Potential Drugs Against COVID-19 Revealed by Gene Expression Profile, Molecular Docking and Molecular Dynamic Simulation

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Research article

Keywords: COVID-19, drug, molecular docking, bioinformatics, gene expression

Posted Date: November 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-108452/v1

Version of Record: A version of this preprint was published at Future Virology on August 1st, 2021. See the published version at https://doi.org/10.2217/fvl-2020-0392.

Abstract

Background

SARS-CoV-2 coronavirus, an emerging Betacoronavirus, is the causative agent of the severe acute respiratory distress syndrome outbreak in 2019 (COVID-19). Currently, there are neither specific and selective antiviral drugs for the treatment nor vaccines to prevent contagion. Here we propose a bioinformatic approach in order to test *in silico* the efficacy of existing drugs for COVID-19.

Results

In the first step of our study we identified, through a gene expression analysis, several drugs that could act on the biological pathways altered in COVID-19. In the second step, we performed a docking simulation in order to test the properties of the identified drugs to target the 3CL main protease of SARS-CoV-2. The drugs that showed higher binding affinity are bardoxolone (-8.78 kcal/ mol), Irinotecan (-8.40 kcal/mol) and Pyrotinib (-8.40 kcal/mol). Molecular dynamics simulations were carried out on the three selected drugs to validate the stability and interactions of the complexes. Among other promising drugs we found also AZD-8055, Olaparib, Tyrphostin AG 879, Topotecan Hydrochloride, MP-412, S-222611, Allitinib, 7-Ethyl-10-Hydroxy-Camptothecin, and Falnidamol.

Conclusions

We suggested some drugs that could efficient in COVID-19. However further studies are suggested to confirm the affinity of these drugs with 3CL main protease of SARS-CoV-2.

Background

A state of global health emergency has been declared by World Health Organization (WHO) for Coronavirus disease (COVID-19). The causative agent is SARS-CoV-2, which like SARS-CoV and MERS-CoV, belongs to the Betacoronavirus genus [1].

The most functionally relevant proteins in SARS-CoV-2 are the spike glycoprotein and 3CL main protease. The spike protein binds to a host cell membrane and allows the entry of the virus into the host cell. It has been described that SARS-CoV entrance is mediated by angiotensin-converting enzyme 2 (ACE2) that is particularly over-expressed by epithelial cells in oral mucosa and intestine [1].

Previous studies have shown that the interaction between the spike glycoprotein and ACE2 has a key role in SARS pathogenesis. Thus, initially, these two proteins have been proposed as possible and promising drug targets. However, anti-viral agents targeting ACE2 and spike glycoprotein did not proceed clinically due to significant side effects [2, 3].

Several studies also demonstrated the potential of the 3CL main protease as a promising drug target [4, 5]. 3CL main protease is responsible for the regulation of the polyproteins that are translated from the

viral RNA [6]. The inhibition of this enzyme could block the replication processes of the virus, which makes it a potential target for drug discovery. Recently X-ray crystal structure of the 3CL main protease was determined providing an excellent basis for structure-based drug discovery [7]. To date, no effective SARS-CoV-3CL inhibitor has been proposed yet to treat COVID-19.

In order to identify new potential target to block COVID-19 disease, *in silico* drug discovery approaches have been demonstrated effective, efficient and cost-saving and can follow two main strategies. The first strategy is based on the analysis of gene expression profiles of patients affected by COVID-19, to identify new potential drug targets acting on molecular mechanisms altered by SARS-CoV-2. Several studies based on this approach allowed researchers to discovery candidate drugs for different viruses, such as SARS-CoV-2, MERS-CoV, Ebola and Zika virus [8-11]. The second strategy is to test existing drugs with a virtual screening approach based on the study of the 3D structures of the target protein. This approach, called molecular docking, can predict how drug candidates bind to a receptor of known 3D structure [12], generating a score (binding energy value) that defines the binding affinities between ligand and target protein. There have been successful applications that demonstrated how molecular docking is becoming a powerful tool in the discovery of drug candidates [13, 14]. However, most of these studies tested the drugs without considering the effect of drugs on genes deregulated as effect of diseases [1, 15]. Although docking programs are fast and easy to use, they are affected by too low accuracy and stability. Indeed, several studies reported that binding free energies based on molecular dynamic simulations are much more reliable [16].

In this study we proposed a bioinformatic approach based on molecular docking, molecular dynamic simulations and gene expression profiles of COVID-19 to be used *in silico* to test existing drugs as therapies against COVID-19.

Materials And Methods

In the first step of our study we identified, through a gene expression analysis, several drugs that could act on the biological pathways altered by COVID-19. In particular, we selected differentially expressed genes between COVID-19 and healthy samples, obtained from two published datasets. In the second step, we performed a docking simulation in order to test the properties of these drugs to target the 3CL main protease of SARS-CoV-2. Figure 1 shows the workflow of the proposed approach.

Gene expression data

We analyzed the gene expression levels of COVID-19 positive and negative patients from 2 Gene Expression Omnibus (GEO) datasets: GSE150316 and GSE147507.

Specifically, we selected 5 negative control samples of lung tissues and 16 COVID-19 positive lung samples from GSE150316; and 2 lung biopsies from COVID-19 negative controls and 2 lung biopsies from postmortem COVID-19 positive patients from GSE147507.

We defined differentially expressed genes between COVID-19 negative and positive samples if the genes have an absolute value of log fold change >0.5 and adjusted *p*-value < 0.05. Pre-processing and differential expression analysis were performed with the Bioconductor package TCGAbiolinks [17].

Drug interactions and over-representation analysis

Matador and DGIdb database were used to identify the interactions between the drug and protein [18, 19].

We performed a Fisher's Test to verify if there is an over-representation of drug targets in the list of differentially expressed genes. We considered drugs acting on the differentially expressed genes if FDR <0.05. *p*-values were adjusted with Benjamini-Hochberg procedure for multiple testing correction [20].

Molecular modelling

We performed molecular docking studies in order to identify potential agents with antiviral properties against COVID-19. Molecular modelling was performed with AutodockTools-1.5.6 [21].

Drugs considered in the over-representation analysis were selected and PubChem database was used to get 3D structure of the selected drugs [22]. Discovery studio was used to prepare ligand (drugs) and generate protein data bank (PDB) format [23]. PDB format is a standard representation for molecular structure data originated from X-ray diffraction.

The crystal structure of COVID-19 main protease in complex with an inhibitor N3 was extracted from Protein Data Bank (PDB IDs: 6LU7). PDB ID 6LU7 consists of two chains: chain A and chain C. The chain C, representing the complex bound inhibitor to the protein receptor molecule, was removed. During the pre-processing step of protein polar hydrogen atoms were added and water molecules were removed.

Molecular docking identifies the amino acids that interact between the selected protein and drugs. Minimum energy binding of the ligand with the receptor was considered. Binding energy is described as a decreasing of the overall energy of the complex when a drug is associated with a target protein. Binding energy also defines the ligand binding affinity.

Molecular Dynamics

The investigated main protease of SARS-CoV-2 and screened molecules resulting from molecular docking were subjected to molecular dynamic simulation. The results obtained through the molecular docking were extended, as molecular dynamics are considered a more solid analysis for molecular study of ligand recognition.

Molecular Dynamic simulations of the main protease of SARS-CoV-2 with screened molecules were performed using BIOVIA Discovery Studio Client [23]. Molecular dynamics simulations were carried out using the Standard Dynamics Cascade protocol in four steps: (i) minimization; (ii) heating; (iii) equilibration run; and (iv) production run. We set simulation time (ps) parameters to 20 and 200 in equilibration and production step, respectively. For the other parameters we used the default values.

Minimization of the 3D structures to get the most stable confirmations of the complexes was performed using the default algorithms: the Steepest Descent algorithm and the Adopted Basis NR algorithm.

After the minimization step, all the complexes were subjected to gradual heating using the default values.

In the equilibration phase the system was stabilized at a target temperature, as the energy has to be distributed appropriately among all system.

Furthermore, complexes were subjected to the production run at constant-temperature, using constant-volume ensemble (NVT). The results of this step are stored in the simulation trajectory.

The average binding energy was calculated for equilibrated molecular dynamic trajectory. Trajectory file contains 100 conformations. The binding energy was calculated by:

$\Delta G_{bind} = \Delta G_{complex} - \Delta G_{protein} - \Delta G_{ligand}$ (1)

where $\Delta G_{complex}$, $\Delta G_{protein}$ and ΔG_{ligand} are the total free energy of protein-ligand complex, protein and ligand in solvent, respectively.

Pharmacokinetics parameters

Lipinski's rule is an algorithm consisting of a set of rules to be considered for the design and development of a drug [24]. It is based on 4 criteria which must be respected: molecular mass should be less than 500 Daltons; the drug should not have more than 5 hydrogen bond donors in its skeleton; the drug should not have more than 5 hydrogen bond acceptors in its skeleton; and the fat solubility of the molecule expressed by the partition coefficient must be less than 5. In summary, Lipinski's rule considers two main concepts: absorption (drug candidate should be better absorbed if small in size) and permeation (drug candidate should better pass through the cell membranes if it is not too hydrophilic). We used Swiss ADME to verify if the selected drugs respect the criteria above reported [24]. We used ChEBI database to download mol file for the selected drugs [25]. Mol file, a common-used chemical structure file format is used as input for Swiss ADME tool.

Results

Gene expression analysis

We obtained 198 differentially expressed genes from GSE150316 and 2670 genes from GSE147507. We found 47 genes that are differentially expressed in both datasets.

We then identified 38 drugs (FDR<0.0211) that have an over-representation of targets in the list of 47 differentially expressed genes. Some of these drugs showed an antiviral property against different viral disease such as bardoxolone, irinotecan, olaparib, inosine, and anthraquinone. The mechanism of action

of these drugs are different such as inhibitor of protein kinase, inhibitor of epidermal growth factor receptor and inducer of apoptosis.

27 out of 38 drugs were tested for docking analysis and are depicted in the Table 1. The 3D structure of Hemay-022, Lapuleucel-T, T-DM1, Ertumaxomab, Seribantumab, Onartuzumab, Depatuxizumab Mafodotin, MDX-210, MM-302, Trodusquemine and Margetuximab are not available from PubCHEM.

Docking studies

In order to identify potential drugs for treating COVID-19 patients, a molecular docking analysis was performed on the 27 drugs revealed by the differential expression analysis.

The results from the SARS-CoV-2 3CL Main Protease are reported in Table 2. out of 27 drugs obtained a binding energy less than -7 kcal/mol (AZD-8055, Olaparib,Irinotecan, Tyrphostin AG 879, Topotecan Hydrochloride, MP-412, S-222611, Allitinib, 7-Ethyl-10-Hydroxy-Camptothecin, Falnidamol, Pyrotinib and Bardoxolone). The study revealed no violation of Lipinski's rule for these drugs. Tyrphostin AG 879, MP-412, S-222611, Allitinib, Pyrotinib, Bardoxolone are not available in ChEBI.

The best binding energy values were obtained for bardoxolone (-8.78 kcal/ mol), irinotecan (-8.40 kcal/mol) and pyrotinib (-8.40 kcal/mol). Figure 2 shows the protein-ligand complex for bardoxolone, irinotecan and pyrotinib.

Bardoxolone has 4 types of interactions with protein residues: van der Waals, hydrogen bonds (conventional and carbon hydrogen bonds), alkyl and pi-alkyl interactions. In particular, the residues LEU282 and LYS5 form alkyl interactions with aromatic ring of the molecule. Moreover, the residue GLN127 interacts with a hydrogen bond with the cyano group of the molecule.

Irinotecan interacts with protein structure through van der Waals, hydrogen bonds, alkyl, pi-alkyl interactions, pi-sigma, and pi-pi stacked. The residue TYR126 forms pi-pi stacked and alkyl interactions with aromatic ring of the molecule. Hydroxyl group interacts with GLU288.

Pyrotinib interacts with protein structure through van der Waals, hydrogen bonds, halogen, pi-pi stacked and alkyl interactions. In particular, chloro of the ligand interacts with the residue THR111 through halogen interaction and with the residue ASN151 through hydrogen interaction. PHE294 forms pi-pi stacked interactions with the aromatic rings of the molecule.

Molecular dynamic simulation

COVID-19 main protease (PDB IDs: 6LU7) in complex with the top three (bardoxolone, irinotecan and pyrotinib) docked ligands have been selected to compute dynamic properties through molecular dynamic simulations. The calculated binding energies are presented in Table 3. All 3 ligands obtained negative binding energies. Pyrotinib exhibited the lowest binding energy (-291 kcal/mol). Irinotecan achieved a binding energy of -240 kcal/mol and bardoxolone of -160 kcal/mol.

Discussion

In this study we proposed a computational approach integrating gene expression analysis and molecular docking to test *in silico* existing drugs against the new COVID-19. In the first step of the study we analysed gene expression profiles of COVID-19 patients from 2 published GEO datasets. We identified 47 common differentially expressed genes in COVID-19 patients compared to healthy subjects, and we verified the over-representation of these genes in the list of drug targets. We obtained 38 drugs as potential promising treatment for COVID-19 patients.

As the 3D structure for several drugs was not available, 27 out of 38 drugs were tested for docking analysis. Docking results suggested 3 potential protease inhibitors namely bardoxolone (binding energy: -8.78 kcal/ mol), irinotecan (binding energy: -8.40 kcal/mol) and pyrotinib (binding energy: -8.40 kcal/mol). However, the best binding energy was obtained by bardoxolone. Based on differential expression and over-representation analysis, bardoxolone appears to be the best drug for treating COVID-19 patients. However, as 12 out of 27 drugs obtained a binding energy less than -7 kcal/mol (AZD-8055, Olaparib,Irinotecan, Tyrphostin AG 879, Topotecan Hydrochloride, MP-412, S-222611, Allitinib, 7-Ethyl-10-Hydroxy-Camptothecin, Falnidamol, Pyrotinib and Bardoxolone), these drugs can be considered all potential protease inhibitor ligands.

Bardoxolone was suggested as drug with novel potent antiviral properties against hepatitis B and C viruses in human hepatocyte cell culture systems and herpesvirus [26, 27]. In particular, Bardoxolone could act as an agonist of activator of nuclear factor-erythroid factor 2 (NRF2) impairing viral replication [26, 27]. NRF2 induces heme oxygenase-1 (HO-1) that was shown suppress genome replication in hepatitis B and C. High NRF2 activity suggests the role of NRF2 as cellular defence against the progression of the infection. Indeed, several studies reported that the cells with a high level of NRF2 are less likely to progress the infection. NRF2 activity was also associated with a decreasing virus production in rotavirus [28].

Irinotecan (CPT-11) is a semisynthetic plant alkaloid derived from Camptothecin and acts as an anticancer due to its ability to inhibit topoisomerase I during S phase; it is used to treat various cancers (i.e. colorectal cancer, pancreatic cancer,...), but its side effects limits its use. Nevertheless, it has been demonstrated in *in vitro* experiments to be effective in blocking Herpes simplex virus-1 (HSV-1) replication and lytic oncolysis [29]. Several nanoparticle- or lipid-based formulations have been developed to be used in different viral infectious disease, such as hepatitis A or influenza [30] carriers it is necessary to control the possible reactivation of the virus to avoid the development of impaired liver function or liver cancer. About 20% or HBV carriers developed reactivation [31]. In these subjects, irinotecan is used as a palliative treatment for advanced colorectal cancer, and its combination with lamivudine that inhibits HBV reverse transcription, could be helpful in controlling the virus reactivation [31].

Pyrotinib (Irene) is an anti-HER2 therapeutic target drug. This drug acts by covalently binding to ATP binding sites of the kinase domain: in this way the drug inhibits the formation of homo/heterodimer and auto-phosphorylation of HER family, blocking the signalling of the downstream pathway

(RAS/RAF/MEK/MAPK, PI3K/AKT) and stopping the cells in G1. With its mechanism of action, this drug is effective on metastatic breast cancer [32]. Being an irreversible tyrosine kinase inhibitor, it blocks signal transduction through the erythroblastic leukemia viral oncogene homolog (erbB) receptors. Although no publication is present in the literature regarding the possible use of pyrotinib as antiviral agent, it is possible that this molecule interferes with viral entry by inhibiting tyrosine kinase activity of surface receptor. Indeed, it has been already reported that tyrosine kinase activity of Abl protein is necessary for the viral spike S-induced syncytia formation prior to the hemifusion step during infectious bronchitis virus (IBV) infection [33]. Moreover, the authors demonstrated that Abl inhibitor drugs are able to block IBV infection process.

Another promising drug against COVID-19 patients that achieved a binding energy of – 7.39 kcal/mol is Olaparib. It is utilized in current clinical use as PARP inhibitors. Poly(ADP-ribose) polymerase (PARPs/ARTDs) enzymes are involved in a wide range of cellular processes such as antiviral response. PARP inhibitors have been shown to be effective in several models of acute respiratory distress syndrome [34]. Furthermore, a consequence of SARS-CoV-2 infection can be inflammatory response flaring out of control induced by Interleukin-1 (IL-1) and 6. PARP inhibitors can reduce the expression of IL-1 and 6 decreasing the inflammatory response [35].

In conclusion, we proposed several existing drugs with antiviral activity that, alone or in combination, could be considered as drugs against COVID-19. However, it is vital to better understand the clinicopathological features of COVID-19 to design treatment plans leading to favourable outcomes in SARS-CoV-2 infected patients.

Conclusions

In this study we have demonstrated that an *in silico* approach, based on integration of gene expression analysis of COVID-19 specific genes and molecular docking of therapeutic drugs on target proteins, revealed a new antiviral use of existing drugs on the base of the interaction between each drugs and specific domains on SARS-CoV-2 viral proteins. Dynamic simulation studies confirmed the promising role of three drugs: bardoxolone, irinotecan and pyrotinib.

Abbreviations

WHO World Health Organization

COVID-19 Coronavirus disease

ACE2 angiotensin-converting enzyme 2

Declarations

Acknowledgements

IBFM-CNR thanks the generous support from Dassault Systèmes BIOVIA for the use of BIOVIA Discovery Studio Academic Research Suite in this work. This work was supported by Italian Ministry of University and Research (MIUR) SYSBIONET-Italian ROADMAP ESFRI Infrastructures.

Authors' contributions

CC has made substantial contribution to the acquisition, analysis and interpretation of data. GB has made substantial contribution to the interpretation of data. IC has revised the manuscript.

Funding

This work was supported by Italian Ministry of University and Research (MIUR) SYSBIONET-Italian ROADMAP ESFRI Infrastructures.

Availability of data and materials

The datasets supporting the conclusions of this article are available in the Gene Expression Omnibus repository (accession numbers: GSE150316 and GSE147507). The crystal structures of SARS-CoV-2 main protease (accession number PDB IDs: 6LU7) was obtained from Protein Data Bank (https://www.rcsb.org/).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Drugs enriched with differentially expressed gene targets

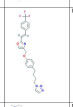
N°	Drug	Structure	Target gene	Mechanism
1	AZD-8055		ERBB2, MAPKAP1	mTOR inhibitor
2	Olaparib		PARP1, ERBB2	inhibitor of the nuclear enzyme poly(ADP-ribose) polymerase (PARP)
3	Irinotecan		ERBB2, TOP1MT	inhibitor of topoisomerase I activity
4	Niraparib		PARP1	inhibitor of poly(ADP-ribose) polymerases
5	Heptanoate		PARP1	plant metabolite
6	Iniparib		PARP1	potential cytotoxic activity
7	Tyrphostin AG 879		ERBB2	tyrosine kinase inhibitor
8	Ethanladi		ERBB2	inhibitor of the human epidermal growth factor receptor tyrosine kinase HAR-2 (also called HER2)
9	Inosine		PARP1	purine nucleoside
10	Anthrapyrazole		ERBB2	polycyclic aromatic hydrocarbon derived from anthracene or phthalic anhydride
11	Doxorubicin		GADD45A, ERBB2	inhibitor of protein synthesis
12	Gemtuzumab Ozogamicin Sodium		NFKB1	inhibitor of topoisomerase II
13	Topotecan Hydrochloride		TOP1MT	inhibitor of topoisomerase
14	TAK-285		ERBB2	inhibitor of epidermal growth factor receptor
15	Mubritinib		ERBB2	protein kinase inhibitor
16	MP-412		ERBB2	protein kinase inhibitor
17	Canertinib		ERBB2	protein kinase inhibitor
18	INSM-18		ERBB2	inhibitor of two receptor tyrosine kinases (RTKs), the insulin-like growth factor receptor (IGF-1R) and the c-erbB2/HER2/neu receptor
19	S-222611		ERBB2	protein kinase inhibitor
20	Afatinib		ERBB2	inhibitor of the epidermal growth factor receptor (EGFR) 1 and human epidermal receptor 2 (ERBB2)
21	7-Ethyl-10-Hydroxy-Camptothecin		TOP1MT	apoptosis inducer
22	Talazoparib Tosylate		PARP1	inhibitor of the nuclear enzyme poly(ADP-ribose) polymerase (PARP)
23	Varlitinib		ERBB2	inhibitor of the epidermal growth factor receptor (EGFR) 1 and human epidermal receptor 2 (ERBB2)
24	Fulvalamid		ERBB2	epidermal growth factor receptor inhibitor
25	Pyrotinib		ERBB2	inhibitor of the epidermal growth factor receptor (EGFR) 1 and human epidermal receptor 2 (ERBB2)
26	GP-724714		ERBB2	inhibitor of human epidermal receptor 2 (ERBB2)
27	Bardoxolone		NFKB1	apoptosis inducer

Table 2. Docking study results for SARS-CoV-2 3CL Main Protease

Drug	Binding energy (kcal/mol)
AZD-8055	-7.5
Olaparib	-7.39
Irinotecan	-8.4
Niacinamide	-4.43
Heptanoate	-4.19
Iniparib	-6.28
Tyrphostin AG 879	-7.67
Irbinitinib	-6.6
Inosine	-4.58
Anthraquinone	-6.2
Doxorubicin	-6.14
Omtriptolide Sodium	-6.62
Topotecan Hydrochloride	-7.4
TAK-285	-5.43
Mubritinib	-6.51
MP-412	-7.93
Canertinib	-6.04
INSM-18	-6.05
S-222611	-7.15
Allitinib	-7.1
7-Ethyl-10-Hydroxy-Camptothecin	-7.07
Talazoparib Tosylate	-6.43
Varlitinib	-6.2
Falnidamol	-7.64
Pyrotinib	-8.4
CP-724714	-6.43
Bardoxolone	-8.78

Table 3. Binding energies based on molecular dynamic simulations for three drugs: bardoxolone, irinotecan and pyrotinib.

Ligands	ΔG kcal/mol
bardoxolone	-160,887 ± 5,877
irinotecan	-240,473 ± 12,521
pyrotinib	-291,571 ± 15,627

Figures

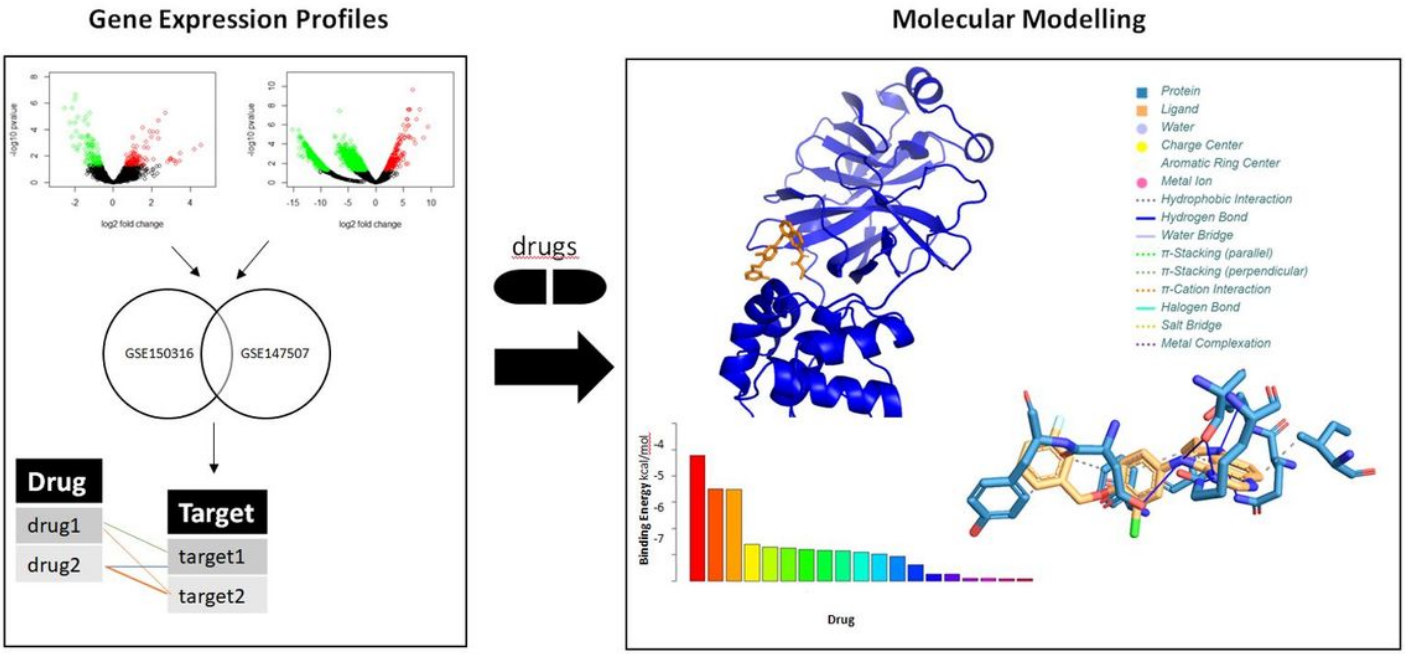


Figure 1

Workflow of the proposed approach. A computational pipeline based on gene expression data analysis, molecular docking and molecular dynamic simulation was adopted for in silico drug discovery.

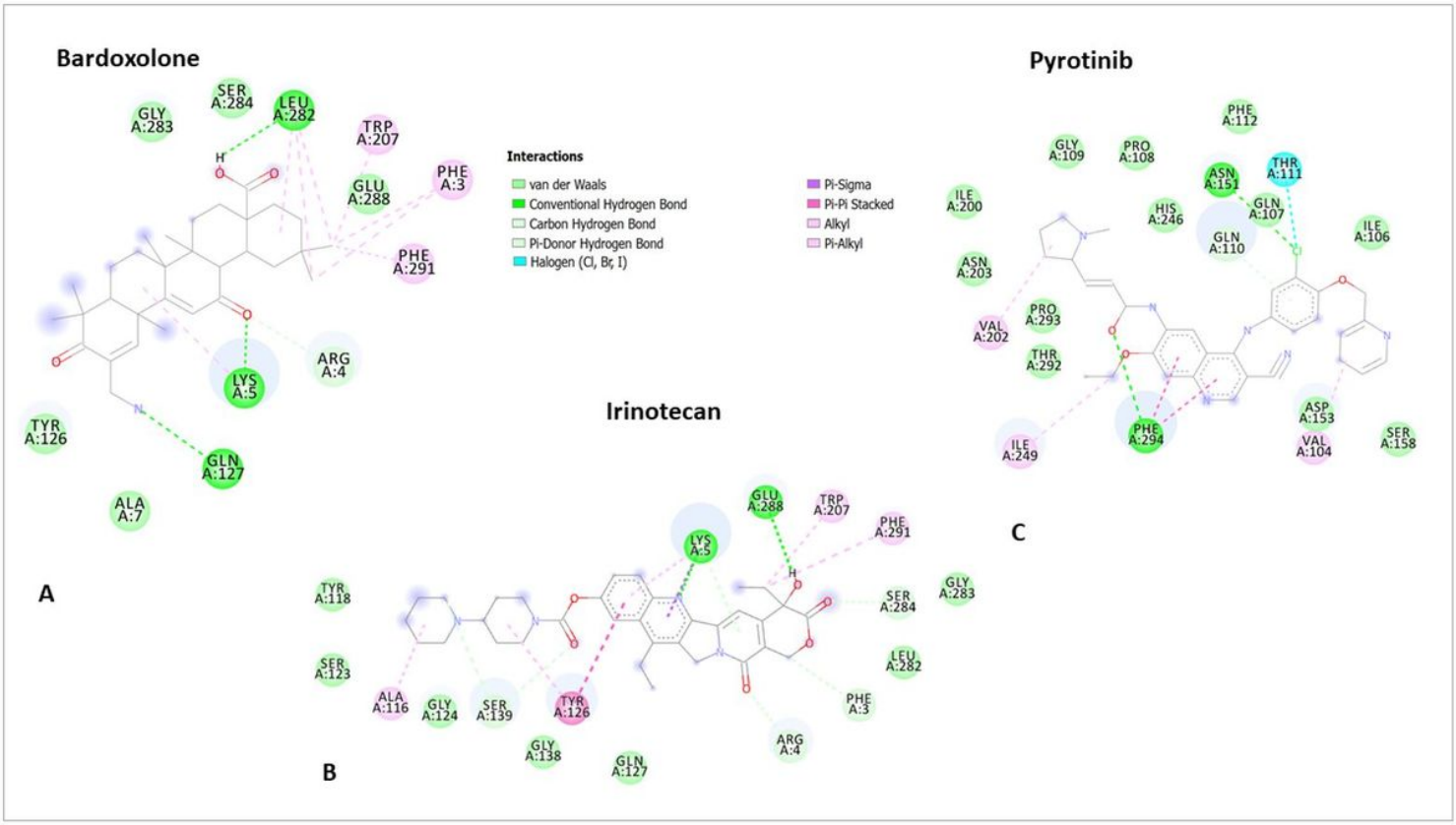


Figure 2

Interaction diagram of 3 drugs (bardoxolone, irinotecan and pyrotinib) with the main protease of SARS-CoV-2. The figure shows detailed information about protein residues, involved amino acid and type of interactions.