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Article/Communication

Ellagic acid derivatives: possible drugs against metapneumovirus?

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Abstract: Human metapneumovirus is one of major causes of common cold among children, especially infants. Its key enzyme is RNA-dependent RNA-polymerase, which performs both replication and transcription, including capping and cap methylation. The goal of the work is to find possible inhibitors of RNA-dependent RNA-polymerase across the active compounds of Rosaceae plants. The candidates were selected by molecular docking to cap-transferring domain of RNA-polymerase (PDB ID: 4UCZ) in Autodock VINA. Among all the substances tested by docking, ellagic acid derivatives showed the most promising results (affinity values below -10 kcal/mol). Hence, they could be treated as possible candidate drugs against metapneumoviral infection after experimental examination. The main advantage of using these substances should be their low toxicity, which is quite uncommon for selective RNA polymerase inhibitors used in clinical practice. Occurrence of ellagic acid derivatives among the plants from Rosaceae family like raspberry could explain their effect during common cold.

Keywords: metapneumovirus; molecular docking; phenolic compounds; glycosides; ellagic acid; RNA-dependent RNA-polymerase

1. Introduction

Human metapneumovirus was isolated in 2001 from nasopharingheal aspirates of children in Netherlands {Citation}[1]. It is one of the major causes of higher respiratory tract infections affecting mainly children; however, sometimes it is also found in immunosuppressed adults and elderly patients [2]. It is a virus belonging to Mononegavirales order, like Filoviridae and Bornaviridae viruses, ant its genome consists of single negative-sense RNA molecule. The family Pneumoviridae includes two genera, Metapneumovirus and Orthopneumovirus, the latter including human respiratory syncytial viruses (RSVs), HRSV-A1, HRSV-A2, HRSV-B1 and HRSV-B2 [3].

Comparison of clinical manifestations of human metapneumovirus infection and human respiratory syncytial virus infection revealed high similarity of these two diseases. However, significant differences were observed in the severity of infection among different age groups of infants. Whereas in the age group of infants between 0 and 6 months HRSV infection had much higher severity score, in the age group of children between 12 and 23 months the situation was the opposite, i.e. severity of HMPV exceeded that of HRSV[4].

The crucial enzyme of metapneumovirus life cycle is RNA-dependent RNA-polymerase (Uniprot ID : Q6WB93) [5]. It is a large protein (2005 Aa) with several functional domains. The functions performed by this enzyme are replicase and

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transcriptase activities, two capping-related processes (cap addition and cap methylation), and polyadenylation of subgenomic viral mRNAs [6]. Different activities are performed by certain domains: Cr-I - CrIII perform polymerization of RNA molecules; it was first supposed that Cr-V domain performs capping, and Cr-VI methylates the cap of viral mRNA.

However, one of the subunits of crystallized cap-binding domain (CRVI+) of RNA-dependent RNA-polymerase in 4UCZ PDB entry contains guanosine triphosphate, whereas another one contains free guanosine. Since these are the substrate and product of the guanylyl transferase reaction, respectively, one can expect that blockade of substrate-binding site by a strongly binding substance could inhibit the capping activity and hence be fatal for virus replication [7].

Beside X-ray diffraction studies, the polymerase of metapneumovirus has been intensively studied by cryo-electron microscopy. Pan and colleagues explored the mode of binding between polymerase (L-protein) and regulatory phosphoprotein P during polymerization reaction and suggested a dynamic model of this process [8]. However, for the purpose of virtual screening of inhibitors, the results of X-ray analysis seem to be more reliable due to higher spatial resolution.

The initial purpose of this work was to search for compounds capable of binding to CrVI+ domain of RNA-dependent RNA-polymerase of human metapneumovirus among the active components of Rosaceae family representatives. However, after the initial search in *Geum rivale*, an interesting finding was developed further in searching the possible RNA-polymerase inhibitors among ellagic acid derivatives.

2. Materials and Methods

2.1 Preparation of files for docking

3D structure of cap-binding domain of human metapneumovirus RNA-dependent RNA polymerase was downloaded from Protein Data Bank (PDB ID: 4UCZ) [7]. After that, the files were processed in PyMol: bound ligands (GMP and GTP) were removed from chains A and B, respectively, after the coordinates of their centers of masses were measured and written down to center docking boxes. The processing of cleaned PDB files was performed in AutodockTools-1.5.7 [9]: Kollman charges [10] and polar hydrogen atoms were added, AD4 atom types were assigned, and the files (separate molecules for chain A and chain B) were saved in PDBQT format.

Ligands for docking were downloaded from PubChem database [11] as .sdf files, converted in PyMol to .pdb files and then processed in AutodockTools-1.5.7: Gasteiger charges [12]and polar hydrogens were added, torsion degrees of freedom and AD4 atom types were assigned automatically.

2.2 Docking calculation

Autodock Vina [13] was used for docking calculations. Docking box was centered on the centers of masses of GMP and GTP in the initial protein file (4UCZ); each of the box sides was 50 Å long. The exhaustiveness parameter was set to 20; all the other parameters were set by default.

2.3. Analysis of docking results

Docking results were filtered by their affinity values (Gibbs energy of ligand-receptor binding) based on the data in docking log files. For the final analysis, only compounds with binding energy below -10 kcal/mol (for both receptor subunits) were used.

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The obtained output .pdbqt files containing several (9) ligand positions were superposed with the files of corresponding protein subunits with their substrates included. If the compound molecules were not overlapped with substrate molecules in any of the binding modes, the compound was assigned as "non-inhibiting"; if at least one of the tested positions overlapped with the substrate, the compound was regarded as "inhibiting" and its inhibitory potence was measured by the binding affinity of the corresponding position.

To calculate K_i value from binding affinity, the following formula was used:

$$Ki = e^{\Delta G/RT} \quad (1),$$

where Ki is inhibition constant, ΔG is binding affinity in J/mol, R = 8.314 J/(mol·K), T is absolute temperature (310 K). To switch from kcal/mol to J/mol, thermochemical calorie value (4184 J/kcal) was used.

2.4. Validation of docking results

Validation of docking results for the components that passed the filtration procedure was carried out via docking onto other conformers of hMPV RNA-polymerase CrVI(+) domain presented in Protein Data Bank: 4UCI; 4UCJ; 4UCK; 4UCL; 4UCY; 4UD0 [7]. Their heteroatomic groups were removed, the coordinates of GTP ligands (centers of masses) were used for centering the docking boxes. All the other procedures were carried out the same way as for 4UCZ conformer.

3. Results

3.1. Docking of Geum rivale compounds

Docking of active phenolic compounds from *Geum rivale* [14] onto both protein chains from the crystal of capping domain revealed that ellagic acid could be regarded as a promising starting compound to find strong inhibitors of capping activity of RNA-dependent RNA-polymerase (Table 1).

Table 1. Docking of active phenolic compounds from *Geum rivale*.

Compound name	PubChem ID	Affinity, kcal/mol chain A / chain B	Ki[1], μM chain A / chain B
Gallic acid	370	-6.0 / -6.1	58.864 / 50.044
Protocatechuic acid	72	-6.3 / -6.4	36.170 / 30.750
P-hydroxybenzoic acid	135	-6.4 / -6.2	30.750 / 42.545
Vanillic acid	8468	-6.0 / -5.8	58.864 / 81. 443
Caffeic acid	689043	-7.3 / -7.1	7.134 / 9.870
Syringic acid	10742	-5.8 / -6.1	81.444 / 50.044
p-coumaric acid	637542	-6.8 / -6.5	16.046 / 26.142
Ferulic acid	445858	-6.3 / -6.4	36.170 / 30.750
Sinapic acid	637775	-6.3 / -6.4	36.170 / 30.750
Salicylic acid	338	-6.4 / -6.4	30.750 / 30.750
Ellagic acid	5281855	-8.4 / -8.2	1.196 / 1.655

[1] Here and below: inhibitor binding constant is regarded as Ki.

3.2. Comparison of ellagic acid with nucleotides

To find out whether the affinity shown in the previous subsection for ellagic acid (-8.4 - -8.2 kcal/mol) such affinity is enough to inhibit enzymatic activity of the capping domain of RNA-polymerase, we carried out docking of nucleoside triphosphates to the active site of the enzyme. The results are listed in Table 2.

Compound name	PubChem ID	Affinity, kcal/mol	Ki, μM

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		chain A / chain B	chain A / chain B
Ellagic acid	5281855	-8.4 / -8.2	1.196 / 1.655
Adenosine triphosphate	5957	-8.9 / -8.4	0.531 / 1.196
Cytosine triphosphate	6176	-8.8 / -8.1	0.625 / 1.947
Guanosine triphosphate	135398633	-9.4 / -9.1	0.236 / 0.384
Uridine triphosphate	6133	-8.5 / -8.6	1.017 / 0.865

As one can see from table 2, all the nucleoside triphosphates have higher binding affinities, than ellagic acid (or at least comparable ones, like cytosine triphosphate). The lowest binding constant was shown for guanosine triphosphate, which is in accordance with physiological function of the capping domain of RNA-dependent RNA-polymerase, i.e. GTP-hydrolysing activity.

3.3. Docking of ellagic acid derivatives

There were 17 ellagic acid derivatives found in PubChem database [11]. After standard preparation, they all were docked into both chains of the RNA-dependent RNA-polymerase. The results are shown in Table 3.

Table 3. Results of docking of ellagic acid derivatives to RNA-dependent RNA-polymerase of human metapneumovirus. The compounds for which docking onto both chains of the enzyme showed affinity values below -10 kcal/mol are shown in bold type.

Compound name	PubChem ID	Affinity, kcal/mol chain A / chain B	Ki, μM chain A / chain B
Ellagic acid	5281855	-8.4 / -8.2	1.196 / 1.655
2,3,8-Tri-O-methylellagic acid	5281860	-8.2 / -7.7	1.655 / 3.727
Ellagic acid 4-O-xylopyranoside	5487461	-10.3 / -9.7	0.055 / 0.145
Eschweilenol C (4-(alpha-rhamnopyranosyl)-ellagic acid)	**10026656**	**-10.2 / -10.8**	**0.064 / 0.024**
Ellagic acid 4-O-xylopyranoside 3,3'-dimethyl ether	10457042	-9.2 / -9.2	0.326 / 0.326
3,7-Di-O-methylducheside A (ellagic acid 4-O-xylopyranoside 3,3',4'-trimethyl ether)	10838304	-9.1 / -9.3	0.334 / 0.278
4-O-(4''-O-Acetyl-alpha-L-rhamnopyranosyl)-ellagic acid	**10951064**	**-10.1 / -10.2**	**0.076 / 0.064**
Ellagic acid tetraacetate	14432358	-8.6 / -8.4	0.865 / 1.196
(3,10-Dioxo-6,13,14-trisulfooxy-2,9-dioxatetracyclo[6.6.2.04,16.011,15]hexadeca-1(15),4,6,8(16),11,13-hexaen-7-yl) hydrogen sulfate	14777411	-8.9 / -9.4	0.531 / 0.236
Ellagic acid 4-xyloside	54004380	-9.8 / -9.7	0.123 / 0.145
Ducheside A (3'-O-Methyl-4-O-(beta-D-xylopyranosyl)ellagic acid)	78384860	-10.4 / -9.7	0.047 / 0.145
Ellagic acid-4-acetylarabinoside	**101129363**	**-11.4 / -11.1**	**0.0092 / 0.0149**
Ellagic acid-4-arabinoside	101757026	-10.4 / -9.8	0.047 / 0.123
Ellagic acid-4-acetylxyloside	**101757027**	**-10.0 / -10.1**	**0.089 / 0.076**
3'-O-Methyl ellagic acid 4-xyloside	101949535	-10.4 / -9.7	0.047 / 0.145
Ellagic acid 2-O-(6-O-alpha-L-rhamnopyranosyl-beta-D-glucopyranoside)	**102148497**	**-11.0 / -10.8**	**0.018 / 0.024**
Carboxyl ellagic acid	**102390018**	**-10.3 / -10.4**	**0.055 / 0.047**

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Among all the tested ellagic acid derivatives, six displayed binding affinities below -10.0 kcal/mol when docked into both chains of RNA polymerase capping domain. Corresponding binding constants are in nanomolar range. That means that they might be good inhibitors, but in order to ensure that, we performed filtration of docking results.

3.4. Filtration of docking results

The docking results were filtered via superposition of the ligand poses from output .pdbqt files with the initial PDB files of the enzyme containing a bound nucleotide. The criterion for effective binding was overlapping of nucleotide atoms and ligand atoms. The affinity of effective binding could be weaker than that of non-effective in case when the docking pose with the best affinity did not overlap with the nucleotide atoms. The numerical results of such filtration are listed in Table 4.

Table 4. Filtration of docking results.

Compound name	Effective binding has the lowest energy (yes/no, chainA/chainB)	Effective affinity, kcal/mol (Ki, μM), chain A	Effective affinity, kcal/mol (Ki, μM), chain B
Eschweilenol C (4-(alpha-rhamnopyranosyl)-ellagic acid) 10026656	Yes/No	-10.2 (0.064)	-9.4 (0.236)
4-O-(4''-O-Acetyl-alpha-L-rhamnopyranosyl)-ellagic acid 10951064	No/Yes	-10.0 (0.089)	-10.2 (0.064)
Ellagic acid-4-acetylarabinoside 101129363	No/No	-10.5 (0.0396)	-10.5 (0.0396)
Ellagic acid-4-acetylxyloside 101757027	Yes/No	-10.0 (0.089)	-9.4 (0.236)
Ellagic acid 2-O-(6-O-alpha-L-rhamnopyranosyl-beta-D-glucopyranoside) 102148497	Yes/Yes	-11.0 (0.018)	-10.8(0.024)
Carboxyl ellagic acid 102390018	Yes/Yes	-10.3(0.055)	-10.4(0.047)

According to the table, compounds number 102148497 and 102390018 should be regarded as the most promising candidates for experimental verification of their RNA-polymerase inhibiting activity, because the effective binding mode is the strongest one for both chains of the enzyme, so there is no significant competition between effective and ineffective binding pathways. As for other compounds, presence of ineffective binding modes stronger than effective ones means that binding of these substances would preferably occur via ineffective binding mode rather than via effective one, so the real effective binding constant could differ from the calculated one. All the selected compounds demonstrated calculated binding affinity in submicromolar range, so each of them deserves experimental investigation.

The binding modes of the best compounds are shown in figure 1 and 2.

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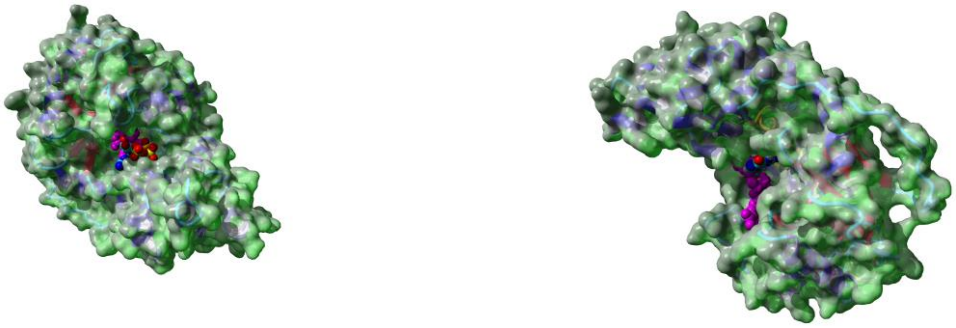
(**a**) (**b**)

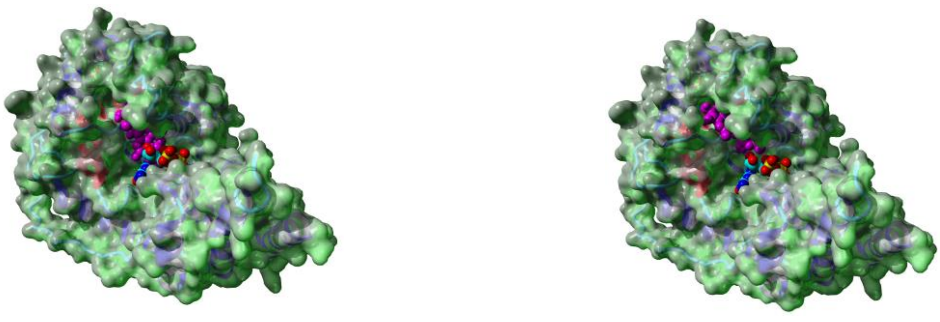
Figure 1. Binding of ellagic acid 2-O-(6-O-alpha-L-rhamnopyranosyl-beta-D-glucopyranoside) (PubChem ID: 102148497) to RNA-dependent RNA-polymerase of human metapneumovirus. (a) – chain A; (b) – chain B. Docking complexes are superposed with GTP-binding modes of chain A and chain B. GTP molecule is colored by standard element scheme: carbon,cyan; nitrogen, blue; oxygen, red; phosphorus, yellow. Protein molecule is shown in ribbon mode (blue alpha-helices, red beta-sheets and cyan coils) with semi-transparent green surface.



(**a**) (**b**)

Figure 2. Binding of Carboxyl ellagic acid (PubChem ID: 102390018) to RNA-dependent RNA-polymerase of human metapneumovirus. (a) – chain A; (b) – chain B. Docking complexes are superposed with GTP-binding modes of chain A and chain B. GTP molecule is colored by standard element scheme: carbon,cyan; nitrogen, blue; oxygen, red; phosphorus, yellow. Protein molecule is shown in ribbon mode (blue alpha-helices, red beta-sheets and cyan coils) with semi-transparent green surface.

Differences between effective and ineffective binding modes are shown in figures 3, 4, 5, 6.



(**a**) (**b**)

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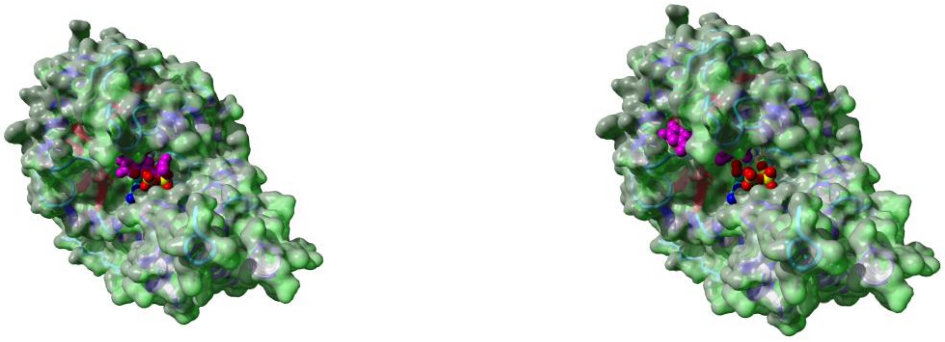
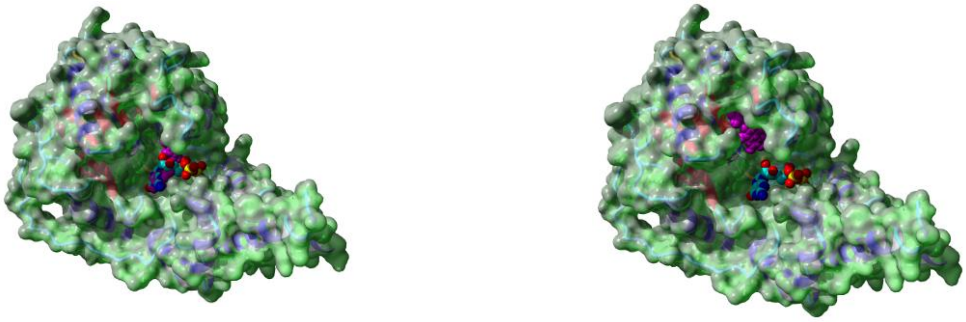
Figure 3. Binding of Eschweilenol C (4-(alpha-rhamnopyranosyl)-ellagic acid) (PubChem ID: 10026656) to RNA-dependent RNA-polymerase of human metapneumovirus. (a) – effective binding mode (atoms of inhibitor and substrate overlap after superposition); (b) – ineffective binding mode (atoms of inhibitor and substrate do not overlap after superposition).



(**a**) (**b**)

Figure 4. Binding of 4-O-(4''-O-Acetyl-alpha-L-rhamnopyranosyl)-ellagic acid (PubChem ID: 10951064) to RNA-dependent RNA-polymerase of human metapneumovirus. (a) – effective binding mode (atoms of inhibitor and substrate overlap after superposition); (b) – ineffective binding mode (atoms of inhibitor and substrate do not overlap after superposition).



(**a**) (**b**)

Figure 5. Binding of ellagic acid-4-acetylarabinoside (PubChemID: 101129363) to RNA-dependent RNA-polymerase of human metapneumovirus. (a) – effective binding mode (atoms of inhibitor and substrate overlap after superposition); (b) – ineffective binding mode (atoms of inhibitor and substrate do not overlap after superposition).

(**a**) (**b**)

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Figure 6. Binding of ellagic acid-4-acetylxyloside (PubChem ID: 101757027) to RNA-dependent RNA-polymerase of human metapneumovirus. (a) – effective binding mode (atoms of inhibitor and substrate overlap after superposition); (b) – ineffective binding mode (atoms of inhibitor and substrate do not overlap after superposition).

3.5. Final validation of docking results: docking to other conformers of CRVI(+)-domain of RNA-polymerase

The selected compounds that passed the filtration were tested on the other conformers of RNA-polymerase CRVI(+) domain. The receptors taken for validation were (PDB IDs): 4UCI; 4UCJ; 4UCK; 4UCL; 4UCY; 4UD0. They were prepared for docking in the same manner as 4UCZ; after that, the ligands were docked into the boxes centered on the centers of masses of GTP ligands. The results are listed in Table 5.

Table 5. Validation of docking results on multiple conformers of RNA-polymerase CRVI(+) domain. The data are represented as: affinity, chain A, kcal/mol ; affinity, chain B, kcal/mol (Ki, chain A, μM / Ki, chain B, μM)

Compound name	4UCI	4UCJ	4UCK	4UCL	4UCY	4UD0
Eschweilenol C (4-(alpha-rhamnopyranosyl)-ellagic acid) 10026656	-9.8/-9.7 (0.123/0.145)	-9.2/-8.4 (0.326/1.196)	-9.6/-10.0 (0.171/0.089)	-9.1/-9.7 (0.384/0.145)	-9.6/-9.1 (0.171/0.384)	-/-9.9 (-/0.105)
4-O-(4''-O-Acetyl-alpha-L-rhamnopyranosyl)-ellagic acid 10951064	-10.1/-10.1 (0.076/0.076)	-9.0/-8.8 (0.452/0.625)	-8.9/-10.2 (0.531/0.064)	-9.6/-9.3 (0.171/0.278)	-10.1/-9.8 (0.076/0.123)	-/-9.8 (-/0.123)
Ellagic acid-4-acetylarabinoside 101129363	-10.8/-10.8 (0.024/0.024)	-9.5/-9.6 (0.201/0.171)	-10.0/-11.5 (0.089/0.008)	-11.0/-10.5 (0.018/0.040)	-10.6/-11.1 (0.034/0.015)	-/-11.1 (-/0.015)
Ellagic acid-4-acetylxyloside 101757027	-10.0/-10.0 (0.089/0.089)	-8.7/-8.3 (0.735/1.407)	-9.2/-10.0 (0.326/0.089)	-9.5/-9.4 (0.201/0.236)	-9.6/-9.2 (0.171/0.326)	-/-10.2 (-/0.064)
Ellagic acid 2-O-(6-O-alpha-L-rhamnopyranosyl-beta-D-glucopyranoside) 102148497	-10.6/-10.5 (0.034/0.040)	-8.7/-9.6 (0.735/0.171)	-9.7/-11.0 (0.145/0.018)	-10.4/-10.6 (0.047/0.034)	-10.5/-10.2 (0.040/0.064)	-/-10.5 (-/0.040)
Carboxyl ellagic acid 102390018	-10.3/-10.3 (0.055/0.055)	-9.7/-9.9 (0.145/0.105)	-9.5/-10.3 (0.201/0.055)	-10.8/-10.9 (0.024/0.021)	-10.9/-10.6 (0.021/0.034)	-/-10.1 (-/0.076)

As one can see from the table, almost all the listed ellagosides show theoretical ability to bind to human metapneumovirus DNA with submicromolar affinities.

4. Discussion

Ellagic acid derivatives have demonstrated their possibility to bind to RNA-dependent RNA-polymerase capping domain with submicromolar affinity in the currrent docking study. Hence, these compounds should be tested as possible inhibitors of metapneumovirus activity and valuable pharmacological agents.

The ellagic acid glycosides are widely distributed among plants. Particularly, they are common for Rosaceae family. For example, *Geum rivale* and *Geum urbanum* contain ellagic acid and rare ellagic acid sulphate derivatives (3,3′-dimethoxy-4-sulphoxyellagic acid potassium salt and 3,3′,4′-trimethoxy-4-sulphoxyellagic acid potassium salt) [14,15] . Other genera of Rosaceae containing ellagosides, ellagitannins and free ellagic acid are

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Filipendula [16], *Alchemilla* [17], *Potentilla* [18,19], *Sanguisorba* [20,21], *Rubus* [22,23], *Fragaria* [24], *Rosa* [25].

Eschweilenols A, B and C are ellagic acid derivatives isolated from the bark of *Eschweilera coriacea* [26], a plant from Suriname rainforest belonging to the family *Lecythidaceae.* According to the literature, Eschweilenol C is a potent anti-fungal and anti-inflammatory substance present also in *Terminalia fagifolia* and *Terminalia bentzoë*, species of Combretaceae family from Brazilian dry forest [27,28].

Pharmacological effects of ellagic acid and its derivatives such as ellagosides and ellagitannins include anti-atherogenic, antioxidant and free radical-scavenging actions [29–33]. Beside that, they displayed antifungal activity [34,35], antimicrobial activity and antiviral activity as well.

Antiviral activity of ellagic acid was demonstrated on several models. Bovine papillomavirus infection of C127 cells results in elevated genome instability, which can be significantly reduced by several antioxidants including ellagic acid [36]. Human papillomavirus, an oncogenic virus with DNA genome, is also sensitive to ellagic acid-containing complex therapy in *in vivo* clinical studies of infected women [37]. Another clinical study in humans (men) infected by papillomavirus reflected that combination of ellagic acid with *Annona muricata* extract is effective against viral replication, significantly decreasing the content of virions of high-risk papillomaviruses in the seminal fluid [38]. Human polyomaviruses BK and JC are the representatives of Papovaviridae family usually causing mild illness in children [39]. Their replication can be attenuated by ellagic acid derivatives as well [40]. A screening study of antiviral activity of chebulagic acid and punicalagin, the derivatives of ellagic acid, showed their high potency against human cytomegalovirus (HCMV), hepatitis C virus (HCV), dengue virus (DENV), measles virus (MV), and respiratory syncytial virus (RSV) [41].

An experimental work that can be regarded as the closest to the current study was carried out on HeLa cells infected by human rhinoviruses 2,3 and 4. Human rhinoviruses have non-segmented RNA genomes, and their replication is mediated by RNA-dependent RNA-polymerase. According to IC50 values, the inhibiting potency of ellagic acid extracted from Lagerstroemia speciosa was 1.8, 2.3 and 2.2. times higher against HRV-2, HRV-3 and HRV-4, respectively, than that of ribavirin, and continuous presence of ellagic acid during the infection decreased viral replication by 70% [42]. Meanwhile, ribavirin is quite a strong antiviral medication with serious adverse effects [43,44], and its administration during mild infections caused by rhinovirus, respiratory syncitial virus and metapneumovirus, seems to be excessive.

Thus, the experimental background showing prospects of clinical application of ellagic acid and its derivatives is quite convincing. Our study demonstrates the possibility of effective use of ellagic acid derivatives against yet another viral infection, that deserves experimental validation.

5. Conclusions

Thus, we have demonstrated that the derivatives of ellagic acid deserve attention as possible inhibitors of human metapneumovirus RNA-dependent RNA-polymerase. The statistics of docking results was reproduced on several crystal structures of the enzymes, providing reliable information on the probability of strong binding to the enzyme. Since ellagic acid is a compound with low toxicity, its derivatives could be used as medications against common cold in children after experimental validation of their properties as antiviral substances.

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and A.V.A.; resources, A.V.A.; data curation, A.V.A.; writing—original draft preparation, E.A.A. and A.V.A.; writing—review and editing, A.V.A.; visualization, E.A.A. and A.V.A.; supervision, A.V.A.; project administration, A.V.A.

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References

1. van den Hoogen, B.G.; de Jong, J.C.; Groen, J.; Kuiken, T.; de Groot, R.; Fouchier, R.A.; Osterhaus, A.D. A Newly Discovered Human Pneumovirus Isolated from Young Children with Respiratory Tract Disease. Nat Med 2001, 7, 719–724, doi:10.1038/89098.

2. van den Hoogen, B.G.; Osterhaus, D.M.E.; Fouchier, R.A.M. Clinical Impact and Diagnosis of Human Metapneumovirus Infection. Pediatr Infect Dis J 2004, 23, S25-32, doi:10.1097/01.inf.0000108190.09824.e8.

3. Rima, B.; Collins, P.; Easton, A.; Fouchier, R.; Kurath, G.; Lamb, R.A.; Lee, B.; Maisner, A.; Rota, P.; Wang, L.; et al. ICTV Virus Taxonomy Profile: Pneumoviridae. J Gen Virol 2017, 98, 2912–2913, doi:10.1099/jgv.0.000959.

4. Moe, N.; Krokstad, S.; Stenseng, I.H.; Christensen, A.; Skanke, L.H.; Risnes, K.R.; Nordbø, S.A.; Døllner, H. Comparing Human Metapneumovirus and Respiratory Syncytial Virus: Viral Co-Detections, Genotypes and Risk Factors for Severe Disease. PLoS One 2017, 12, e0170200, doi:10.1371/journal.pone.0170200.

5. UniProt Consortium, T. UniProt: The Universal Protein Knowledgebase. Nucleic Acids Research 2018, 46, 2699–2699, doi:10.1093/nar/gky092.

6. Fearns, R.; Plemper, R.K. Polymerases of Paramyxoviruses and Pneumoviruses. Virus Res 2017, 234, 87–102, doi:10.1016/j.virusres.2017.01.008.

7. Paesen, G.C.; Collet, A.; Sallamand, C.; Debart, F.; Vasseur, J.-J.; Canard, B.; Decroly, E.; Grimes, J.M. X-Ray Structure and Activities of an Essential Mononegavirales L-Protein Domain. Nat Commun 2015, 6, 8749, doi:10.1038/ncomms9749.

8. Pan, J.; Qian, X.; Lattmann, S.; El Sahili, A.; Yeo, T.H.; Jia, H.; Cressey, T.; Ludeke, B.; Noton, S.; Kalocsay, M.; et al. Structure of the Human Metapneumovirus Polymerase Phosphoprotein Complex. Nature 2020, 577, 275–279, doi:10.1038/s41586-019-1759-1.

9. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. J. Comput. Chem. 2009, 30, 2785–2791, doi:10.1002/jcc.21256.

10. Singh, U.C.; Kollman, P.A. An Approach to Computing Electrostatic Charges for Molecules. J. Comput. Chem. 1984, 5, 129–145, doi:10.1002/jcc.540050204.

11. Kim, S.; Chen, J.; Cheng, T.; Gindulyte, A.; He, J.; He, S.; Li, Q.; Shoemaker, B.A.; Thiessen, P.A.; Yu, B.; et al. PubChem in 2021: New Data Content and Improved Web Interfaces. Nucleic Acids Res 2021, 49, D1388–D1395, doi:10.1093/nar/gkaa971.

12. Gasteiger, J.; Marsili, M. A New Model for Calculating Atomic Charges in Molecules. Tetrahedron Letters 1978, 19, 3181–3184, doi:10.1016/S0040-4039(01)94977-9.

13. Trott, O.; Olson, A.J. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. J Comput Chem 2010, 31, 455–461, doi:10.1002/jcc.21334.

14. Owczarek, A.; Gudej, J. Investigation into Biologically Active Constituents of Geum Rivale L. Acta Pol Pharm 2013, 70, 111–114.

15. Owczarek, A.; Olszewska, M.A. Quantitative Determination of Ellagic Acid and Gallic Acid in Geum Rivale L. and G. Urbanum L. Acta Biologica Cracoviensia. Series Botanica 2014, 56, 74–78.

16. Bijttebier, S.; Van der Auwera, A.; Voorspoels, S.; Noten, B.; Hermans, N.; Pieters, L.; Apers, S. A First Step in the Quest for the Active Constituents in Filipendula Ulmaria (Meadowsweet): Comprehensive Phytochemical Identification by Liquid Chromatography Coupled to Quadrupole-Orbitrap Mass Spectrometry. Planta Med 2016, 82, 559–572, doi:10.1055/s-0042-101943.

17. Ilić-Stojanović, S.; Nikolić, V.; Kundaković, T.; Savić, I.; Savić-Gajić, I.; Jocić, E.; Nikolić, L. Thermosensitive Hydrogels for Modified Release of Ellagic Acid Obtained from Alchemilla Vulgaris L. Extract. International Journal of Polymeric Materials and Polymeric Biomaterials 2018, 67, 553–563, doi:10.1080/00914037.2017.1354202.

18. Tomczyk, M.; Bazylko, A.; Staszewska, A. Determination of Polyphenolics in Extracts of Potentilla Species by High-Performance Thin-Layer Chromatography Photodensitometry Method. Phytochem. Anal. 2009, n/a-n/a, doi:10.1002/pca.1174.

19. Morikawa, T.; Imura, K.; Akagi, Y.; Muraoka, O.; Ninomiya, K. Ellagic Acid Glycosides with Hepatoprotective Activity from Traditional Tibetan Medicine Potentilla Anserina. J Nat Med 2018, 72, 317–325, doi:10.1007/s11418-017-1137-y.

20. Gao, X.; Wu, J.; Zou, W.; Dai, Y. Two Ellagic Acids Isolated from Roots of Sanguisorba Officinalis L. Promote Hematopoietic Progenitor Cell Proliferation and Megakaryocyte Differentiation. Molecules 2014, 19, 5448–5458, doi:10.3390/molecules19045448.

21. Tan, Y.H.; Shudo, T.; Yoshida, T.; Sugiyama, Y.; Si, J.Y.; Tsukano, C.; Takemoto, Y.; Kakizuka, A. Ellagic Acid, Extracted from Sanguisorba Officinalis, Induces G1 Arrest by Modulating PTEN Activity in B16F10 Melanoma Cells. Genes Cells 2019, 24, 688–704, doi:10.1111/gtc.12719.

22. Zafrilla, P.; Ferreres, F.; Tomás-Barberán, F.A. Effect of Processing and Storage on the Antioxidant Ellagic Acid Derivatives and Flavonoids of Red Raspberry (Rubus Idaeus) Jams. J. Agric. Food Chem. 2001, 49, 3651–3655, doi:10.1021/jf010192x.

23. Sójka, M.; Janowski, M.; Grzelak-Błaszczyk, K. Stability and Transformations of Raspberry (Rubus Idaeus L.) Ellagitannins in Aqueous Solutions. Eur Food Res Technol 2019, 245, 1113–1122, doi:10.1007/s00217-018-3212-3.

24. Gasperotti, M.; Masuero, D.; Guella, G.; Palmieri, L.; Martinatti, P.; Pojer, E.; Mattivi, F.; Vrhovsek, U. Evolution of Ellagitannin Content and Profile during Fruit Ripening in Fragaria Spp. J. Agric. Food Chem. 2013, 61, 8597–8607, doi:10.1021/jf402706h.

25. Tumbas, V.T.; Čanadanović-Brunet, J.M.; Četojević-Simin, D.D.; Ćetković, G.S.; Đilas, S.M.; Gille, L. Effect of Rosehip (Rosa Canina L.) Phytochemicals on Stable Free Radicals and Human Cancer Cells. J. Sci. Food Agric. 2012, 92, 1273–1281, doi:10.1002/jsfa.4695.

26. Yang, S.-W.; Zhou, B.-N.; Wisse, J.H.; Evans, R.; van der Werff, H.; Miller, J.S.; Kingston, D.G.I. Three New Ellagic Acid Derivatives from the Bark of Eschweilera Coriacea from the Suriname Rainforest. J. Nat. Prod. 1998, 61, 901–906, doi:10.1021/np980046u.

27. Rodrigues de Araújo, A.; Iles, B.; de Melo Nogueira, K.; Dias, J. do N.; Plácido, A.; Rodrigues, A.; Albuquerque, P.; Silva-Pereira, I.; Socodatto, R.; Portugal, C.C.; et al. Antifungal and Anti-Inflammatory Potential of Eschweilenol C-Rich Fraction Derived from Terminalia Fagifolia Mart. Journal of Ethnopharmacology 2019, 240, 111941, doi:10.1016/j.jep.2019.111941.

28. Apel, C.; Bignon, J.; Garcia-Alvarez, M.C.; Ciccone, S.; Clerc, P.; Grondin, I.; Girard-Valenciennes, E.; Smadja, J.; Lopes, P.; Frédérich, M.; et al. N-Myristoyltransferases Inhibitory Activity of Ellagitannins from Terminalia Bentzoë (L.) L. f. Subsp. Bentzoë. Fitoterapia 2018, 131, 91–95, doi:10.1016/j.fitote.2018.10.014.

29. Larrosa, M.; García-Conesa, M.T.; Espín, J.C.; Tomás-Barberán, F.A. Ellagitannins, Ellagic Acid and Vascular Health. Molecular Aspects of Medicine 2010, 31, 513–539, doi:10.1016/j.mam.2010.09.005.

30. Priyadarsini, K.I.; Khopde, S.M.; Kumar, S.S.; Mohan, H. Free Radical Studies of Ellagic Acid, a Natural Phenolic Antioxidant. J. Agric. Food Chem. 2002, 50, 2200–2206, doi:10.1021/jf011275g.

31. Kumar, A.; Kaushik, P.; Incerpi, S.; Pedersen, J.Z.; Goel, S.; Prasad, A.K.; Rohil, V.; Parmar, V.S.; Saso, L.; Len, C. Evaluation of the Free Radical Scavenging Activities of Ellagic Acid and Ellagic Acid Peracetate by EPR Spectrometry. Molecules 2021, 26, 4800, doi:10.3390/molecules26164800.

32. Dalvi, L.T.; Moreira, D.C.; Andrade, R.; Ginani, J.; Alonso, A.; Hermes-Lima, M. Ellagic Acid Inhibits Iron-Mediated Free Radical Formation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2017, 173, 910–917, doi:10.1016/j.saa.2016.10.034.

33. Anwaier, G.; Lian, G.; Ma, G.-Z.; Shen, W.-L.; Lee, C.-I.; Lee, P.-L.; Chang, Z.-Y.; Wang, Y.-X.; Tian, X.-Y.; Gao, X.-L.; et al. Punicalagin Attenuates Disturbed Flow-Induced Vascular Dysfunction by Inhibiting Force-Specific Activation of Smad1/5. Front. Cell Dev. Biol. 2021, 9, 697539, doi:10.3389/fcell.2021.697539.

34. Li, Z.-J.; Guo, X.; Dawuti, G.; Aibai, S. Antifungal Activity of Ellagic Acid In Vitro and In Vivo: ANTIFUNGAL ACTIVITY OF ELLAGIC ACID IN VITRO AND IN VIVO. Phytother. Res. 2015, 29, 1019–1025, doi:10.1002/ptr.5340.

35. Zhou, L.; Li, D.; Jiang, W.; Qin, Z.; Zhao, S.; Qiu, M.; Wu, J. Two Ellagic Acid Glycosides from Gleditsia Sinensis Lam. with Antifungal Activity on Magnaporthe Grisea . Natural Product Research 2007, 21, 303–309, doi:10.1080/14786410701192702.

36. Stich, H.F.; Siu Sing Tsang; Palcic, B. The Effect of Retinoids, Carotenoids and Phenolics on Chromosomal Instability of Bovine Papillomavirus DNA-Carrying Cells. Mutation Research/Genetic Toxicology 1990, 241, 387–393, doi:10.1016/0165-1218(90)90069-E.

37. Morosetti, G.; Criscuolo, A.A.; Santi, F.; Perno, C.F.; Piccione, E.; Ciotti, M. Ellagic Acid and Annona Muricata in the Chemoprevention of HPV-Related Pre-Neoplastic Lesions of the Cervix. Oncology Letters 2017, 13, 1880–1884, doi:10.3892/ol.2017.5634.

38. La Vignera, S.; Basile, L.; Aversa, A.; Calogero, A.E.; Grillo, A.; Cannarella, R.; Mongioì, L.M.; Condorelli, R.A. The Use of Ellagic Acid and Annona Muricata Improves Semen Quality in Men with High-Risk Papillomavirus Infection. J Clin Med 2022, 11, 4691, doi:10.3390/jcm11164691.

39. Pinto, M.; Dobson, S. BK and JC Virus: A Review. J Infect 2014, 68 Suppl 1, S2-8, doi:10.1016/j.jinf.2013.09.009.

40. Goodwin, E.C.; Atwood, W.J.; DiMaio, D. High-Throughput Cell-Based Screen for Chemicals That Inhibit Infection by Simian Virus 40 and Human Polyomaviruses. J Virol 2009, 83, 5630–5639, doi:10.1128/JVI.00203-09.

41. Lin, L.-T.; Chen, T.-Y.; Lin, S.-C.; Chung, C.-Y.; Lin, T.-C.; Wang, G.-H.; Anderson, R.; Lin, C.-C.; Richardson, C.D. Broad-Spectrum Antiviral Activity of Chebulagic Acid and Punicalagin against Viruses That Use Glycosaminoglycans for Entry. BMC Microbiol 2013, 13, 187, doi:10.1186/1471-2180-13-187.

42. Park, S.W.; Kwon, M.J.; Yoo, J.Y.; Choi, H.-J.; Ahn, Y.-J. Antiviral Activity and Possible Mode of Action of Ellagic Acid Identified in Lagerstroemia Speciosa Leaves toward Human Rhinoviruses. BMC Complement Altern Med 2014, 14, 171, doi:10.1186/1472-6882-14-171.

43. Lee, D.-U.; Je, S.H.; Yoo, S.J.; Kwon, T.; Shin, J.Y.; Byun, J.J.; Park, J.-H.; Jeong, K. -w.; Ku, J.-M.; Lyoo, Y.S. Hematological Adverse Effects and Pharmacokinetics of Ribavirin in Pigs Following Intramuscular Administration. J. vet. Pharmacol. Therap. 2017, 40, 561–568, doi:10.1111/jvp.12394.

44. Martin, P.; Jensen, D.M. Ribavirin in the Treatment of Chronic Hepatitis C. J Gastroenterol Hepatol 2008, 23, 844–855, doi:10.1111/j.1440-1746.2008.05398.x.