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Old drugs as lead compounds for a new disease? Binding analysis of SARS coronavirus main proteinase with HIV, psychotic and parasite drugs

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Abstract—The SARS-associated coronavirus (SARS-CoV) main proteinase is a key enzyme in viral polyprotein processing. To allow structure-based design of drugs directed at SARS-CoV main proteinase, we predicted its binding pockets and affinities with existing HIV, psychotic and parasite drugs (lopinavir, ritonavir, niclosamide and promazine), which show signs of inhibiting the replication of SARS-CoV. Our results suggest that these drugs and another two HIV inhibitors (PNU and UC2) could be used as templates for designing SARS-CoV proteinase inhibitors.

1. Introduction

Reemergence of severe acute respiratory syndrome (SARS) is a distinct possibility. Currently neither antiviral therapy nor vaccine is available. Viral replicase and protease are preferred targets for the screening and design of antiviral compounds and have been successfully targeted in several viral diseases. The SARS-associated coronavirus (SARS-CoV) main proteinase (Mpro or 3CL pro) plays a key role in proteolytic processing of the replicase polyproteins 1a and 1ab, which makes it an attractive target for developing drugs against this new disease. Recent report indicated that the proteinase inhibitor kaletra, a mixture of protease inhibitors—lopinavir and ritonavir, approved for treating HIV in 2000, shows signs of effectiveness against the SARS virus.[1] In particular, researchers in Taiwan discovered that two existing medicines, which have significant effect in inhibiting the replication of SARS-CoV (http://www.etaiwannews.com/Taiwan/2003/10/31/1067562739.htm). One is an anti-parasite drug niclosamide, and another is anti-psychotic drug promazine. The purpose of this study is to analyze whether the SARS-CoV main proteinase could be the target of these existing drugs. We performed in silico binding studies of the drugs using the recently identified crystal structure of Mpro,[2,3] to provide information for anti-SARS inhibitor design.

2. Materials and methods

The atomic coordinates of SARS-CoV main proteinase were downloaded from Protein Data Bank (PDB ID 1Q2W). Another crystal structure of SARS-CoV main proteinase is also available (PDB ID 1UJ1), the superposition of 1Q2W A chain and 1UJ1 A chain is shown in Figure 1, they overlap very well (rmsd = 0.64), here we chose 1Q2W as docking studies, which was released early. The overall structure of a monomer of SARS-CoV main proteinase is composed of three domains: domain I (residues 1–101), domain II (residues 102–200) and III (residues 201–303), represented by green, pink and white trace in Figure 1. The cleft between domains I and II is its substrate-binding site.

Except four drugs (lopinavir, ritonavir, niclosamide and promazine), we also conducted the docking studies of two other molecules, PNU and UC2, for their molecular formulas are close to those of niclosamide and promazine, respectively (Fig. 2), and they both are the inhibitors of HIV-1 reverse transcriptase.[4,5] The program Hex was employed to conduct the docking of the ligands to the SARS-CoV main proteinase, its basic approach to the docking problem is to model each molecule using 3D

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Figure 1. Superposition of two crystal structures from SARS-CoV main proteinase: 1Q2W A chain and 1UJ1 A chain. Domain I (residues 1–101, green trace), domain II (residues 102–200, pink trace) and III (residues 201–303, white trace).

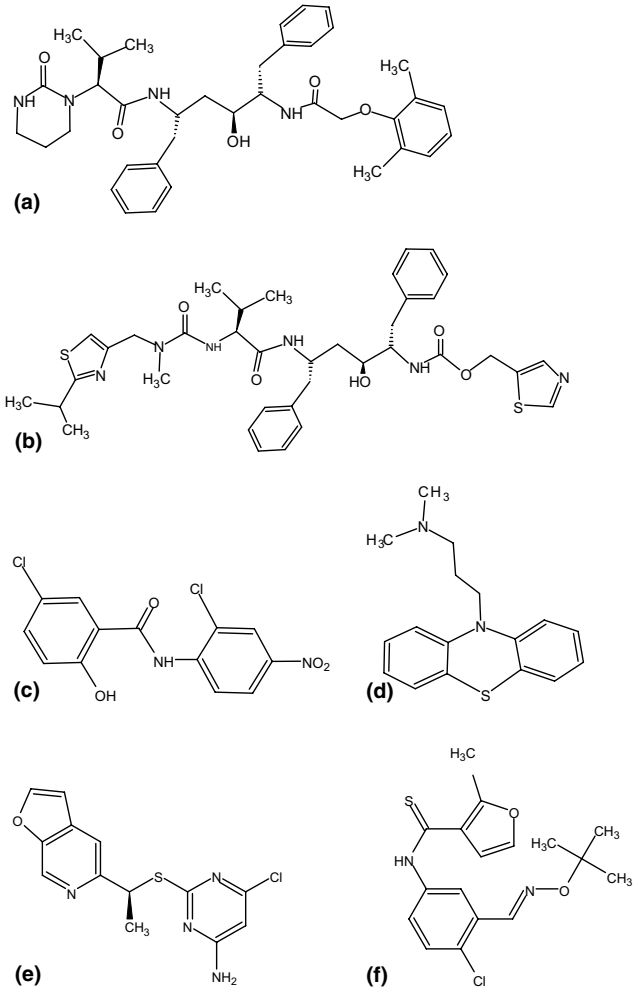

Figure 2. Chemical structures of drugs and inhibitors mentioned in this study: (a) lopinavir ($C_{37}H_{48}N_4O_5$), (b) ritonavir ($C_{37}H_{48}N_6O_5S_2$), (c) niclosamide ($C_{13}H_8Cl_2N_2O_4$), (d) promazine ($C_{17}H_{20}N_2S$), (e) PNU ($C_{13}H_{11}ClN_4OS$), (f) UC2 ($C_{17}H_{19}ClN_2O_2S$).

parametric functions, which encode both surface shape, electrostatic charge and potential distributions. The surface shape representation uses a novel 3D surface skin model of protein topology, and a novel soft molecular mechanics energy minimization procedure is used to refine the candidate docking solutions. Unlike conventional 3D fast Fourier transform (FFT) docking approaches, Hex uses spherical polar Fourier correlations to accelerate the docking between 10 and 100 times faster than FFT docking algorithm.[6] Here we used the following parameter set: correlation type = shape + three probes, post-processing = MM minimization, steric scan = 20 (maximum), final search = 32 (maximum), the others are default set. The structural comparison was performed by LGA.[7] The visualization of 3D structure was generated by PROTEINEXPLORER (http://www.proteinexplorer.org).

3. Results and discussion

Figure 3 displays the overall structures of docking for four drugs (lopinavir, ritonavir, niclosamide and promazine) and two inhibitors (PNU and UC2) to SARS-CoV main proteinase. The binding pockets of these compounds in SARS-CoV main protease are shown in Table 1, which is defined by those residues that have at least one heavy atom (other than hydrogen) with a distance less than 5 Å from a heavy atom of inhibitors, as described by Chou et al.[8] The results show that the binding pockets of six compounds can be divided into three classes: (1) residues 40–86 and 181–192 for four drugs/inhibitors (lopinavir, niclosamide, promazine and PNU); (2) residues 41–51 and 164–194 for UC2 inhibitor; (3) residues 19–57 and 117–193 for drug ritonavir. All these pockets locate in domain I (residues 8–101), domain II (residues 102–184) and a long loop region (residues 185–200) connecting domains I and II in SARS-CoV main proteinase. Thus, the four drugs and two inhibitors studied here can basically bind to the active site of SARS-CoV main proteinase, a cleft between domains I and II.

To estimate the binding affinities of each compound, the inhibitory constant (K_i, mole) was calculated from the equation:

$$\Delta G = -RT \ln K_i$$

where ΔG is the free energy of binding (kJ/mol) (here refers to the final docked energy), R is the gas constant 8.31 J/K/mol and T is the absolute temperature (at 300 K), as did in Jenwitheesuk and Samudrala.[9] The results indicate that the inhibitory constants of six compounds are: 8.7×10^{-20} (lopinavir), 5.6×10^{-25} (ritonavir), 4.2×10^{-22} (niclosamide), 6.2×10^{-21} (promazine), 3.5×10^{-23} (PNU), 2.1×10^{-19} (UC2). It is noted that these values are too low, for example, the inhibitory constant of lopinavir was determined as $\sim10^{-7}$ by Jenwitheesuk and Samudrala.[10] The reason for this difference is that the docked energy value from Hex program is a pseudo-energy, which is designed to give reasonably consistent units with conventional energy calculations, not based on experimentally derived

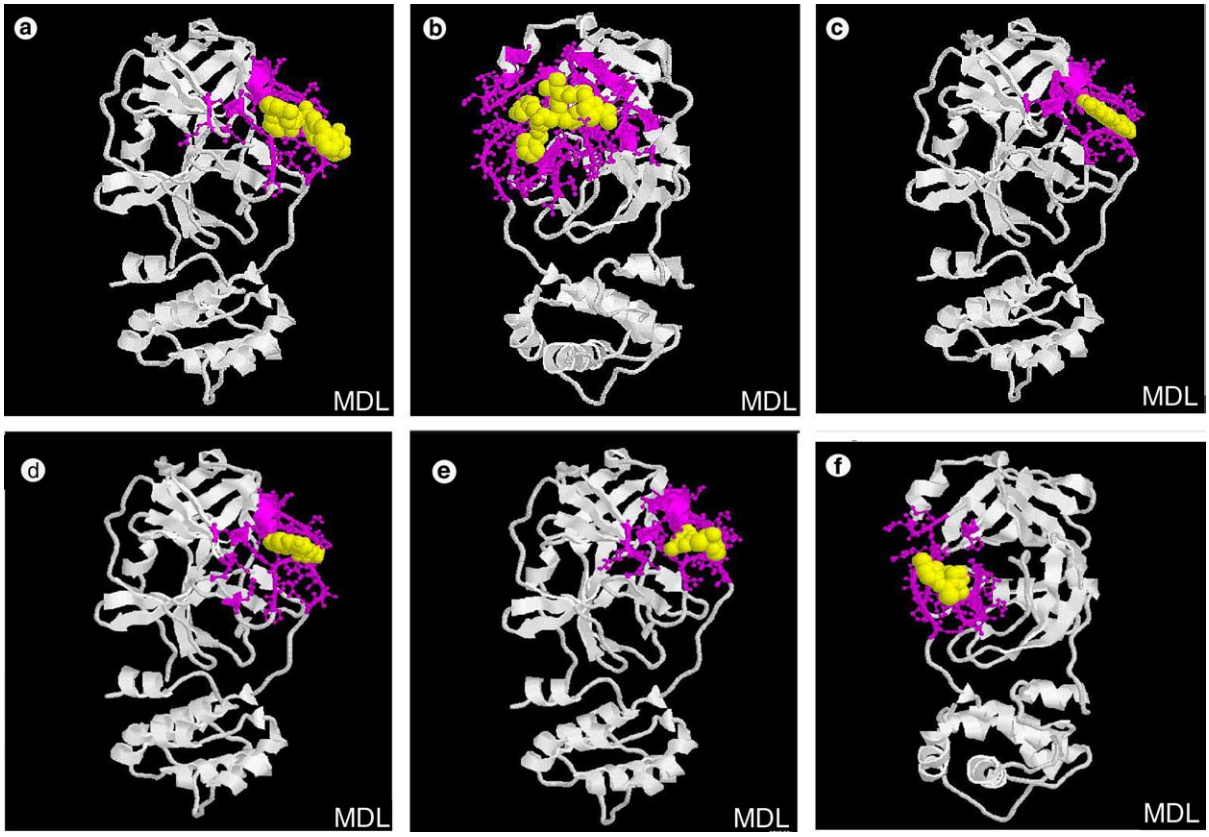

Figure 3. The binding pockets (pink ball-stick) of SARS-associated coronavirus main proteinase (white cartoon) with drugs and inhibitors (yellow spacefill): Anti-HIV drugs lopinavir (a) and ritonavir (b), anti-parasite drug niclosamide (c), anti-psychotic drug promazine (d), HIV inhibitors PNU (e) and UC2 (f).

Table 1. Binding pockets for SARS-CoV main proteinase with different drugs

Lopinavir	Ritonavir		Niclosamide	Promazine	PNU	UC2
ARG 40	GLN 19	SER 123	ARG 40	ARG 40	ARG 40	HIS 41
CYS 44	VAL 20	PHE 140	MET 49	ILE 43	HIS 41	MET 49
MET 49	THR 21	LEU 141	LEU 50	CYS 44	ILE 43	LEU 50
LEU 50	CYS 22	ASN 142	ASN 51	MET 49	CYS 44	ASN 51
ASN 51	GLY 23	GLY 143	PRO 52	LEU 50	MET 49	HIS 164
PRO 52	THR 24	SER 144	ASN 53	ASN 51	LEU 50	MET 165
ASN 53	THR 25	CYS 145	TYR 54	PRO 52	ASN 51	GLU 166
TYR 54	THR 26	GLY 146	GLU 55	ASN 53	PRO 52	LEU 167
GLU 55	LEU 27	SER 147	ASP 56	TYR 54	ASN 53	PRO 168
ASP 56	ASN 28	HIS 163	LEU 57	GLU 55	TYR 54	THR 169
LEU 57	PRO 39	HIS 164	MET 82	ASP 56	GLU 55	GLY 170
MET 82	ARG 40	MET 165	VAL 186	LEU 57	ASP 56	VAL 171
ASN 84	HIS 41	GLU 166	ASP 187	LEU 58	LEU 57	HIS 172
CYS 85	VAL 42	LEU 167	ARG 188	MET 82	LEU 58	ALA 173
PHE 181	ILE 43	PRO 168	GLN 189	ASN 84	MET 82	PHE 181
PHE 185	CYS 44	VAL 171	THR 190	CYS 85	GLN 83	PRO 184
VAL 186	MET 49	HIS 172		VAL 186	ASN 84	PHE 185
ASP 187	LEU 50	ALA 173		ASP 187	CYS 85	VAL 186
ARG 188	ASN 51	GLY 174		ARG 188	LEU 86	ASP 187
GLN 189	PRO 52	PHE 181		GLN 189	VAL 186	ARG 188
THR 190	ASN 53	PHE 185		THR 190	ASP 187	GLN 189
ALA 191	TYR 54	VAL 186			ARG 188	THR 190
GLN 192	LEU 57	ASP 187			GLN 189	ALA 191
	CYS 117	ARG 188			THR 190	GLN 192
	TYR 118	GLN 189				ALA 193
	ASN 119	THR 190				ALA 194
	GLY 120	ALA 191				
	SER 121	GLN 192				
		ALA 193				

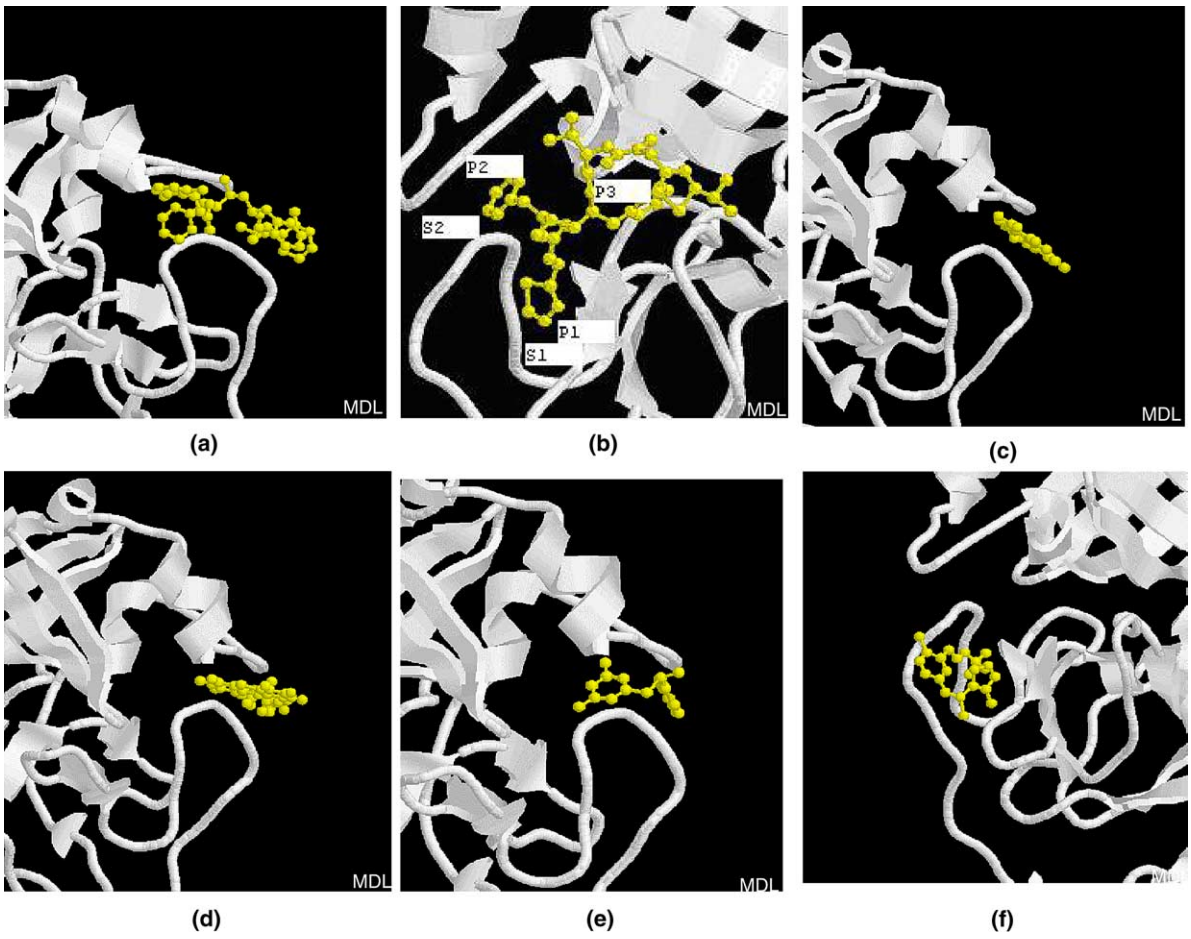

Figure 4. A close view of the interactions between SARS-associated coronavirus main proteinase (white cartoon) with drugs and inhibitors (yellow ball-stick): (a) lopinavir, (b) ritonavir, (c) niclosamide, (d) promazine, (e) PNU and (f) UC2.

parameters, and as a theoretical reference value only when performing the docking algorithm. Thus we do not expect these values are the genuine representations of inhibitory constants and we use them primarily for qualitative comparison among the drugs/inhibitors studied here. Because the lower the K_i is, the greater the binding affinity is, hence HIV drug ritonavir is the compound that bind to the substrate binding site of SARS-CoV proteinase with the highest binding affinity, followed by HIV inhibitor PNU and anti-parasite drug niclosamide, and UC2 is the compound with the lowest binding affinity. Moreover, the inhibitory constants of ritonavir, PNU, niclosamide, promazine and UC2 are about 10^{-5}, 10^{-3}, 10^{-2}, 10^{-1} and 10-fold inhibitory constant of lopinavir, respectively, if we assume that a value of 10^{-7} mol for lopinavir's inhibitory constant is correct, the inhibitory constants of ritonavir, PNU, niclosamide, promazine and UC2 could be estimated as 10^{-12}, 10^{-10}, 10^{-9}, 10^{-8} and 10^{-6} mol, respectively.

The close views of the interactions between SARS-CoV main proteinase and these drugs/inhibitors are exhibited in Figure 4. The results show that half of lopinavir is left outside the catalytic site (Fig. 4a), for ritonavir, the thiazole group (P1) and a benzene group (P2) are inserted into S1 and S2 specificity pockets, respectively, while another benzene side chain (P3) might be too long to fit the substrate binding pocket perfectly (Fig. 4b), there is similar situation in the inhibitor AG7088,[11] which has been experimentally shown to not bind with high affinity to the SARS-CoV proteinase (http://www.nature.com/nsu/030512/030512-11.html). Thus the efficacy of lopinavir/ritonavir could be poor. Indeed, consistent with our predictions, experimental observation data indicated that both lopinavir and ritonavir individually have only a weak in vitro activity against SARS-CoV. However, the addition of lopinavir/ritonavir to ribavirin and corticosteroid treatment regimens appears to reduce incubation and mortality rates, especially when administered early.[12] Similarly, the half of niclosamide or promazine is left outside the active site (Fig. 4c and d), obviously the propane side chain in promazine is too long. For PNU inhibitor, seems it can basically fit into the active cleft, except the dihydrofuran side chain is a little bit long (Fig. 4e). Finally, the inhibitor UC2 binds to a position that is slightly away from the active centre (Fig. 4f), its neopentane or methylfuran side chain is a little long and makes it unable to insert into the active pocket properly. Indeed UC2 is the compound with lowest binding affinity as mentioned above. Taken together, our study illustrates that existing drugs/inhibitors may be used as starting points for the discovery of rationally designed anti-SARS proteinase drugs.

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