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In Silico Identification of Multi-target Anti-SARS-CoV-2 Peptides from Quinoa Seed Proteins

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Abstract

Peptides are promising antagonists against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). To expedite drug discovery, a computational approach is widely employed for the initial screening of anti-SARS-CoV-2 candidates. This study aimed to investigate the potential of peptides from quinoa seed proteins as multi-target antagonists against SARS-CoV-2 spike glycoprotein receptor-binding domain, main protease, and papain-like protease. Five quinoa proteins were hydrolyzed in silico by papain and subtilisin. Among the 1465 peptides generated, seven could interact stably with the key binding residues and catalytic residues of the viral targets, mainly via hydrogen bonds and hydrophobic interactions. The seven peptides were comparable or superior to previously reported anti-SARS-CoV-2 peptides based on docking scores. Key residues in the seven peptides contributing to binding to viral targets were determined by computational alanine scanning. The seven peptides were predicted in silico to be non-toxic and non-allergenic. The peptides ranged between 546.66 and 3974.87 g/mol in molecular mass, besides exhibiting basic and cationic properties (isoelectric points: 8.26–12.10; net charges: 0.1–4.0). Among the seven peptides, VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS was found to bind the largest number of key residues on the targets. In conclusion, seven putative non-toxic, non-allergenic, multi-target anti-SARS-CoV-2 peptides were identified from quinoa seed proteins. The in vitro and in vivo efficacies of the seven peptides against SARS-CoV-2 deserve attention in future bench-top testing.

Keywords Antiviral peptide · Bioinformatics · COVID-19 · Quinoa seed protein · SARS-CoV-2

Introduction

The coronavirus disease 2019 (COVID-19) outbreak posed a significant threat to human health worldwide (Huang et al. 2020). The receptor-binding domain (RBD) of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) spike glycoproteins plays a vital role in facilitating entry into the host cell via its interactions with the human angiotensin-converting enzyme 2 (hACE2) (Wang et al. 2020; Yi et al. 2020). Mutations of nine key binding residues of RBD, namely Leu455, Phe456, Ser459, Gln474, Ala475, Phe486, Phe490, Gln493, and Pro499, have been empirically demonstrated to abolish the binding affinity of SARS-CoV-2 spike glycoproteins to hACE2 (Yi et al. 2020). This supports the hypothesis that targeting the key binding residues could block the binding of SARS-CoV-2 spike glycoproteins to hACE2, thus precluding the infections of host cells (Yi et al. 2020). Following the release of the viral genome into the host cytoplasm and the synthesis of viral polyproteins, SARS-CoV-2 main protease (M^{pro}) and papain-like protease (PL^{pro}) cleave the polyproteins into functional fragments that are crucial for viral replication. It was expected that impeding the action of the two proteases by targeting the catalytic residues of M^{pro} (His41 and Cys145) and PL^{pro} (Cys111, His272, and Asp286) could suppress SARS-CoV-2 replication and the spread of infection (Ortega et al. 2020; Wlodawer et al. 2020). To date, no human proteases with similar cleavage specificities as M^{pro} and PL^{pro} are known. Thus inhibitors against SARS-CoV-2 M^{pro} and PL^{pro} are likely non-toxic

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to human cells (Zhang et al. 2020). Taken together, the SARS-CoV-2 spike glycoprotein RBD, M^{pro}, and PL^{pro} make attractive targets for the discovery and development of effective antiviral drugs against COVID-19.

Since the COVID-19 outbreak, many research groups have started searching for potential anti-COVID-19 agents, adopting the in silico approach. Through virtual screening and molecular docking, several natural and artificial inhibitors, as well as repurposed drugs that potentially block the entry of SARS-CoV-2 into human cells, were discovered (Choudhary et al. 2020; Luo et al. 2020; Wong et al. 2020a, 2021). Likewise, computational discoveries of inhibitors of M^{pro} and PL^{pro} were made (Ortega et al. 2020; Wong et al. 2021; Kandeel et al. 2020).

Food-derived bioactive peptides have drawn attention for their potential applications as functional food ingredients, therapeutics, vaccines, and diagnostic agents (Haggag et al. 2018; Chai et al. 2017; Wong et al. 2020b). Plant seeds are good sources of antiviral peptides (Sonawane and Arya 2018). To the best of our knowledge, there are yet reports of antiviral peptides from quinoa seeds. However, the discovery of bioactive peptides, such as antioxidant, anti-angiotensin-converting-enzyme-I, anti-inflammatory, and anti-dipeptidyl-peptidase-IV peptides, from quinoa seeds are well documented (Aluko and Monu 2003; Nongonierma et al. 2015; Galante et al. 2020; Ren et al. 2017). The release of bioactive peptides from food proteins is often accomplished by enzymatic hydrolysis (Chai et al. 2017; Wong et al. 2020b). Papain and subtilisin could effectively release bioactive peptides from quinoa proteins (Aluko and Monu 2003; Nongonierma et al. 2015). Therefore, the two enzymes were chosen for in silico release of peptides from quinoa seed proteins in this study. To date, several peptide-based compounds, namely N3, VIR251, and SBP1, were demonstrated empirically as antagonists against SARS-CoV-2 M^{pro}, PL^{pro}, and spike glycoprotein RBD, respectively (VanPatten et al. 2020; Jin et al. 2020; Rut et al. 2020). Compared to phytochemicals, peptides are still underexplored as SARS-CoV-2 inhibitors. Therefore, the objective of this study was to computationally investigate the potential of quinoa-derived peptides as multi-target antagonists against three key SARS-CoV-2 targets: spike glycoprotein RBD, M^{pro}, and PL^{pro}. The multi-target approach would be efficacious in dealing with the high mutation rates of RNA viruses-associated diseases, as is expected of COVID-19 (Pachetti et al. 2020). In this study, computational alanine scanning was performed to investigate the significance of each residue of the putative multi-target peptides towards the stability of their binding to viral targets. In silico prediction of physicochemical properties, pharmacokinetics, druglikeness, medicinal chemistry, toxicity and allergenicity of the short-listed multi-target peptides were also carried out to provide hints for their antiviral potential and safety risk.

Methods

In Silico Proteolysis

The sequences of five major storage proteins of the quinoa seed (2S albumin-like, 11S seed storage globulin, 11S globulin seed storage protein 2-like, 13S globulin seed storage protein 1-like, and 13S globulin seed storage protein 2-like) were downloaded from NCBI (https://www.ncbi.nlm.nih.gov) (Clark et al. 2015) on 14 August 2020. These protein sequences were subjected to in silico hydrolysis by papain (EC 3.4.22.2) and subtilisin (EC 3.4.21.62) separately by using the BIOPEP-UWM web-server (Minkiewicz et al. 2019).

Molecular Docking Analyses with HPEPDOCK

The crystal structures of three SARS-CoV-2 targets, namely spike glycoprotein RBD (PDB ID: 6LZG) (Wang et al. 2020), M^{pro} (PDB ID: 6LU7) (Jin et al. 2020), and PL^{pro} (PDB ID: 6WX4) (Rut et al. 2020), were retrieved from Protein Data Bank (http://www.rcsb.org/pdb) (Berman et al. 2000; Burley et al. 2018) on 20 July, 22 July, and 28 August 2020, respectively. The proteins were prepared for molecular docking using BIOVIA Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, BIOVIA Discovery Studio Visualizer, Version 20.1.0.192, San Diego: Dassault Systèmes, 2020). Co-crystallized water, heteroatom, and ligand were removed, whereas polar hydrogens were added. The bound ligands hACE2, N3, and VIR251 in complex with the crystal structures of spike glycoprotein RBD, M^{pro}, and PL^{pro}, respectively, were separated and prepared in the same manner as protein preparation. The optimized structures saved in the pdb format were taken for HPEPDOCK analysis (Zhou et al. 2018). The three prepared viral proteins were uploaded as receptor inputs, whereas the prepared hACE2, N3, and VIR251 were uploaded as binding site references for analysis on spike glycoprotein RBD, M^{pro}, and PL^{pro}, respectively. Quinoa peptides released by in silico papain and subtilisin hydrolyses were entered in the FASTA format as peptide inputs. Reference peptides previously reported to potentially bind with SARS-CoV-2 viral proteins were mostly entered in the FASTA format as peptide inputs, except for reference peptides p18, p28, and VIR250, which were uploaded as in the pdb format. Tables S1–S3 show previously reported peptides that we used as reference peptides to compare with quinoa seed peptides.

The docking scores computed for the quinoa and reference peptides were recorded. Three dimensional (3D) diagram of protein-peptide docking models were visualized

by using BIOVIA Discovery Studio Visualizer. The two dimensional (2D) diagram of docked models with lower (more negative) docking scores relative to SBP1, N3 and/or VIR251 were visualized with LigPlot+ v.2.2. (Wallace et al. 1995; Laskowski and Swindells 2011). The molecular interactions, including hydrogen bonds, hydrophobic interactions, salt bridges, and external bonds, between the peptides and viral proteins were checked.

Computational Alanine Scanning

Computational alanine scanning was performed using the BUDE alanine scan server (https://balas.app) (Wood et al. 2020) to identify peptide residues that are critical for binding to the three target viral proteins. The models of selected peptides docked to the viral proteins were uploaded to the server. The approach involves substituting each residue with alanine in turn, followed by computation of the resultant change (ΔΔG) in the overall binding energy (Ibarra et al. 2019).

Prediction of Physicochemical Properties, Pharmacokinetics, Druglikeness, Medicinal Chemistry, Toxicity, and Allergenicity

SwissADME (http://www.swissadme.ch) was used to calculate physicochemical descriptors and predict ADME criteria, pharmacokinetics, druglike nature, as well as medicinal chemistry acceptance (Daina et al. 2017). The SMILES format of the peptides were obtained from the NovoPro server (https://www.novoprolabs.com). Further, the molecular mass, isoelectric point, and net charge at neutral pH of selected quinoa peptides were predicted by using an online peptide property calculator (https://pepcalc.com). The potential toxicity of peptides was predicted by using the ToxinPred server (http://crdd.osdd.net/raghava/toxinpred) (Gupta et al. 2013) as previously described (Chai et al. 2019). AllerTOP v.2.0 (http://www.ddg-pharmfac.net/AllerTOP) (Dimitrov et al. 2014) was used for allergenicity prediction.

Results and Discussion

In silico proteolysis of quinoa seed storage proteins by using papain and subtilisin produced 883 and 582 peptide fragments, respectively (Table S4). The peptides can be collectively reduced into 654 unique sequences of 2–33 residues (data not shown). HPEPDOCK analysis of the unique sequences on M^{pro}, PL^{pro}, and spike glycoprotein RBD returned docking scores ranging from − 36.954 to − 251.813, − 41.689 to − 223.713, and − 36.904 to − 210.374, respectively (Table S5). By selecting for peptides with lower (more negative) docking scores than those computed for N3, VIR251, and/or SBP1, we narrowed down the 654 peptides to 18 (data not shown). N3, VIR251, and SBP1 are peptide-based inhibitors whose inhibition of M^{pro}, PL^{pro} and spike glycoprotein RBD were demonstrated empirically (VanPatten et al. 2020; Jin et al. 2020; Rut et al. 2020). Next, by selecting for peptides which could bind to at least one key binding residue on the spike glycoprotein RBD, at least one catalytic-dyad residue in M^{pro}, and at least one catalytic-triad residue in PL^{pro}, we narrowed down the 18 peptides to eight potential trifunctional peptides (Table 1, Tables S6–S8). All of the eight peptides listed in Table 1 could bind more stably to PL^{pro} in comparison with VIR251, as suggested by their lower scores relative to that of VIR251. Furthermore, PHWNIN and ERHHRGGRGRQS could also bind more stably to M^{pro} when compared to N3. None of the 654 quinoa peptides analyzed had a lower docking score than SBP1 when docked against the spike glycoprotein RBD (Table S5). Considering their predicted binding to key binding/catalytic residues in the three target proteins, the eight peptides are potential trifunctional inhibitory peptides against SARS-CoV-2. Targeting multiple proteins associated with crucial pathways of SARS-CoV-2 infection is more likely to control COVID-19 conditions in contrast to targeting only a single viral protein (Zhou et al. 2020).

In this study, we also analyzed the docking scores of several reference peptides compared to the quinoa seed peptides. The docking score of VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS was up to 1.32-fold lower than those of reference peptides QRPR, p28, and LPIY when docked against M^{pro} (Table S5), and up to 1.53-fold lower than VIR251, p28, VIR250, and p18 when docked against PL^{pro} (Table S5). When docked against the spike glycoprotein RBD, the docking score of VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS was up to 1.67-fold lower than reference peptides PQQQF, PISCR, VPW, and VQVVN (Table S5). Hence, based on the docking score, VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS potentially bind more stably to M^{pro}, PL^{pro}, and the spike glycoprotein RBD when compared with some anti-COVID-19 peptides reported in the literature.

The elucidation of intermolecular interactions between the quinoa peptides and the three viral proteins is necessary to establish the significance of the docking scores obtained. Among the eight potential trifunctional peptides in Table 1, VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS could form the highest number of interactions with three target proteins. It could potentially bind to His41 and Cys145 on M^{pro} via hydrophobic interaction (Fig. 1). His41 of M^{pro} plays the important role of deprotonating Cys145, activating the latter for its nucleophilic attack on the peptide bond of SARS-CoV-2 polyproteins. Thus, disruption of the two catalytic-dyad residues could potentially dampen M^{pro} activity

Table 1 Docking scores computed for eight potential trifunctional peptides and their number of interactions with the target proteins

Proteases used for hydrolysis	Peptides released[a]	Docking scores based on viral proteins[b]			Number of key binding/catalytic residues the peptides interacting with[c]		
		M^{pro}	PL^{pro}	RBD	M^{pro}	PL^{pro}	RBD
Papain	PNWKIN	− 200.405	**− 200.518**	− 192.529	2	1	3
	PHYNN	− 212.036	**− 190.318**	− 172.688	2	1	2
	PHWNIN	**− 243.337**	**− 210.290**	− 190.799	1	1	2
Subtilisin	VEDKGMMHQQRM-MEKAMNIPRMCGT-MQRKCRMS	− 211.578	**− 223.713**	− 207.146	2	3	4
	TKHGGRINTL	− 206.586	**− 188.343**	− 169.248	2	1	4
	PKRF	− 182.408	**− 191.522**	− 145.040	2	2	2
	ERHHRGGRGRQS	**− 217.103**	**− 202.493**	− 207.936	1	2	3
	AIRAMPL	− 188.547	**− 189.679**	− 146.175	2	1	1

[a]Quinoa peptides derived from each protease treatment were sorted in descending order based on the total number of interactions they were predicted to have with the viral proteins

[b]Values in bold are lower (more negative) than those computed for N3 (− 215.634) and VIR251 (− 186.078), the inhibitors complexed to the M^{pro} and PL^{pro} crystal structures, respectively

[c]Key binding residues of spike glycoprotein RBD (Leu455, Phe456, Ser459, Gln474, Ala475, Phe486, Phe490, Gln493 and Pro499) are those critical for binding between RBD and hACE2. The catalytic-dyad residues in the active site of M^{pro} are His41 and Cys145. The catalytic-triad residues in the active site of PL^{pro} are Cys111, His272 and Asp286

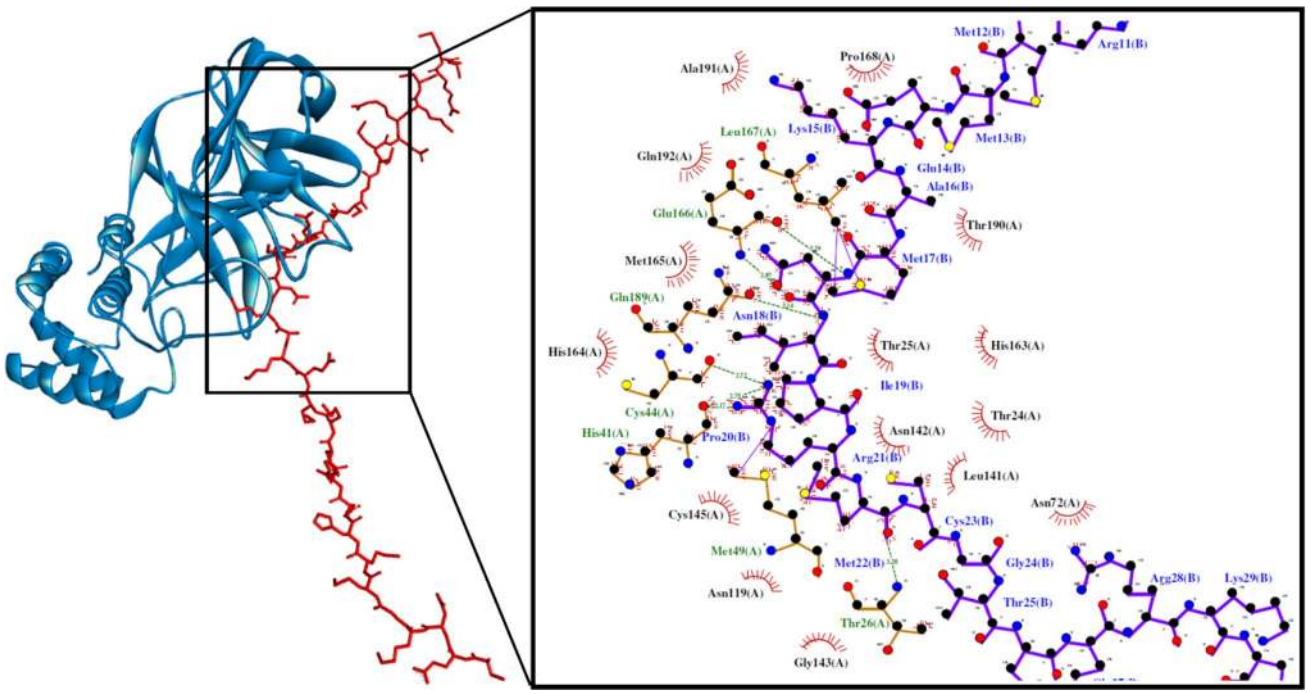

Fig. 1 The M^{pro}-VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS docked model presented in 3D (left) and 2D (right) diagrams. The protein and peptide structures are represented in blue and red, respectively. In the 2D diagram, the protein bonds are in orange, whereas that of a peptide is purple. The hydrophobic bonds, external bonds, hydrogen bonds, and salt bridges are displayed in red spoked arcs, purple lines, green and red dashed lines. The projected view displays only the interacting residues at the binding interface (colour figure online)

(Ramos-Guzmán et al. 2020). VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS could also form five hydrogen bonds with His41, Glu166, and Gln189, which are in the substrate-binding pocket of M^{pro} (Jin et al. 2020). Multiple hydrogen bonds formed between the peptide and residues in the M^{pro} substrate-binding pocket could lock the peptide inside the pocket, inhibiting M^{pro} activity (Jin et al. 2020). Notably, among all peptides analyzed in this study, VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS is the only peptide that could bind to all three catalytic-triad residues of PL^{pro} (Table S7). It was predicted to bind to Cys111 and His272 on PL^{pro} via hydrophobic interaction and binding to Asp286 via the salt bridge (Fig. 2). Its three catalytic-triad residues mediate the proteolysis of viral polyproteins by PL^{pro}: Cys111 as a nucleophile, His272 as a general acid, with the assistance of Asp286 (Henderson et al. 2020). In light of those mentioned above, the cleavage of SARS-CoV-2 polyproteins by M^{pro} and PL^{pro} could potentially be inhibited by VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS. Moreover, the peptide was predicted to form hydrophobic interactions with key binding residues Leu455, Phe456, Phe486, and Gln493 on RBD (Fig. 3). The four residues are crucial for hACE2-SARS-CoV-2 complex formation, a prerequisite for viral attachment to the host cell (Yi et al. 2020). Meanwhile, the high affinity between SARS-CoV-2 spike glycoprotein and hACE2 is accounted for by significant hydrophobic attractions between the hydrophobic surfaces of the two proteins at their binding interface (Li et al. 2020). Hence, VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS could potentially compromise the binding of SARS-CoV-2 spike glycoprotein RBD with hACE2.

Based on the in silico alanine scanning experiment on the eight trifunctional peptides (Table S9), the residues that formed a more significant number of interactions with the viral proteins were often the more critical residues for binding and stabilizing the peptide-protein complex. For instance, Arg of AIRAMPL was computed by LigPlot+ to form four salt bridges, three hydrogen bonds, and hydrophobic interactions with PL^{pro} residues; alanine substitution of the residue increased ΔΔG by 20.2079 kJ/mol. Contrarily, those residues predicted by LigPlot+ to have no interactions with the viral proteins had little or no effects on ΔΔG after alanine substitution. Our analysis on spike glycoprotein RBD agrees with Baig et al. (2020) that Phe and His are essential residues for binding to the viral protein. For instance, substituting Phe of PKRF with Ala increased ΔΔG by 23.5656 kJ/mol. Alanine substitution of His of TKHGGRINTL increased ΔΔG by 8.3854 kJ/mol.

Furthermore, substituting Leu of AIRAMPL with Ala increased ΔΔG by 8.5420 kJ/mol. This observation agrees with that of Baig et al. (2020) that a C-terminal Leu likely plays a significant role in stabilizing the interactions between peptides and spike glycoprotein RBD. Information gathered from the in silico alanine scanning experiment could guide

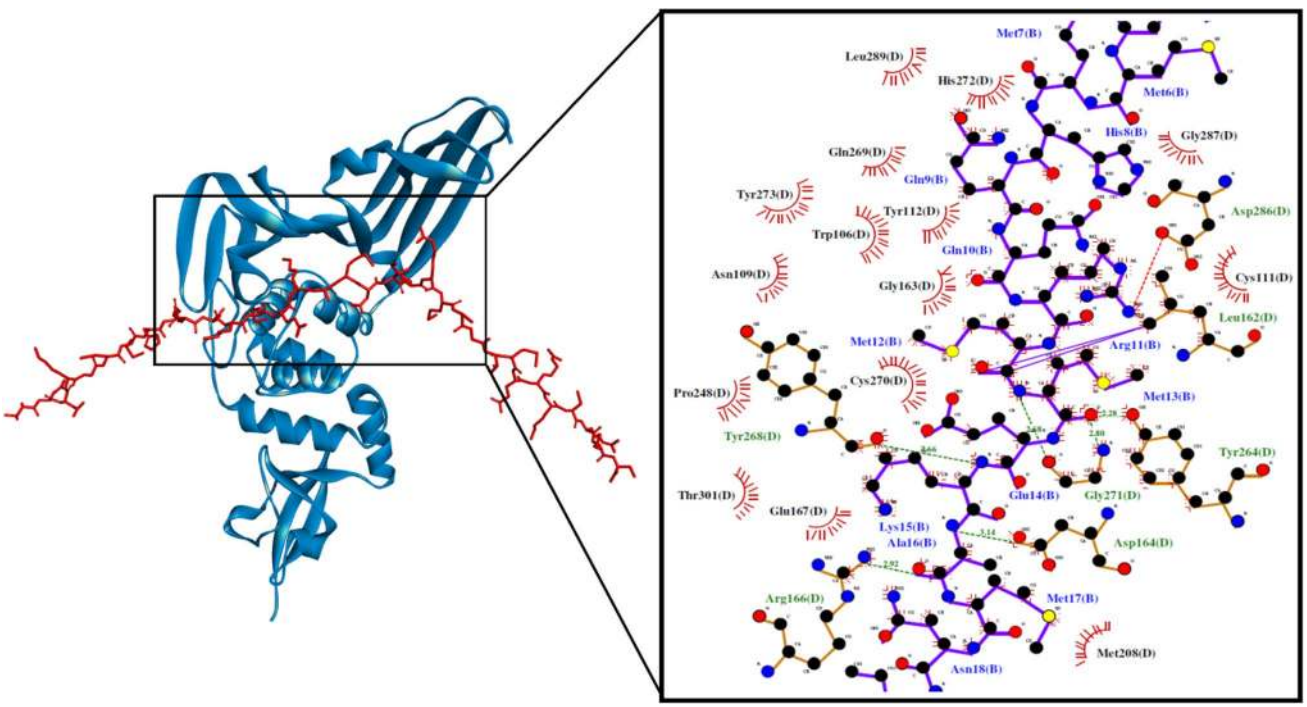

Fig. 2 The PL^{pro}-VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS docked model presented in 3D (left) and 2D (right) diagrams. Descriptions for the 2D and 3D diagrams are the same as those in Fig. 1 (colour figure online)

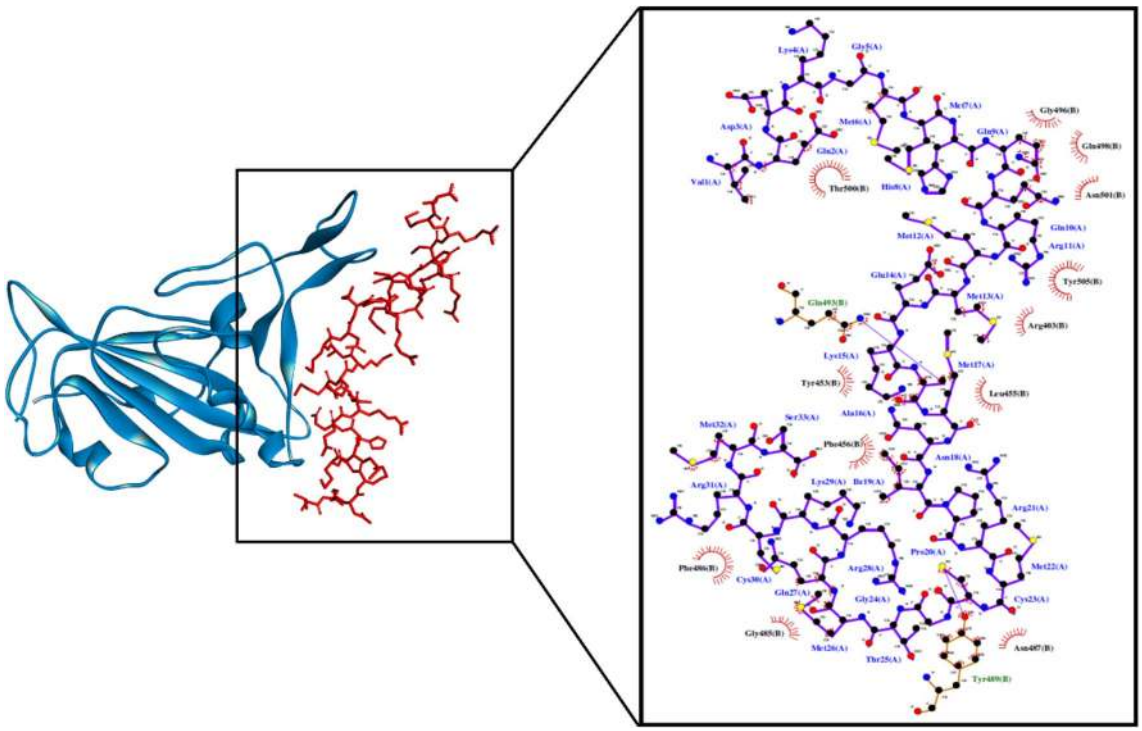

Fig. 3 The RBD-VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS docked model presented in 3D (left) and 2D (right) diagrams. Descriptions for the 2D and 3D diagrams are the same as those in Fig. 1 (colour figure online)

future studies to design more effective anti-COVID-19 peptides from the eight candidates reported in this study.

All eight potential trifunctional peptides were basic and cationic (Table 2). As indicated by their isoelectric points (7.99–12.10), the basic properties of the peptides are accounted for by the presence of basic residues. The eight potential trifunctional peptides are comprised of at least 14.3% of basic residues in their sequences. NGAICWGPCPTAFRQIGNCGHFKVRCCKIR is an antiviral peptide derived from mouse beta-defensin-4. The peptide was effective against respiratory viruses, including severe acute respiratory syndrome coronavirus type 1 (SARS-CoV-1) and middle-east respiratory syndrome coronavirus (MERS-CoV) (Zhao et al. 2016). The abundance of basic amino acids in NGAICWGPCPTAFRQIGNCGHFKVRCCKIR was proposed to facilitate late endosomal acidification inhibition, thus precluding viral RNA release into host cells (Zhao et al. 2016).

On the other hand, the electrostatic affinity to the negatively-charged viral surface is a possible mechanism by which some cationic antiviral peptides could disrupt the viral envelope (Mahendran et al. 2020; Skalickova et al. 2015). SARS-CoV-2, SARS-CoV-1, and MERS-CoV share similarities in their infection mechanisms (Zhu et al. 2020). Moreover, VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS and

Table 2 Predicted physicochemical properties, toxicity, and allergenicity of eight potential trifunctional peptides

Peptides	Mass (g/mol)	Isoelectric point	Net charge	Toxicity	Allergenicity
PKRF	546.66	11.52	2.0	Non-toxin	Probable non-allergen
PHYNN	643.65	7.99	0.1	Non-toxin	Probable allergen
PNWKIN	770.88	10.57	1.0	Non-toxin	Probable non-allergen
AIRAMPL	770.99	10.90	1.0	Non-toxin	Probable non-allergen
PHWNIN	779.84	8.26	0.1	Non-toxin	Probable non-allergen
TKHGGRINTL	1096.24	11.39	2.1	Non-toxin	Probable non-allergen
ERHHRGGRGRQS	1432.51	12.10	3.2	Non-toxin	Probable non-allergen
VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS	3974.87	10.40	4.0	Non-toxin	Probable non-allergen

NGAICWGPCPTAFRQIGNCGHFKVRCCKIR have comparable physicochemical characteristics in terms of their basic properties and net charges (data not shown). Thus whether the quinoa peptide VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS could also inhibit SARS-CoV-2 by dampening endosomal acidification and disrupting viral envelope, besides acting directly on the three target proteins, is an interesting question to be addressed in future research.

In this study, the eight potential trifunctional peptides ranged between 4 and 33 residues in length and between 546.66 and 3974.87 g/mol in molecular mass (Table 2). Overall, these peptide lengths fall within the range of 2–39 residues reported for FDA-approved peptide drugs (including both marketed drugs and clinical candidates) as reviewed by Santos et al. (2016). The same authors revealed that a majority of the peptide drugs have molecular masses of up to 1800 g/mol (Santos et al. 2016). Hence, with the exception of VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS, the other seven trifunctional peptides in this study also fit the molecular mass range of of the aforementioned FDA-approved peptide drugs. However, molecular mass alone should not render VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS (3974.87 g/mol) unworthy of further consideration; the peptide is still clearly smaller than two FDA-approved antiviral peptide drugs (tifuvirtide, 5037 g/mol; enfuvirtide, 4565 g/mol) (Santos et al. 2016; National Center for Biotechnology Information 2021a, 2021b). In general, in terms of peptide length and molecular mass, the eight potential trifunctional peptides do not differ considerably from currently known peptide drugs.

All the eight peptides listed in Table 2 were predicted to be non-toxic. Only antiviral peptides that could impair viral infection without being toxic to human cells can be considered useful and desired therapeutic agents (Mulder et al. 2013). Our results also agree with Gupta et al. (2013) that non-toxic peptides are often comprised of Ala, Arg, Gln, Ile, Leu, Lys, Met, Thr, and Val. For instance, 64% of the residues of VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS are composed of the residues above. Except for PHYNN, the seven other potential trifunctional peptides are probable non-allergenic (Table 2). These seven non-allergenic peptides are of interest because of the predicted low risks of sensitization and allergic reactions. Allergic inflammation may reduce the production of innate interferons and delay antiviral responses in the host cells (Edwards et al. 2017). Hence, at least based on in silico analysis, the antiviral effects of the seven non-allergenic peptides are less likely to be compromised by allergenicity when compared with PHYNN. Furthermore, the ability of the seven shortlisted peptides to target key proteins that are involved in pre- and post-cell-entry viral activities implies their prophylactic and therapeutic potential.

The usefulness of the SwissADME web tool in predicting the physicochemical properties, lipophilicity, druglikeness and pharmacokinetics of hypotensive dipeptides derived from flaxseeds was reported by Ji et al. (2020). In this study, we also used SwissADME for a similar analysis (Table 3). Predictors of the potential of a drug to be orally bioavailable include the ratio of sp^3 hybridized carbons over the total carbon count of the molecule (Fraction Csp3), number of rotatable bonds, number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD) and topological polar surface area (TPSA) (Santos et al. 2016). According to Santos et al. (2016), a majority of the FDA-approved peptide drugs that are orally available have up to 20 rotatable bonds, with fraction Csp3 of up to 0.55. Among the eight potential trifunctional peptides, only PHYNN, with 21 rotatable bonds and fraction Csp3 of 0.43 (Table 3), could be taken as generally matching the two criteria. Meanwhile, except for ERHHRGGRGRQS and VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS, the other six trifunctional peptides were found to have between 8 and 18 HBA and 9–19 HBD. These six peptides have lipophilicity (log $P_{o/w}$) of − 0.36 to − 4.62 (Table 3). Thus the six peptides may predictably be orally available as most orally available peptide drugs have up to 50 HBA and up to 25 HBD, in addition to lipophilicity between − 5 and 8 (Santos et al. 2016). Furthermore, except for TKHGGRINTL, ERHHRGGRGRQS and VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS, the other five trifunctional peptides have TPSA between 224.55 and 325.48 $Å^2$. This points to the chance of these five peptides being orally available as majority of orally available peptide drugs have TPSA of up to 400 $Å^2$ (Santos et al. 2016). To sum up, based on the six descriptors above, PHYNN is the most likely to be orally available.

The eight trifunctional peptides may not be bioavailable following oral ingestion due to poor GI absorption. However, there is chance that PKRF and PHYNN may still be taken up by lung cells, the site of SARS-CoV-2 invasion, if these peptides could be effectively introduced into the body non-orally. For PKRF and PHYNN to bind to the SARS-CoV-2 spike glycoproteins when the virus is extracellular, the uptake of the peptides into the human cells is not crucial. But for the two peptides to bind to the intracellular targets M^{pro} and PL^{pro}, their uptake into the human cells is a prerequisite. On the other hand, the eight trifunctional peptides in this study were predictably not inhibitors of all five cytochrome P450 (CYP) isozymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4) (Table 3). The five CYP isozymes play key roles in Phase I biotransformation and their inhibition by a drug may lead to bioaccumulation and subsequent toxicity (Sychev et al. 2018). Thus, this observation may further support the non-toxicity of the eight

Table 3 Physicochemical properties, lipophilicity, water solubility, pharmacokinetics, druglikeness and medicinal chemistry friendliness of the eight potential trifunctional peptides as analyzed by using SwissADME

Parameters		Peptides							
		PKRF	PHYNN	PNWKIN	AIRAMPL	PHWNIN	TKHG-GRINTL	ERHHRGG RGRQS	VEDKGM-MHQQRM-MEKAMNI-PRMCGT-MQRKCRMS
Physico-chemical properties	Number of heavy atoms	39	46	55	53	56	77	101	267
	Number of aromatic heavy atoms	6	11	9	0	14	5	10	5
	Fraction Csp3	0.58	0.43	0.56	0.76	0.47	0.67	0.56	0.70
	Number of rotatable bonds	21	21	28	30	26	47	65	180
	Number of H-bond acceptors	8	11	11	10	11	18	24	55
	Number of H-bond donors	9	10	11	10	11	19	30	58
	Molar refractivity	149.93	160.79	202.96	206.63	202.46	273.98	350.66	1001.83
	TPSA ($Å^2$)	224.55	300.82	322.82	316.33	325.48	525.37	789.00	1944.79
Lipophilicity	Consensus log $P_{o/w}$	− 0.80	− 3.14	− 1.60	− 0.36	− 1.50	− 4.62	− 8.94	− 12.55
Water solubility	Log *S* (ESOL)	0.33	0.79	− 0.45	− 0.59	− 0.71	1.14	3.98	2.50
	Class (ESOL)	Highly soluble	Highly soluble	Very soluble	Very soluble	Very soluble	Highly soluble	Highly soluble	Highly soluble
Pharmacokinetics	GI absorption	Low	Low	Low	Low	Low	Low	Low	Low
	P-gp substrate	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	CYP1A2 inhibitor	No	No	No	No	No	No	No	No
	CYP2C19 inhibitor	No	No	No	No	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No	No	No	No
	CYP3A4 inhibitor	No	No	No	No	No	No	No	No
Druglikeness	Lipinski's rule (number of violations)	No (3)	No (3)	No (3)	No (3)	No (3)	No (3)	No (3)	No (3)
	Bioavailability score	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17

Table 3 (continued)

Parameters		Peptides							
		PKRF	PHYNN	PNWKIN	AIRAMPL	PHWNIN	TKHG-GRINTL	ERHHRGG RGRQS	VEDKGM-MHQQRM-MEKAMNI-PRMCGT-MQRKCRMS
Medicinal chemistry	Leadlike-ness (number of viola-tions)	No (2)	No (2)	No (2)	No (2)	No (2)	No (2)	No (2)	No (2)
	Synthetic accessibil-ity	4.87	5.37	6.52	7.16	6.49	9.34	10.00	10.00

Fraction Csp3 ratio of sp^3 hybridized carbons over the total carbon count of the molecule, *H-bond* hydrogen bond, *TPSA* topological polar surface area, *log* $P_{o/w}$ partition coefficient between *n*-octanol and water, *ESOL* estimated solubility, *GI* gastrointestinal, *P-gp* permeability glycoprotein, *CYP1A2* cytochrome P450 1A2, *CYP2C19* cytochrome P450 2C19, *CYP2C9* cytochrome P450 2C9, *CYP2D6* cytochrome P450 2D6, *CYP3A4* cytochrome P450 3A4

trifunctional peptides as predicted by ToxinPred (Table 2). Concerning druglikeness and leadlikeness, the eight trifunctional peptides are not favorable. However, as emphasized by Santos et al. (2016), many orally bioavailable peptide drugs, marketed and in clinical trials, do not conform to the existing criteria for oral bioavailability that is applicable to small molecule drugs. For example, tifuvirtide and enfuvirtide, two FDA-approved antiviral peptide drugs, fail to comply with the Lipinski's rule-of-five (Santos et al. 2016). Meanwhile, Mishra and Dey (2019) reported that peptides that violated the Lipinski's rule-of-five could exhibit drug-like properties, including penetration of cellular membranes, in experimental studies in vitro. Udenigwe (2014) noted that physical experiments are indispensable for validating the outcomes of in silico discovery of food-derived bioactive peptides. Thus, to sum up, wet-lab experiments should be conducted in the future to verify the bioavailability, toxicity, allergenicity, and druglikeness of the eight potential trifunctional peptides obtained in this study, besides confirming their inhibitory effects on the three SARS-CoV-2 targets M^{pro}, PL^{pro} and spike glycoproteins.

Conclusion

Overall, from quinoa seed proteins, this study has computationally identified seven non-toxic and likely non-allergenic peptides that could serve as trifunctional inhibitory peptides against SARS-CoV-2. They were predicted to bind to critical binding residues of the spike glycoprotein and critical catalytic residues in the active sites of M^{pro} and PL^{pro}. Hence, they could potentially block the entry of SARS-CoV-2 into the host cell and suppress viral replication. The seven peptides also have desirable physicochemical properties typical of other known antiviral peptides. Among the seven, VEDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS that could form the highest number of interactions with key residues in the viral proteins. Thus, this peptide might be the most promising candidate for the future development of peptide-based therapeutics or prophylactics to combat COVID-19.

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Author Contributions FCW and DTK were involved in designing the experiments. TTC, DTK and JHO carried out the acquisition, analysis, and the interpretation of the data. JHO, TTC and FCW prepared the manuscript. All the authors approved the manuscript.

Data Availability Data of the study are included in the supplementary information files.

Declarations

Conflict of interest The authors declare no financial or commercial conflict of interest.

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