Docking Adenosine Receptor Ligands to SARS-CoV2 mRNA Cap Guanine-N7 Methyltransferase

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Abstract

The identification of an adenosine binding pocket in the SARS-CoV2 mRNA cap guanine-N7 methyltransferase (nsp16) represents a promising lead in the development of new drugs to combat the devastating pandemic, Covid-19. There are therapeutic agents and drug-like molecules that bind adenosine receptors and hence are likely to bind other adenosine binding proteins such as SARS-CoV2 nsp16. This study explores the docking of known adenosine receptor ligands to SARS-CoV2 nsp16. Among the compounds successfully docked to SARS-CoV2 nsp16 is the anti-inflammatory compound Piclidenoson, which is already in clinical trials for treatment of Covid-19. Energy minimization of the Piclidenoson/nsp16 complex suggests that Piclidenoson binding can induce conformational changes to nsp16.

Keywords: SARS-CoV2 mRNA cap guanine-N7 methyltransferase, nsp16, Covid-19, docking, adenosine receptor ligands

Introduction

Coronaviruses cause a wide variety of diseases ranging from mild colds and gastrointestinal upset to severe respiratory syndromes including Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS).[1, 2] The SARS-CoV2 virus is unique among coronaviruses in that it is both highly transmissible and the disease it causes, Covid-19, has high morbidity and mortality rates.[3] Not surprisingly, the SARS-Cov2 virus has several properties that are unique among coronaviruses.

Like many viruses, coronavirus genomes encode enzymes that cap and otherwise modify viral RNA so that it resembles host mRNA. In particular, coronavirus genomes encode a mRNA cap guanine-N7 methyltransferase, nsp16 (in this paper, nsp16 will refer to SARS-CoV2 nsp16 unless otherwise specified), that modifies capped, viral RNA. The SARS-CoV2 mRNA cap guanine-N7 methyltransferase is unique in that it allosterically binds adenosine.[4] While the *in vivo* significance of this site is not yet known, this site is an attractive target for antiviral therapies. One reason this site is an attractive target is that, since adenosine functions as a neurotransmitter with well characterized receptors, many available pharmaceuticals and pharmaceutical lead compounds are known to bind adenosine binding proteins.[5, 6] For instance, one of the most widely used drugs, caffeine, binds adenosine receptors. [6]

This paper reports the results of docking 35 compounds – known adenosine receptor binders, adenosine derivatives and β-D-fructopyranose – to a crystal structure of the SARS-CoV2 mRNA cap guanine-N7 methyltransferase (PDB ID 6W4H) with β-D-fructopyranose [7] in the site which Viswanathan, *et al*., identified as binding adenosine.[4] 12 compounds, with known affinity for adenosine receptors, are predicted to bind the adenosine binding site in nsp16 better than does adenosine itself. Energy minimized structures for ligand/nsp16 complexes suggest that some of the putative ligands identified in this docking study can perturb nsp16 structure.

Methods

Identification of Putative Ligands and Docking to Nsp16

Searching adenosine receptor binders in the ZINC database[8] identified putative nsp16 binders. Putative ligands selected for this docking study included the top 4 highest affinity substances and the top 4 “drugs substances” for each adenosine receptor subtype (ADORA1, ADORA2A, ADORA2B and ADORA3): due to the same substance being present on multiple “top 4” lists, this process gleaned only 27 compounds. Filling out the list of putative ligands were three well known adenosine receptor binders (caffeine, D-limonene and lactucin), one compound similar to one of those established binders (intybin, also known as lactucopicrin, which is a lactucin derivative), three adenosine nucleotides (cAMP, AMP and ATP) and β-D-fructopyranose (BDF).

Docking was performed on chain A (nsp16) obtained from a high resolution (1.80 Å) structure of the nsp16/nsp10 complex (PDB ID 6W4H) using iGEMDOCK v2.1.[9] Both unrestricted docking and docking to the region around (binding site radius 10 Å) the β-D-fructopyranose (BDF) molecule bound to nsp16 (chain A in 6W4H).

Energy Minimization

Energy minimizations were performed on the docked adenosine receptor binder/protein complexes with iGEMDOCK binding energies at least as negative as that for adenosine. Each complex consisted of the docked ligand, chain A (nsp16) from 6W4H and all ligands bound to chain A (except for BDF, which is in the adenosine binding pocket). In order to evaluate comparable structures, the crystal structure 6W4H was also subjected to energy minimization; two systems were considered: one with both chains and all ligands present in the crystal structure and another with only chain A (nsp16) and with the β-D-fructopyranose ligand removed. The Schrödinger Maestro, version 11.0,[10] Protein Prepwizard performed initial preparation of the protein/ligand complexes using default settings except for converting selenomethionine residues to methionines and not creating disulfide bonds. This study only considered atoms with alternate conformations in their maximally occupied positions. A pH of 7.5 was used to adjust protonation states.

The system to be energy minimized included a minimum volume of TIP4PEW water (using a orthorhombic box with a buffer distance of 10Å on each side), Na^+ or Cl^- ions for neutralization and 0.15 M NaCl. Minimization used default parameters in DESMOND v. 4.8,[11] ran within the Schrödinger Maestro interface.

Analysis of Energy Minimized Structures

The MatchAlign tool in UCSF Chimera[12] superimposed energy minimized structures for the 6W4H chain A structure without BDF (6W4HΔBDF) as well as the structures with docked, putative ligand, onto the energy minimized 6W4H structure. Use of the Match->Align tool facilitated extraction (via a header in the displayed sequence alignment) extraction of RMSD values (calculated for all heavy atoms on a per-residue basis) for each superimposition. UCSF Chimera was also used to produce cartoons of protein structures. Both Excel and MATLAB[13] proved invaluable in collating and plotting RMSD values.

Results and Discussion

Docking

iGEMDOCK indicated that 15 ligands bound at or near the putative adenosine binding site as well as adenosine itself (Table 1, Supplementary Table 1). Those ligands include ATP, AMP and the natural product intybin, a derivative of the adenosine receptor binding natural product lactucin. Also included were the drug Prazosin and a drug candidate compound Piclidenoson (also known as CF-101 and IB-MECA).

Both Prazosin[14] and Piclidenoson[1, 15] have anti-inflammatory effects. In fact, Konig, *et al*., have already suggested the use of Prazosin in treating Covid-19 associated cytokine storms[14] , and Piclidenoson is already in Phase-2 clinical trials for use in treating Covid-19.[16-18] Should either drug prove useful in inhibiting SARS-CoV2 mRNA cap guanine-N7 methyltransferase, they will have two beneficial effects in treating Covid-19: directly inhibiting the viral lifecycle and providing symptomatic relief of disease associated inflammation. Similarly, Intybin has known analgesic effects.[19] Should it prove useful in inhibiting SARS-CoV2 mRNA cap guanine-N7 methyltransferase, it will also have two beneficial effects in treating Covid-19.

Table 1: Best nsp16 Binders as Ranked by iGEMDOCK Binding Energy

Ligand ID	Name	Binding "Energy"
3924085		-127.865
49872226		-114.615
71332259		-113.749
4261765	ATP	-110.042
26014679		-109.926
26015531		-109.101
95616601	Prazosin	-107.192
49877703		-103.369
103269598		-103.190
13475415		-102.223
3811810	Piclidenoson	-102.108
42888212		-98.4758
2516024		-95.9594
898598	Intybin	-95.3088
3860156	AMP	-94.5175
2169830	Adenosine	-92.9955

The best ranked binder identified in this study is ZINC ID 3924085. This compound is not only known to bind adenosine receptors but is also predicted, according to the SEA[20] predictions in its ZINC database entry, to bind two other methyltransferase enzymes. It may be that SARS-CoV2 mRNA cap guanine-N7 methyltransferase is not the only adenosine binding methyltransferase; or, more generally, it may be that there is a correlation between binding at adenosine binding sites and ability to bind methyltransferase enzymes.

Analysis of Energy Minimized Structures

While neither the 6W4HΔBDF structure nor many of the energy minimized ligand/protein complexes showed much deviation from the energy minimized version of PDB ID 6W4H, four structures showed moderate deviations (e.g. median per-residue, calculated using all heavy atoms, RMSD > 0.15 Å, Figure 1). One of those four ligand/protein complexes is the complex of nsp16 with Piclidenoson. Piclidenoson has already been identified (*vide supra*) as a potential therapeutic agent in Covid-19 treatment, presumably based on its anti-inflammatory properties, but this study suggests it may also exert a therapeutic effect via nsp16 inhibition.

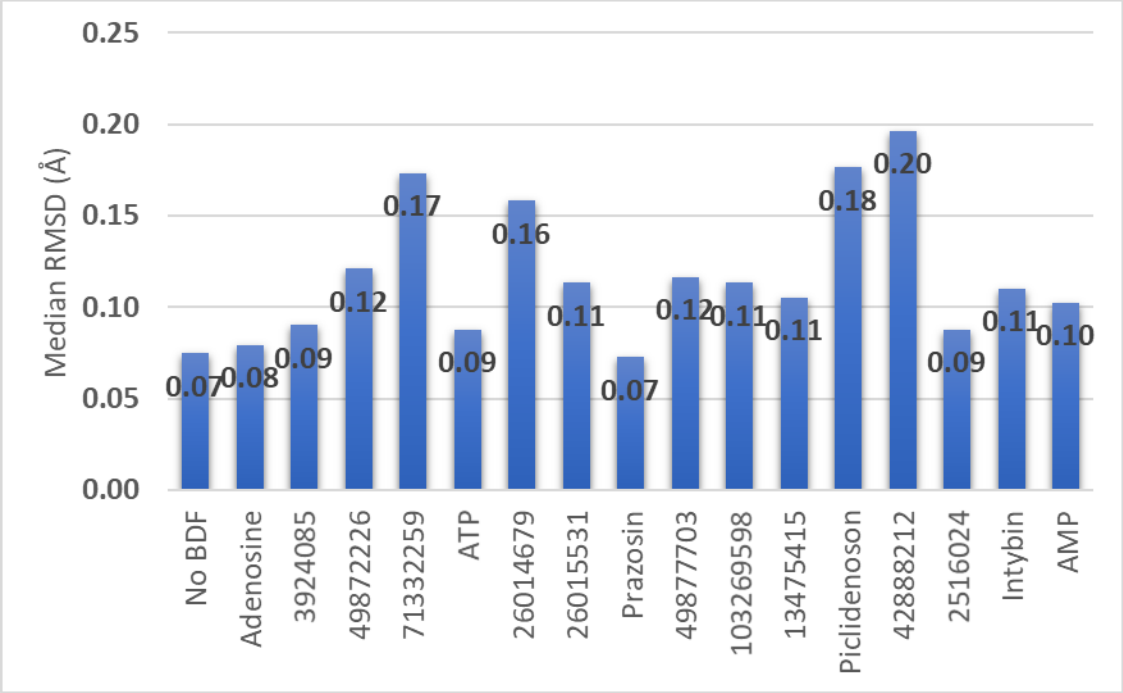

Figure 1: Median per-residue, heavy atom RMSD values from superimpositions of protein/ligand complexes (labeled by either the ligand name or ZINC accession number).

Each of the four ligand/protein complexes, whose energy minimized structures greatly (median per-residue RMSD > 0.15 Å) deviated from the energy minimized 6W4H structure, displayed large scale conformational changes (per residue RMSD > 0.5 Å) in the nsp16/nsp10 interface (Figure 2). In particular, the energy minimized ZINC ID 71332259/6W4H chain A complex (Figure 2C) has extensive regions of deviation from the energy minimized 6W4H structure in the region of the

nsp16/nsp10 interface, which is not surprising given the ligand binding site in this complex is nearer to the nsp16/nsp10 interface than is the adenosine binding site identified by Viswanathan, *et al*.[4] In the energy minimized structures shown in Figure 2, all but the ZINC ID 42888212 complex (Figure 2A), which actually has the greatest median per-residue RMSD from the energy minimized 6W4H structure, have ligands with only a small fraction of binding occurring in the adenosine binding site identified by Viswanathan, *et al*.

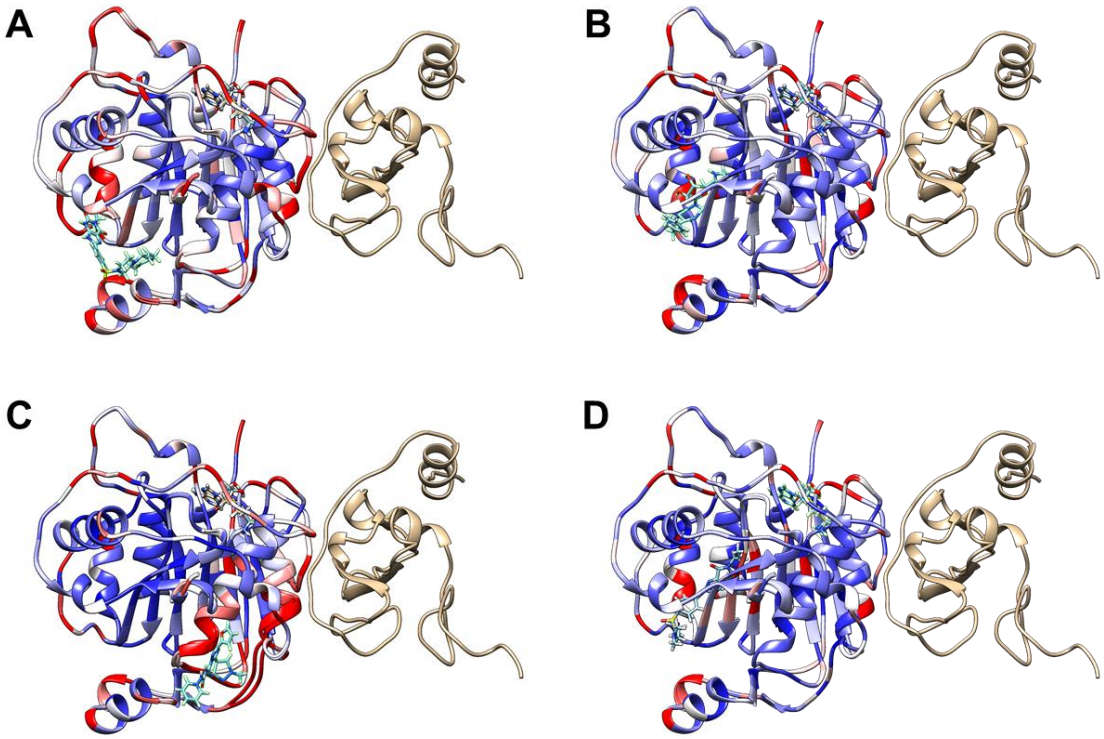

Figure 2: Superimpositions of energy minimized protein ligand complexes and the energy minimized 6W4H structure. In each panel, nsp16 coloring tracks the per-residue, heavy atom RMSD between the superimposed structures with red indicating RMSD > 0.5 Å, white indicating RMSD = 0.25 Å and blue indicating low RMSD values. Ligands (both the docked ligand and S-Adenosyl-Methionine) are rendered as ball and stick models: the adenosine binding site identified by Viswanathan, *et al*. [4] is in the lower left corner of each panel (where the ligand is bound in panel A). Complexes, identified by the ZINC ID of the ligand or by name where such a name exists, shown are those with maximal median per-residue RMSD values as plotted in Figure 1: (A) 42888212, (B) Piclidenoson (ZINC ID 3811810), (C) 71332259, and (D) 26014679.

Discussion and Conclusions

Given that adenosine receptors are an important target in the treatment of inflammatory diseases [5, 15] and the role that an over-active immune response plays in the pathogenesis of Covid-19,[14] the identification of an adenosine binding pocket in a critical SARS-CoV2 protein[4] suggests the possibility of finding drugs that target both SARS-CoV2 induced inflammatory responses and the reproduction of the SARS-CoV2 virus itself. This study identified multiple adenosine receptor binders likely to also bind at or near the SARS-CoV2 mRNA cap guanine-N7 methyltransferase (nsp16) adenosine binding pocket.

Among those compounds identified are two molecules already being studied for treatment of Covid-19. Another molecule (ZINC ID 71332259), binding near the nsp16 adenosine binding pocket, potentially (as judged by changes in energy minimized structures with the ligand bound vs. with β-D-fructopyranose bound) can disrupt the nsp16/nsp10 interface necessary for SARS-CoV2 replication.[21] Since this molecule docks at the edge of the adenosine binding pocket unique to SARS-CoV2 nsp16, it may prove useful in disrupting nsp16/nsp10 interactions in multiple coronavirus species. The identification of a molecule hypothetically interfering with protein/protein interactions by docking to an allosteric site suggests that docking, combined with energy minimization, is a useful tool for identifying molecules that potentially interfere with protein/protein interactions.

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Declaration of Interests

The authors have no conflicts of interest to declare.

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Data Availability

The energy minimized complexes between proteins and docked ligands are available from the corresponding author upon request.

Supplemental Data

Supplementary Table 1: Compounds Studied in this Docking Study. Receptor binding for nucleotides is not indicated as those compounds (blue) were not selected based on (potential) adenosine receptor binding. Sources of information about which receptors the given ligand binds are (a) CHemBL 20 (as indicated in the compound's page in the ZINC database), (b) the compound's page on CHEBI and (c) the compound's page on PUBMED. The justifications for including the compound in this study are that it is indicated in the ZINC database as being one of the top 4 adenosine receptor – (i) 1 (ADORA1), (iia) 2A (ADORA2A), (iib) 2B (ADORA2B) and (iii) 3 (ADORA3) – binders, being one of the top 4 adenosine receptor – (I) 1 (ADORA1), (IIa) 2A (ADORA2A), (IIb) 2B (ADORA2B) and (III) 3 (ADORA3) – binding drugs, (g) based on general knowledge, (n) that it is an adenosine nucleotide and (p) it is present in a crystal structure in the binding pocket being probed.

		Binding "Energy" from iGEMDOCK				
Ligand ID	Name	BDF Site	Unconstrained	Receptors	Source	Rationale
3924085		-127.865	-132.176	ADORA1, ADORA2A	(a)	(iia)
49872226		-114.615	-118.832	ADORA1, ADORA2A, ADORA2B	(a)	(iib)
71332259		-113.749	-136.032	ADORA3	(a)	(iii)
4261765	ATP	-110.042	-142.907	N/A	N/A	(n)
26014679		-109.926	-113.676	ADORA1	(a)	(i)
26015531		-109.101	-107.098	ADORA1	(a)	(i)
95616601	Prazosin	-107.192	-120.318	ADORA3, etc.	(a)	(iii)
49877703		-103.369	-127.484	ADORA2A, ADORA2B	(a)	(iib)
103269598		-103.19	-118.902	ADORA1, ADORA2A, ADORA2B, ADORA3	(a)	(iib)
13475415		-102.223	-129.190	ADORA1, ADORA2A, ADORA2B, ADORA3	(a)	(iii)
3811810	Piclidenoson	-102.108	-105.614	ADORA1, ADORA2A, ADORA2B, ADORA3	(a)	(iii)
42888212		-98.4758	-119.854	ADORA1, ADORA2A, ADORA2B	(a)	(iib)
2516024		-95.9594	-93.7110	ADORA1, ADORA2A	(a)	(i)
898598	Intybin	-95.3088	-112.137	?	(b)	(g)
3860156	AMP	-94.5175	-112.766	N/A	N/A	(n)
2169830	Adenosine	-92.9955	-98.9584	ADORA1, ADORA2A, ADORA3, etc.	(a)	(p),(i),(iia)
49784476		-92.3989	-121.047	ADORA1, ADORA2A, ADORA2B, ADORA3	(a)	(iii)
3873977	cAMP	-91.5504	-101.337	N/A	N/A	(n)
4216238		-87.1933	-88.8634	ADORA1, ADORA2A	(a)	(i),(iia)
49072969		-81.4281	-98.5377	ADORA1, ADORA2A	(a)	(i)
1530776	Eht0201	-81.397	-97.7419	ACHE, ADORA2B	(a)	(IIB)
19144216	Pyrilamine	-79.567	-94.4813	ADORA3, etc.	(a)	(III)
3814423	Cyproterone	-79.4722	-105.083	ADORA1, etc.	(a)	(I)
96913999		-79.2381	-83.9763	ADORA2A	(a)	(iia)
49069606		-78.1205	-97.6733	ADORA1, ADORA2A	(a)	(iia)
96914006		-77.9239	-80.8353	ADORA2A	(a)	(iia)
897089	Mefloquin	-74.2772	-75.2000	ADORA1, ADORA2A, ADORA3, KCNH2	(a)	(IIA),(IIB)
18043251	Theophylline	-74.0092	-74.4111	ADORA1, ADORA2A, ADORA2B, ADORA3	(a)	(I),(IIA),(IIB)
403567	Enprofylline	-73.9011	-83.7338	ADORA2A, ADORA2B	(a)	(IIB)

389747	Naloxone	-72.0337	-88.3865	ADORA3, etc.	(a)	(III)
519270	Lactucin	-71.1499	-92.4700	Unidentified Adenosine Receptor(s)	(b)	(g)
1530764	Protriptyline	-69.7285	-86.1253	ADORA3, SLC6A2	(a)	(III)
1532739	β-D-fructopyranose	-66.1619	-74.5833	N/A	N/A	(p)
1084	Caffeine	-65.2476	-78.3291	ADORA2A, etc.	(a)	(g)
968226	D-Limonene	-48.5445	-54.5227	ADORA2A	(c)	(g)

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