Network Pharmacology Analysis of Orally Bioavailable SARS-CoV-2 Protease Inhibitor Shows Synergistic Targets to Improve Clinical Efficacy

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ABSTRACT
Introduction: Orally bioavailable SARS-CoV-2 antiviral drugs will significantly improve the clinical management of the disease. PF07321332 (PF32) one such orally bioavailable SARS-CoV-2 protease inhibitor which can be helpful to prevent viral replication in the host. Materials 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-CoV-2 proteases without any affinity against SARS-CoV-2 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.

Key words: Molecular interaction, Protease inhibitor, SARS-CoV-2, Antiviral, Coronavirus.

INTRODUCTION

The pandemic caused by Coronavirus (SARS-CoV-2) which originated in the Wuhan region of China has caused over 150 million human infections (COVID-19) globally with a mortality rate of ~2%. 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-CoV-2 replication by targeting its proteases. Several synthetic and natural compounds have shown variable efficacy against SARS-CoV-2 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-CoV-2 replicase polyprotein 1ab which is a multifunctional protein essential to transcription and replication of the coronavirus. 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.swistargetprediction.ch) using its SMILES identity. 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. 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. The reported affinity of homologous structure of SARS-CoV-2 protease 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. In similar lines the affinity of PF32 against SARS-CoV-2 targets was also estimated as reported before.

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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 in silico 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).

<table>
<thead>
<tr>
<th>PF32 Targets (Common Name)</th>
<th>PDB ID</th>
<th>IC$_{50}$(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast activation protein alpha (FAP)</td>
<td>1Z68</td>
<td>73.2±0.5</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IV (DPP4)</td>
<td>4A5S</td>
<td>4745±20.2</td>
</tr>
<tr>
<td>Dipeptidyl peptidase VIII (DPP8)</td>
<td>6EOP</td>
<td>NA</td>
</tr>
<tr>
<td>Prolyl endopeptidase (PREP)</td>
<td>3DDU</td>
<td>34.4±0.2</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IX (DPP9)</td>
<td>6EOR</td>
<td>NA</td>
</tr>
<tr>
<td>Baculoviral IAP repeat-containing protein 2 (BIRC2)</td>
<td>4HY4</td>
<td>79.1±0.4</td>
</tr>
<tr>
<td>Leukocyte elastase (ELANE)</td>
<td>5ABW</td>
<td>NA</td>
</tr>
<tr>
<td>HLA class I histocompatibility antigen A-3 (HLA-A)</td>
<td>1DUZ</td>
<td>35.4±0.1</td>
</tr>
<tr>
<td>Thyrotropin-releasing hormone receptor (TRHR)</td>
<td>NR</td>
<td>CBE</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme (ACE)</td>
<td>4UFA</td>
<td>26.7±0.1</td>
</tr>
<tr>
<td>Plasma kallikrein (KLKB1)</td>
<td>6O1G</td>
<td>NA</td>
</tr>
<tr>
<td>Thrombin (F2)</td>
<td>1H8D</td>
<td>34.6±0.2</td>
</tr>
<tr>
<td>Trypsin 1 (PRSS1)</td>
<td>2RA3</td>
<td>41.3±0.4</td>
</tr>
<tr>
<td>11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1)</td>
<td>1XU9</td>
<td>28.6±0.1</td>
</tr>
<tr>
<td>Inhibitor of apoptosis protein 3 (XIAP)</td>
<td>4J44</td>
<td>158.2±1.2</td>
</tr>
<tr>
<td>11-beta-hydroxysteroid dehydrogenase 2 (HSD11B2)</td>
<td>NR</td>
<td>CBE</td>
</tr>
<tr>
<td>Pepsinogen C (PGC)</td>
<td>1HTR</td>
<td>677.9±4.1</td>
</tr>
<tr>
<td>Dipeptidyl peptidase II (DPP7)</td>
<td>4EBB</td>
<td>NA</td>
</tr>
<tr>
<td>TRAIL receptor-1 (TNFRSF10A)</td>
<td>5CIR</td>
<td>197.7±1.6</td>
</tr>
</tbody>
</table>

NR: not reported. NA: no affinity, CBE: cannot be estimated.
PF32 is recently developed orally bioavailable SARS-CoV-2 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-CoV-2 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.

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 towards Angiotensin-converting enzyme (ACE) was maximum. Considering the reports of SARS-CoV-2 using the ACE2 as receptor for entering into host cell, 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-CoV-2, 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-CoV-2 main protease as well as its Replicase polyprotein 1ab. This selective inhibition of SARS-CoV-2 proteases but not its RNA polymerase or spike protein with higher affinity (IC50: 45 to 60 nM) together with its bioavailability/cell permeability may potentiate clinical safety and efficacy of PF32. However unlike the SARS-CoV-2 targets, the identified targets of PF32 in human tissues had a wider tissue expression profile, which paralaxs 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-CoV-2, which may have synergistic effects for superior clinical efficacy.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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