In Silico Prediction of the Action of Ivermectin-like Compounds on Binding Sites of the SARS-CoV-2 Spike Protein and Receptor-binding Domain of ACE2

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Abstract:

Background: Ivermectin (IVM), a macrocyclic lactone anthelmintic drug, is a promising lead compound that may disrupt the binding interface of the SARS-CoV-2 spike protein with the protein-binding domain of angiotensin-converting enzyme 2 (ACE2), and so could present an opportunity for further drug development of anti-COVID-19 medication.

Objective: This study aimed to determine and predict the most effective IVM-based analogs against the SARS-CoV-2 spike protein and human angiotensin-converting enzyme 2 by using computational analysis.

Method: This study performed a rational *in silico* study to screen ivermectin-like compounds with a similarity score less than 0.70 and then screened these for acceptable pharmacokinetic properties, to further examine molecular docking analysis of SARS-CoV-2 spike protein and protein-binding domain of angiotensin-converting enzyme 2.

Result: The results showed that compound **14**, with a similar score of 0.722, exerted the most binding affinity with both targets, with a binding energy of -8.32 and -7.98 kcal/mol to the SARS-CoV-2 spike protein and the protein-binding domain of angiotensin-converting enzyme 2 respectively, showing better values than that of ivermectin.

Conclusion: Our study confirms the possibility that the ivermectin-like compound **14** may be a most promising candidate drug, acting on the SARS-CoV-2 spike protein and angiotensin-converting enzyme 2, so should be studied further as part of a drug discovery and development process.

Keywords: Ivermectin, *in silico* analysis, COVID-19, SARS-CoV-2 Spike protein, Angiotensin-converting enzyme 2

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Introduction

Since 2019, the world has suffered from the emergence of the coronavirus disease 2019 (COVID-19) outbreak, which is a major public health issue and a cause of high levels of morbidity and mortality.[1] It has affected more than 205 million people worldwide, including 4 million deaths.[2] To reduce the harmful sequelae of COVID-19 infection, such as respiratory failure, or multi-organ dysfunction, treatment involving antiviral agents is one of the promising therapeutic approaches for this emerging infectious disease.[1]

Of the ongoing drugs in the COVID-19 pipeline, ivermectin (IVM) is a most interesting compound because it exhibits a broad spectrum of antiviral activity *in vitro* apart from its well documented anti-parasitic activities.[3-7] We believe that IVM could be a potential anti-COVID-19 lead candidate for further drug development because it has been reported that IVM inhibits the SARS-CoV-2 virus *in vitro*[3-4] and *in vivo*.[8] Furthermore clinical studies have also revealed that IVM is associated with a lower mortality rate in hospitalized COVID-19 patients.[9-11] One of the postulations of the mechanism of action of IVM toward SARS-CoV-2 virus is inhibition and disruption of the binding of the SARS-CoV-2 spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor.[12] An *in-silico* analysis demonstrated that IVM disrupted the binding interface between the Leu91 of SARS-CoV-2 spike protein and the His378 of host cell ACE2.[13-14]

This finding inspired our idea that chemistry containing structural moieties similarly to IVM, in terms of IVM-like analogs, could be possible compounds with efficacy and safety for COVID-19 pharmacotherapy. The objectives of this study were to determine the most effective IVM-based analogs and their favorable pharmacokinetic properties, by using computational analysis of the SARS-CoV-2 spike protein and human ACE2 receptors.

Methodology

1. Selection and preparation of IVM analogs.

IVM was submitted in the Simplified Molecular Input Line Entry System (SMILES) format, to calculate similarity scores, by using SwissSimilarity, a free web tool that can compute the similarity of all compounds that are available in the Sigma Aldrich library.[15] The top compounds that had a similarity score of more than 0.70 were included and these IVM analogs were submitted into SwissADME (http://www.swissadme.ch/index.php), to compute their physicochemical and pharmacokinetic properties.[16] All compounds that were judged to be orally active drugs with favorable pharmacokinetic properties were included in this analysis.[17] Structures of IVM and selected IVM-like analogs were initially constructed using ChemDraw Professional 16.0, followed by three-dimensional (3D) structure transformation, using Chem3D Professional 10.0.

2. Preparation of structure of SARS-CoV-2 Spike protein and human ACE2.

A Crystal structure of a SARS-CoV-2 spike receptor-binding domain, bound with ACE2 (PDB ID: 6M0J),[18] was prepared by removing all water molecules, any solvent, and the ligand.

3. Molecular docking analysis.

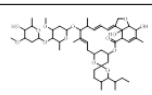
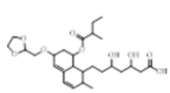
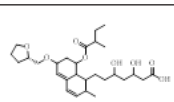
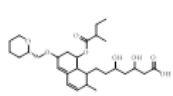
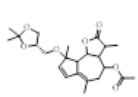
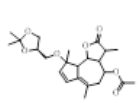
The binding free energy and inhibitory constant of IVM and its analogs were docked and then analyzed by using AutoDock 4.2.6 software.[19] Each energy-minimized IVM and its analogs were submitted into the well-prepared targets with default parameters of docking procedures. The binding site sphere for IVM and its analogs interaction

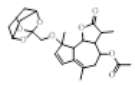
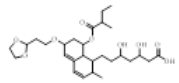
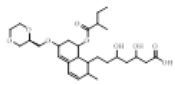
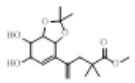
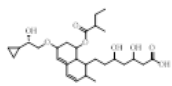
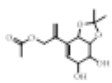
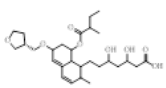
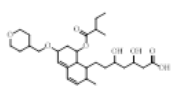
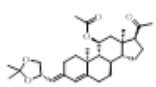
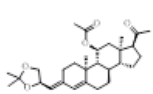
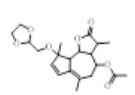
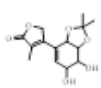
was defined according to the previous studies. The molecular docking protocol was obtained from the active site of the SARS-CoV-2 spike protein and ACE2 receptor with a molecular grid at 0.375 Å grid spacing. Docking results of all analogs with SARS-CoV-2 spike protein and ACE2 receptor were evaluated using the best binding free energy (BE, kcal/mol) and inhibitory constant from all clusters of each conformational structure. Virtual analysis of the best results was then viewed and analyzed by using UCSF Chimera.[20]

Results and discussion

The SwissSimilarity results showed 75 compounds from the Sigma Aldrich library having similarity scores more than 0.7 (the range of 0.71-0.83), which were further submitted to SwissADME. All of the pre-selected IVM-like compounds belonged within Lipinski's rule of five criteria.[17] Serious neurological adverse drug reactions of IVM have been documented and there is a need to avoid harm to patients in situations of overdose. The mechanisms of this adverse drug reaction are unclear but might be due to IVM inhibiting the P-glycoprotein drug pump (MDR-1) of the BBB (blood brain barrier) causing CNS toxicity.[21] We then excluded 9 of the pre-selected IVM-preselected compounds exhibiting increased BBB permeability and 36 other selected compounds that were classified as P_{gp} substrate. Finally, we obtained 17 lead compounds that showed favorable physiochemical and pharmacokinetic properties as shown in Table 1.

Table 1 Physiochemical properties of IVM and IVM-based compounds

Compound	Chemical structure	Similarity	MW	HBA	HBD	*c*LogP
IVM		1.0	875.09	14	3	4.37
1		0.772	510.62	9	3	2.72
2		0.768	508.64	8	3	3.3
3		0.768	522.67	8	3	3.56
4		0.765	420.5	7	0	2.64
5		0.765	420.5	7	0	2.68

Compound	Chemical structure	Similarity	MW	HBA	HBD	*c*LogP
6		0.765	460.52	8	0	2.51
7		0.762	524.64	9	3	3
8		0.748	524.64	9	3	2.71
9		0.742	326.38	6	2	1.6
10		0.738	508.64	8	4	3.1
11		0.729	284.31	6	2	0.59
12		0.728	508.64	8	3	3.22
13		0.728	522.67	8	3	3.49
14		0.722	470.64	5	0	4.83
15		0.722	470.64	5	0	4.84
16		0.715	392.44	7	0	2.09
17		0.711	282.29	6	2	0.4
Required parameters[a]	-	-	< 500	< 10	< 5	2-5

[a] Required parameters necessary to fulfill appropriate physiochemical properties as judged appropriate according to Lipinski's rules.[17]

IVM was docked with the SARS-CoV-2 spike protein and ACE2 in the region of the receptor-protein binding interface. The binding energy of IVM to SARS-CoV-2 spike protein and ACE2 were -6.60 and -4.84 kcal/mol, with an estimated inhibition constant (Ki) of 14.54 and 283.49 μM, respectively. It was noted that IVM favored binding to the SARS-CoV-2 spike protein compared with ACE2. According to molecular docking analysis, the compounds that exerted binding free energy greater than that of the IVM towards SARS-CoV-2 spike protein were compound **4-6, 14,** and **15,** while binding with ACE2 were compounds **4-6, 11, 14-15,** and **16-17** (Table 2). The compound with the best binding affinity toward both SARS-CoV-2 spike protein and ACE2 was compound **14** which provided binding energies of -8.32 and -7.98 kcal/mol respectively.

Table 2 Molecular docking analysis of IVM and IVM-based compounds toward SARS-CoV-2 spike protein and ACE2

Compound	SARS-CoV-2 spike protein		ACE2	
	BE (kcal/mol)[a]	Inhibition Constant (μM)[a]	BE (kcal/mol)[a]	Inhibition Constant (μM)[a]
IVM	-6.60	14.54	-4.84	283.49
1	-4.09	999.74	-4.73	341.18
2	-4.96	230.62	-4.02	1.13 mM
3	-4.39	603.20	-3.63	2.19 mM
4	-6.70	12.20	-7.08	6.43
5	-6.63	13.86	-6.61	14.27
6	-6.57	15.38	-6.92	8.46
7	-4.22	805.28	-3.80	1.63 mM
8	-3.76	1.77 mM	-3.23	4.31 mM
9	-5.69	67.99	-4.69	362.60
10	-3.51	2.65 mM	-2.73	10.04 mM
11	-5.40	110.21	-5.33	123.87
12	-3.99	1.19 mM	-3.62	2.22 mM
13	-4.27	737.17	-4.60	422.19
14	-8.32	0.79	-7.98	1.40
15	-7.62	2.60	-7.60	2.67
16	-6.37	21.36	-6.70	12.23
17	-6.23	27.04	-5.88	48.88

[a] Binding free energy and inhibitory constant results were obtained from AutoDock 4.2.6 software.[19]

For binding mode analysis of compound **14** with ACE2, the heteroatoms of compound **14** interact via H-bonding interaction with the H-bond donor amino acid including Arg403, Tyr453, and Ser494; and by hydrophobic interaction with Tyr449, Leu452, Leu455, Phe490, Leu492, Gln493, Tyr495, Gly496, and Tyr505 (Figure 2).

The interaction of SARS-CoV-2 spike protein, both IVM and compound **14** were found to interact via H-bonding interaction with Lys26, and Gln96 but the bond distance in the case of IVM was 2.85, and 2.95 Å, respectively, whereas in the case of compound **14** it was 2.74, and 3.02 Å, respectively (Figure 2). The hydrophobic interactions between SARS-CoV-2 spike protein and IVM were found to interact with Glu22, Asp30, Asn33, Asn90, Val193, and Pro389, whereas the hydrophobic interactions of compound **14** were found to interact with Glu23, Thr27, Asp30, Asn33, and Pro389 (Figure 2).

Compound **14**, a triterpene analog, appears to be a promising compound that effectively binds to both the SARS-CoV-2 spike protein and to ACE2. This compound could have a potential role in inhibiting viral entry, so may be considered as a possible antiviral agent to fight SARS-CoV-2 infection. The results demonstrate that this rationale of *in silico* prediction of IVM-based compounds is one of the approaches that can be used to screen and design drug candidates, which is less time consuming and provides essential information to prioritize drug discovery and development processes in the ongoing COVID-19 situation. However, this computational analysis still requires experimental studies to further confirm this *in silico* hypothesis.

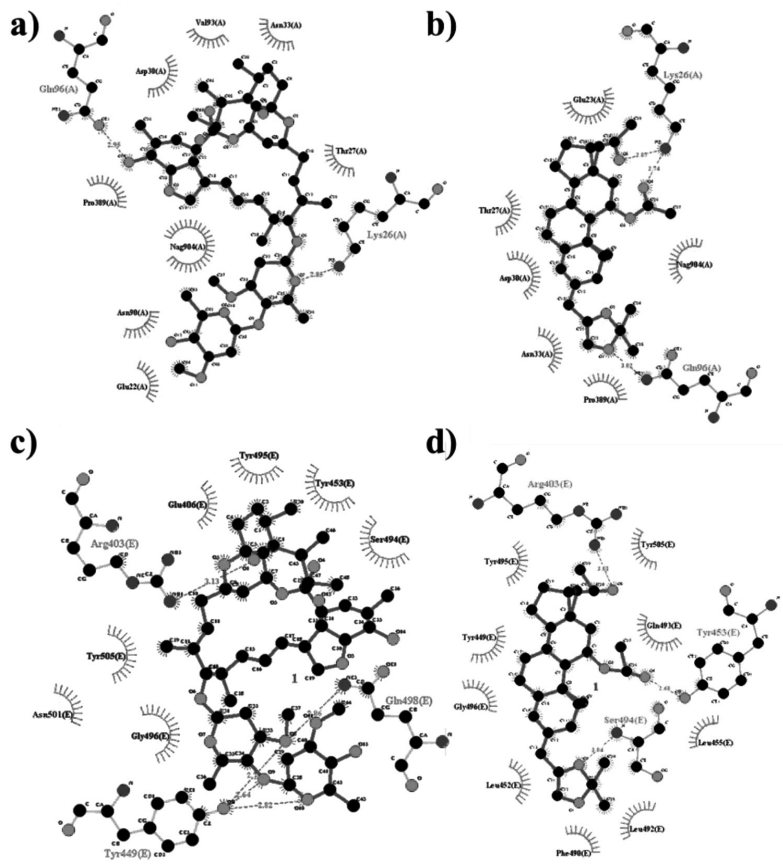


Figure 1 Binding mode results of IVM (a) and compound **14** (b) toward SARS-CoV-2 spike protein (6M0J); IVM (c) and compound **14** (d) toward ACE2 receptor. The green dashed line denoted H-bonding interaction.

Conclusion

This computational analysis revealed that compound **14** shown the best binding affinity towards both the SARS-CoV-2 spike protein and ACE2 receptor, with higher values than IVM, whilst also exhibiting acceptable physicochemical characteristics and pharmacokinetic properties. This result suggests that *in silico* analysis has proved to be an advantageous tool for drug design, reducing the time required to ratify rational strategies for anti-COVID-19 drug development. However, preclinical studies are required to further evaluate its efficacy and toxicity.

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Conflicts of Interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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