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Флавоноиды как потенциальные ингибиторы коронавируса SARS-CoV-2: исследование *in silico*

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РЕЗЮМЕ

Введение. Вирус SARS-CoV-2 (Severe Acute Respiratory Syndrome CoronaVirus 2) обладает одним из крупнейших геномов, который кодирует 16 неструктурных белков (NSP: Non-Structural Protein), необходимых для репликации и преодоления защитных механизмов организма-хозяина. Флавоноиды представляют интерес в качестве объектов исследования при разработке препаратов для комплексной терапии COVID-19 (Corona Virus Desease 2019). Представители этой группы характеризуются широким спектром биологической активности и высоким профилем безопасности.

Цель работы – провести виртуальный скрининг флавоноидов на возможность ингибирования жизненно важных белков коронавируса SARS-CoV-2.

Материалы и методы. Структуры белков SARS-CoV-2: ADP-связывающего домена NSP3, основной протеазы NSP5, РНК-зависимой-РНК-полимеразы NSP12, эндорибонуклеазы NSP15 получены из Protein Data Bank (PDB). Структуры 163 флавоноидов различных групп, взяты из базы данных ZINC. Процессинг моделей белков осуществляли в программе AutoDockTools, а лигандов – в Raccoon | AutoDock VS. Виртуальный скрининг и ре-докинг проводили в AutoDock Vina.

Результаты. В ходе валидации установлено совпадение конформации нативных лигандов в исходной структуре и при ре-докинге, что позволяет судить о применимости методики виртуального скрининга. Флавоноиды взаимодействовали с ключевыми аминокислотными остатками во всех исследованных белках. Наилучшую энергию аффинитета продемонстрировали 3,7-дигидроксифлавон и 6*S*-кокцинеон Б, обладающий мультимодальным эффектом.

Заключение. Полученные результаты могут быть использованы в разработке фитопрепаратов для комплексной терапии COVID-19.

Ключевые слова: SARS-CoV-2, COVID-19, флавоноиды, молекулярный докинг, виртуальный скрининг, кокцинеон Б

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Flavonoids as potential inhibitors of SARS-CoV-2 infection: *in silico* study

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ABSTRACT

Background. SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has one of the largest genomes. It encodes 16 non-structural proteins that are necessary for replicating and overcoming host defense mechanisms. Flavonoids are of interest as research objects in developing drugs for comprehensive COVID-19 therapy. This group of compounds is characterized by a wide range of biological activity and a high safety profile.

Aim. To perform virtual screening of flavonoids for possible inhibition of proteins of the SARS-CoV-2 infection.

Materials and methods. Structural proteins of SARS-CoV-2 infection, such as ADP-binding domain NSP3, main protease NSP5, RNA-dependent RNA-polymerase NSP12, and endoribonuclease NSP15, were obtained from Protein Data Bank (PDB). Flavonoid structures were obtained from the ZINC database. Protein models were processed using AutoDockTools software, and ligands were processed in Raccoon | AutoDock VS. Virtual screening and re-docking were performed in AutoDock Vina.

Results. Validation showed agreement between native and re-docked conformations, indicating the applicability of the virtual screening method. Flavonoids interacted with the key amino acid residues in all the studied proteins. The highest binding energy was demonstrated by 3,7-dihydroxyflavone and 6S-coccineone B, the latter having a multimodal effect.

Conclusion. The results of the study may be used for the development of phytomedicines for comprehensive therapy for COVID-19.

Keywords: SARS-CoV-2, COVID-19, flavonoids, molecular docking, virtual screening, coccineone B

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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INTRODUCTION

Coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) belongs to the *Betacoronavirus* genus, which is a member of the *Coronaviridae* family. SARS-CoV-2 is characterized as an enveloped, positive-sense, single-stranded (+RNA) RNA virus. It has one of the largest genomes among the entire domain, which includes about 30,000 nucleobases. This allows to assume that it has a wide range of biological targets [1]. The genome encodes two overlapping polyproteins that contain 16 non-structural proteins (NSP). Some of them are involved in the replication and life cycle of the virus, while others are necessary to overcome host defense mechanisms [2].

Flavonoid compounds are of particular interest in the development of new drugs for COVID-19 (CoronaVirus Disease 2019) treatment. They are characterized by antiradical [3], antiviral [4], capillary protective [5], and anti-inflammatory effects [5, 6].

The aim of the study was to perform virtual screening of flavonoids for possible inhibition of proteins of the SARS-CoV-2 infection.

MATERIALS AND METHODS

To search for antiviral drugs, we selected four biological targets of SARS-CoV-2 with a resolution of at least 2.5 Å. These targets are presented in the Protein Data Bank (PDB) (date of access: 25.04.2020): ADP-binding domain NSP3 (PDB ID: 6W6Y), main

protease NSP5 (PDB ID: 6LU7), RNA-dependent RNA polymerase NSP12 (PDB ID: 7BV2), endoribonuclease NSP15 (PDB ID: 6VWW). The protein characteristics are presented in Table 1. Processing of protein structures for virtual screening was performed in AutoDockTools Version 1.5.6 (The Scripps Research Institute; USA) [7]. During the processing, water and ligand molecules were removed, missing hydrogen atoms and partial atomic charges were added according to the Gasteiger partial charge calculation method.

The coordinates of native ligands were chosen as centers for constructing GRID maps with dimensions of 25 × 25 × 25 Å (see Table 1). The center of the active site in endoribonuclease NSP15 was determined according to the literature [8].

The structures of 163 flavonoids were obtained from the ZINC database. Preparation of ligands for the study was carried out by adding partial charges in the Raccoon | AutoDock VS version 1.0 (The Scripps Research Institute; USA) [9]. The processing of native ligands for validation re-docking was performed by a similar method.

Table 1

SARS-CoV-2 proteins included in the study

Biological target	Resolution of the resulting protein, Å	Native ligand	GRID-map center, Å
ADP-binding domain NSP3	1.45	AMP	X: 10.567 Y: -8.238 Z: 17.980
Main protease NSP5	2.16	N3 inhibitor	X: -12.149 Y: 14.097 Z: 69.719
RNA-dependent RNA polymerase NSP12	2.50	Remdesivir metabolite	X: 90.089 Y: 93.714 Z: 102.212
Endoribonuclease NSP15	2.20	–	X: -52.239 Y: 30.584 Z: 31.357

Re-docking of native ligands and virtual screening of all compounds were carried out with the AutoDock Vina 1.1.2 software (The Scripps Research Institute; USA) [10] using the Lamarckian genetic algorithm (LGA). The results of molecular modeling were visualized in the Discovery Studio Visualizer v19.1.0.18287 (BIOVIA; USA). To describe the distribution of virtual screening results, the binding energies of flavonoids were chosen that bind better than 10, 50, and 90% of the compounds in the sample.

RESULTS

Positions of ligands during the re-docking almost repeated the geometry in the initial protein structures, which allows to suggest the validity of the research method. The results of the virtual screening of the compounds were ranked according to the scoring function value in the AutoDock Vina 1.1.2 software in the form of binding energy. The obtained data were compared with the binding energy of native ligands. The activity threshold was a scoring function value of 7.1 kcal / mol, as a marker of reversibility of the protein – ligand complex.

In general, the lowest binding energy in the studied flavonoids was noted for endoribonuclease NSP15: successful docking took place for 20 compounds. In contrast, RNA-dependent RNA polymerase NSP12 bound to all 163 virtual structures. ADP-binding domain NSP3 and main protease NSP5 had medium binding energy: 111 and 108 flavonoids, respectively. Within the samples of ligands that were successfully docked into the active centers of biological targets, the median binding energies were –7.4; –7.4; –8.9, and –7.3 kcal / mol for the ADP-binding domain NSP3, main protease NSP5, RNA-dependent RNA polymerase NSP12, and endoribonuclease NSP15, respectively (Table 2).

Table 2

Distribution of flavonoid binding affinity to biological targets

Value characterizing the sample	Binding energy, kcal / mol			
	ADP-binding domain NSP3	Main protease NSP5	RNA-dependent RNA polymerase NSP12	Endoribonuclease NSP15
X_{10}*	–7.1	–7.1	–7.4	–7.1
X_{50}*	–7.4	–7.4	–8.9	–7.3
X_{90}*	–7.8	–7.9	–10.2	–7.6

*X_{10}, X_{50}, X_{90} – binding energy of flavonoids, where 10, 50, and 90% is a proportion (%) of compounds with binding energy that is less in modulus.

The results of virtual screening for the leader compounds are presented in Table 3. Flavonoids were shown to form non-covalent bonds with the same amino acid residues as native ligands. For example, for endoribonuclease NSP15, the following amino acid residues were involved: His 235, His 250, Lys 290, Ser 294, Thr 341, and Tyr 343. The best scoring function values were obtained for 6S-coccineone B and 3,7-dihydroxyflavone. The docking results with the leader compounds are shown in the Figure.

Table 3

Results of re-docking of native ligands and docking of 3 leader compounds into the active sites of SARS-CoV-2 proteins

Ligand	Scoring function (binding energy), kcal / mol	Protein – ligand interaction
ADP-binding domain NSP3		
AMP	–8.7	H-bonds: Ala 21, Asp 22, Ile 23, Val 49, Ile 131. Hydrophobic: Ala 38, Gly 48, Ala 50, Ala 52, Pro 125, Leu 126, Gly 130, Phe 132, Ala 154, Val 155, Phe 156, Leu 160. π-stacking: Ile 23, Val 49.
6*S*-coccineone B	–8.3	H-bonds: Ala 21, Gly 130. Hydrophobic: Asp 22, Ile 23, Gly 48, Pro 125, Leu 126, Pro 136, Ala 154, Phe 156, Leu 160, Leu 164. π-stacking: Val 49, Ala 52, Ala 129, Val 155.
6*R*-coccineone B	–8.0	H-bonds: Gly 130. Hydrophobic: Ala 21, Asp 22, Ile 23, Pro 125, Pro 136, Ala 154, Asp 157. π-stacking: Gly 48, Val 49, Ala 52, Leu 126, Ala 129, Val 155, Phe 156, Leu 160.
7,8-dihydroxyflavone	–8.0	H-bonds: Ile 23, Gly 48. Hydrophobic: Ala 21, Asp 22, Gly 130, Pro 136, Ala 154, Phe 156, Leu 160. π-stacking: Val 49, Ala 52, Leu 126, Ala 129, Val 155.
Main protease NSP5		
N3 inhibitor	–8.4	H-bonds: Phe 140, Asn 142, Gly 143, His 163, His 164, Glu 166, Gln 189, Thr 190. Hydrophobic: Thr 24, Thr 25, Thr 26, Met 49, Tyr 54, Ser 144, Cys 145, Met 165, His 172, Asp 187. π-stacking: His 41, Leu 141, Pro 168, Ala 191. Covalent: Cys 145.
6*S*-coccineone B	–8.5	H-bonds: Ser 144, Cys 145. Hydrophobic: Thr 25, Thr 26, His 41, Phe 140, Leu 141, Asn 142, Gly 143, His 163, His 164, Met 165, Glu 166. π-stacking: Leu 27, Cys 145.
Scutellarein	–8.1	H-bonds: Leu 141, Ser 144, Cys 145, Glu 166. Hydrophobic: His 141, Met 49, Phe 140, Asn 142, His 163, His 172, Arg 188, Gln 189, Thr 190. π-stacking: Met 165.
2-(1,3-benzodioxol-5-yl)-6-hydroxy-4*H*-chromen-4-one	–8.1	H-bonds: His 41, Phe 141, His 163, Asp 187. Hydrophobic: Pro 52, Tyr 54, Leu 141, Ser 144, His 164, Met 165, Glu 166, His 172, Arg 188, Gln 189. π-stacking: Cys 145, Met 49, His 41.
RNA-dependent RNA polymerase NSP12		
Remdesivir metabolite	–8.3	H-bonds: U(T) 10, U(P) 20, Asp 760. Hydrophobic: Lys 545, Val 557, Cys 622, Asp 623, Ser 682, Thr 687, Ala 688, Ser 757, Ser 759. π-stacking: A(11) 11, U20. Covalent: U(P) 20. Coulombic: Mg 101, Mg 1004.
3,7-dihydroxyflavone	–10.4	H-bonds: U(T) 12, U(P) 20, Gly 590. Hydrophobic: A(T) 11, A(P) 19, Val 588, Thr 591, Ser 592, Trp 598, Met 601, Ala 688, Gln 815. π-stacking: Ile 589, Lys 593, Leu 758, Cys 813.
2-(2,5-dimethoxy-phenyl)-3-hydroxy-chromen-4-one	–10.3	H-bonds: Gly 590, Thr 591. Hydrophobic: U(T) 12, A(T) 14, A(P) 19, U(P) 20, Ser 592, Phe 594, Trp 598, Met 601, Phe 812, Gln 815. π-stacking: A(T) 13, Ile 589, Lys 593, Leu 758, Cys 813.
Pinobanksin 3-*O*-propanoate	–10.3	H-bonds: U(P) 20, Gly 590. Hydrophobic: U(T) 12, A(T) 14, U(P) 18, A(P) 19, Thr 591, Ser 592, Phe 594, Trp 598, Gln 815. π-stacking: A(T) 13, Ile 589, Lys 593, Leu 758, Cys 813.
Endoribonuclease NSP15		
6*S*-coccineone B	–7.9	H-bonds: His 250, Val 292, Tyr 343. Hydrophobic: Lys 290, Tyr 343. π-stacking: His 235, Gly 248, Cys 293, Thr 341, Leu 346.
6*R*-coccineone B	–7.6	H-bonds: Val 292, Tyr 343. Hydrophobic: His 235, Gly 247, His 250, Cys 293, Thr 341, Leu 346. π-stacking: Lys 296, Tyr 343.
Calycosin	–7.6	H-bonds: His 235, Lys 290, Ser 294, Tyr 343. Hydrophobic: Gly 248, His 250, Cys 293, Trp 333, Glu 340, Leu 346. π-stacking: Trp 333, Thr 341, Tyr 343.

Note: the scoring values obtained as a result of three repetitions were identical. The native ligand is in italics.

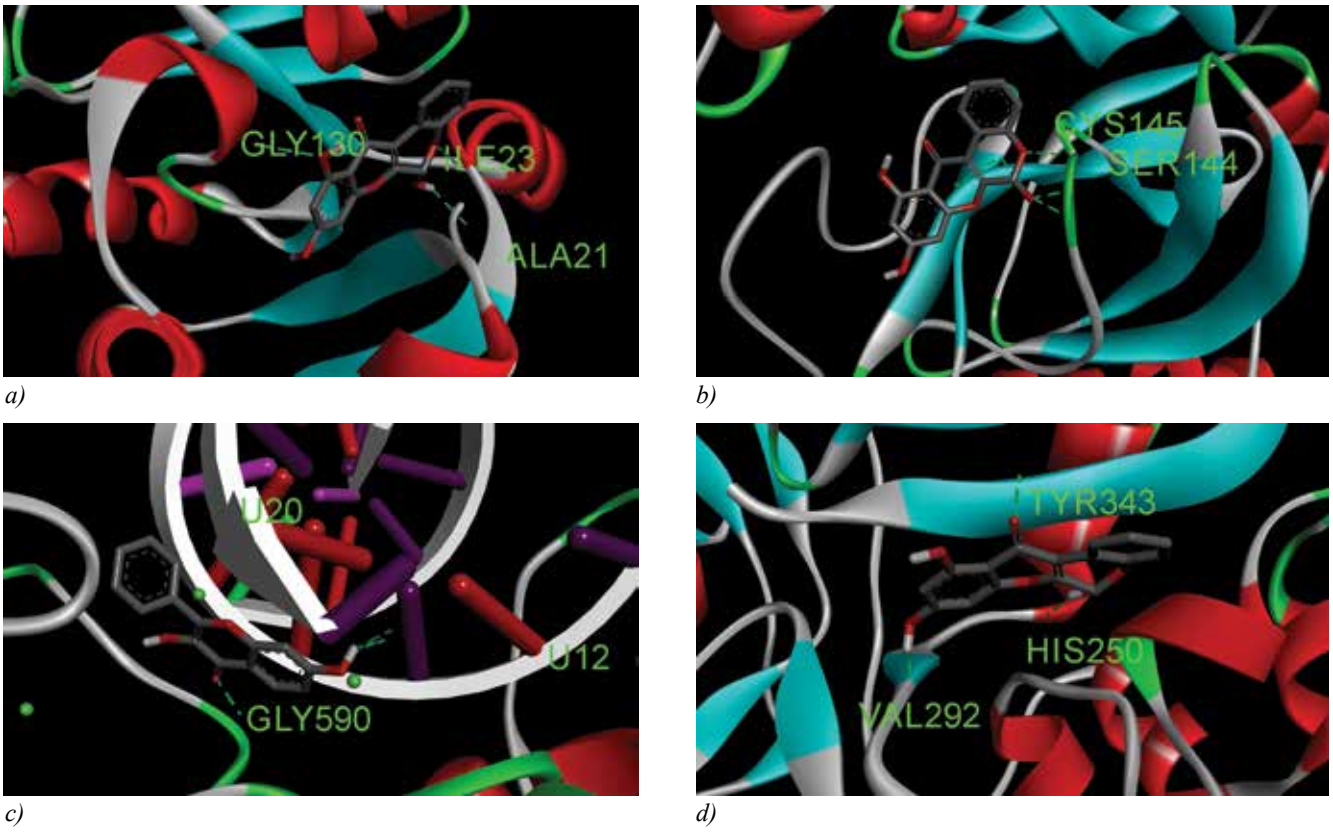

Figure. Docking: *a)* 6*S*-coccineone B into ADP-binding domain NSP3, *b)* 6*S*-coccineone B into main protease NSP5, *c)* 3,7-dihydroxyflavone into RNA-dependent RNA polymerase NSP12, *d)* 6*S*-coccineone B into endoribonuclease NSP15. Hydrogen bonds are shown as green dotted lines

It is interesting to note that the spatial structure of the ligand affects the qualitative and quantitative characteristics of docking. Thus, for 2*R*,3*R*-dihydroquercetin, better binding to main protease NSP5 was found compared with its other stereoisomers.

DISCUSSION

The median flavonoid binding energies to the ADP-binding domain NSP3, main protease NSP5, and endoribonuclease NSP15 had similar values (Table 2), which can be explained by belonging of these proteins to a class of hydrolases. In contrast, the median value of binding affinity to RNA-dependent RNA polymerase NSP12 belonging to a class of transferases was higher than that of the three above-mentioned proteins (Table 2).

The interaction of these compounds with the ADP-binding domain NSP3 can presumably block the ability of coronavirus to hide from host defense mechanisms [11]. Inhibition of main protease NSP5 and RNA-dependent RNA polymerase NSP12 can lead to prevention of assembly of new virions. The interaction of flavonoids with the uridylate-specific site of endoribonuclease NSP15 presumably blocks protein interference with the host innate immune response [12]. COVID-19 affects the lungs and is accompanied by inflammation [13]. Given a wide spectrum of pharmacological activities of flavonoids, which have proven to be effective capillary protectors [5] and anti-inflammatory agents [6], and their multitarget antiviral effect, these natural compounds can find application in the treatment of this disease.

CONCLUSION

In the course of the study, flavonoids were found to have a virucidal effect on SARS-CoV-2. One of them, 6S-coccineon B, is able to show high activity against several biological targets of SARS-CoV-2. The obtained results can be used to develop phytomedicines for comprehensive therapy of COVID-19.

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