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EXPERIMENTAL PAPER

In silico studies of selected xanthophylls as potential candidates against SARS-CoV-2 targeting main protease (Mpro) and papain-like protease (PLpro)

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Summary

Introduction: The main protease (Mpro) and the papain-like protease (PLpro) are essential for the replication of SARS-CoV-2. Both proteases can be targets for drugs acting against SARS-CoV-2.

Objective: This paper aims to investigate the *in silico* activity of nine xanthophylls as inhibitors of Mpro and PLpro.

Methods: The structures of Mpro (PDB-ID: 6LU7) and PLpro (PDB-ID: 6W9C) were obtained from RCSB Protein Data Bank and developed with BIOVIA Discovery Studio. Active sites of proteins were performed

using CASTp. For docking the PyRx was used. Pharmacokinetic parameters of ADMET were evaluated using SwissADME and pkCSM.

Results: β-cryptoxanthin exhibited the highest binding energy: –7.4 kcal/mol in the active site of Mpro. In PLpro active site, the highest binding energy had canthaxanthin of –9.4 kcal/mol, astaxanthin –9.3 kcal/mol, flavoxanthin –9.2 kcal/mol and violaxanthin –9.2 kcal/mol. ADMET studies presented lower toxicity of xanthophylls in comparison to ritonavir and ivermectin.

Conclusion: Our findings suggest that xanthophylls can be used as potential inhibitors against SARS-CoV-2 main protease and papain-like protease.

Key words: ***coronavirus, COVID-19, pandemics, computer-aided drug design, antiviral***

Słowa kluczowe: ***koronawirus, COVID-19, pandemia, projektowanie leków wspomagane komputerowo, działanie przeciwwirusowe***

INTRODUCTION

The SARS-CoV-2 is classified into the Betacoronaviruses genus, in the Riboviria kingdom, Nidovirales order, Coronaviridae family and the Orthocoronavirinae subfamily [1]. SARS-CoV-2 is a large, enveloped virus, which contains positive single-stranded, non-segmented RNA. It has four structural proteins: spike protein (S), nucleocapsid protein (N), membrane glycoprotein (M), and envelope protein (E) [2]. In SARS-CoV-2 are two open reading frames: ORF1a and ORF1ab. ORFs encode polyproteins pp1a and pp1ab, which form the non-structural proteins (NSP 1-16) [3]. NSPs are essential for viral replication. This process is facilitated by main protease (Mpro), also known as 3C-like protease (3CLpro), and the papain-like protease (PLpro). Both proteases can be targets for anti-SARS-CoV-2 drugs [4]. Recent studies show that main protease can be inhibited by ritonavir and papain-like protease by ivermectin [5, 6].

The SARS-CoV-2 coronavirus is responsible for recent pandemics. The virus causes a disease known as COVID-19. As of May 5, 2021, there were 153 738 171 confirmed cases of COVID-19 worldwide, including 3 217 281 deaths [7]. Vaccinations against SARS-CoV-2 infection were introduced very quickly [8] research on new ones is still being performed. However, there are no treatment guidelines for COVID-19. Various drugs are used with varying degrees of success. So far, remdesivir is the only drug approved by the Food and Drug Administration (FDA) to treat COVID-19 [9].

Xanthophylls are a group of pigments belonging to carotenoids found in plants and algae. The more common ones are fucoxanthin, astaxanthin, violaxanthin, zeaxanthin, and lutein [10]. Xanthophylls have multi-focal activity. Among others, their antibacterial [11], antibiofilm [12], antioxidant [13, 14], anti-inflammatory [13, 15] and anticancer [16] activities have been described. The latest publications indicate that xanthophylls may also have a protective effect in the course of COVID-19 [17, 18]. However, their mechanism of action against SARS-CoV-2 is not known.

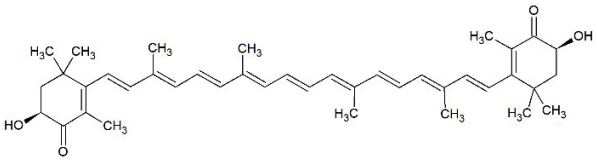
One of methods of drug discovery is *in silico* research, namely computer-aided drug design (CADD). CADD is a cost-effective and fast tool, as compared to traditional methods. It allows the prediction of protein structure and function, identification of small molecule (ligand) interactions, active site residues and the study of protein-ligand interactions. Designing and binding ligands to a protein (target) is referred to as docking. Docking identifies specific hit molecules from among multiple ligands [19, 20]. *In silico* studies also determine the safety of a potential drug by prediction of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles [21].

This paper aims to investigate the *in silico* activity of selected xanthophylls as inhibitors of the main protease (Mpro) and the papain-like protease (PLpro) of SARS-CoV-2.

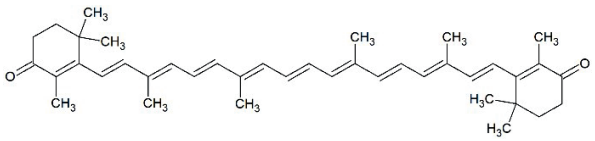
MATERIALS AND METHODS

Preparation of ligands and receptor

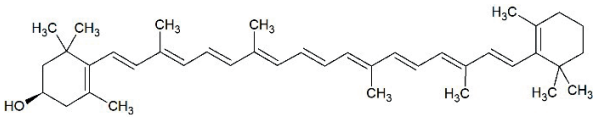
The 3D SDF structures of nine xanthophylls (astaxanthin, canthaxanthin, β-cryptoxanthin, flavoxanthin, fucoxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin) was downloaded from the PubChem database (fig. 1). Ritonavir and ivermectin were used as control compounds. Using the PyRx 0.8 [23] compounds, energy was minimized and files were converted to the PDBQT format for



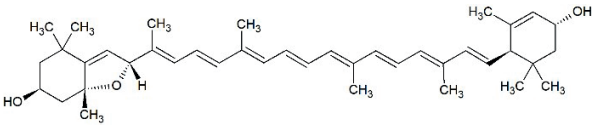
astaxanthin



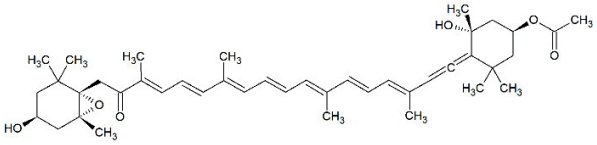
canthaxanthin



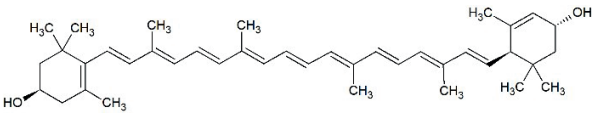
β-cryptoxanthin



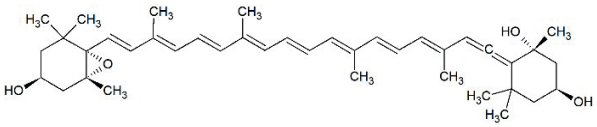
flavoxanthin



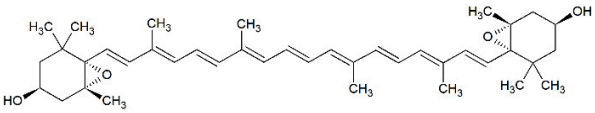
fucoxanthin



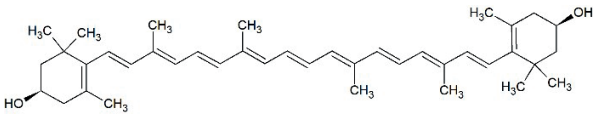
lutein



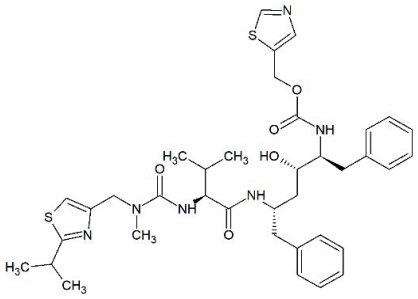
neoxanthin



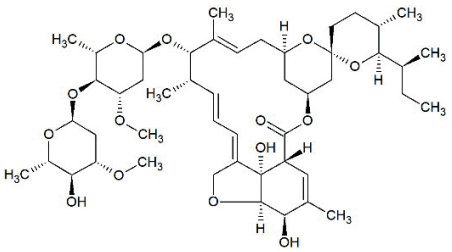
violaxanthin



zeaxanthin



ritonavir



ivermectin

Figure 1.

Chemical structures of the selected molecules for computational studies

docking. The structures of the SARS-CoV-2 main protease (PDB-ID: 6LU7 at a resolution of 2.16 Å) and the papain-like protease (PDB-ID: 6W9C at a resolution of 2.70 Å) were obtained from RCSB Protein Data Bank [24]. The hetero-atoms, water, and ligand groups were removed from the structures using BIOVIA Discovery Studio DS2021 [25].

Docking

Active sites of proteins were performed using the Computed Atlas of Surface Topography of proteins (CASTp) [26]. The active site of 6LU7 was in chain A and the active site of 6W9C was between A, B and C chains.

For docking, the Autodock Vina tool was used compiled in the PyRx 0.8 [23]. The grid boxes had the following values: for 6LU7 dimensions x, y, z: 25.7459 Å, 29.2824 Å, 30.5270 Å, centre x, y, z: -11.1833 Å, 14.7388 Å, 68.9308 Å, and for 6W9C dimensions x, y, z: 86.9085 Å, 88.8525 Å, 95.9205 Å, centre x, y, z: -36.8549 Å, 11.6758 Å, 38.9205 Å.

In silico drug-likeness and ADMET prediction

Drug-likeness properties were calculated using Lipinski's rule of five [27]. According to this rule, the orally active substance should have no more than one violation of the following criteria:

- no more than 5 H bond donors (OH, NH, and SH);
- no more than 10 H bond acceptors (N, O, and S atoms);
- molecular weight less than 500 Da;
- octanol-water partition coefficient (log P) lower than 5.

Pharmacokinetic parameters of absorption, distribution, metabolism, excretion, and toxicity (ADMET) were evaluated using SwissADME [29].

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

In silico studies allow for fast prediction of activity and toxicity of natural compounds. SARS-CoV-2 computational analyses are important in searching for active drugs or designing vaccines [30–34]. The main protease (Mpro) and the papain-like protease (PLpro) can be targets for anti-SARS-CoV-2 drugs. Research in this direction is prevalent and concerns mainly common natural compounds or repurposing of already used drugs [35–38]. Studies presented in this article are first concerning the activity of xanthophylls against SARS-CoV-2.

Results indicate that some xanthophylls exhibited binding energies similar to control drugs. In the case of the main protease, the binding energy of ritonavir was –7.7 kcal/mol, whereas *β*-cryptoxanthin was –7.4 kcal/mol. In papain-like protease, the binding energy of ivermectin was –9.5 kcal/mol, whereas for canthaxanthin was –9.4 kcal/mol, for astaxanthin –9.3 kcal/mol, and for both flavoxanthin and violaxanthin was –9.2 kcal/mol (tab. 1). Amino acid residues involved in interactions between viral proteases and leads with the best binding energies are presented in figures 2–7.

It was found that all the tested xanthophylls violated two rules of Lipinski, namely molecular weight and Log P. This means that all compounds are poorly soluble in water and have low gastrointestinal absorption. Therefore, it would be necessary to create, for example, nanoparticles to increase the availability of xanthophylls. Ritonavir and ivermectin, used as controls, also have two violations of Lipinski's rules (tab. 2).

All the tested xanthophylls do not demonstrate AMES toxicity, hepatotoxicity and skin sensitization. Xanthophylls have oral rat acute and chronic toxicity similar to these of control drugs. What is interesting, ritonavir and ivermectin show hepatotoxicity. It means that xanthophylls are less toxic than these two FDA-accepted drugs (tab. 3).

Table 1.

Free binding energies of the selected xanthophylls and control compounds against SARS-CoV-2

Compound	PubChem ID	Main protease (6LU7)	Papain-like protease (6W9C)
		Binding energy [kcal/mol]	
Astaxanthin	5281224	–6.1	**–9.3**
Canthaxanthin	5281227	–6.1	**–9.4**
β-Cryptoxanthin	5281235	**–7.4**	–8.8
Flavoxanthin	5281238	–6.6	**–9.2**
Fucoxanthin	5281239	–6.3	–8.3
Lutein	5281243	–6.2	–9.0
Neoxanthin	5281247	–6.9	–8.8
Violaxanthin	448438	–6.5	**–9.2**
Zeaxanthin	5280899	–6.4	–8.2
Ritonavir	392622	**–7.7 (control)**	–8.7
Ivermectin	6321424	–8.1	**–9.5 (control)**

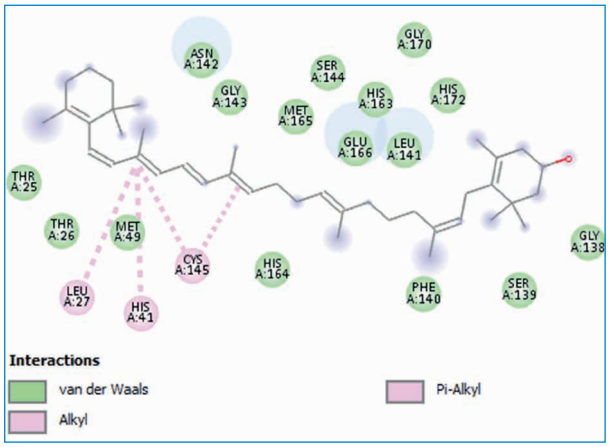


Figure 2.

Interactions of β-cryptoxanthin docked into the main protease

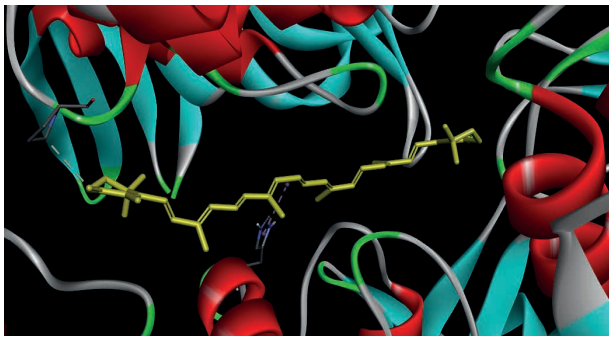


Figure 3.

Canthaxanthin docked into the active site of the papain-like protease

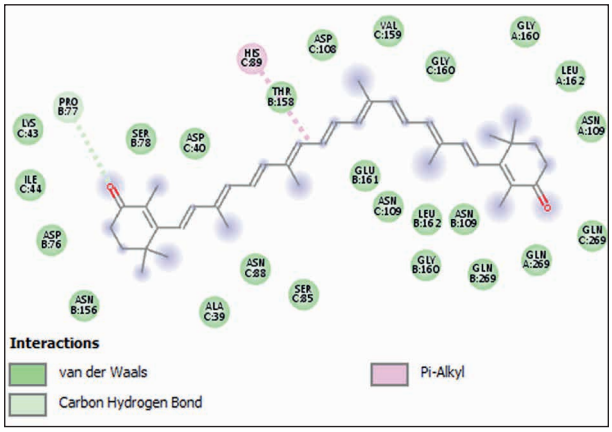


Figure 4.

Interactions of canthaxanthin docked into papain-like protease

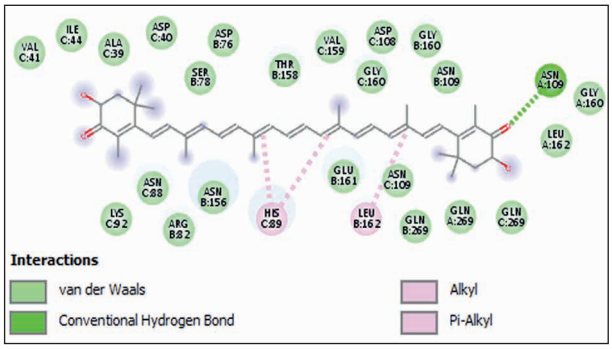


Figure 5.

Interactions of astaxanthin docked into papain-like protease

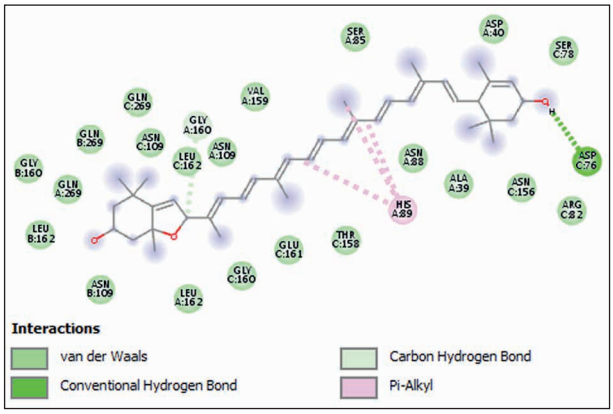


Figure 6.

Interactions of flavoxanthin docked into papain-like protease

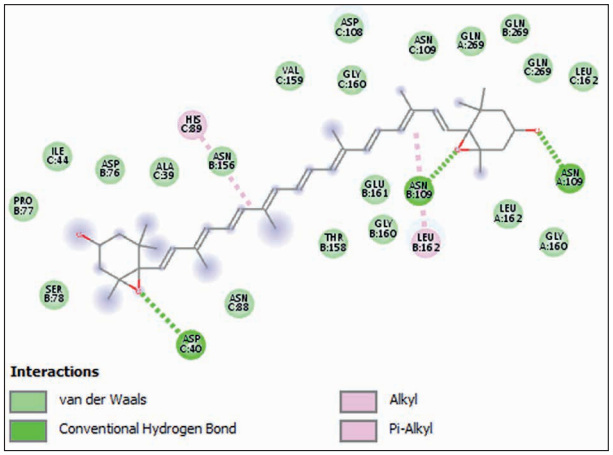


Figure 7.

Interactions of violaxanthin docked into papain-like protease

Table 2.

Physicochemical properties of the tested xanthophylls

Compound	Lipinski's rule of five			
	Molecular weight (Da)	Partition coefficient (Log P)	Hydrogen bond donors (HBD)	Hydrogen bond acceptors (HBA)
Astaxanthin	596.84	8.24	2	4
Canthaxanthin	564.84	9.64	0	2
β-Cryptoxanthin	552.87	10.20	1	1
Flavoxanthin	584.87	8.50	2	3
Fucoxanthin	658.91	7.72	2	6
Lutein	568.87	9.21	2	2
Neoxanthin	600.87	7.88	3	4
Violaxanthin	600.87	8.39	2	4
Zeaxanthin	568.87	9.31	2	2
Ritonavir	720.94	5.04	4	7
Ivermectin	875.09	4.35	3	14

Table 3.

Predicted toxicity of the tested xanthophylls

Name	AMES toxicity	Oral rat acute tox. LD_{50}	Oral rat chronic tox. LOAEL	Hepatotoxicity	Skin sensitization
Astaxanthin	No	3.515	2.173	No	No
Canthaxanthin	No	2.188	2.568	No	No
β-Cryptoxanthin	No	2.333	0.517	No	No
Flavoxanthin	No	2.218	2.225	No	No
Fucoxanthin	No	2.428	1.146	No	No
Lutein	No	3.491	2.572	No	No
Neoxanthin	No	2.350	2.077	No	No
Violaxanthin	No	2.132	2.054	No	No
Zeaxanthin	No	3.496	2.603	No	No
Ritonavir	no	2.703	2.231	Yes	No
Ivermectin	no	3.013	1.883	Yes	No

CONCLUSIONS

Some xanthophylls exhibited binding energies similar to drugs used in the treatment of SARS-CoV-2 infection. We identified β-cryptoxanthin as a potent inhibitor of SARS-CoV-2 main protease, and simultaneously canthaxanthin, astaxanthin, flavoxanthin and violaxanthin as inhibitors of papain-like protease. ADMET studies presented that xanthophylls have lower toxicity than ritonavir and ivermectin. Our findings suggest that xanthophylls can be used as potential inhibitors against SARS-CoV-2 main protease and papain-like protease.

Conflict of interest: Authors declare no conflict of interest.

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