INT. J. BIOL. BIOTECH., 18 (1): 5-9, 2020.

DIFFERENTIAL BINDING OF REPURPOSED HIV PROTEASE INHIBITORS FOR SARS-CoV-2

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ABSTRACT

Herein, molecular structure of SARS-CoV-2 main protease (Mpro) was used for docking or binding energy analysis with medicinally known anti-HIV protease inhibitor drug molecules at changing pH (4-8). Variance analysis performed on binding energies between pH 4-8 confirmed that the binding energy and mode of interaction of some ligands were variable with pH. Among the selected repurposed protease inhibitors, indinavir and saquinavir showed to have the highest differential binding energy with the changing pH. Using the variance analysis, we also proposed novel structural derivative of saquinavir as a pH sensitive specific protease inhibitor with higher pH related selectivity. Hence, the most pH selective protease inhibitors for treatment of SARS-CoV-2 were identified.

Keywords: SARS-CoV-2; Docking; HIV; Protease inhibitors.

INTRODUCTION

SARS-CoV-2 has enveloped structure consisting of single strand RNA (Hui *et al.*, 2020). So far the efforts to find a molecular solution to SARS-CoV-2 has involved identification of molecules for binding with the human cell reception sites that serve as hosts to SARS-CoV-2 such as main protease (Mpro), RNA polymerase (RdRp) (Li, De, 2020), receptor-binding domain (RBD) and angiotensin-converting enzyme II (ACE2). In this work, we are focusing on SARS-CoV-2 related human cell protease receptor sites for binding or docking at range of pH. Mpro protease is a potential drug target or reception site for anti-viral drug molecules for SARS-CoV (Blanchard *et al*., 2004; Chu *et al*., 2004; Lu *et al*., 2006). The structure of main protease related to SARS-CoV-2 is released in pdb with code 6LU7. It also can be used for identification and structure-based protease inhibitor drug molecular design (Khadim *et al*., 2020). We used binding energies at range of pH starting from neutral to acidic. The reason is that, oxidative metabolism in virus-infected cells form Adenosine triphosphate ATP. Glycolysis also contributes to further acidity also by converting glucose to pyruvate at high rate ($C_6H_{12}O_6 \rightarrow CH_3COCOO^- + H^+$) that converts into lactate. Thus, pH of the human cell turns acidic. Due to the acidic environment inside virus infected cell, pH of the protease itself or environment becomes acidic (Liu *et al*., 2016) and evidence shows that HIV protease catalyze acidic pH (Do *et a*l., 1991). We used the pH as a differential character of the virus infected cells to evaluate binding energies of anti-HIV protease inhibitors **(1a-10a)** at pH 4-8 on main protease (PDB 6LU7). This enabled us to design single step synthetic derivatives of protease inhibitor (**6a'**) with high selectivity for binding at low pH. This molecule can serve as future synthetic target for specific SARS-CoV-2 protease inhibitor with higher selectivity.
Hence, this research work identifies pH selective repurposed commercially available protease **(1a-10a).**

MATERIALS AND METHODS

Docking simulations were performed using molecular operating environment (MOE version 2015.10) software. Structures of all drug molecules **(1a-10a)** were obtained from PubChem data in .sdf format. These structures were converted to. mol2 format by using Open Babel software (O'Boyle *et al*., 2011). For drug target receptor sites, the structure of the SARS-COV-2 Mpro with N3 inhibitor (PDB 6LU7) with the resolution 2.16 Å (Liu *et al*, 2020) were obtained from the Protein Data in .pdb formats. Docking simulations were performed for protease inhibitor molecules **(1a-10a)** on 6LU7 at pH (4-8). Prior to the docking energy minimization of drug molecules was carried out using force field MMFF94x. Through the preparation function, all the atoms of the 3D structure of the drug

molecule and substrate were protonated and temperature was maintained at 300 K. Substrate was also subjected to reduced pH as its Lewis basic sites were protonated as well. Docking was carried out in force field Amber10: EHT with R-Field 1:80 solvation. Binding score was based on hydrogen bonds, Van der Waals and π-stacking interactions.

RESULTS AND DISCUSSION

Binding energies of selected repurposed drug molecules (**1a-10a**) at pH between 4 to 8 are given in Table 1. Details of ligand and receptor interaction for protease inhibitors (**6a**) are given in Table 2. Data in Table 1 showed that all the selected protease inhibitors (**1a-10a**) bind with energy in between the range of 7.5-10 -kcal/mol. Variance analysis showed that all selected protease inhibitors (**1a-10a**) gave variable binding energy with changing pH. All the selected molecules (**1a-10a**) for protease inhibition are known and commercially available HIV related protease inhibitors. These molecules can serve as drugs for treatment of SARS-CoV-2 infection on basis of their binding with the sites on 6LU7 main protease. The hydrogen bond forming Lewis basic sites on protease are bind with the selected drug molecules (**1a-10a**). Variance analysis of the binding energy values of the molecules **(1a-10a)** within pH range of 4-8 revealed the drug molecules which have the most consistent or pH dependent binding interaction reception sites on protease. Substrate or the reception site was also subjected to change in pH (4-8) as the Lewis basic sites on the substrate were protonated as well. By variance analysis performed on binding energies across pH, Tipranavir (**8a**) gave the least variance or most consistent binding energies across pH range of 4-8, whereas Indinavir (**3a**) showed high variance with reduced binding energy at acidic pH. Saquinavir (**6a**) also showed high variance in binding energy values with the protease (6LU7) when the pH changes from 8 to 4. This implies that acidity coming from glycolysis in the cell (Liu *et al.*, 2016) resulted in different binding behavior as shown by Indinavir (**3a**) and Saquinavir **(6a).** This can form the basis of a selective drug molecule serving as a protease inhibitor for SARS- CoV-2 with less interaction with neutral pH of healthy cell, hence least side effects. Fig. **1** shows the binding energy as function of pH for **(1a-10a)** in form of a plot which shows that, the highest variation in binding energy values is shown by (**3a**) and (**6a**) while Saquinavir (**6a**) showed higher binding energy values than Indinavir (**3a**) at lower pH. Based on this, we chose Saquinavir (**6a**) as the base molecule for structural manipulation (**6a'**). Details of the changing mode of interaction of Saquinavir **(6a)** with changing pH are provided in Table 2.

Table 1. Data of binding energy values (-kcal/mol) of molecules (**1a-10a**) on 6LU7 protease.

S. No	Drug	Binding energy (pH 4)	Binding energy (pH 5)	Binding energy (pH 6)	Binding energy (pH 7)	Binding energy (pH 8)	Variance
1a	Lopinavir	-8.7	-8.6	-8.7	-9.1	-8.9	0.1
2a	Ritonavir	-9.6	-9.5	-9.0	-9.6	-9.2	0.1
3a	Indinavir	-8.5	-8.2	-9.1	-9.9	-9.4	0.5
4a	Atazanavir	-9.3	-9.2	-8.9	-8.9	-9.7	0.1
5a	Nelfinavir	-8.4	-8.2	-8.3	-9.1	-8.2	0.1
6a	Saquinavir	-9.1	-9.1	-9.1	-10.3	-9.7	0.3
7a	Darunavir	-8.3	-8.8	-8.8	-8.4	-8.3	0.1
8a	Tipranavir	-7.8	-8.3	-8.0	-8.1	-8.1	0.03
9a	Amprenavir	-8.8	-8.3	-8.6	-8.4	-8.2	0.1
10a	Fosamprenavir	-9.0	-8.7	-8.4	-8.3	-8.4	0.1

Proposed structure (6a')

Based on the variance analysis from data in Table 2, it is seen that in case of interaction with (**3a**), even though the variance was high across the pH range 4-8, the binding energy was getting reduced with reduction of acidic pH. Reverse was true for (**6a**) as it not only proved to be pH sensitive binding structure yet also gave higher binding energies with reduced pH. Hence we chose (**6a**) to be manipulated into (**6a'**) (Fig. **2**). From Table 3 it has been found out that in case of interaction with (**6a**), the receptor binding sites O GLN 189 and SD ME 165 serve as hydrogen bond acceptors towards the N11 on drug molecule (**6a**) but as the pH lowers to values below 7 these Lewis basic sites get protonated and as a result they can't accept or form hydrogen bonds from the protonated or the Bronsted acidic sites on drug molecule (**6a**). Hence, in simulated structure (**6a'** in Fig. **2**) N11 of the (**6a**) was dialkylated to inhibit its interactions with SD ME 165 at neutral pH 7-8. This resulted in reduced binding energy at neutral pH. As a result, higher differential of up to 1.8 was achieved in favor of increased binding energy at acidic

energy. (**6a'**) can act as a synthetic target as a derivative of saquinavir. (**6a'**) gives binding energy of 9.59 at pH 5 and 7.80 at pH 7. Difference is almost 2.21 in favor of stronger binding at low pH of infected cell. Details of interaction between ligand and receptor i.e. (**6a'**) and 6LU7 protease are given in Table 3. Difference of mode of interaction from the details at pH 5 is noticeable (Table 2).

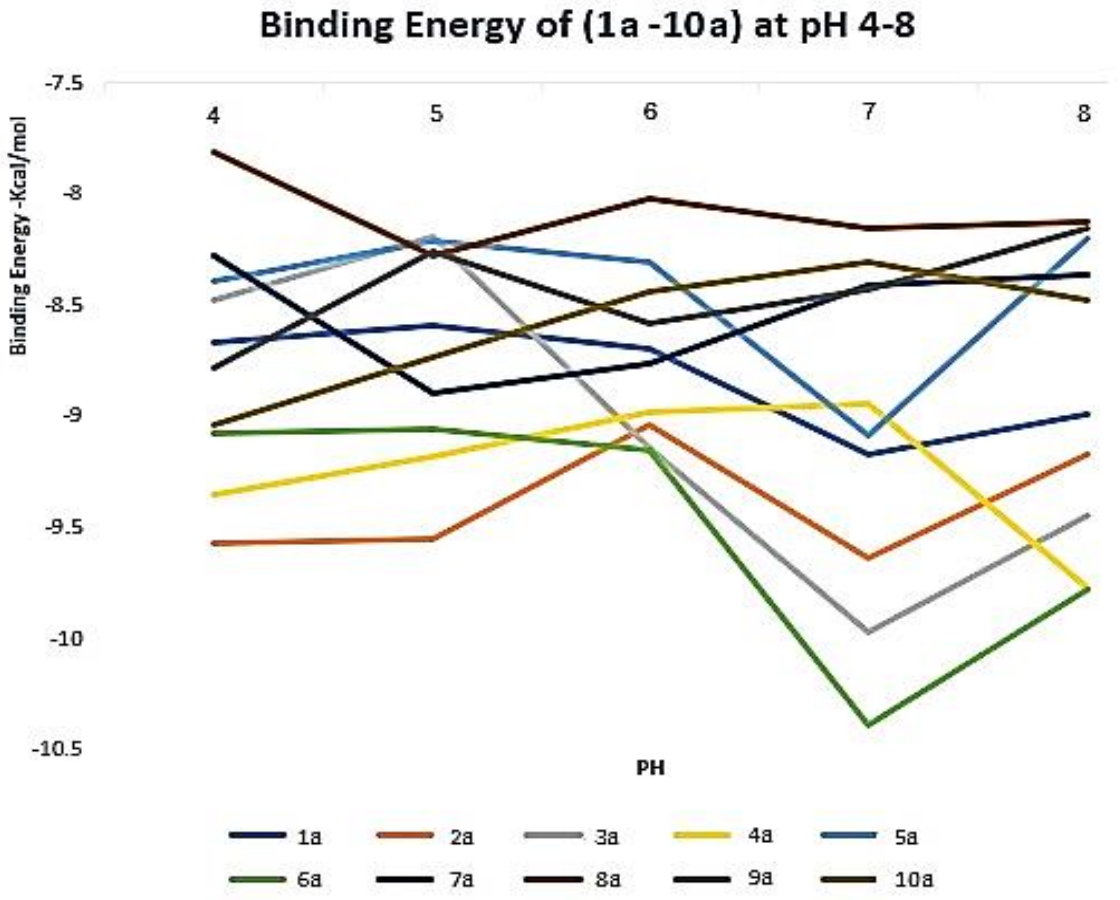

Fig. 1. Plot of binding energy values of (1a-10a) at pH 4-8.

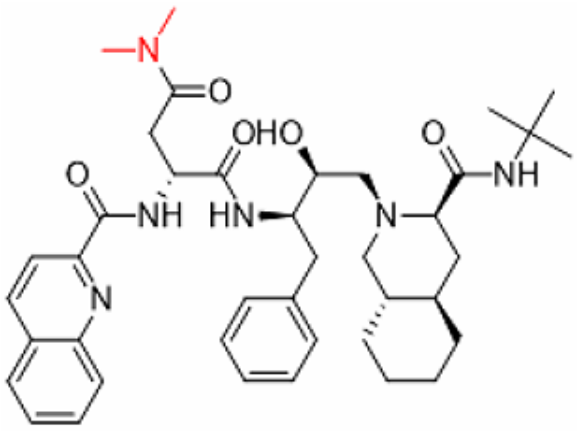

Fig. 2. (**6a'**) derivative. The N11 (highlighted in red) site is dialkylated.

Table 2. Details of interaction between ligand (**6a**) and receptor 6LU7 protease.

pH	Total binding energy (-kcal/mol)	Interaction site on drug	Type of Interaction	Interaction site on receptor	Distance (A)	Binding energy (-kcal/mol)
8	-9.78	N10	H-donor	O GLU 166	3.5	-0.6
		N11	H-donor	SD ME 165	3.4	-2.3
7	-10.39	O2	H-donor	SG CYS 145	3.6	-1.2
		O5	H-donor	SD MET 165	3.4	-0.2
		N9	H-donor	SG CYS 145	3.8	-1.5
		N11	H-donor	OE1 GLN 189	3.2	-1
6	-9.1467	O2	H-donor	OE1 GLN 189	2.8	-2.8
		N9	H-donor	OE1 GLN 189	3.1	-3.3
		C26	H-donor	SD MET 49	4.2	-0.8
		O3	H-acceptor	SG CYS 145	3.7	-0.8
5	-9.056	O2	H-acceptor	N GLU 166	2.9	-2.4
4	-9.075	N6	H-donor	SG CYS 145	4.1	-2.3
		C21	H-donor	O HIS 164	3.3	-1.6
		C24	H-donor	SD MET 49	4.2	-0.7
		O5	H-acceptor	N THR 26	3.01	-1.7

Table 3. Details of ligand and receptor interaction of proposed (**6a'**) and 6LU7 protease.

pH	Total Binding Energy (-kcal/mol)	Interaction site on drug	Type of Interaction	Interaction site on receptor	Distance (A)	Binding energy (-kcal/mol)
7	-7.8	O17	H-acceptor	NE2 GLN 189 (A)	3.0	2.0
5	-9.5	O49	H-donor	OE1 GLN 189 (A)	2.9	1.0
		6-ring	π-H	CA PRO 168 (A)	4.3	0.6

CONCLUSION

We used SARS-CoV-2 related protease as a drug target reception site for binding with the repurposed commercially available and medicinally approved anti-HIV protease inhibitors **(1a-10a)** within pH 4-8. It was found that both the binding energy values along with the modes of interaction were functions of variation in pH for some anti-HIV repurposed protease inhibitors at increasingly acidic pH to simulate acidic environment of infected cell. Indinavir (**3a**) and saquinavir **(6a)** showed the most variable binding energy values with the variation in pH. Using mode of interactions, we also proposed single step simulated synthetic derivative (**6a'**) with higher pH related selectivity. This proposed structure can serve as retrosynthesis targets for SARS-CoV-2 specific protease inhibitor.

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(*Accepted for publication December 2020*)