental investigation to investigate the efficacy of luteolin as a potential antiviral agent against HCMV.

Keywords: HCMV, Luteolin, Molecular docking, Glycoprotein N, Glycoprotein H, Antiviral

Introduction

Human cytomegalovirus (HCMV) is the most common viral infection globally, impacting a significant portion of the population, affecting approximately 60% populations in developing countries and the seroprevalence ranges up to 99% in developing countries[6,22]. Primary infection by HCMV was usually asymptomatic or mild in immunocompetent individuals, however it can be sometime fatal in immunocompromised persons and congenital infection in new-borns[3,14,25]. Once infected, HCMV can cause latent infection that lasts for the entire life of the host escaping from the host immune system[12,41]. It is one of the most common prenatal infections that can cause mental retardation and sensorineural hearing loss in neonates[18,29]. In organ transplant recipients, HCMV causing serious disease cause morbidity and mortality[23].

The HCMV is a beta-herpesvirus and consists of a double-stranded linear DNA molecule with an inner core encased in a thick layer of tegument protein which is surrounded in a lipid bilayer envelope. Its genome is approximately 240 kbp in size, with over 750 open reading frames (ORFs) with distinguishable long (UL) and short (US) segments that are flanked by internal and terminal repeats[23,27]. HCMV envelope glycoproteins responsible for binding to the host cell, viral entry and the majority of the envelope glycoproteins shows greater antigenic properties[2,19]. The most prevalent glycoproteins in the HCMV envelope are gB, gN and gH and elicits strong immune response in humans and induces the production of neutralizing antibodies[11, 26, 38]. Targeting on these glycoproteins in the development of antiviral drugs and vaccines will significantly aid in reducing HCMV infections.

Ganciclovir, valganciclovir (ganciclovir prodrug) is the most common treatment for CMV disease while foscarnet or cidofovir are substitutes[37], but challenges persist, including issues related to toxicity. Further, prolonged use of these antivirals medications has led to the emergence of HCMV resistance strains. The rising occurrence of antiviral drug resistance underscores the urgent need for development of new antiviral therapies. In these scenarios, the antiviral activities of phytochemical such as flavonoids may prove beneficial as novel antiviral agent. Plants, fruits and seeds contain high concentrations of flavonoids, a class of secondary chemical compounds that exhibit bioactive properties that are associated with a variety of health benefits including anti-inflammatory, anti-cancer, anti-aging, immunomodulatory, antidiabetic, antibacterial and antiviral activity among others[8,13].

Selected studies have shown luteolin, a natural flavonoid found in many plants showing antiviral properties, effectively inhibiting coronavirus and influenza virus, rotavirus among other viruses[21]. Recent developments in computational approaches have accelerated the process of drug design, discovery and development by analysing the biological and pharmacokinetic characteristics of lead compounds prior to entering pre-clinical or clinical trials[7,32].

Drug development can be performed more rapidly and at a cheaper cost by early detection of compounds with both desirable and undesirable properties utilising computational approaches. Computational approaches such as molecular docking and molecular dynamics are widely used in drug design, discovery and development to better understand the molecular processes[4,42]. In this *in silico* study, we examined the antiviral activity of phytocompound luteolin a plant-derived flavonoid luteolin, as a potential antiviral agent by molecular docking targeting Human Cytomegalovirus (HCMV) glycoproteins N and glycoprotein H. This approach will enable us to evaluate phytocompound's ligand-protein binding affinity for a specific viral surface protein.

Material and Methods

Human cytomegalovirus target selection: A comprehensive literature review was conducted to facilitate the selection of specific consensus target in human cytomegalovirus using Merlin AY446894 strain encoding target eliciting neutralizing antibodies. Human cytomegalovirus glycoproteins N (HCMV UL73) and glycoprotein H (UL75) consensus region were used as a target selected for analysis. Structural details of these targeted proteins were obtained from Uniport database [https://www.uniprot.org/][1,5]. The domain regions of the glycoproteins were determined using InterProScan server[30]. Three-dimensional structure generated using MODELLER tool (version 10.4) and 3-D structures were validated using Ramachandran plot[9,10,15]. Further the targeted proteins were subjected to an *in silico* analysis.

Identification and selection of candidate compounds: To identify phytocompounds with antiviral activities, a comprehensive literature review was conducted. Promising compounds identified through this review were subsequently selected for further analysis. Detailed information about the selected compound was retrieved using the PubChem database [https:// pubchem.ncbi.nlm.nih.gov/][16]. The naturally occurring flavonoid, Luteolin [PubChem ID: 5280445] based on previous study was selected as the compound for examining the antiviral activity[21,40].

Molecular docking using AutoDock 4.2 tool: A molecular docking analysis was performed using Auto Dock Vina (Scripps Research, USA) to evaluate the interactions of selected compound with target proteins in Human Cytomegalovirus (strain Merlin)[36]. The phytocompound was converted to .pdb format utilizing the Discovery studio tool[28]. The active binding site of these proteins was identified through the CastP server[35]. Auto Dock 4 tool was used where proteins were given Kollman charges and the grid's size and spacing were established[31]. Binding energies of the protein-ligand complex were deduced. The presence of intermolecular interactions between protein-ligand complexes was shown using the LigPlot+tool[17].

Druglikeness Prediction: The SwissADME platform (http://www.Swiss) was used for drug likeness prediction following Lipinski's rule of five which limits the molecular weight (MW) to 500 Da, H-bond donors to 5, log P to 5 and H-bond acceptors to ten[20].

Results

Homology modelling and Structural validation: For the *in silico* analysis, two specific proteins from HCMV, namely envelope glycoprotein N and envelope glycoprotein H, were selected. The detail information of these selected proteins was obtained from the UniProt database (Table 1). For envelope glycoprotein N, the UniProt id was found to be F5HHQ0 with a sequence length of 135 amino acid and no 3D-structure was idenitifed for this protein. The envelope glycoprotein H, the UniProt id was Q6SW67 with long sequence length of 742 amino acid and the 3D structure was derived from PDB database with PDB id 7LBG.

Domain region of envelope glycoprotein H sequence was determined using the InterProScan server which was found between 570-708 amino acids (Figure 1). The three-dimensional model of envelope glycoprotein N was generated using the MODELLER tool (version 10.4) (Figure 2 and Robetta server respectively). After modeling, the 3-D structures were validated using the Saves server by plotting the Ramachandran plot.

In Ramachandran plot of Envelope glycoprotein N, it was found that residues in most favored regions are 113 (91.1%), residues in additional allowed regions are 10 (8.1%), residues in generously allowed regions are 1 (0.8%) and there were no residues in the disallowed region, number of non-proline and non-glycine residues are 124 (100%), number of end-residues are (excluding Gly and Pro), number of glycine residues are (shown as triangles) in 4 and number of proline residues are 5 out of total 135 number of residues analyzed in Ramachandran plot (Figure 3).

Table 1
Target proteins information of human cytomegalovirus glycoprotein N and glycoprotein H

S.N.	Protein name	Entry ID (UNIPROT)	Gene Name	Organism
1.	Envelope glycoprotein N	F5HHQ0	UL 73	Human cytomegalovirus (strain Merlin) (HHV-5) (Human herpesvirus 5)
2.	Envelope glycoprotein H	Q6SW67	UL 75	Human cytomegalovirus (strain Merlin) (HHV-5) (Human herpesvirus 5)

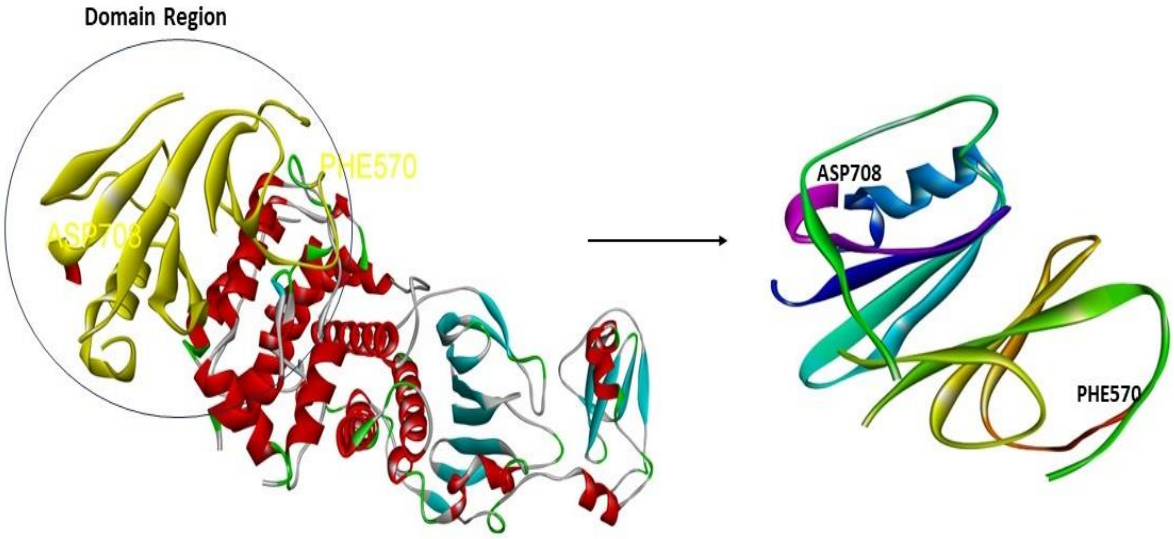


Figure 1: Domain region of the human cytomegalovirus envelope glycoprotein H

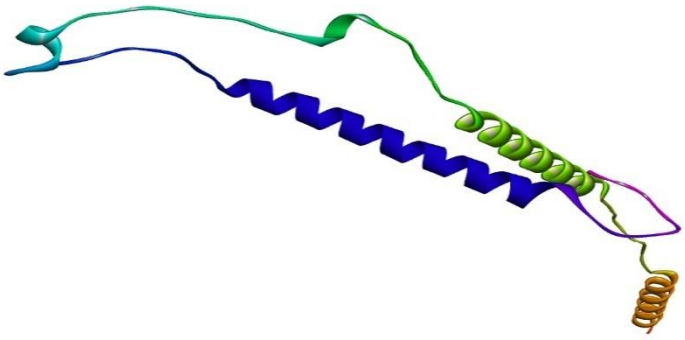


Figure 2: Three-dimensional structure of the human cytomegalovirus envelope glycoprotein N

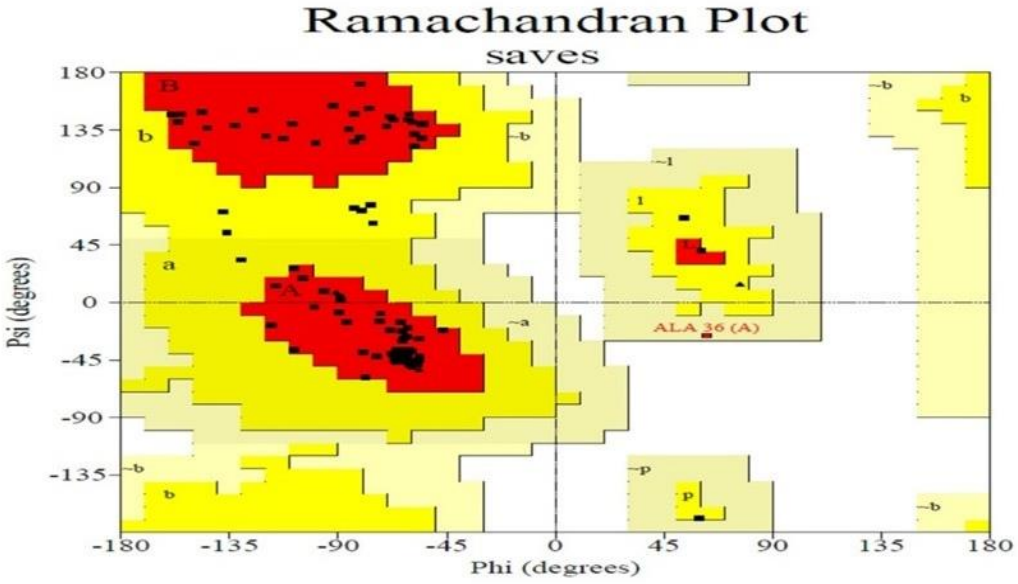


Figure 3: Ramachandran plot of human cytomegalovirus envelope glycoprotein N showing the favored region (in red)

Molecular docking: Molecular docking tests were conducted on luteolin for antiviral activity against HCMV. The phytocompound was converted into .pdb format utilizing the Discovery studio tool while the active binding sites of the corresponding proteins were identified using CastP server. For envelope, glycoprotein N active sites were Lys63, Pro64, Gly65, Ser66, Thr68, His69, Asp70, Pro71, Asn72, Tyr92, Leu94, Ser98, Phe99, Ala101, Trp102, Met105 and for envelope, glycoprotein H active sites were Phe570, Cys571, Leu572, Ala580, Thr582, Ser588, Ile590, Ile597, Lys598, Thr618, Asp619, Lys623, Cys624, Glu625. The grid box dimensions' values for Envelope glycoprotein N are X – 40, Y-68, Z-82. The grid box dimensions' values for Envelope glycoprotein H are X – 52, Y-54, Z-52.

Molecular docking results of luteolin against HCMV envelope glycoprotein N where luteolin exhibits a binding energy of -6.4 kcal/mol (Table 2). In case of envelope glycoprotein N, it was found that luteolin forms one hydrogen bond with Thr52(A), Thr135(A) and cys116(A) respectively (Figures 4a and 4b). Similarly, the docking results of luteolin envelope glycoprotein H (Table 2). In the docking study, it was found that the compound luteolin showed binding affinity of -7.1 kcal/mol against envelope glycoprotein H. Luteolin forms two hydrogen bonds with Ser577 (A) and one hydrogen bond with Ile666(A) of HCMV envelope glycoprotein H (Figures 5a and 5b).

The ADME assessment: The compound luteolin fulfilled Lipinski's criteria for absorption, distribution, metabolism and excretion. Lipinski's parameters for efficient drug absorption and penetration in the body include a maximum of five H-bond donors, a molecular weight of 286 Da, a log P of 2.1251 and no more than ten H-bond acceptors. Further, the Swiss ADME study indicates that the phytocompound could be successfully absorbed in humans for effective pharmacological activity (Table 3).

Discussion

There is no approved vaccine for HCMV. Many studies have reported in the rise of HCMV drug resistance in one or more approved antiviral medications due to prolonged use and thus there is an urgent need for the development of the new antiviral medications. Molecular docking, as a powerful computational tool, shows a cost-effective and time-efficient approach for *in silico* screening of potential drugs by predicting their binding position and affinities and interactions with specific viral protein targets. In this study, we employed molecular docking to investigate the inhibitory potential of phytocompound luteolin against envelope glycoprotein N and envelope glycoprotein H of HCMV.

A previous study by Munafo et al[24] provided evidence supporting the antiviral properties of luteolin against SARS-CoV-2. Further study by Wang et al[39] elucidated luteolin's mechanism of action against SARS-CoV-2, demonstrating its capacity to target about 64 associated genes and affecting 20 signaling pathways through molecular docking. Beyond SARS CoV-2, luteolin's antiviral properties extend to other viral families. In addition to the molecular docking, the *in vitro* studies also demonstrated antiviral activity of luteolin against influenza A virus by inhibiting the viral replication in the initial stage of infection.

Furthermore, luteolin enhances immune response due to its anti-inflammatory property by regulating signalling pathways of MAPK, TLR4, NF-kB associated with viral replication[21,43]. Moreover, Wang et al[40] highlighted luteolin's inhibitory effect on another herpesvirus, HSV-1 by activating cGAS-STING pathway[40]. Collectively, these studies, coupled with our *in silico* findings, strongly suggest that luteolin possesses significant antiviral activity among a wider range of viral families and could be a potential antiviral against HCMV.

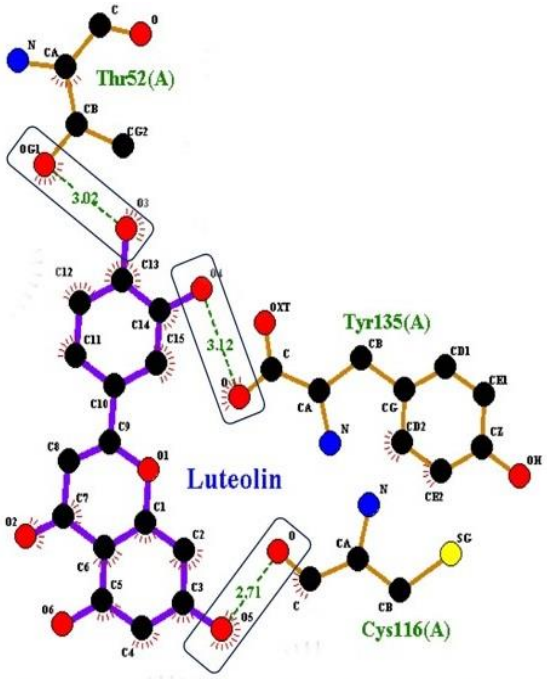
Specifically, luteolin exhibits binding affinities to both HCMV envelope glycoprotein N and glycoprotein H, indicating strong interactions with these viral surface envelope proteins. The formation of hydrogen bonds with specific amino acid residues in both glycoproteins further explains its potential inhibitory mechanism. Further this compound also fulfilled the Lipinski criteria for its bioactivity.

Table 2
Molecular docking of envelope glycoprotein N and H of human cytomegalovirus (strain Merlin) (HHV-5) (Human herpesvirus 5) using Autodock vina

S.N.	Protein name	Phytocompound	PubChem CID	Binding Energy(kcal/Mol)
1.	Envelope glycoprotein N	Luteolin	5280445	-6.4
2.	Envelope glycoprotein H	Luteolin	5280445	-7.1

Table 3
Lipinski's parameters of the phytochemical compound luteolin

S.N.	Phyto-chemicals Name	MR (Molar Refractivity) (40-130)	Molecular Weight (<=500 D)	HBD-Hydrogen bond donor (<=5)	HBA1-Hydrogen bond acceptors (<=10)	LogP (<=5)	Lipinski rule of five
1.	Luteolin	72.4786	286.00	4	6	2.1251	100%



Envelope Glycoprotein N -Luteolin complex

Figure 4a: Two-dimensional interaction of luteolin with human cytomegalovirus envelope glycoprotein N

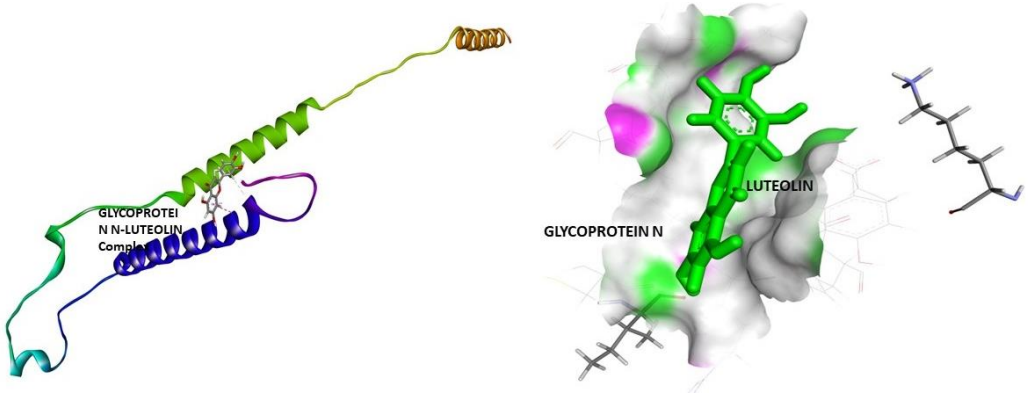
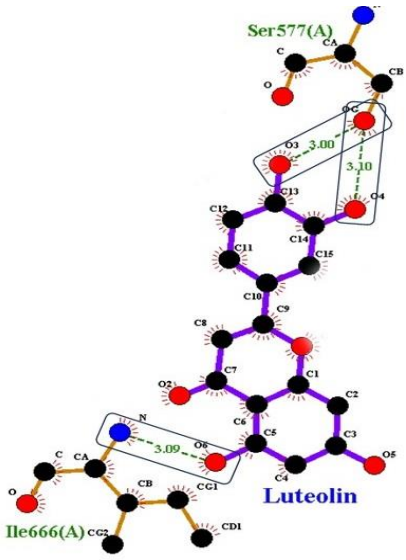


Figure 4b: Three-dimensional interaction of luteolin with the human cytomegalovirus envelope glycoprotein N



Envelope Glycoprotein H -Luteolin complex

Figure 5a: Two-dimensional interaction of luteolin with human cytomegalovirus envelope glycoprotein H

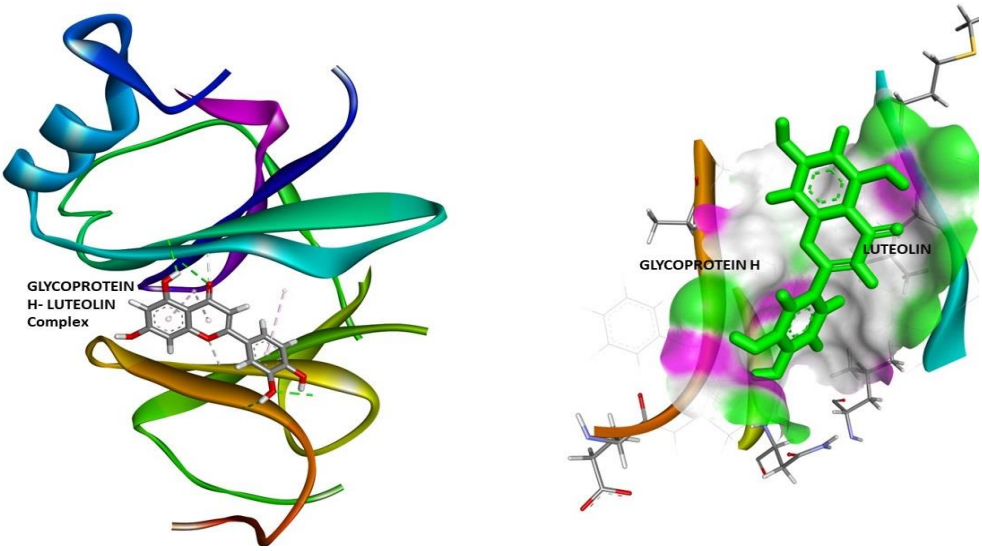


Figure 5b: Three-dimensional interaction of luteolin with human cytomegalovirus envelope glycoprotein H

Additionally, studies have reported that luteolin is non-mutagenic. It is also significant to note that free luteolin form can be detected in human serum following administration[34]. This study is consistent with other studies in supporting luteolin's antiviral potential against dengue, SARS- CoV-2, Influenza among other viruses[33.44].

While our *in silico* molecular docking study suggests that luteolin has stronger antiviral binding affinities against HCMV, it is important to acknowledge the limitations of this computational approach. Molecular docking provides theoretical predictions based on models and may not fully capture the complexities of a cellular environment. We need more data on *in vitro* and *in vivo* on antiviral efficacy and this study is valuable and reports a crucial step in the discovery of antiviral candidate, luteolin's promising potential and plays a significant role in formulating intervention strategies.

Conclusion

Molecular docking analysis demonstrated luteolin exhibiting higher binding affinities for the envelope glycoprotein N and glycoprotein H of HCMV. This study highlights luteolin as a promising antiviral against human cytomegalovirus, exhibiting its ability to target viral envelope glycoproteins effectively. This underscores the need for further experimental studies to evaluate the potential of luteolin as an antiviral agent against human cytomegalovirus.

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