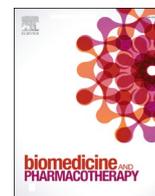




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## Review

## Virtual screening of substances used in the treatment of SARS-CoV-2 infection and analysis of compounds with known action on structurally similar proteins from other viruses

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## ARTICLE INFO

## Keywords:

Virtual screening  
Bibliometric analysis  
Treatment of SARS-CoV-2 infection  
Proteins  
Viruses  
Molecular docking

## ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is considered the etiological agent of the disease that caused the COVID-19 pandemic, and for which there is currently no effective treatment. This pandemic has shown that the rapid identification of therapeutic compounds is critical (when a new virus with high transmissibility occurs) to prevent or reduce as much as possible the loss of human lives. To meet the urgent need for drugs, many strategies were applied for the discovery, respectively the identification of potential therapies / drugs for SARS-CoV-2. Molecular docking and virtual screening are two of the *in silico* tools/techniques that provided the identification of few SARS-CoV-2 inhibitors, removing ineffective or less effective drugs and thus preventing the loss of resources such as time and additional costs. The main target of this review is to provide a comprehensive overview of how *in-silico* tools have been used in the crisis management of anti-SARS-CoV-2 drugs, especially in virtual screening of substances used in the treatment of SARS-CoV-2 infection and analysis of compounds with known action on structurally similar proteins from other viruses; also, completions were added to the way in which these methods came to meet the requirements of biomedical research in the field. Moreover, the importance and impact of the topic approached for researchers was highlighted by conducting an extensive bibliometric analysis.

### 1. Introduction

Since the development of the modern digital era, numerous scientific fields have made a sudden transition to data-dependent research methods [1]. Bioinformatics is a field of science that was developed as a result of this progress [2,3]. Molecular docking, virtual screening (VS), and molecular dynamics represent important tools that can be used in bioinformatics. Experiments conducted *in vivo* or *in vitro* are expensive and time-consuming, but they provide accurate results. By comparison, *in silico* ones do not yield information that is as accurate. However, they are faster and less expensive, making them widely used in research in the pharmaceutical industry and other scientific fields [2,4].

Molecular docking studies, as a part of *in silico* analysis, are carried

out using a biomedical informatics program that attempts to predict as accurately as possible how a ligand (a small molecule or a protein) interacts with a receptor represented by proteins or genetic materials [5]. These studies are used to identify compounds with therapeutic potential. Furthermore, they accurately determine the interaction between the ligand and the protein, and help establish a link between the chemical structure and the activity of the studied compound [6]. The Protein Data Bank (PDB) is a repository of three-dimensional structures of proteins that are used as receptors for molecular docking studies [7]. Selection of the receptor is a crucial step in the docking process, and if there are multiple sources for a protein, the higher resolution receptor will be selected [8].

The docking of numerous molecules on a target protein and the

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<https://doi.org/10.1016/j.bioph.2022.113432>

Received 27 May 2022; Received in revised form 7 July 2022; Accepted 15 July 2022

Available online 18 July 2022

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assessment of these compounds based on the score supplied using an algorithm is the basis of VS. Molecular dynamics allows the introduction of variables such as protein flexibility, behavior in the presence of water and ions and the evolution of the system over time. By associating VS / molecular docking with molecular dynamics, the obtained results are significantly closer to the in-vivo ligand-protein interaction mode [9]. Although investment in drug research and development is on an upward trend, the number of new drugs introduced into therapy declined significantly in recent years [10]. The regulatory agencies authorize only a small percentage of the drugs tested. Therefore, pharmaceutical companies tend to use drugs that are already used in therapy by finding new indications for them. A recent example is the repositioning of drugs to treat SARS-CoV-2 infection since the onset of the pandemic [11–13].

The present research aims to provide an overview of how *in silico* tools have been used in the crisis management of anti-SARS-CoV-2 drugs, especially in virtual screening of substances used in the treatment of SARS-CoV-2 infection, also considering the analysis of compounds with known action on structurally similar proteins from other viruses, and the way these methods were usable in the biomedical research. Moreover, the importance of the topic for researchers and the impact of the information in the field were underlined by conducting an extensive bibliometric analysis. Using scientific mapping technology to visually assess knowledge structure and research trends facilitates the access of future authors to the most prolific journals while also revealing the most relevant and productive researchers in the field. Identifying the terms with a high occurrence and the citation average for the articles in which they are contained highlights the latest developments in this field. The keywords representing antivirals and other chemical substances are useful tools in understanding the current trends in molecules used for docking or included in databases subjected to virtual screening.

## 2. Bibliometric analysis

In order to assess the importance of the topic, an extensive bibliometric analysis was performed, highlighting the most important parameters describing literature such as data source, journal, impact factor, number of citations, most prolific authors, etc.

### 2.1. Data source

A scientific literature search was performed on the Scopus database, the largest abstract and citation database to date. The following search parameters were used: articles that were written in English with the following keywords in the title, abstract, and keywords: SARS-CoV-2, molecular docking, virtual screening. 575 results were identified, of which 539 (93.74 %) were original articles, 16 (2.78 %) reviews, and the rest of them were included in the following categories: book chapter, conference paper, and letter. Most of the articles were classified in the following subject areas: “Biochemistry, Genetics and Molecular Biology-304 articles”, “Chemistry-175”, “Pharmacology, Toxicology, and Pharmaceuticals-171”, “Computer Science-117”, and “Medicine-104”; other subject areas had < 100 articles.

### 2.2. Leading countries in publishing SARS-CoV-2 and molecular docking papers

India, the United States, China, and Saudi Arabia published most of the articles (75.88 %) in this field. Despite ranking third in terms of the number of published articles, China has a far higher average citation/document than India and the United States. Although Italy has only 25 published articles, the average citation/document is 20. Pakistan also stands out with an average citation/document of 26.41 for 22 articles. India had a high total link strength (TLS) score because it collaborated with a significant number of countries (48) in the publication process. Table 1 shows all the countries that had more than 20 published papers in the topic.

**Table 1**  
Countries with >20 published papers.

Country	Papers No/ %	Citations	Average citation / document	TLS
India	200/37.11	2885	14.43	104
United States	76/14.10	1321	17.38	70
China	69/12.80	1918	27.80	48
Saudi Arabia	64/11.87	552	8.63	91
Egypt	31/5.75	288	9.29	40
Brazil	26/4.82	345	13.27	10
Iran	25/4.64	133	5.32	7
Italy	25/4.64	500	20.00	20
United Kingdom	25/4.64	282	11.28	44
Germany	22/4.08	380	17.27	26
Pakistan	22/4.08	581	26.41	15

Legend: TLS, total link strength: a measure of the degree of collaboration with other countries.

VOSviewer was used to create the network map of country co-authorship for articles matching the presented search terms (Fig. 1). Countries that frequently co-author articles are included in different clusters:

- The red cluster includes Bangladesh, China, Germany, Iran, Italy, Pakistan, and Spain.
- The green cluster includes Egypt, Nigeria, South Africa, Turkey, and the United Kingdom.
- The blue cluster includes India, Saudi Arabia, and South Korea.
- The yellow cluster includes the United States and Brazil.

The bubble size reflects the TLS, which means that more collaborative countries have a larger bubble. The strength of the link (LS) between two countries is shown by the line's thickness. The line connecting two countries will be thicker if they frequently publish together because the LS score for those publications is higher. Researchers from India (TLS 104), Saudi Arabia (91), and the United States (70) closely collaborated with a significant proportion of foreign academics. Indian researchers had the highest level of collaboration with researchers from Saudi Arabia, followed up by experts from the United States and South Korea.

### 2.3. Journals

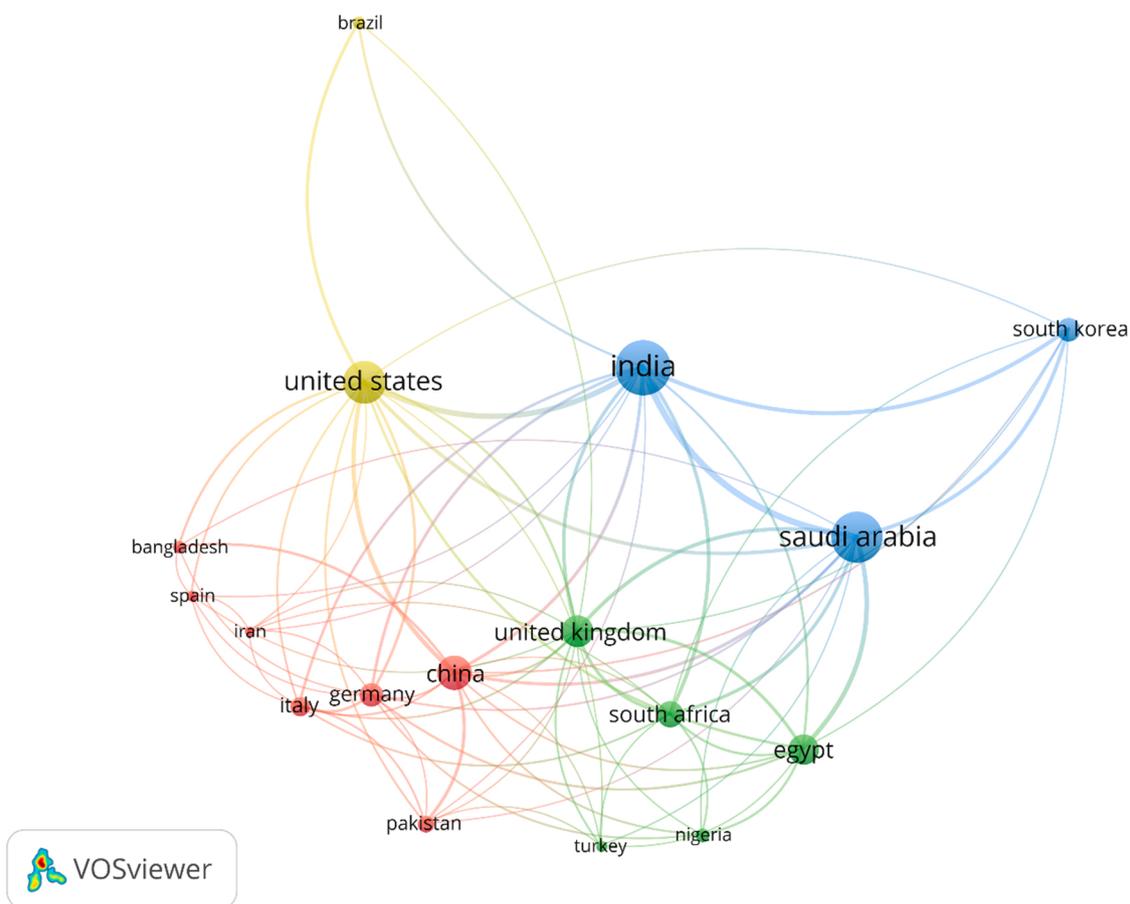
Over 200 journals have published at least 1 article matching the search terms. The journals that showed the most interest regarding in-silico studies are presented in Table 2. All the information regarding the impact factor (IF) was obtained using the Journal Citations Reports website. When analyzed in terms of the IF, the highest-ranking journal was the International Journal of Molecular Sciences (5.924) and the lowest ranking was the Journal of Molecular Graphics and Modeling (2.518).

Furthermore, to assess whether molecular docking and virtual screening are within the scope of interest of these journals outside of the context of SARS-CoV-2, the term SARS-CoV-2 has been removed from the search criteria, and the number of papers from 2012 to the present is analyzed (Fig. 2). Several variables are considered while selecting a journal to publish a scientific paper, including the topic area in which the journal shows interest, in order to increase the chances of successful publication.

### 2.4. Prolific authors

Of the 539 articles analysed, 2868 authors were identified. Table 3 presents the top ten writers ordered by the number of published papers. Furthermore, they have 82 (15.2 %) publications with 1393 citations.

Fig. 3 shows the network map of co-authorship authors, created by VOSViewer; only authors with a minimum of 5 articles were represented. The bubble map shows the relationship between the authors,



**Fig. 1.** Bubble map of co-authorship by the country for the selected search terms. Only countries with > 10 published articles were presented (17 countries in 4 clusters).

**Table 2**  
Top 10 journals according to the number of articles published that fit the search parameters.

Journal	Papers	Total citations	Average citation / document	Publisher	Impact factor <sup>a</sup>	TLS
Journal of Biomolecular Structure and Dynamics	104	1966	18.90	Taylor and Francis Ltd.	–	46
Computers in Biology and Medicine	21	191	9.10	Elsevier	4.589	24
Molecules	18	274	15.22	Multidisciplinary Digital Publishing Institute (MDPI)	4.412	21
Journal of Chemical Information and Modeling	15	373	24.87	ACS Publications	4.956	39
International Journal of Molecular Sciences	12	188	15.67	MDPI	5.924	13
Molecular Diversity	11	89	8.09	Springer	2.943	10
Journal of Molecular Graphics and Modeling	10	89	8.90	Elsevier	2.518	7
Frontiers in Chemistry	9	42	4.67	Frontiers Media	5.221	12
Pharmaceuticals	9	60	6.67	MDPI	5.863	5
Scientific Reports	8	112	14.00	Nature	4.380	6

<sup>a</sup> Impact factors are given according to year 2020.

highlighting the groups of authors who published together. There were generated six clusters (e.g., the red cluster including 11 authors, the yellow cluster 4, the green cluster 9, the blue cluster 5, the purple cluster 3, and the cyan cluster 1). The most extensive set of interrelated data featured 29 interconnected writers.

**2.5. Most cited articles**

Table 4 shows the most cited papers, the authors’ names, the journal in which they published, and the publication’s IF. 7 out of the 10 articles were published in 2020, in journals with IF ranging from 3.39 (Journal

of Biomolecular Structure and Dynamics) to 11.614 (Acta Pharmaceutica Sinica B). Considering that nearly all authors require access to high-quality information when writing a paper, the number of citations is a good measure for quantifying an article’s quality. The IF of the journal in which an article is published is also a reliable predictor of the quality of the material contained.

**2.6. Co-occurrence of the keywords**

Topics with high citations included drug screening (178 publications, 29.35 average citation/publication), drug repositioning (115,

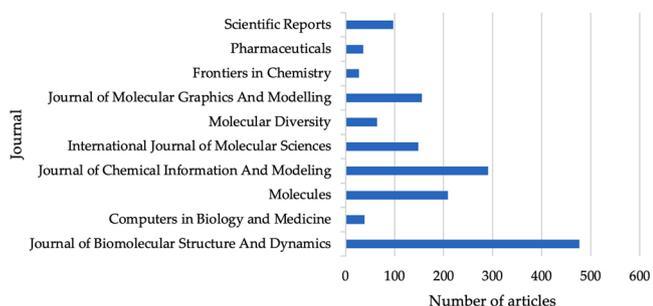


Fig. 2. The number of articles fitting the following search terms “molecular docking, virtual screening” from 2012 to present in the journals presented in Table 2.

Table 3  
Prolific authors based on the number of articles published.

Author	Papers	Total citations	Average citation / document
Kumar S.	12	285	23.75
Sharma P.	9	150	16.67
Sharma S.	9	109	12.11
Chandra S.	8	134	16.75
Joshi T.	8	134	16.75
Prakash A.	8	285	35.63
Ahmad S.	7	46	6.57
Islam M.A.	7	57	8.14
Kumar V.	7	65	9.29
Wei D.-Q.	7	128	18.29

25.57), and molecular dynamics simulation (171, 13.32). Antiviral drugs were of high-interest terms like remdesivir (52, 36.9), favipiravir (19, 74.84), darunavir (21, 82.90), lopinavir (37, 55.57), having a high average citation rate/document > 35, but also a high occurrence. Chloroquine (29, 55.86) and hydroxychloroquine (32, 14.81) had a high occurrence among the terms related to drugs). Other drug terms presented nelfinavir (24, 23.92), cobicistat (14, 25.93), simeprevir (15, 32.67), paritaprevir (12, 26.00), saquinavir (17, 30.53), amprenavir (10, 21.40), atazanavir (12,17.25) indinavir (20, 21,10), nilotinib (11, 25.91), raltegravir (12, 46.42), grazoprevir (12, 19.67), tipranavir (14, 27.93). However, they had lower presence in publications, but still a high average citation score/ publication. Umifenovir (11, 6.82), ribavirin (16, 11.69), and sofosbuvir (10, 9.40) had low occurrences and average citation scores/ publication compared to the other drug terms. The assessment of the words related to phytochemicals (30, 12.57) concluded that flavonoids (14, 8.57), luteolin (10, 7), quercetin (16, 7.31), rutoside (15, 9.13), Chinese medicine (11, 21.91) had on the average, lower occurrence and lower average citation score/ document compared to synthetic drugs.

### 3. The origin of SARS-CoV-2

*Coronaviridae*, *Arteriviridae*, *Mesoniviridae*, and *Roniviridae* comprise the order Nidovirales, of which coronaviruses are the largest group. The *Coronaviridae* family is further classified into two subfamilies, *Torovirinae* and *Coronavirinae*. The *Coronavirinae* subfamily is subdivided into 4 genera [25], as it is presented in Fig. 5. The form of presentation has been chosen as to highlight the taxonomic hierarchy in biological classification of SARS-CoV-2.

HCoV-229E and HCoV-NL63 of the genus *Alphacoronavirus* usually

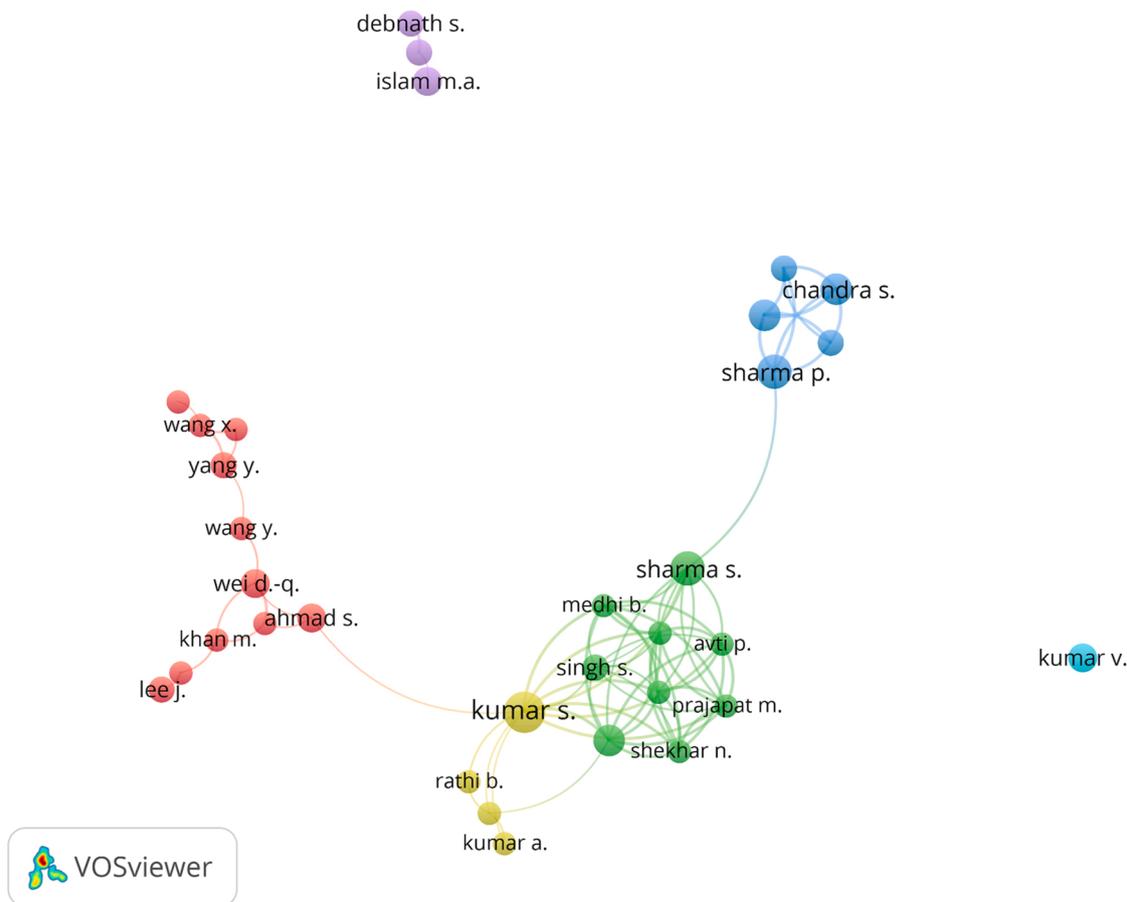


Fig. 3. Bubble map of co-authorship. Only authors with > 5 published articles were presented (33 authors).



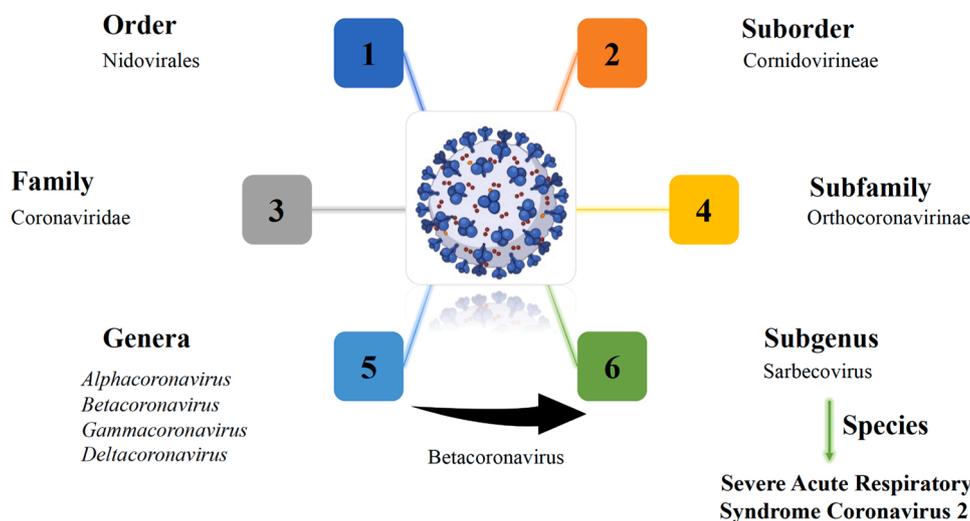


Fig. 5. A brief taxonomy of coronaviruses.

cause common colds, but they can produce complications in immunocompromised patients or the elderly. Betacoronaviruses like HCoV-OC43 and HCoV-HKU1 are non-virulent, while SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) may cause severe symptoms. *Alphacoronavirus* and *Betacoronavirus* also contain animal viruses. Furthermore, only the viruses found in animals have been identified as *Gammacoronavirus* and *Deltacoronavirus* [26–28].

SARS-CoV-2 was identified by genetic sequencing [29]. Formerly known as the novel coronavirus 2019 (2019-nCoV), SARS-CoV-2 is a new betaCoV belonging to the same subgenus as SARS-CoV and MERS-CoV viruses that caused epidemics with fatality rates of 10 % and 35 %, respectively [30].

The origin of SARS-CoV-2 remains unknown, and the generally accepted hypothesis is that the disease was transmitted from animal to human (zoonotic transmission). In silico-related studies describing the interactions between human ACE-2 receptor homologues, TLRs and SARS-CoV-2 spike glycoprotein [31] showed that the glycoprotein can be considered phylogenetically as close to the coronavirus found in bats, binding mightily to the ACE2 receptor protein (both animal – bat, and human). In the same study, it was revealed that cell surface TLRs (i.e., TLR4) are probably the most involved in recognizing the patterns of molecules induced by SARS-CoV-2, which produce the characteristic inflammatory responses. Thus, the data come to support the zoonotic origin (from a bat) hypothesis of SARS-CoV-2 [32].

The genome of the new coronavirus is 96.2 % similar to RaTG13, a  $\beta$ -CoV found in bats, and 79.5 % to SARS-CoV [33,34]. The majority of the SARS-CoV-2 genome is represented by open reading frames (ORF1a and ORF2b), which will be translated into two pp1a and pp1ab polyproteins. The two polyproteins will be cleaved by papain-like protease (PL<sup>pro</sup>) and main-protease viral proteases (3CL<sup>pro</sup>, M<sup>pro</sup>) to create 16 non-essential proteins (nsp). The rest of the genome will be translated into four structural proteins spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N) [35].

#### 4. Drug repositioning

The process of developing new drugs is costly and time-consuming. The pharmaceutical industry is experiencing issues due to the low number of novel pharmaceuticals launched onto the market in recent years. To address this issue, pharmaceutical companies have resorted to the following methods: improved formulation (modified release), improved bioavailability by co-administration of two pharmaceuticals (e.g. pharmacokinetic enhanced protease inhibitors with ritonavir or cobicistat [36]), and drug repositioning (Fig. 1) (finding new indications

for drugs already approved for human use) [10,37].

The latter method was used at the onset of the COVID-19 pandemic when in the absence of a viable treatment, the only therapeutic option was to use drugs already on the market. Many of the compounds used against SARS-CoV-2 have proven ineffective (e.g. ivermectin), and the efficacy of others has decreased due to the evolution of the virus (sotrovimab has low activity against the subvariant Omicron BA.2.) [12, 38]. Remdesivir was the only repositioned drug that remained as a first-line treatment among the drugs included in the WHO therapeutic guidelines for COVID-19 therapy. Ritonavir-boosted Nirmatrelvir, Bebtelovimab, Molnupiravir, and Sotrovimab were developed de novo for the treatment of SARS-CoV-2 infection [38,39].

#### 5. Molecular docking

Molecular docking is the process of simulating how a receptor will interact with a ligand. The ligand is usually a small molecule that is thought to have a pharmacological effect, while the receptor is a protein or nucleic acid molecule [5]. Because molecular docking predicts the three-dimensional structure of the ligand-protein and the orientation of the ligand, this method can be used in the discovery of new drugs or in drug repositioning. The binding potential of the ligand to the receptor is calculated using different algorithms that consider possible interactions (e.g., columbic, van der Waals) [40,41].

The foundations of molecular docking have been laid since 1980, and considerable progress has been made to date. The software is far more accurate and accessible. Thus, academic researchers conduct molecular docking studies more frequently (Fig. 7) [42–44]. If the target protein's structure has not been identified, the homology modeling approach can be utilized, which entails building a protein with an unknown structure from a homologous one whose structure is known. [41].

The way a ligand binds to a certain protein (position prediction) gives information about the interactions between them. Binding energy is often used to compare ligands with similar chemical structures (the hypothesis being that an analog or a substance with a higher affinity than the native ligand may cause competitive inhibition). Furthermore, the goal of VS is to discover new ligands with therapeutic potential [32, 45].

Following the docking studies, a checkup is necessary to see if the approach used or the software functioned appropriately. The verification process involves re-docking the native ligand, and calculating root mean square deviation (RMSD) [45]. Calculating the RMSD determines the distance between a pair of superimposed atoms, the value of which is calculated for heavy backbone atoms. The docking protocol is

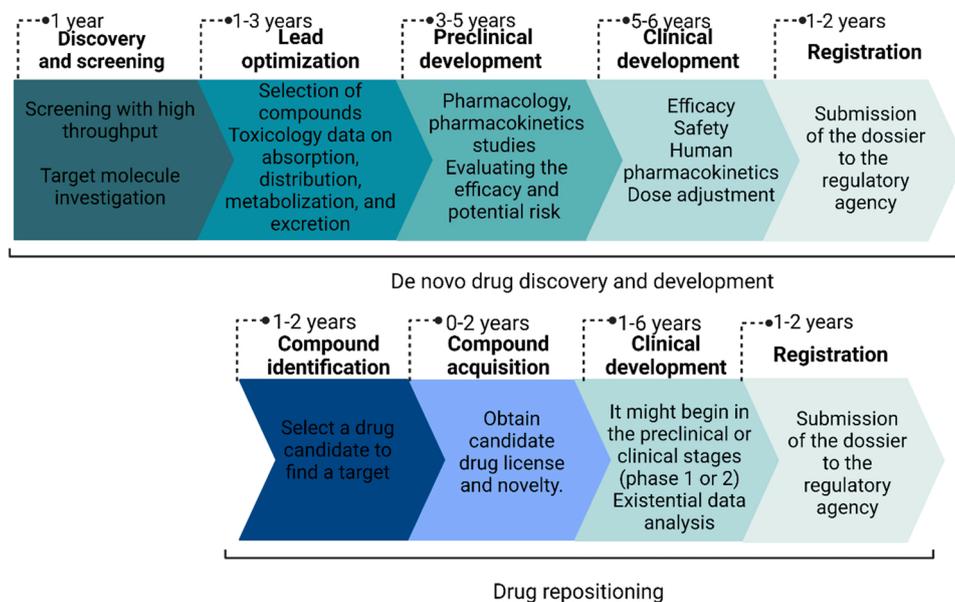


Fig. 6. The advantages of drug repositioning compared to de novo drug design.

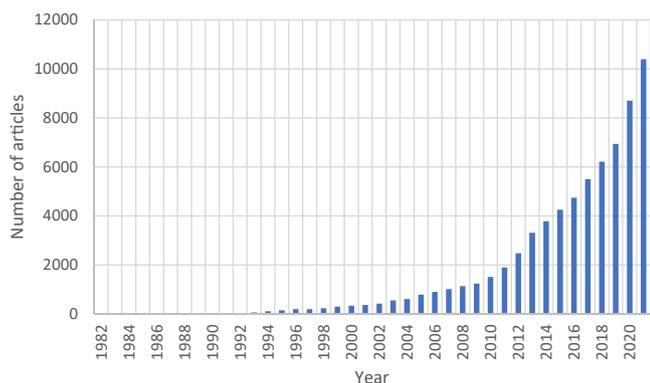


Fig. 7. Number of articles/years regarding molecular docking (data from PubMed).

considered better in molecular docking when the RMSD value is low. [46].

The pandemic has brought to light a significant issue affecting humanity, namely the absence of therapies for new and quickly spreading illnesses. In such cases, one alternative is to find new therapeutic indications for authorized drugs, and molecular docking is a helpful tool to achieve this. [47].

## 6. Virtual screening (VS)

Pharmaceutical companies employ high-throughput screening (HTS) to identify novel compounds. HTS is an automated process that uses robots paired with high-sensitivity sensors to examine a large number of molecules in a short time [48]. However, HTS is an expensive method that requires special equipment, which has led to the need to find a more economical alternative. Thus, VS has been developed, through which numerous compounds are docked on a protein, being previously downloaded from a database (Table 5) that can be open access. A protein whose crystallographic structure is known is commonly used, but there is also the possibility of generating a protein by homology modeling [49–51].

The most significant benefit of VS is the reduction in the number of substances that must be subjected to expensive experiments. The most

Table 5  
Compound databases.

Database	Availability	No. of compounds	
PubChem	Open access	11,000,000	
ChEMBL		2200,000	
ZINC		2300,000	
DrugBank		14,624	
SWEETLEAD		4442	
TCMSP		3311	
Asinex		575,302	
CAS Antiviral COVID19		50,000	
ChemDiv		Commercial	1522,032
Enamine			2157,315
Princeton Biomolecular Research	1533,024		

significant disadvantage is that a compound with pharmacologic potential can be omitted by the algorithm [52]. VS methods are usually classified into ligand-based VS (LBVS) and structure-based VS (SBVS). In the case of LBVS, a ligand is used as a reference, and the database is searched for molecules with similar characteristics. SBVS approaches are substantially more complex since they consider a protein macromolecule. Ligand classification is based on the degree of the ligand fit in the protein active site (ligand-protein match) [53,54].

## 7. Molecular dynamics

Molecular dynamics simulations are in-silico methods that predict the behavior of a system over time. The rapid evolution of computer hardware has also benefited the field of molecular dynamics. Simulations, which once needed the most advanced computers of the time, can be done today on a computer with mid-range specifications [55,56]. The increased availability of these simulations is mirrored in the number of published articles (Fig. 8).

Docking or VS experiments usually have a mobile ligand-rigid protein, although proteins are mobile in the physiological environment. The association of these studies with molecular dynamics is used to improve the accuracy of the results [57,58].

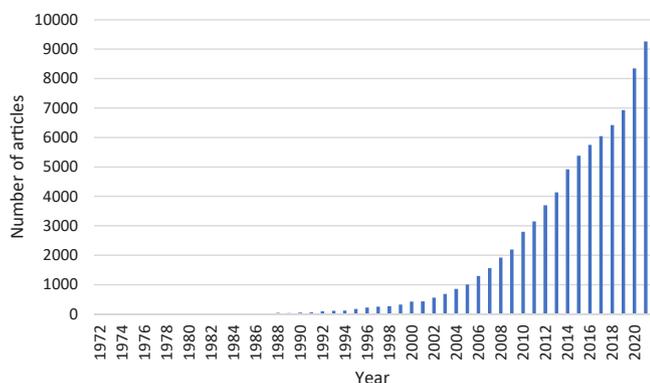


Fig. 8. Number of articles/years related to molecular dynamics (data from PubMed).

## 8. Targeted proteins using *in silico* methods

### 8.1. 3-chymotrypsin like protease (3CL<sup>pro</sup>, M<sup>pro</sup>)

The SARS-CoV-2 genome contains at least six ORFs, of which ORF1ab encodes two polyproteins, pp1a and pp1ab. These will be cleaved by the 3CL<sup>pro</sup> and PL<sup>pro</sup> (Fig. 9) and as a result, 16 non-structural proteins (nsp) which are essential to the virus's life cycle are formed [59, 60]. 3CL<sup>pro</sup> is a dimer composed of two symmetrical protomers further subdivided into three domains. Domain I is represented by residue 8–101, domain II by residue 102–184, and domain III by residue 201–303. A catalytic dyad represented by histidine41 (belongs to domain I) and cysteine145 (belongs to domain II) forms the active site of 3CL<sup>pro</sup> [61].

3CL<sup>pro</sup> has no known structural analog in the human body, so discovering a therapeutic agent that targets the mechanism of action of 3CL<sup>pro</sup> will not show negative effects in the host cell [62–64]. Table 5 summarizes some of the SBVS studies performed searching for compounds with inhibitory potential on 3CL<sup>pro</sup>. The PDB ID of the target protein and the software used for molecular docking were coupled with molecular dynamics.

The crystal structure of 3CL<sup>pro</sup> SARS-CoV-2 with the PDB ID: 6LU7 was published on 2020–02–05 by Jin et al., 6LU7 has a resolution of 2.16 Å and is co-crystallized with a molecule that functions as a protease inhibitor. This molecule was obtained by computer-aided drug design, following the analysis of a large number of compounds (over 10,000) [85]. Many protease inhibitors used in the treatment of HIV/SIDA and hepatitis C (Table 6) have been identified as possible inhibitors of 3CL<sup>pro</sup> protease by docking studies and docking studies combined with VS [86].

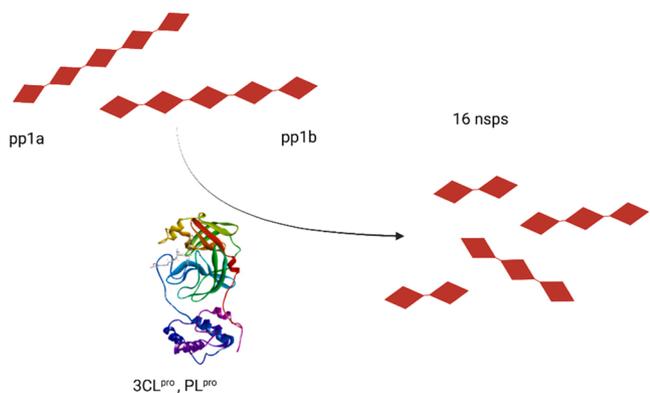


Fig. 9. Mechanism of action of 3-chymotrypsin like protease- 3CL<sup>pro</sup> and papain-like protease PL<sup>pro</sup>, pp1a and pp1ab- polyprotein 1a and 1ab, nsp- non-structural proteins.

Protease inhibitors that are conventionally used in the treatment of HIV / AIDS have a relatively high affinity for 3CL<sup>pro</sup>, making them suitable candidates for inhibiting the SARS-CoV-2 protease. Darunavir and Saquinavir have affinities of  $-7.7 / -9.5$  kcal/mol, respectively. Grazopavir and Paritaprevir, two NH<sub>3</sub> / 4 A protease inhibitors used to treat HCV, have affinities to 3CL<sup>pro</sup> of  $-8.1 / -9.5$  kcal/mol, respectively. According to docking studies on 3CL<sup>pro</sup> and the fact that they act on the protease of another RNA virus (HCV/ HIV/SIDA), these compounds may also interfere with SARS-CoV-2 3CL<sup>pro</sup>.

#### 8.1.1. A quick look at nirmatrelvir

The mechanism of action is specific to this class and prevents 3CL<sup>pro</sup> from processing the pp1a and pp1ab polyproteins [106]. It is coupled with ritonavir, a human immunodeficiency virus type 1 (HIV-1) protease inhibitor that plays a vital role in lowering the metabolization rate of Nirmatrelvir, by inhibition of the CYP3A isoenzymes [107].

According to WHO, it is the most effective therapy for high-risk patients to date (e.g., unvaccinated or immune-suppressed patients). The drug is administered orally in the early stages of the disease. The pharmaceutical concept of the drug is not new, and it is based on a compound developed for the treatment of SARS-CoV. The Emergency use authorization was granted within a year after Phase 1 clinical studies got started. [108].

In terms of *in-silico* docking, Nirmatrelvir was docked to the 6Y2F protein, and the authors observed that it binds to the following residues through hydrogen bonds: Cys145, Glu166, and Gln189 [109]. Another docking study on the protein with the PDB ID: 7SI9 reported that Nirmatrelvir interacts with the protein via hydrogen bonds with the following amino acids: Gly143, Phe140, and Glu 166 [110].

### 8.2. Papain-like protease (PL<sup>pro</sup>)

Another SARS-CoV-2 protease that anti-virals might target is PL<sup>pro</sup> because it is involved in processing the viral polyproteins (Fig. 4) of SARS-CoV-2, allowing viral spread [111]. It has been demonstrated that it also interfere with host cell proteins by modulating the host viral response to facilitate the spread of the virus [112].

Proteases are one of the primary targets that researchers are examining in the battle against viral infections. *In silico* studies on PL<sup>pro</sup> have been conducted to discover inhibiting drugs for this dual-effect protease. Table 7 presents a series of *in-silico* studies aimed at discovering PL<sup>pro</sup> inhibitors.

### 8.3. RNA-dependent RNA polymerase (RdRp)

RdRp is a non-structural protein with a key role in genomic transcription and replication. The analysis of the crystallographic structure of RdRp shows the distinct shape of the right hand consisting of a palm, fingers, and thumb. [129,130]. Involved in genomic transcription and replication, RdRp is considered a valuable therapeutic target (Fig. 11). RdRp has no structural homologs in the human cell, so drugs that act specifically on this protein should, in theory, not cause side effects induced by the drug's mechanism of action [131,132].

In the research process of RdRp inhibitors, the most promising drug class is that of nucleoside analogs [133]. VS studies sought to find RdRp inhibitors, and the most promising compounds were the already approved anti-virals and used as nucleoside analogs in RNA viral infections. The fact that the active sites of these enzymes retain their fundamental properties in most RNA viruses increases the interest in RdRp-targeted anti-viral for the treatment of SARS-CoV-2 infection [134,135]. Until the crystallographic structure of RdRp was identified, molecular docking studies were based on homology models to build the protein needed to perform these studies [136–138]. After the publication of the crystallographic structure of RdRp (e.g. PDB ID: 7BW4 [139] 6M71 [140]), homology models have diminished in significance. Table 8 reviews SBVS studies focused on finding RdRp inhibitors.

**Table 6**A summary of in-silico studies targeting 3CL<sup>pro</sup>.

Target	Docking software	Molecular dynamics	Drugs tested	Promising anti-viral ligands	Ref.
6LU7	AutoDock Vina	–	ZINC database. 10 FDA-approved drugs	ZINC32960814, ZINC12006217, ZINC03231196	[65]
6LU7	Autodock Vina	–	TCMSP database	Puerarin, bicuculline, luteolin	[66]
6LU7	Glide	DESMOND	Seaweed Metabolite Database	Nigriganoside A, Nigriganoside B, Callophysin A	[67]
6M2N	AutoDock Vina	YASARA	32 phytochemicals	Amentoflavone, Gallocatechin	[68]
6LU7	AutoDock Vina	GROMACS	92 phytochemicals	Hyperin, lupinifolin, rutin	[69]
6LU7	LeDock software	GROMACS	ZINC database	Viomycin, capastat	[70]
6LU7	AutoDock Tool	AMBER 16	Phytochemicals	Demethoxycurcumin, Bisdemethoxycurcumin, Scutellarin, Quercetin, Myricetin	[71]
6Y2F	AutoDock Vina	–	263 phytochemicals, 75 FDA-approved drugs	Alloyohimbine Gummadiol, Asparagamine A Vincapusine, Simeprevir	[72]
6LU7	MOE, Autodock Vina	–	FDA-approved Drug Library from Selleckchem	Ledipasvir, Paritaprevir, Glecaprevir, Daclatasvir	[73]
6LU7	AutoDock Vina	–	154 phytochemicals	Oxytetracycline, Naringin, Kanamycin, Cefpiramide, Salvianolic Acid B, Teniposide, Etoposide, Doxorubicin	[74]
6M03	AutoDock Vina	GROMACS	PubChem database.	Benzoylgedunin, Glycyrrhizic Acid, Limonin, Obacunone	[75]
6LU7	DOCK 6	AMBER 16	SWEETLEAD database of drug molecules	6-Deaminosinefungin, UNII-09H5KY11SV	[76]
6LU7	AutoDock Vina	GROMACS	Zinc database, drugbank database	Indinavir, Ivermectin, Cephalosporin-Derivatives, Neomycin and Amprenavir	[77]
6LU7	Schrödinger's Covalent Docking	AMBER 18	Asinex Focused Covalent library 116- FDA-approved drugs	Tideglusib, Nilotinib, Amentoflavone	[78]
6LU7	The smina software	AMBER 18	CAS Antiviral COVID19 database	Simeprevir, Paritaprevir	[79]
6LU7	Maestro	AMBER 18	31 FDA approved drugs TCM	4-(morpholin-4-yl)- 1,3,5-triazin-2-amine derivatives: 2001083–68–5 and 2001083–69–6	[80]
6LU7	Glide	Schrödinger software	Over 1000 analogs were prepared by the Maestro tool.	Saquinavir	[81]
6LU7	UCSF Chimera platform	AMBER 18	97 compounds from marine and terrestrial fungi	TCM5280805, TCM5280445, TCM5280343, TCM5280863, TCM5458190,	[82]
6LU7	AutoDock vina	–	Bioactive alkaloids (62) and terpenoids (100)	25 unnamed Hydroxyethylamine Analogs	[83]
6LU7	Autodock4	GROMACS	93 ligands Generative Adversarial Network	Quinadoline, Scedapin C, Polyketide isochoaetochromin D1	[84]
				0-Hydroxyusambarensine, Cryptoquindoline, 6-oxoisoguesterin, 22-hydroxyhopan3-one	
				6 unnamed compounds	

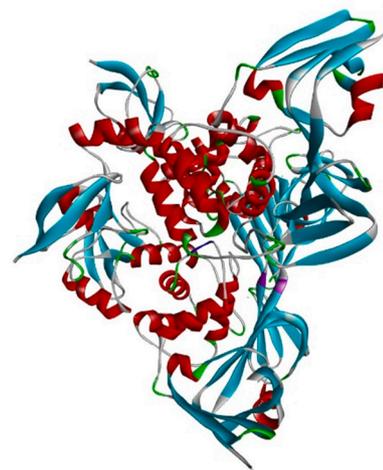
Legend: FDA, Food and Drug Administration; MOE, Molecular Operating Environment; TCMSP, Traditional Chinese Medicine System Pharmacology Database; YASARA, Yet another scientific artificial reality application

**Table 7**

A summary of protease inhibitors docked on 6LU7.

Docking software	Ligand	Affinity	Mechanism	Used in	References
Target					
AutoDock	Ciluprevir	-9,1	NH3/4 A	Hepatitis	[87–89]
Vina	Grazoprevir	-8,1	protease	C	[87,90]
6LU7	Simeprevir	-9,0, – 9,0	inhibitor		[87,91]
	Paritaprevir	-9,5			[87,92]
	Faldaprevir	-8,4		Of interest in treating hepatitis.	[87,93]
	Indinavir	-8,5 -8,3	Protease inhibitor	HIV/AIDS	[87,94,95]
	Saquinavir	-8,5, -8,9 -9,5			[87,94,96]
	Nelfinavir	– 9,1 -7,9			[97,98]
	Atazanavir	-8,1			[94,99]
	Darunavir	-7,7			[94,100]
	Ritonavir	-7,3 -7,6			[101,102]
	Breacanavir	-8,1			[87,103]
	Lopinavir	-8,1, – 9,3, – 8,4 -7,9 – 8,2			[87,94,97, 101,104, 105]

Legend: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.

**Fig. 10.** The 3D structure of PL<sup>pro</sup> (PDB ID: 6W9C).

### 8.3.1. Remdesivir

Remdesivir is a nucleotide analog designed to target RBA viruses (e. g., hepatitis C). It is a prodrug that is bioactivated in the body and functions as an adenosine triphosphate analog (ATP). Fig. 5 summarizes the mechanism of action. The suggested administration model is one dose on the first day, followed by two more on subsequent days. According to research, it must be administered in the early stages of the infection [154,155].

Since the pandemic's beginning, in-silico studies that recommended

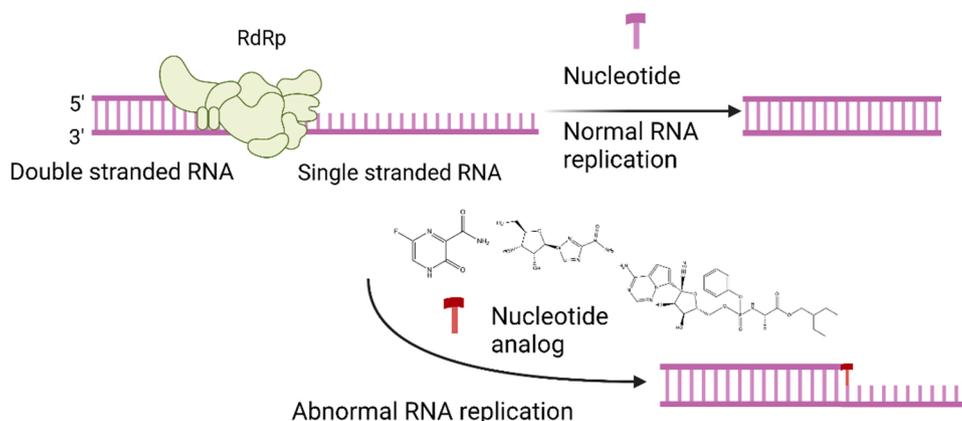


Fig. 11. Nucleoside analogs mechanism of action. RNA, ribonucleic acid; RdRp, RNA-dependent RNA polymerase.

Table 8

A summary of in-silico studies targeting 3CL<sup>PRO</sup>.

Target	Docking software	Molecular dynamics	Drugs tested	Promising anti-viral ligands	Ref.
6W9C	AutoDock Tools	GROMACS	Multiple drug databases	MFC00832476, MFC02180753, Bemcentinib, Pacritinib Ergotamine	[113]
7JN2	Maestro	Desmond	Database generated by AI integration	A3659, A3777, A3777	[114]
7CMD	MOE	GROMACS	In-house database contains 35,000 compounds	Unnamed	[115]
6WX4	Maestro	AMBER	149 polyphenols	Leucopelargonidin, Taxifolin, Morin, Eriodictyol, Myricetin, Enterodiol	[116]
6WX4	YASARA	YASARA	14,000 phytochemicals	Baicalin, Hesperidin, Naringin, Flemiflavanone D, Euchrestaflavanone A	[117]
7CJM	AutoDock Vina	AMBER	FoodComEx database	Triamterene, Estrone, Ibuprofen, Chlorpheniramine	[118]
6W9C)	AutoDock Tools	GROMACS	ChEBML database	Azadirachtin-H Azadirachtin-I Azadirachtin-Q Azadirachtin	[119]
7JN2	Glide	Desmond	SuperNatural Database	SN00334175, SN00162745	[120]
6W9C)	AutoDock Vina	LARMD	Phytochemicals of <i>Nigella sativa</i>	Campesterol, Cycloleucalenol, Alphaspinasterol, Taraxerol,	[121]
6W9C	AutoDock Vina too	GROMACS	40 related to <i>S. Sonchifolius</i> and <i>L. Meyenii</i>	Rutin	[122]
6XAA	AutoDock Vina	AMBER	MPD3 database + reported natural anti-viral compounds	Gycyrrhizin, Azadirachtani, Mycophenolic acid, Kushenol-w, 6-azauridine	[123]
6WX4	Maestro	–	ChEMBL 2390 phase III and IV clinical trial drugs	Curcumin, Afatinib	[124]
6W9C	AutoDock Vina	GROMACS	ZINC15 drug database – 291 FDA approved drugs	Thiamine, Levodopa Naloxone	[125]
7JIW	AutoDock Vina	AMBER	FoodB database	<b>Diosbulbinoside D, Estrone-2,3-quinone, Cyanidin 5-O-β-D-glucoside</b>	[118]
7JRN	CDOCKER	–	9032 drugs from TCM database	Hinokiflavone, Morelloflavone, Methylchloflavon, Amentoflavone, Ginkgetin, Isoginkget, Sciadopitysin, Podocarpusflavone A, Cryptomerin	[126]
6W9C	UCSF Chimera platform	AMBER	97 terrestrial fungi metabolites	Norquinadoline A, Scedapin C, Quinadoline B, Cytochalasin Z8	[127]
6W9C	Chimera-AutoDock Vina plugin	Desmond	46 bioactive flavonoids	Naringin, Hesperidin	[128]

FDA, Food and Drug Administration; MOE, Molecular Operating Environment; TCMSP, Traditional Chinese Medicine System Pharmacology Database; YASARA, Yet another scientific artificial reality application.

remdesivir for the treatment of COVID-19 have begun to emerge, using the keywords, "molecular docking" and, remdesivir". Table 9 summarizes several studies involving remdesivir. Most of these studies used remdesivir as a ligand control.

## 9. Conclusions and future perspectives

In-silico docking is continually changing, and previously unavailable approaches due to hardware limitations or a lack of target proteins have become widely available. Researchers' interest in this topic has grown due to the large number of macromolecules published on the Protein Data Bank (190404) and the fast advancement of hardware components. In order to discover new, innovative drug substances, pharmaceutical companies are increasingly using HTS, but the high costs make this method require a more economical alternative. The in-silico counterpart

of HTS is VS, which enables the test of a large number of compounds in a short amount of time.

VS, more specifically, SBVS in combination with molecular dynamics, has the potential to play a vital role in the reuse or even development of medicinal compounds. Several experiments were conducted to find pharmacological compounds that may act on this RNA virus during the pandemic. Drugs that act on the target structures of other RNA viruses were among the drugs considered. Drugs such as protease inhibitors used in treating HIV / AIDS or hepatitis C have been subjected to docking studies. Compounds of natural origin have been rigorously tested against all SARS-CoV-2 targets in the hope of finding a potential inhibitor.

Currently, in-silico studies are being utilized to limit the number of more costly in-vitro / in-vivo candidates. The introduction of a standard approach is a first step that must be made before the usefulness of in-

**Table 9**  
A summary of in-silico studies targeting RdRp.

Target	Docking software	Molecular dynamics	Drugs tested	Promising anti-viral ligands	Ref.
7BV2	AutoDock Vina, Glide, rDock	GROMACS	1615 FDA-approved drugs, ZINC database	Leucal, Natamycin, Folic Acid	[101]
7BW4	DockThor, Autodock Vina, PatchDock	GROMACS	48 antivirals	Ribavirin, Zanamivir, Penciclovir	[141]
6M71	AutoDock Vina	GROMACS	92 phytochemicals	Hesperidin, Rutin, Quercetin	[69]
7BTF	AutoDock Vina	NAMD	Novel adenosine derivatives	4 unnamed adenosine derivatives	[142]
6M71	Autodock 4.0.1.	–	65 FDA approved small molecule	Raltegravir, Indinavir, Tipranavir, Dolutegravir, Etravirine	[143]
6M71	Moe-Dock	GROMACS	63 anti-viral drugs approved by the FDA	Ledipasvir, Remdesivir, Paritaprevir	[144]
6M71	AutoDock Vina	–	Flavonoids, phenolic acids, and terpenes	p-Coumaric Acid, Ellagic Acid, Kaempferol, Quercetin	[145]
6M71	Discovery studio	Desmond	Zinc15 DataBank	Paritaprevir, Glecaprevir, Velpatasvir, Remdesivir, Ribavirin	[146]
7BTF	Autodock Vina	–	FDA Drug library	Ketazolam, Methylnaltrexone, Ethynodiol Diacetate	[147]
7BV2	Maestro	GROMACS	480 tested polyphenols were retrieved from Phenol-Explorer 3.6	Cyanidin 3-O-rutinoside, Petunidin 3,5-O-diglucoside, Delphinidin 3-O-rutinoside	[148]
6M71	Autodock vina	YASARA	ZINC database	Grazoprevir, Ledipasvir, Galidesivir	[149]
7BV2	AutoDock Vina	GROMACS	Asinex EliteSynergy and BioDesign libraries	Las 51620435, Las 51620429	[150]
7BV2	Schrodinger Glide	Desmond	FDA drugs drugbank	Rosoxacin, Levomefolic, Etodolac	[151]
6M71	Schrodinger Glide	GROMACS	75 FDA approved antiviral drugs	Ritonavir, Dolutegravir, Tenofovir, Tinofovirafenamide, Boceprevir, Catechin and Zanamivir	[152]
6M71	Schrodinger Glide	–	FDA approved drug database	Ompipressin, Otosiban, Lanreotide, Argiprestocin, Demoxytocin, Carbetocin, Lypressin, Examorelin, Colistin, Polymyxin B1	[153]

Legend: FDA, Food and Drug Administration; MOE, Molecular Operating Environment; YASARA, Yet another scientific artificial reality application.

**Table 10**  
Docking studies that include Remdesivir.

Target	Molecular docking software	Binding affinity Kcal/mol	Hydrogen bond with	Ref
6M71	Autodock Vina	-9	ASN781, HIS133, SER709, TYR129 LYS A47, ASP711	[156]
6M71	Autodock Vina	- 8	LYS47, TYR129, SER709, ASP711, ASN781	[146]
6M71	Autodock Vina	- 7.1	Lys621, Cys622, Asp761, Lys798, Glu811.	[157]
7VH8	AutoDock 4.2	-8.56	GLY143 CYS145 LEU167 TYR54 CYS145 GLU166	[158]
6M71	AutoDock Vina	-7.8	Gly143, Ser144 (2), Cys145, Glu166, Asn142	[159]
7BV2	AutoDockVina	-7.2	ILE23, LEU126, GLY48	[160]
7BTF	DOCK 6	-8.8	ALA 550, LYS 55, ARG 55, CYS 813, SER814, GLN 815	[161]

Legend: ASN, asparagine; HIS, histidine; SER, serine; TYR, tyrosine; LYS, lysine; ASP, aspartic acid; ALA, alanine; ARG, arginine; CYS, cysteine; GLN, glutamine; GLY, glycine; LEU, leucine; GLU, glutamic acid; ILE, isoleucine.

in-silico procedures rises, especially when it comes to molecular docking. Because most programs employ different algorithms and scoring techniques, reproducibility of findings from one program to another is nearly impossible. Artificial intelligence (AI) and deep learning (DL) have become more prevalent in recent years, and public interest in these technologies has increased. One of the most significant advancements in in-silico research is the incorporation of AI into the drug development process. These approaches are becoming more widely available and are highly accurate. However, it has been observed that *in silico* methods are extremely useful when it comes to researching the therapeutic potential of different molecules, analyzing the interactions between viral targets and their potential/possible inhibitors, as well as designing new agents usable in prevention or therapy. The applicability of these methods of drug screening, is of real use both in the field of designing innovative drugs for treating various aspects of COVID-19 and regarding their applicability in the event of a future pandemic. In summary, the

development of all these biomedical tools mentioned above opens new opportunities in testing and discovering innovative therapies, contributing to obtaining fundamental results with immediate usability in adopting precise intervention strategies / protocols in cases of need or emergency.

## Funding

The APC was funded by the University of Oradea. The research was funded by Romanian Ministry of Research, Innovation and Digitisation through Programme 1—Development of the National Research and Development System, Subprogramme 1.2—Institutional Performance—Projects for funding the excellence in RDI, Contract No. 29 PFE/30.12.2021 with University of Oradea, Oradea, Romania.

## CRedit authorship contribution statement

**Paul Andrei Negru:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Denisa Claudia Miculas:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Tapan Behl:** Supervision, Methodology. **Alexa Florina Bungau:** Visualization, Data curation, Investigation. **Simona Gabriela Bungau:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

## Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

All data used in this research are supported by the References inserted in the References section.

## Acknowledgments

The authors also thank the University of Oradea, for supporting the publication of this manuscript.

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