Network Pharmacology analysis of orally bioavailable SARS-COV2 protease inhibitor shows synergistic targets to improve clinical efficacy

Arun Kumar (arun.kumar@ucd.ie)
University College Dublin https://orcid.org/0000-0001-8422-0219

Research Article

Keywords: molecular interaction, protease inhibitor, SARS-CoV2, antiviral, coronavirus

Posted Date: May 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-513595/v1

Abstract

Introduction: Orally bioavailable SARS-CoV2 antiviral drugs will significantly improve the clinical management of the disease. PF07321332 (PF32) one such orally bioavailable SARS-CoV2 protease inhibitor which can be helpful to prevent viral replication in the host.

Material and methods: Hence this study evaluated the network pharmacology of PF32 using established methods to predict its potential safety and efficacy.

Results: PF32 was selective against SARS-CoV2 proteases without any affinity against SARS-CoV2 RNA polymerase or its spike protein. While PF32 showed pharmacologically relevant affinity against several targets in human tissues. The target profiling of PF32 indicated a fourfold selectivity towards several proteases in human tissues with an affinity (IC_{50}) ranging from 26 to 41 nM.

Conclusion: The predicted inhibitory effects of PF32 against both host and viral proteases may have synergistic effects for superior clinical efficacy.

Introduction

The pandemic caused by coronavirus (SARS-CoV2) which originated in the Wuhan region of China has caused over 150 million human infections (COVID-19) globally with a mortality rate of ~2%.[1-4] Several measures to treated the COVID-19 continues to be explored amid the spread of various mutants collateral to the mass vaccination efforts. One such approach is the development of drugs to inhibit SARS-CoV2 replication by targeting its protease.[4-7] Several synthetic and natural compounds have shown variable efficacy against SARS-CoV2 protease. Recently PF07321332 (PF32) is reported as an orally bioavailable protease inhibitor and is currently in clinical trials (Clinical trial ID NCT04756531; http://www.clinicaltrials.gov) to evaluate its safety and efficacy in the treatment of SARS-CoV-2 infections. PF32 specifically inhibits SARS-CoV2 replicase polyprotein 1ab which is a multifunctional protein essential to transcription and replication of the coronavirus.[3, 8, 9] The orally bioavailability of PF32 will be major advantage in the clinical management of COVID-19. Like all drugs, PF32 may also have off target effects. Understanding of these off targets is essential to envisage potential synergistic and/or adverse effects. Hence in this study network pharmacology of PF32 was assessed using well established tools to get an insight into the all potential targets of PF32 in human cells.

Materials And Methods

Drug structure and target analysis

The structure of PF32 (figure 1A) was reconstructed in Swiss target prediction database (http://www.swisstargetprediction.ch) using its SMILES identity.[10, 11] The database was searched for the all human specific targets of PF32 and the probability scores of the targets were analysed.

Protein structure and molecular docking analysis:

The protein data bank (PDB; https://www.rcsb.org) was searched for the 3D structures of identified targets of PF32 and the data was processed as reported before.[7, 12] The PDB file of PF32 was generated using the Chimera software and used for the analysis of its molecular interactions (number of hydrogen bonds) with all its identified targets as reported before.[12-15] The reported affinity of homologous structure of PF32 with Fibroblast activation protein alpha (PDB ID IZ68) was used as reference and the affinity of the PF32 with its identified targets was predicted based on the differences in the ratio of hydrogen bonds compared to that of the reference standard as reported before.[7, 12, 16] In similar lines the affinity of PF32 against SARS-COV2 targets was also estimated as reported before.[12, 16]

Expression of PF32 targets in human tissues

Protein expression of PF32 targets in various human tissues was assessed from the human protein atlas database (https://www.proteinatlas.org) on 6th May 2020 as described before [17, 18].

Results

A majority (49%) of the PF32 targets in humans tissues are proteases, which is followed by electrochemical transporters (12%) and family A G protein coupled receptors (11%) (figure 1B). The insilco analysis of the PF32 structure (figure 1A), indicated several drug/lead like features including its oral bioavailability (figure 1C). In the swiss target prediction analysis, 19 proteins showed probability scores ranging from 0.11- 0.12 (figure 1D), which will be referred to as identified targets of PF32. These identified targets were further analysed for their molecular interactions (hydrogen bonds) with PF32 in Chimera software. The number of hydrogen bonds (figure 1E, 2A) between PF32 and its identified targets did not correlate with their respective probability scores, suggesting other molecular interaction (probably Van der Waals forces) may also influence these interactions. The number of hydrogen bonds between PF32 and its identified targets ranged from 0 to 178 (figure 1E). Previous reports have indicated an affinity (IC_{50}) of 73.2±0.5 between PF32 homologue and Fibroblast activation protein alpha (FAP) and this was used as a reference to predict the affinity of PF32 with its identified targets (figure 1f). Affinity (IC_{50}) of PF32 against the various receptors ranged from 26 to 4745 nM.

The following proteins showed higher affinity (4UFA, 1XU9, 3DDU, 1H8D, 1DUZ, 2RA3) with their IC_{50} values ranging from 26 to 41 nM (table 1). PF32 showed higher affinity with Identified targets 4UFA and 1XU9 and least affinity with 4A5S (table 1). Nevertheless the affinities predicted with 11 of the Identified targets were in pharmacologically relevant concentrations (table 1).

The affinity of PF32 against various SARS-COV2 specific targets were also assessed. Representative images of molecular interaction of PF32 against selected identified and SARS-COV2 specific targets are shown in figure 2A. PF32 showed highest affinity (IC_{50} 45-60 nM) against SARS-COV2 main protease and Replicase polyprotein 1ab (figure 2B). As several 3D structures of Replicase polyprotein 1ab are reported

in the PDB databased, a representative of each of the variable 3D structure reported was screened in this study. PF32 did not show any affinity against SARS-COV2 spike protein, RNA polymerase and some variable structures of Replicase polyprotein 1ab (table 2).

The expression pattern of the identified targets of PF32 was evaluated in the human tissues. Expression pattern of representative identified targets (ACE, HSD11B1, PREP and FAP) are shown in figure 3. While some identified targets were expressed only in selected tissues (ACE and HSD11B1) others were ubiquitously expressed (PREP and FAP) (figure 3).

Discussion

PF32 is recently developed orally bioavailable SARS-COV2 protease inhibitor which has entered clinical safety and efficacy evaluation phase (Clinical trial ID NCT04756531) (https://go.drugbank.com/drugs/DB16691). This study reports the network pharmacology analysis to identify human tissue and SARS-COV2 specific targets the PF32 molecule can interact with. Knowledge of these interactions will be essential to understand the safety and efficacy of PF32 as a supplement to that identified in clinical trials.[19-21] The target profiling of PF32 indicated a fourfold selectivity towards proteases with an affinity (IC_{50}: 26 to 41 nM) which was pharmacologically relevant. In the human tissue the affinity of PF32 was maximum towards Angiotensin-converting enzyme (ACE). Considering the reports of SARS-COV2 using the ACE2 as receptor for entering into host cell,[3, 22] the affinity of PF32 towards ACE may evince synergistic effects by both inhibiting the virus multiplication as well as preventing virus entry into host cells. Besides ACE, several other proteases were also overserved to have pharmacologically relevant affinity with PF32. Although the clinical relevance of these interactions are unclear at present, considering the systemic inflammation evinced by SARS-COV2,[23-25] the broader protease inhibitory potential of PF32 observed in this study may facilitate synergistic clinical benefits.

Similar to the broader affinity of PF32 against several human tissue specific proteases, PF32 was observed to have pharmacologically relevant affinity against SARS-COV2 main protease as well as its Replicase polyprotein 1ab. This selective inhibition of SARS-COV2 proteases but not its RNA polymerase or spike protein with higher affinity (IC_{50}: 45 to 60 nM) together with its bioavailability/cell permeability may potentiate clinical safety and efficacy of PF32. However unlike the SARS-CoV2 targets, the identified targets of PF32 in human tissues had a wider tissue expression profile, which paraphs reflects the wider pharmacological profile of PF32. Depending on the tissue specific virus presence and considering associated systemic inflammation the diffused pharmacological profile of PF32 may prove to be clinically beneficial.

In summary the network pharmacology analysis of PF32 in this study identifies its relevant targets in human tissues and SARS-CoV2, which may have synergistic effects for superior clinical efficacy.

Declarations

Declaration of Conflict of interest: none

Acknowledgement: Research support from University College Dublin-Seed funding/Output Based Research Support Scheme is acknowledged.

References

1. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Biomed. 2020. 91;(1). 157-160.
2. Fauci AS, Lane HC, Redfield RR. Covid-19 - Navigating the Uncharted. N Engl J Med. 2020. 382;(13). 1268-1269.
3. Stower H. Virological assessment of SARS-CoV-2. Nat Med. 2020. 26;(4). 465.
4. Kılroy D, Kumar A. Anatomical perspective on the loss of smell and taste sensation in SARS-CoV-2 infection. . Anatomy. 2020. 14(2). 145-149.
5. Kumar AHS. Pharmacology of Chloroquine: Potential Mechanism of Action against Coronavirus. BEMS Reports. 2020. 6;(1). 9-10.
6. Kumar AHS, Sharma V. Acetamido-Propanoic Acid Derived Compounds as Protease Inhibitors to Target Coronaviruses. BEMS Reports. 2019. 5;(2). 20-22.
7. Sagar VK, Kumar AHS. Efficacy of Natural Compounds from Tinospora cordifolia against SARS-CoV-2 Protease, Surface Glycoprotein and RNA Polymerase. BEMS Reports. 2020. 6;(1). 6-8.
8. Beck BR, Shin B, Choi Y, et al. Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. Comput Struct Biotechnol J. 2020. 18. 784-790.
9. Choy KT, Wong AY, Kaewpreedee P, et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. Antiviral Res. 2020. 178. 104786.
10. Gfeller D, Grosdidier A, Wirth M, et al. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res. 2014. 42;(Web Server issue). W32-8.
11. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019. 47;(W1). W357-W364.
12. Kumar AHS, Molecular profiling of Neprilysin expression and its interactions with SARS-CoV-2 spike proteins to develop evidence base pharmacological approaches for therapeutic intervention., Research Square, Online, 2021, pp. 1-16.
13. Goothy SSK, Kumar AHS. Network Proteins of Angiotensin-converting Enzyme 2 but Not Angiotensin-converting Enzyme 2 itself are Host Cell Receptors for SARS-Coronavirus-2 Attachment. BEMS Reports. 2020. 6;(1). 1-5.
14. Kumar AHS. Molecular Docking of Natural Compounds from Tulsi (Ocimum sanctum) and neem (Azadirachta indica) against SARS-CoV-2 Protein Targets. BEMS Reports. 2020. 6;(1). 11-13.
15. Yang Z, Lasker K, Schneidman-Duhovny D, et al. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. J Struct Biol. 2012. 179;(3). 269-78.

16. Kumar A. Modelling the efficacy of Neprilysin from various species in degrading different Amyloid-beta peptides: Potential application in therapeutics of Alzheimers disease. . BioRxiv. 2021. 1-16.
17. Hruz T, Laule O, Szabo G, et al. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformatics. 2008. 2008. 420747.
18. Uhlen M, Fagerberg L, Hallstrom BM, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015. 347;(6220). 1260419.
19. Casas AI, Hassan AA, Larsen SJ, et al. From single drug targets to synergistic network pharmacology in ischemic stroke. Proc Natl Acad Sci U S A. 2019. 116;(14). 7129-7136.
20. Khanal P, Patil BM. Integration of network and experimental pharmacology to decipher the antidiabetic action of Duranta repens L. J Integr Med. 2021. 19;(1). 66-77.
21. Khanal P, Duyu T, Patil BM, et al. Screening of JAK-STAT modulators from the antiviral plants of Indian traditional system of medicine with the potential to inhibit 2019 novel coronavirus using network pharmacology. 3 Biotech. 2021. 11;(3). 119.
22. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003. 426;(6965). 450-4.
23. Katopodis P, Kerslake R, Davies J, et al. COVID19 and SARSCoV2 host cell entry mediators: Expression profiling of TMRSS4 in health and disease. Int J Mol Med. 2021. 47;(4).
24. Shtaya A, Trippier S, Ghatala R, et al. Comment on "Stroke in patients with SARSCoV2 infection: case series" from a London hospital experience. J Neurol. 2021. 268;(2). 424-430.
25. Mandel M, Harari G, Gurevich M, et al. Cytokine prediction of mortality in COVID19 patients. Cytokine. 2020. 134. 155190.

Tables

Table 1: Affinity of PF32 with its identified targets having a reported probability of interaction in humans.

PF32 Targets (Common Name)	PDB ID	IC50 (nM)
Fibroblast activation protein alpha (FAP)	1Z68	73.2 ± 0.5
Dipeptidyl peptidase IV (DPP4)	4A5S	4745 ± 20.2
Dipeptidyl peptidase VIII (DPP8	6EOP	NA
Prolyl endopeptidase (PREP)	3DDU	34.4 ± 0.2
Dipeptidyl peptidase IX (DPP9)	6EOR	NA
Baculoviral IAP repeat-containing protein 2 (BIRC2)	4HY4	79.1 ± 0.4
Leukocyte elastase (ELANE)	5ABW	NA
HLA class I histocompatibility antigen A-3 (HLA-A)	1DUZ	35.4 ± 0.1
Thyrotropin-releasing hormone receptor (TRHR)	NR	CBE
Angiotensin-converting enzyme (ACE)	4UFA	26.7 ± 0.1
Plasma kallikrein (KLKB1)	6O1G	NA
Thrombin (F2)	1H8D	34.6 ± 0.2
Trypsin I (PRSS1)	2RA3	41.3 ± 0.4
11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1)	1XU9	28.6 ± 0.1
Inhibitor of apoptosis protein 3 (XIAP)	4J44	158.2 ± 1.2
11-beta-hydroxysteroid dehydrogenase 2 (HSD11B2)	NR	CBE
Pepsinogen C (PGC)	1HTR	677.9 ± 4.1
Dipeptidyl peptidase II (DPP7)	4EBB	NA
TRAIL receptor-1 (TNFRSF10A)	5CIR	197.7 ± 1.6

NR: not reported. NA: no affinity, CBE: cannot be estimated.

Table 2: Affinity of PF32 against SARS-COV2 targets.

Target Type	PDB ID	IC50 (nM)
Main protease	6Y84	48.9 ± 1.2
Spike protein	6VSB	NA
RNA polymerase	6M71	NA
3C-like protease	7JPY	206.3 ± 8.4
Replicase polyprotein 1ab	5R82	58.6 ± 1.4
Replicase polyprotein 1ab	5RFC	59.3 ± 1.2
Replicase polyprotein 1ab	5RF9	59.3 ± 1.3
Replicase polyprotein 1ab	5RGJ	56.5 ± 1.1
Replicase polyprotein 1ab	5RL7	NA
Replicase polyprotein 1ab	5RS7	115.7 ± 6.4
Replicase polyprotein 1ab	5RVP	63.3 ± 1.8
Replicase polyprotein 1ab	5S71	NA
Replicase polyprotein 1ab	6W01	NA
Replicase polyprotein 1ab	6X4I	NA
Replicase polyprotein 1ab	6YVF	54.5 ± 1.1
Replicase polyprotein 1ab	7NFV	83.2 ± 2.4
Replicase polyprotein 1ab	7LTJ	NA
Replicase polyprotein 1ab	7KYU	65.9 ± 2.1
Replicase polyprotein 1ab	7JYC	NA
Replicase polyprotein 1ab	7D1M	NA
Replicase polyprotein 1ab	6ZSL	NA

NA: no affinity.

Figures

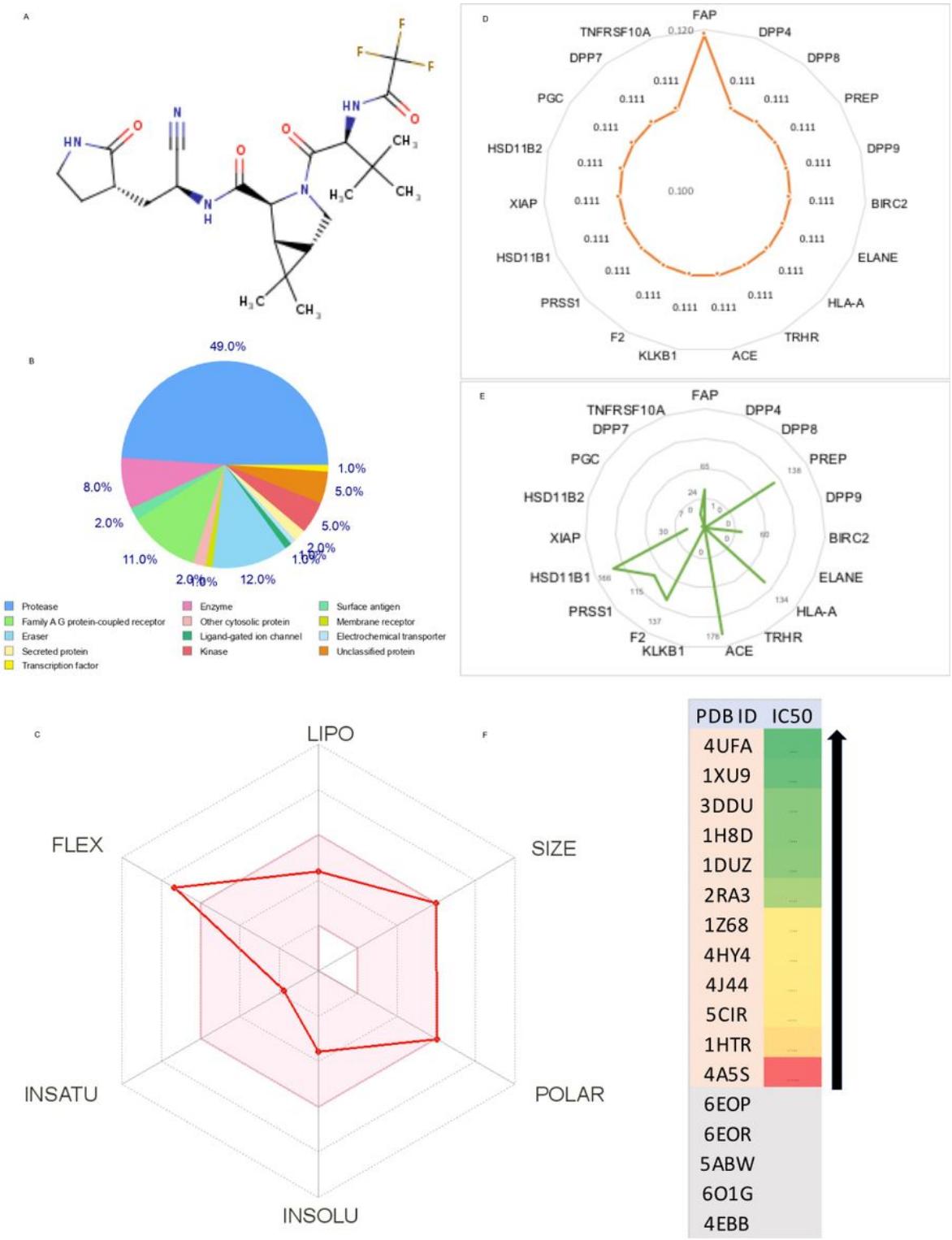

Figure 1

Pharmacological properties of PF07321332 (PF32). A) Structure of PF32. B) Summary of identified targets of PF32 in humans. C) Drug and oral bioavailability properties of PF32 based on its size, lipophilicity (LIPO), structure flexibility (FLEX), polarity, instauration (INSATU) and solubility (INSOLU). D) Probability of interaction score of identified targets of PF32 in humans. E) Number of hydrogen bonds

between PF32 and its identified targets at 10Å distance. F) Affinity (IC50) of PF32 against its identified targets (direction of arrow is from low to high affinity, targets in grey have no affinity).

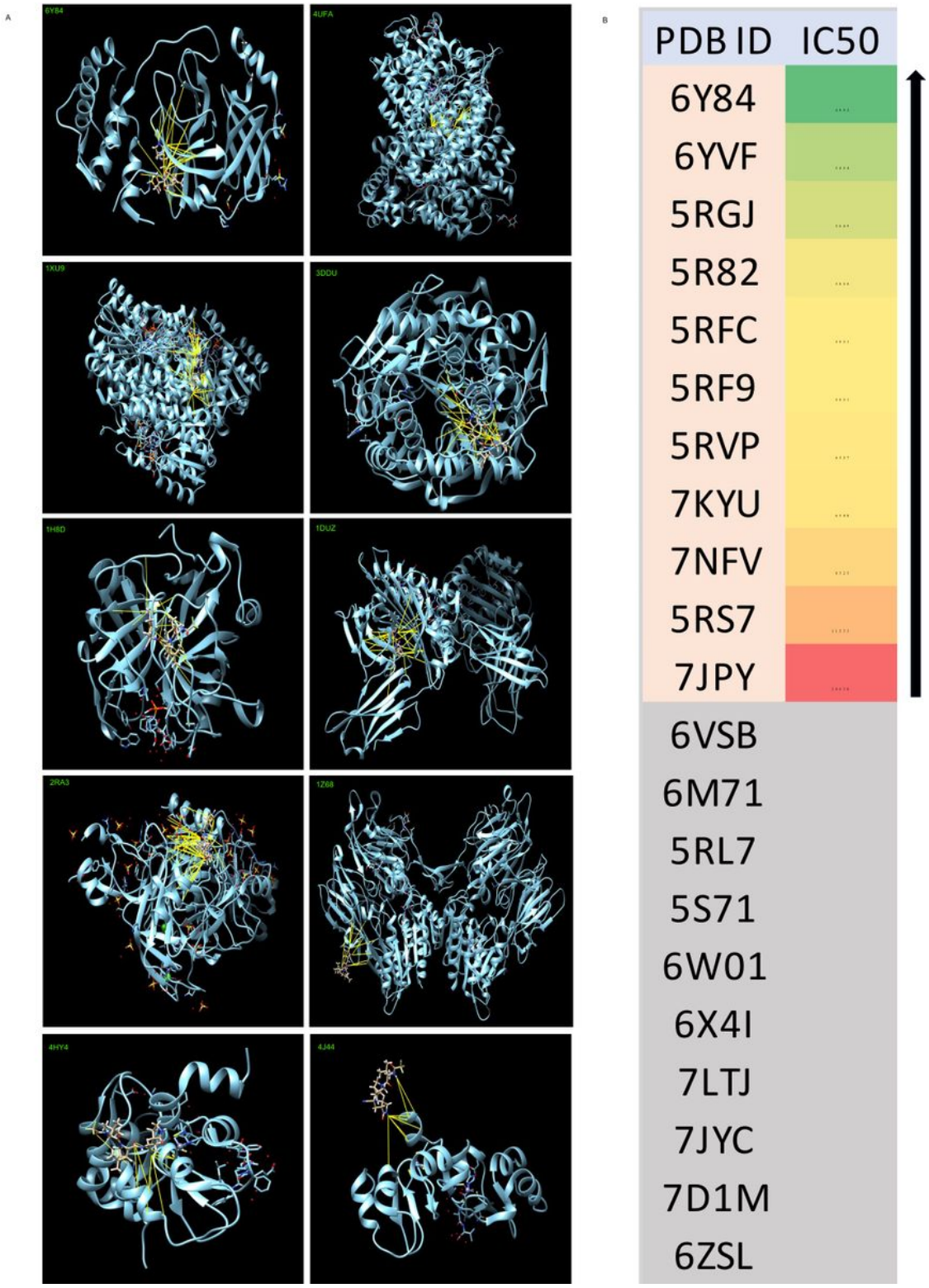

Figure 2

Molecular interactions and affinity of PF07321332 (PF32) with its targets. A) Selected images showing interactions of PF32 with its identified targets in humans and SARS-COV2 (yellow lines indicate the

hydrogen bonds). B) Affinity (IC50) of PF32 against various SARS-COV2 targets (direction of arrow is from low to high affinity, targets in grey have no affinity).

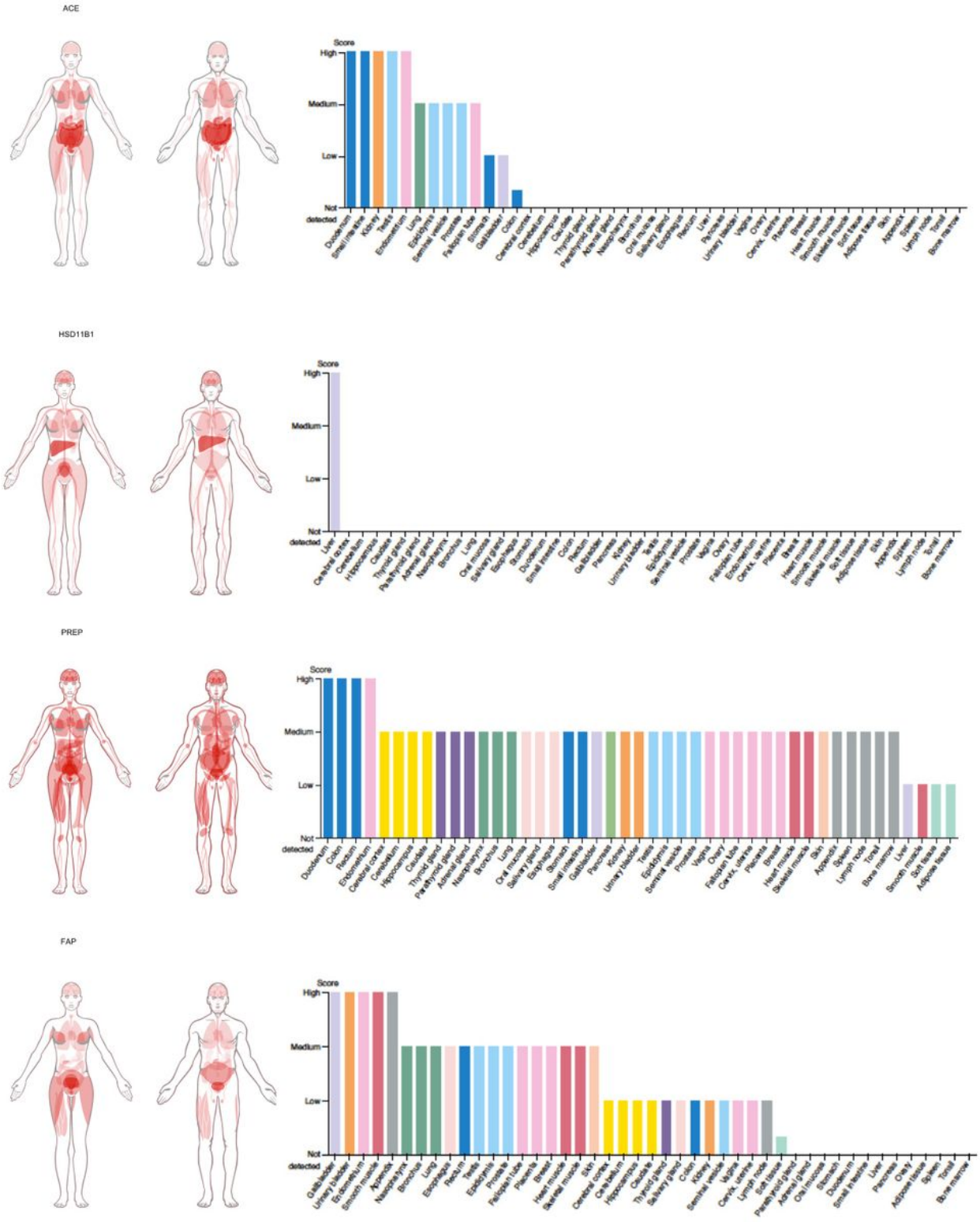

Figure 3

Anatomogram of selected targets of PF32 in human tissues. The graphs show the expression levels of the respective protein in various humans tissues.