Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
In-silico screening and in-vitro assay show the antiviral effect of Indomethacin against SARS-CoV-2

Rajkumar Chakraborty a,1, Gourab Bhattacharje b,1, Joydeep Baral b,1, Bharat Manna c,1, Jayati Mullick d, Basavaraj S. Mathapati d, Priya Abraham d, Madhumathi J e, Yasha Hasija a,***, Amit Ghosh b,***, Amit Kumar Das c,***

a Department of Biotechnology, Delhi Technological University, Main Bawana Road, Shabbad Daulatpur, Delhi, 110042, India
b Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur, 721302, India
c School of Energy Science and Engineering, Indian Institute of Technology Kharagpur, Kharagpur, 721302, India
d ICMR-National Institute of Virology, Pune, 411001, India
e Indian Council of Medical Research, Delhi, 110029, India

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Drug repurposing
Virtual screening
Differential gene expression
Indomethacin

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the worldwide spread of coronavirus disease 19 (COVID-19), and till now, it has caused death to more than 6.2 million people. Although various vaccines and drug candidates are being tested globally with limited to moderate success, a comprehensive therapeutic cure is yet to be achieved. In this study, we applied computational drug repurposing methods complemented with the analyses of the already existing gene expression data to find better therapeutics in treatment and recovery. Primarily, we identified the most crucial proteins of SARS-CoV-2 and host human cells responsible for viral infection and host response. An in-silico screening of the existing drugs was performed against the crucial proteins for SARS-CoV-2 infection, and a few existing drugs were shortlisted. Further, we analyzed the gene expression data of SARS-CoV-2 in human lung epithelial cells and investigated the molecules that can reverse the cellular mRNA expression profiles in the diseased state. LINC L1000 and Comparative Toxicogenomics Database (CTD) were utilized to obtain two sets of compounds that can be used to counter SARS-CoV-2 infection from the gene expression perspective. Indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), and Vitamin-A were found in two sets of compounds, and in the in-silico screening of existing drugs to treat SARS-CoV-2. Our in-silico findings on Indomethacin were further successfully validated by in-vitro testing in Vero CCL-81 cells with an IC\text{50} of 12 μM. Along with these findings, we briefly discuss the possible roles of Indomethacin and Vitamin-A to counter the SARS-CoV-2 infection in humans.

1. Introduction

Coronavirus disease 2019 (COVID-19) has so far infected more than 525.2 million people worldwide and caused more than 6.2 million deaths (www.worldometers.info/coronavirus). Despite significant advancements in designing and dispersing multiple vaccines [18,49,80,109] against SARS-CoV-2, the arrival of newer strains [35,108] had prompted us finding more effective vaccines [110]. Using various methods of drug repurposing, many existing medicines had been tried and tested against SARS-CoV-2 with limited success [79,91,103]. Many vitamins and minerals were also suggested as immunity boosters to prevent or reduce the severity of SARS-CoV-2 infection [44]. Despite significant research, we are yet to achieve any widely accepted drug regimen to treat COVID-19 [67].

Abbreviations: COVID-19, Coronavirus disease 2019; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; ORF, Open reading frame; NSP, Nonstructural protein; PDB, Protein Data Bank; RdRp, RNA-dependent RNA polymerase.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: yashahasija@dtu.ac.in (Y. Hasija), amitghosh@iitkgp.ac.in (A. Ghosh), amitk@bt.iitkgp.ac.in (A.K. Das).
1 These authors contributed equally.
As per our present knowledge, SARS-CoV-2 viruses mediate host cell infection and viral replication through a few key proteins to invade and sabotage the host cell cellular machinery [111]. Among these proteins, four structural proteins, i.e., spike protein, membrane protein, nucleocapsid protein, and envelope protein are common to all coronaviruses and appeared to be attractive targets for therapeutic drug development. In addition, eleven accessory proteins (ORF-3a, 3b, 3c, 3d, 6, 7a, 7b, 8, 9b, 9c, and 10) and sixteen nonstructural proteins (NSPs 1-16) are also being investigated to understand their role in replicating SARS-CoV-2 genome inside the host cell [75]. Among the nonstructural proteins, main protease (NSP5), papain-like protease (a domain of NSP3), RNA-dependent RNA polymerase (RdRp: NSP12 in complex with NSP7 and NSP8), NSP15, and NSP16 had been targeted by many drug developers due to their critical roles in SARS-CoV-2 genome replication. Attempts to find effective inhibitors from already existing drugs [12,61], chemical analogs of antiviral drugs [68], bioactive molecules available from natural sources [2,13,14,45,82,87], and many other approaches resulted in limited success. It is interesting to note that few recent works have identified the interacting partners of these SARS-CoV-2 proteins in human host cells [33]. Identifying the viral protein targets and their interacting host partners are of grave importance to combat SARS-CoV-2 infection. Computational screening is an in-silico method to identify potential lead compounds from a library of chemical compounds against a target molecule [50]. Various small molecule pharmaceutical compound libraries are available, for example, TTD [48,104], IUPHAR/BPS guide to pharmacology [11], VARIDT [28], TCDB [78], INTEDE [114], and many more. Though the use of these diverse pharmaceutical databases might increase the search space for identifying potential inhibitor compounds, drug repurposing strategy could be an interesting option to target the proteins crucial for SARS-CoV-2 infection. Among the various available libraries of chemical compounds, Drugbank [25] is a database often used to screen the chemical lead compounds of FDA-approved drugs against various target receptors.

Data of differentially expressed genes of SARS-CoV-2 infected primary human lung epithelium (NHBE) cells [34] are available. In addition, LINCS L1000 contains gene expression data on the effect of thousands of molecules (http://www.lincsproject.org/). Many useful databases and resources are extracted from L1000-based LINCS data and the search engine ‘L1000 Characteristic Direction Signature’ is one of them. It is designed to search for gene expression signatures against LINCS data to detect and prioritize small molecules that reverse or mimic the input gene expression signature. Therefore, this tool can be used to check the reversibility of SARS-CoV-2 infected gene expression with the candidate drug molecules. Similarly, another database, Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/), can be used to investigate the molecules that influence a list of genes and this analysis may be compared to the list of genes obtained from the NHBE cells.

Our primary focus of this study was to identify the crucial host-pathogen interaction partners by extensive literature survey and data mining and to prepare a list of viral and host target proteins for virtual screening of the already existing drugs. We further aimed to apply computational screening methods of the existing FDA approved drug molecules to find out their interactions with these identified crucial proteins for SARS-CoV-2 infection. In order to investigate if any of the existing drug molecules can bind to important host and viral proteins of SARS-CoV-2 infection, we obtained the chemical structure files of the existing drugs from the DrugBank. Based on the available databases of various compounds and gene expression databases, we also wanted to investigate if any of the available compounds may reverse the genetic changes incurred by SARS-CoV-2. We found that two of the existing drugs, Indomethacin and Vitamin-A, might be effective to target the crucial host-viral interactions and compensate for the gene expression change caused by SARS-CoV-2. Between Indomethacin and Vitamin A, Indomethacin was found to be more involved with the changes in gene expression data in hosts. It motivated us to test the efficacy of Indomethacin against SARS-CoV-2 in vitro and we observed a moderate efficacy (IC50 = 12 μm) of this drug against SARS-CoV-2. Our work corroborates the growing notion that Indomethacin has potential anti-viral properties against SARS-CoV-2 and may be considered in treating COVID-19.

2. Methods

2.1. Data mining

Extensive literature surveys and text mining were performed with the help of the PubTator central tool [106]. The interactome dataset for Cov-2–Human proteins was obtained from the BioGRID database [88]. The interacting host-pathogen partners acquired from the literature as well as from the BioGRID repository were compiled together, and the interactions were shortlisted following various parameters (represented as a flowchart in Fig. 1).

Initially, only the experimentally validated SARS-CoV-2-human interactions were selected from the compiled dataset. Since the experimental validation (from the literature) for majority of the interactions was performed by affinity purification and mass spectrometry (AP-MS), the shortlisting in this level was done by keeping the average spectral count as the deterministic parameter for high confidence interactions [53]. To determine an interaction to be of high confidence, the average spectral count cut-off was kept at 10. Next, the high confidence interacting proteins were screened and selected according to the availability of three-dimensional structures deposited in the Protein Data Bank (PDB, www.rcsb.org). Finally, the active sites for the short-listed interacting partners were identified with the help of UniProt [20] or predicted using PROSITE [86] server, for further analyses.

2.2. Virtual screening

For the Virtual screening, a total of 1918 FDA-approved drugs were obtained from the [25] (www.drugbank.ca) in 3D SDF format. The SDF files were processed to assign Gasteiger partial charge to each ligand atom and generate the corresponding PDBQT files using Open Babel [65]. Besides, all the target proteins were obtained from the Protein Data Bank (www.rcsb.org). The protein atoms were treated with Kollman partial charges using AutoDockTools [63]. All the virtual screenings were performed using AutoDock Vina [97]. The search space for each target protein was defined according to its active site by using a grid box with a spacing of 3.5 Å.

2.3. Gene expression data analysis

RNAseq data of primary human lung epithelium (NHBE) of control vs infected with SARS-CoV-2 (USA-WA1/2020) for 24 h were selected from GSE147507 [15]. The control and treated groups contained independent biological triplets of each kind. The GSE sample IDs are given below in Table 1.

The expression dataset was analyzed using GREIN [55]: GEO RNA-seq Experiments Interactive Navigator. GREIN is an interactive web platform that offers easy-to-use solutions for GEO RNA-seq data exploration and analysis. GREIN is driven by a back-end code pipeline to process RNA-seq data reliably and a large number (>6000) of data sets already processed. There have been many algorithms which have performed excellently in processing OMICS data [27,89,90,112,113]. GREIN automates all Quality Control checks and Data preparation for its users which included data normalization using the trimmed mean of M-values (TMM) approach, where M-values represent empirical fold changes between two samples. GREIN handles all the standard pipelines for identifying differentially expressed genes in RNAseq data. Simultaneously, the L1000 Fireworks Display database, L1000FWD [105] was used for searching and visualizing drug-induced gene signatures. Signature similarity search is used to retrieve signatures that
mimic or oppose the queried up and down-regulated COVID-signature gene set through a trained unsupervised clustering algorithm called k-means on 16,000 small drug molecules induced gene signatures. The Comparative Toxicogenomics Database, CTD [22] was used to retrieve Chemical-Gene interactions and mapped with the COVID-signature gene set (graphically represented in Fig. 2). CTD is a repository, curated and manually verified from scientific literature, contains interaction data among genes, chemicals, diseases, phenotypes, and pathways. We have used “Calculate and draw custom Venn diagrams” tool available at https://bioinformatics.psb.ugent.be/webtools/Venn/ to draw Fig. 5.

2.4. Molecular docking

Common hits from the virtual screening, compounds from L1000 data that complement the gene signature of SARS-infected cells, and chemicals obtained from CTD linked with selected host genes were chosen for additional molecular docking investigations. AutoDock Tools 1.5.6 for AutoDock 4.2 [63] was used to import each protein independently. Afterward, water molecules and hetero-atoms were eliminated, followed by the addition of polar hydrogen and the computation of Gasteiger and Kollman charges. Finally, the proteins were saved in the pdbqt format. The grid size of the receptors was determined by selecting the active site amino acid residue data gathered from literature and Computed Atlas of Surface Topography of proteins (CASTp 3.0) [93]. The grid center and dimensions for each receptor are provided in supplementary file 1. To continue with the Genetic Algorithm, we select 50 runs, with a population size of 300, a number of evals of 250000, and a number of generations of 27000. All other parameters were left at their default levels.

2.5. In-vitro drug testing of Indomethacin

The in-vitro testing and anti-viral assay for Indomethacin against SARS-CoV-2 (NIV2020-770 isolate) infected Vero CCL-81 cell line were performed at the ICMR-National Institute of Virology (ICMR-NIV), Pune-411001, India.

2.5.1. Preparation of the drug

A stock solution of Indomethacin (Sigma Aldrich, Israel; cat #17378) was prepared by dissolving in absolute ethanol at a concentration of 40 mM. Further, it was diluted to the desired concentrations (0.1 μM, 1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 250 μM, 500 μM, 750 μM and 1000 μM) in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Grand Island, USA) culture medium [8].

2.5.2. Cell culture

Vero CCL-81 cell line (ATCC, CCL-81) were cultured at 37 °C in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Grand Island, USA) culture medium [8].

2.5.3. Cytotoxicity assay

Vero CCL-81 cells (100 μL per well) were seeded onto a 96-well plate at a density of 3 × 10^3 cells/mL and grown for 24 h before adding the drug. Vero CCL-81 cells were treated with different concentrations of the drug Indomethacin (0.1 μM, 1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 250 μM, 500 μM, 750 μM and 1000 μM) in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Grand Island, USA) culture medium [8].

| Mock Treated/Control | Infected with SARS-CoV-2 (USA-WA1/2020) for 24 h treatment |
|----------------------|------------------------------------------------------------|
| GSM4432378           | GSM4432381                                                 |
| GSM4432379           | GSM4432382                                                 |
| GSM4432380           | GSM4432383                                                 |

Table 1: GSE sample IDs for control and infected sets.

Fig. 1. Flow chart representing steps followed in shortlisting available SARS-CoV2-Human interacting partners.

Fig. 2. CTD-Gene set data mapping pipeline.
viability) was calculated using the Graph Pad Prism software version 9.

2.5.4. Antiviral assay post drug-treatment

All experiments involving handling of infectious materials were performed in the Biosafety Level-3 (BSL-3) laboratory with appropriate biosafety practices. The Vero CCL-81 cells were seeded in 96 well plates at a density of 2 × 10^4 cells/well and then grown for 24 h before adding the drug. Vero CCL-81 cells were infected with SARS-CoV-2 strain at an MOI (Multiplicity of Infection) of 0.001 for 2 h at 37 °C. The cells were washed with 1XPBS and treated with the drug (1 μM, 10 μM, 50 μM, 100 μM, 250 μM, and 500 μM concentration based on the CC50) in triplicates and incubated for 72 h. Vehicle control had an equal concentration of the vehicle, drug controls had only the respective concentration of the drug without the virus, and cell control (CC) had no virus and drugs. Cells were observed at 48 h and 72 h for CPE (Cytopathic effect). The supernatant was collected after 72 h of treatment of Indomethacin.

Viral RNA was extracted from the supernatants of infected cells using the automated nucleic acid extraction system (Magmax, Thermo Scientific), following the manufacturer’s recommendations. The extracted viral RNA was analyzed by real-time RT-PCR using the SSIII qRT-PCR kit (Invitrogen) on the ABI 7300 Real-Time PCR system. A standard curve was generated by determining the copy numbers from serial dilutions (10^3-10^9 copies) of in vitro transcribed RNA for RdRp-2 gene [19]. IC50 (Concentration showing 50% inhibition) was calculated by using Graph Pad Prism software version 9.

3. Results

3.1. Data mining/Literature survey

The compiled SARS-CoV-2-Human interaction data set from literature and the BioGRID repository consisted of more than 700 protein-protein interactions with more than 300 experimentally validated interactions (AP-MS validation). On further screening for high confidence interactions, 57 host-viral interactions were identified, among which 6 viral and 12 host proteins were identified (Table 2) based on the available 3D structural data (RCSB PDB).

3.2. Virtual screening

From the virtual screening (VS) results, we focused to identify the set of drug molecules that exhibited binding affinity less than −5 kcal/mol with possible interaction with selected viral and host targets. Such common drug molecules were much of interest as they could be potential candidates for multi-target interaction. The list of drug molecules along with their binding affinity with the corresponding targets are provided in supplementary files 2 and 3, and represented in Fig. 3. The total number of such common drug molecules were 1287 and 1381 for the host proteins and viral proteins respectively (Supplementary files 2 and 3). As we obtained a large number of hits, we investigated if any of these drug molecules could perturb the gene expression pattern on the SARS-CoV-2 infected cells, thereby cross-validating the potential of the identified drugs.

3.3. Gene expression data analysis

3.3.1. Analysis of GSE147507 GEO dataset

A total of 317 genes with p-values < 0.05 were found to be differentially expressed (Supplementary file 4). Of these, 90 mRNAs were down-regulated and 227 mRNAs were up-regulated, contributing as a signature of gene expression named ‘COVID-signature’, represented in Fig. 4. This expression signature was fed to the L1000 Firework Display (L1000FWD) to scan for the reverse gene expression signatures associated with small drug molecules. Through this step, 670 potential small drug molecules were identified (Supplementary file 5), which can significantly reverse the gene expression signature of the control vs SARS-CoV-2 infected gene set.

Mapping was done to retrieve chemicals that have a known interaction with the COVID signature gene set. Fisher’s exact test was then applied to each chemical, as it was found that the chemical may interact with multiple genes from the signature gene set. Fisher’s exact test was used to detect non-significant chemical and gene associations. Two hundred and thirty-one chemicals were found to have significant interactions with the COVID signature gene set (Supplementary file 5). Finally, three molecular data sets were created (graphically represented in Fig. 5):

1. Approved drug molecules were retrieved from the Drugbank and screened through virtual screening. One thousand two hundred fifty-one approved drug molecules were analyzed based on the free energy values described in the above sections.
2. Six hundred and seventy-two drug molecules that have a significantly reversed signature expression compared to the COVID signature gene set in cell lines were considered.
3. Two hundred and thirty-one molecules with significant interactions with the COVID signature gene set were retrieved from CTD.

All the three data sets were subjected to the Venn diagram (Fig. 5) to find the common molecules among them. Two molecules, Indomethacin and Vitamin-A, were found common in all the three data sets. Indomethacin showed interactions with 85 genes of the COVID signature gene set, which were mapped from CTD data, whereas Vitamin-A had interactions with 4 genes form the COVID signature gene set. In addition, as Indomethacin was shown to be involved with a significantly greater number of genes in the COVID signature gene set, it was further tested in vitro.

3.4. In vitro drug testing of Indomethacin

To check the efficacy of the computationally predicted drugs in vitro,
we tested Indomethacin on Vero CCL-81 cells infected with SARS-CoV-2. Indomethacin showed dose-dependent cytotoxicity in Vero CCL-81 cells when tested at the following concentrations - 1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 250 μM, and 500 μM (Fig. 8). The CC\textsubscript{50} of Indomethacin was found to be ~490 μM. The IC\textsubscript{50} of 12 μM was observed from the anti-viral efficacy assay. The selectivity index of Indomethacin was calculated to be ~40. One of the widely used drugs used against SARS-CoV-2 is Remdesivir and its IC\textsubscript{50} was found to be 11.41 μM in previous in-vitro studies in Vero cells [42]. This result suggests that Indomethacin has antiviral activity against SARS-CoV-2 as shown by reduced RdRp-2 gene copy numbers.

4. Discussion

In the present study, using in-silico screening of the already existing drugs against crucial viral and host proteins, we have identified Indomethacin and Vitamin-A as potential drug candidates against SARS-CoV-2. Vitamin-A, an important molecule that supports human life, is required for cell growth, differentiation, immune response, and epithelial integrity. A lower level of Vitamin-A was previously observed in the TB patients compared to the healthy patients, indicating the association between lack of Vitamin-A and the occurrence of TB [4,64]. We have observed that Vitamin-A can also be effective in binding both the viral and the host proteins crucial for SARS-CoV-2 infection. It has also been speculated that the epithelial cells lacking Vitamin-A may be more prone to pathogen infection than others [77]. Vitamin-A is also important for the development and regulation of macrophages and neutrophils, migration and homeostasis of T-cells, immunoglobulin production, and B-cell activity [37]. Vitamin-A plays an important role in the formation of epithelial and mucus cells, whereas, coughing had long back been associated with the loss of epithelial cells [107]. In the case of COVID-19, 71.7% of patients were detected with low levels of Vitamin A [95]. Decreased levels of Vitamin-A were associated with increased severity of COVID-19 infection [92]. Analyses of deficiency in micronutrients showed that Vitamin-A was an important element missing from the COVID-19 patients and a lower risk of disease progression was observed with a higher level of Vitamin-A [102]. In another study, it was shown that 37% of COVID-19 patients were Vitamin-A deficient, whereas a high level of Vitamin-A was associated with asymptomatic COVID-19 cases [7]. The same study also showed that 23% decrease in the levels of Vitamin-A in severe cases compared to the asymptomatic COVID-19 patients [7]. Low levels of Vitamin-A in serum is often associated with liver damage, a marker of COVID-19 [57]. It has been speculated that Vitamin-A plays a crucial role in immunomodulatory functions by secreting IgA, which might be crucial in preventing SARS-CoV-2 infection [98]. In another hypothesis, retinol depletion and retinoid signaling pathway have been considered to play a critical role in the COVID-19 pathogenesis [81]. All-trans retinoic acid, a derivative of Vitamin-A, was shown to have an antiviral effect by inhibiting the main protease of SARS-CoV-2 [62]. There is a growing discussion if Vitamin-A can be used as a potential therapeutic/supplement [30,96] or as a nutrient supplementation [44,101]. Our study supports this notion.
Table 3
This table lists the binding energies and inhibition constants calculated during docking with types of interactions involved between host receptors and Indomethacin.

| Host Receptors                                      | Binding Energy (kcal/mol) | Inhibition Constant (K_i) | Interactions |
|------------------------------------------------------|---------------------------|---------------------------|--------------|
|                                                      |                           |                           | No. of Hydrogen Bonds | No. of Hydrophobic Interactions | Salt Bridges | Pi-Stacking | Pi-Cation Interaction |
| Human Insulin-Degrading Enzyme                      | −11.98                    | 1.64 nM                   | 5             | 4             | 3           |             |                |
| MZM REP Domains of Mind bomb 1                      | −10.57                    | 17.87 nM                  | 1             | 7             | 1           | 1           |                |
| Human Glutathione Peroxidase 1                      | −9.65                     | 84.17 nM                  | 4             | 3             |             |             |                |
| Catalytic and Ubiquitin-associated domains of MARK1/ PAR-1 | −9.53                    | 103.34 nM                 | 3             | 6             | 1           |             |                |
| Angiotensin Converting Enzyme-2                      | −9.22                     | 173.61 nM                 | 3             | 4             | 1           | 3           |                |
| Human Sirtuin homolog 5                              | −9.2                      | 173.09 nM                 | 2             | 7             | 2           |             |                |
| Kinase and Ubiquitin-associated domains of MARK3/Par-1 | −9.18                    | 187.71 nM                 | 2             | 8             | 1           |             |                |
| NTF2 domain of Ras GTPase-activating protein-binding protein 1 | −8.67                    | 440.55 nM                 | 3             | 5             |             |             |                |
| Human plakophilin 2 isoform a (PKP2a)                | −8.32                     | 792.90 nM                 | 1             | 6             | 1           | 1           |                |
| SmgGDS-558                                          | −8.2                      | 971.48 nM                 | 2             | 8             |             |             |                |
| Human Heme Oxygenase-1                              | −8.1                      | 1.16 uM                   | 5             | 5             |             |             |                |
| G3BP2 NTF2-like domain in complex with a peptide     | −8.15                     | 4.82 mM                   | 2             | 4             |             |             |                |

Table 4
This table lists the binding energies and inhibition constants calculated during docking with types of interactions involved between viral receptors and Indomethacin.

| Viral receptors                                      | Binding Energy (kcal/mol) | Inhibition Constant (K_i) | Interactions |
|------------------------------------------------------|---------------------------|---------------------------|--------------|
|                                                      |                           |                           | No. of Hydrogen Bonds | No. of Hydrophobic Interactions | Salt Bridges | Pi-Stacking | Pi-Cation Interaction |
| SARS-CoV-2 main protease                             | −11.52                    | 3.62 nM                   | 3             | 7             | 1           |             |                |
| SARS-CoV-2 RNA-dependent RNA polymerase              | −10.9                     | 10.18 nM                  | 4             | 7             |             |             |                |
| SARS-CoV-2 receptor binding domain                   | −9.44                     | 121.24 nM                 | 3             | 2             |             |             |                |
| Papain-like Protease of SARS-CoV-2                   | −8.4                      | 701.79 nM                 | 5             | 1             |             |             |                |
| NSP16 from SARS-CoV-2                                | −8.36                     | 740.66 nM                 | 2             | 10            | 2           |             |                |
| NSP15 Endoribonuclease from SARS-CoV-2               | −8.05                     | 1.13 uM                   | 3             | 4             | 1           |             |                |

Fig. 6. Host targets human heme oxygenase-1, human sirtuin homolog 5, human glutathione peroxidase-1, human insulin degrading enzyme, catalytic and ubiquitin-associated domains of MARK-1/PAR-1, kinase and ubiquitin-associated domains of MARK3/PAR-1, NTF2 domains of Ras GTPase activating protein-binding domain, human plakophilin-2, Ubiquitin-protein ligase Mib1, G3BP2 NTF2-like domain, SmgGDS-558, and Angiotensin-converting enzyme 2 are docked with Indomethacin and their interactive residues at the active sites are shown in the images. Detailed interactions are listed in supplementary file 6.
further by showing that Vitamin-A has the potential to bind the crucial proteins and slight ability to reverse the genetic changes brought upon by SARS-CoV-2.

As can be observed from Table 3, Indomethacin formed strong interactions with human insulin-degrading enzyme, MZM-REP Domains of Mind bomb 1, human glutathione peroxidase and the other host proteins studied here. Indomethacin showed strong hydrophobic interactions and significant H-bond formations with the host proteins. It can be observed (Fig. 6) that the residues His112, Phe115, Leu116, Ser128, Phe820, Asn821, and Arg824 of the Human insulin-degrading enzyme made crucial interactions with Indomethacin. Among these residues, only His112 is not included as it is slightly distant from the active site pocket as analyzed by CASTp 3.0 [93]. Similarly, residues Arg17, Leu71, Asp72, His114, Phe152, Ile181, and His190 from the active site of MZM-REP domains of Mind bomb 1 formed both hydrophobic and H-bonding interactions with Indomethacin. Among the other host receptors, such as Human Glutathione Peroxidase 1, Catalytic and Ubiquitin-associated domains of MARK1/Par-1, Angiotensin-converting Enzyme-2, Human Sirtuin homolog 5, Kinase and Ubiquitin-associated domains of MARK3/Par-1, NTF2 domain of Ras GTPase-activating protein-binding protein 1, human plakophilin 2 isoform a (PKP2a), SmgGDS-558 and Human Heme Oxygenase-1 also showed strong affinity towards Indomethacin. Thus, Indomethacin can play a strong role in binding crucial host proteins involved in SARS-CoV-2 infection. Similarly, from Table 4, it can be observed that Indomethacin formed strong interactions with main protease, RNA-dependent RNA polymerase, receptor binding domain of the spike protein, Papain-like Protease, NSP16, and NSP15 Endoribonuclease of SARS-CoV-2 by employing both hydrophobic interactions and H-bonding interactions. Fig. 7 demonstrates that the residues Val104, Ile106, Gln107, Asn151, Thr292, Asp295, and Arg298 of the main protease interact with Indomethacin. His41 and Cys145 are the two main residues important for the proteolytic activity of this main protease [12,45,61]. However, it should be noted that the other potential inhibitors of the main protease interact with different sets of residues [12, 45, 61]. It can be further inspected if the interactions mentioned may cause any allosteric changes in the main protease of SARS-CoV-2. The residues Phe368, Leu371, Leu372, Ala375, Trp509, Leu514, Tyr515, and Ser518 of RNA dependent RNA polymerase (RdRp) help in binding Indomethacin. Previous studies showed that the residues important for binding Remdesivir and Favipiravir are K551, R553, and R555, and K545, K551, and R553, respectively [14]. The interacting residues with other potential inhibitors also vary slightly in a few other studies [14, 68]. Similarly, detailed analyses should be performed for each host and viral protein to decipher the anti-viral mechanism of Indomethacin against SARS-CoV-2.

Indomethacin is a non-steroidal anti-inflammatory medication (NSAID) that is frequently used in the treatment of rheumatoid arthritis and gout. Indomethacin acts similarly to other NSAIDs, such as aspirin and ibuprofen, by decreasing the activity of cyclooxygenase-1 and 2 (COXs) and inhibiting pro-inflammatory prostaglandin formation [99, 100]. In comparison to steroidal drugs such as betamethasone and hydrocortisone, NSAID Indomethacin inhibited phospholipase A2 significantly and more effectively [54]. In comparison to steroidal drugs such as betamethasone and hydrocortisone, NSAID Indomethacin inhibited phospholipase A2 significantly and more effectively [54]. The host’s active response to viral infection, such as SARS-CoV-2, results in the accumulation of mucus and inflammation, particularly in the lungs, where patients frequently exhibit profuse phlegm, resulting in severe dyspnea [36]. NSAIDs such as Indomethacin can suppress such responses and alleviate respiratory distress in the patient [17]. Indomethacin had also been demonstrated to activate eIF2 double-stranded RNA (dsRNA) dependent protein kinase R (eIF2 kinase PKR) and limit viral multiplication and translation directly, without impairing the host cell translation machinery [9]. Various previous studies had shown that Indomethacin possesses potential anti-viral effects against viruses such as Epstein-Barr virus [21], HIV.
Clinically, Indomethacin treatment had been shown to alleviate headache [8]. Apart from its anti-inflammatory properties, Indomethacin had been demonstrated to treat mild and moderate COVID-19 with reduced viral loads and inflammatory markers [9]. Notably, in this study, we have observed Indomethacin influences 85 genes (Supplementary file 4) associated with the COVID signature, indicating a significant role it may play in host responses. We also identified many host and viral proteins that Indomethacin can bind. Our analyses of gene expression data indicate that Indomethacin may be critical in reversing the effects of SARS-CoV-2. Our in-vitro data on antiviral studies suggested that dose-dependent administration of Indomethacin has an anti-SARS-CoV-2 impact, as determined by mRNA expression. Various possible mechanisms explaining how Indomethacin can be effective against COVID-19 had been hypothesized [10]. One such hypothesis involves the biosynthesis pathway of prostaglandin. Indomethacin had been known to interact with PGES2 (human prostaglandin E synthase type 2) [11], which had been shown to interact with the NSP-7 protein of SARS-CoV-2 [12]. A study of various NSAIDs using a network pharmacology approach revealed a potential role of Indomethacin in inhibiting crucial hub proteins of the RAS signaling pathways, thus reducing SARS-CoV-2 induced excessive inflammation [13]. In our study, we observed that Indomethacin could bind to the NT2F domain of Ras GTPase-activating protein-binding protein 1 (Table 3, Fig. 6), which calls for further investigation in this direction.

The combination of Indomethacin with other lead molecules showed to enhance the antiviral efficacy significantly in another in-vitro study [14]. Indomethacin showed a prominent binding affinity with the main protease of SARS-CoV-2 and had better binding than many other NSAIDs [15]. Derivatives of Indomethacin had been shown to inhibit the main protease of SARS-CoV-2 [16]. It had also been hypothesized whether the use of Indomethacin helps in the recovery from SARS-CoV-2 induced dry cough [17]. A few proteolysis targeting chimeras (PROTAC) derived from Indomethacin were shown to possess better antiviral efficacy than Indomethacin against some coronavirus strains [18]. Our findings collectively support the hypothesis that Indomethacin may be considered as a possible treatment for SARS-CoV-2. When there are no contraindications for its use, Indomethacin may be beneficial to the patient. Clinically, Indomethacin treatment had been shown to alleviate headache in SARS-CoV-2 patients in a recent study [19]. In another clinical study, it had been suggested to treat mild and moderate COVID-19 with Indomethacin [20]. Another in-vitro, animal and model-based simulation study showed Indomethacin can be used against SARS-CoV-2 [21].

Hydroxychloroquine, Remdesivir, and Lopinavir were initially tested against SARS-CoV-2 infection in Vero cells. Antiviral efficacy of Indomethacin (IC50 = 12 μM) was observed to be in the similar ranges of Remdesivir (IC50 = 11.41 μM), Hydroxychloroquine (IC50 = 7.28 μM), and Lopinavir (IC50 = 9.12 μM) [22]. It was also shown that Remdesivir was more effective in a human cell line (IC50 = 1.3 μM) and the selectivity index was 38.5, which is also in the similar range of Indomethacin in this present study (~40). In another study, PF-00835231 showed better antiviral efficacy than Remdesivir in two different cell lines after 24 and 48 h, and had been hypothesized to be a more effective drug against SARS-CoV-2 [23]. IC50 values of the drugs Remdesivir, Lopinavir, and Chloroquine varied in different cell lines [24]. Additional in-vitro studies in other relevant cell lines could be performed to further confirm the antiviral efficacy of Indomethacin. In this study, we showed how Indomethacin may bind to the critical receptor proteins. However, with poor binding, it is difficult to elucidate which proteins are critical for SARS-CoV-2 infection. Hence, we conducted additional in-vitro tests with moderate success. We describe briefly why Vitamin-A may be considered as a supplement in the event of SARS-CoV-2 infection. We also discuss recent findings on Indomethacin against SARS-CoV-2. More in-vitro studies of various Indomethacin analogs in combination with Vitamin-A may be performed in the future to find out better therapeutics against SARS-CoV-2.

Declaration of competing interest

The authors declare no competing financial interest.

Acknowledgements

This work was accomplished in the SAMHAR-COVID-19 hackathon organized by C-DAC, India, in association with NVIDIA and OpenACC (https://samhar-covid19hackathon.cdac.in/). We gratefully acknowledge the technical work by Dinesh Singh, Sachin Keng, Sadhana Kode, Vaishali Tatte, Vaishnavi Bagde, Rameshwar Khedekar, and JPN Babu for the in-vitro testing at the ICMR-NIV, Pune, India and Dr. Kalpana Joshi (ICMR Expert Committee) for reviewing the antiviral data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compbio.2022.105788.

References

[1] K.R.A. Abdellatif, et al., New indomethacin analogs as selective COX-2 inhibitors: synthesis, COX-1/2 inhibitory activity, anti-inflammatory, ultracryogenicity, histopathological, and docking studies, Arch. Pharm. 354 (2021), 2000298.
[2] H.R. Abd El-Mageed, et al., In silico evaluation of different flavonoids from medicinal plants for their potency against SARS-CoV-2, Biologics 1 (2021) 416–434, 1, 416–434 (2021).
[3] Ayman Abo Elmaaty, et al., Computational insights on the potential of some NSAIDs for treating COVID-19: priority set and lead optimization, Molecules 26 (12) (2021) 3772.
[4] O. Albana, et al., Impact of vitamin A and carotenoids on the risk of tuberculosis progression, Clin. Infect. Dis. 65 (2017) 900–909.
[5] R.A. Al-Horani, S. Kar, Potential anti-SARS-CoV-2 therapeutics that target the post-entry stages of the viral life cycle: a comprehensive review, Viruses 12 (2020).
[6] M. Alkotaji, Al-Zidan, R.N. Indomethacin, Can it counteract bradykinin effects in COVID-19 patients? Curr. Pharmacol. Rep. 7 (2021) 102–106.
[7] I. Al-Saleh, et al., Essential metals, vitamins and antioxidant enzyme activities in COVID-19 patients and their potential associations with the disease severity, Biometals 35 (2022) 125–145.
[8] C. Amici, et al., Indomethacin has a potent antiviral activity against SARS coronavirus, Antivir. Ther. 11 (8) (2006) 1021–1030.
[9] C. Amici, et al., Inhibition of viral protein translation by indomethacin in vesicular stomatitis virus infection: role of eIF2alpha kinase PKR, Cell Microbiol. 17 (9) (2015) 1391–1404.
[10] J.F. Armstrong, et al., The IUPHAR/BPS Guide to PHARMACOLOGY in 2020: extending immunopharmacology content and introducing the IUPHAR/MMV
[87] R. Singh, et al., In-silico evaluation of bioactive compounds from tea as potential SARS-CoV-2 nonstructural protein 16 inhibitors, J. Tradl. Complement. Med. 12 (2022) 35–43.

[88] C. Stark, et al., BioGRID: a general repository for interaction datasets, Nucleic Acids Res. 34 (2006). D535-9.

[89] J. Tang, et al., Simultaneous improvement in the precision, accuracy, and robustness of label-free proteome quantification by optimizing data manipulation chains, Mol. Cell. Proteomics 18 (2019) 1683-1699.

[90] J. Tang, et al., ANPELA: analysis and performance assessment of the label-free quantification workflow for metaproteomic studies, Briefings Bioinf. 21 (2020) 621-636.

[91] P. Tarighi, et al., A review of potential suggested drugs for coronavirus disease (COVID-19) treatment, Eur. J. Pharmacol. 895 (2021), 173890.

[92] P.R. Tepasse, et al., Vitamin A plasma levels in COVID-19 patients: a prospective multicenter study and hypothesis, Nutrients 13 (2021) 2173, 2021, Vol. 13, Page 2173.

[93] W. Tian, et al., CASTp 3.0: computed atlas of surface topography of proteins, Nucleic Acids Res. 34 (2006). D535-9.

[94] Tianhong Xu, Xuejuan Gao, Zengbin Wu, Douglas W. Selinger, Zichen Zhou, bioRxiv 2020.04.01.017624; doi: https://doi.org/10.1101/2020.04.01.017624.

[95] T.M. Tomasa-Irriguible, et al., Low levels of few micronutrients may impact COVID-19 disease progression: an observational study on the first wave, Metabolites 11 (2021).

[96] S.E. Trasino, A role for retinoids in the treatment of COVID-19? Clin. Exp. Pharmacol. Physiol. 47 (2020) 1765–1767.

[97] O. Trott, et al., Autodock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2) (2010) 455–461.

[98] F.J. Turrubiates-Hernández, et al., (The involvement of vitamin A in the production of secretory IgA in the respiratory epithelium for potential protection against SARS-CoV-2 infection), Rev. Alerg. Mex. 68 (2021) 185–197.

[99] J.R. Vane, R.M. Botting, Mechanism of action of nonsteroidal anti-inflammatory drugs, Am. J. Med. 104 (3A) (1998) 25–22S.

[100] J.R. Vane, R.M. Botting, Mechanism of action of anti-inflammatory drugs, Int. J. Tissue React. 20 (1) (1998) 3-15.

[101] B. Vlieg-Boerstra, et al., Nutrient supplementation for prevention of viral respiratory tract infections in healthy subjects: a systematic review and meta-analysis, Allergy 77 (2022).

[102] M. Voelkle, et al., Prevalence of micronutrient deficiencies in patients hospitalized with COVID-19: an observational cohort study, Nutrients 14 (2022) 1862.

[103] X. Wang, et al., COVID-19 drug repurposing: a review of computational screening methods, clinical trials, and protein interaction assays, Med. Res. Rev. 41 (1) (2021) 5–28.

[104] Y. Wang, et al., Therapeutic target database 2020: enriched resource for facilitating research and early development of targeted therapeutics, Nucleic Acids Res. 48 (2020) D1031–D1041.

[105] Z. Wang, et al., L1000FWD: fireworks visualization of drug-induced transcriptomic signatures, Bioinformatics (Oxford, England) 34 (12) (2018) 2150–2152.

[106] C. Wei, H. Kao, Z. PubMed Lu, A web-based text mining tool for assisting biocuration, Nucleic Acids Res. 41 (2013) W518–W522.

[107] S.B. Wollbach, P.R. Howe, Tissue changes following deprivation of fat-soluble A vitamin, Nutr. Rev. 42 (1995) 753–777.

[108] T. Xiaolu, et al., On the origin and continuing evolution of SARS-CoV-2, Natl. Sci. Rev. 7 (6) (2021) 1012–1023.

[109] P.D. Yadav, et al., Neutralization of Beta and Delta variant with sera of COVID-19 recovered cases and vaccines of inactivated COVID-19 vaccine BBV152/Govaxin, J. Trav. Med. 28 (7) (2021) taab104, 11.

[110] P.D. Yadav, S. Kumar, Global emergence of SARS-CoV-2 variants: new foresight needed for improved vaccine efficacy, Lancet Infect. Dis. 22 (2022) 298–299.

[111] R. Yan, et al., Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2, Science 367 (2020) 1444–1448.

[112] Q. Yang, et al., NOREVA: enhanced normalization and evaluation of time-course and multi-class metabolomic data, Nucleic Acids Res. 48 (2020) W436–W448.

[113] Q. Yang, et al., A novel bioinformatics approach to identify the consistently well-performing normalization strategy for current metabolomic studies, Briefings Bioinf. 21 (2020) 2142–2152.

[114] J. Yin, et al., INTEDE: interactome of drug-metabolizing enzymes, Nucleic Acids Res. 49 (2021) D1233–D1243.

[115] M. Yuan, et al., A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV, Science 368 (2020) 630–633.

[116] James M. Murphy, et al., Conformational instability of the MARK3 UBA domain compromises ubiquitin recognition and promotes interaction with the adjacent kinase domain, Proc. Natl. Acad. Sci. U.S.A. 104 (36) (2007) 14336–14341, https://doi.org/10.1073/pnas.070301210.

[117] Latesh Lad, et al., Comparison of the Heme-free and -bound Crystal Structures of Human Heme Oxygenase-1, J. Biol. Chem. 278 (10) (2003) 7834–7843, https://doi.org/10.1074/jbc.M211450200.