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Discovery of Taroxaz-104: The first potent antidote of SARS-CoV-2 VOC-202012/01 strain

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2,5-Disubstituted-1,3,4-oxadiazole agent
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ABSTRACT

Polyhydroxyphenols and nitrogenous heterocyclics are two of the most powerful active species of molecules in pharmaceutical chemistry, as each of them is renowned for its various bioactivities for humans. One of their outstanding actions is the antiviral activities, which clearly appear if the principal functional entities of both classes meet into one compound. The recent COVID-19 pandemic pushed us to computationally sift and assess our small library of synthetic 2-(3,4,5-trihydroxyphenyl)-1,3,4-oxadiazoles against the main coronaviral protein/enzymatic targets. Surprisingly, few ligands exhibited interesting low binding energies (strong inhibitory affinities) with some SARS-CoV-2 proteins, mainly the pivotal enzyme RNA-dependent RNA polymerase (nCoV-RdRp). One of these compounds was Taroxaz-104 (5,5′-{5,5′-[(1*R*,2*R*)-1,2-dihydroxyethane-1,2-diyl]bis(1,3,4-oxadiazole-5,2-diyl)}dibenzene-1,2,3-triol), which presented lower binding free energies of about -10.60 and -9.10 kcal/mol (as compared to the reference agent, GS-443902, which presented about -9.20 and -7.90 kcal/mol) with nCoV-RdRp-RNA and nCoV-RdRp alone, respectively. Extensive molecular modeling examination disclosed the potent Taroxaz-104 inhibition of one of the possible active/allosteric sites of nCoV-RdRp, since Taroxaz-104 molecule interacts with at least seven main amino acids of the presumed pocket/cavity of this nCoV-RdRp active site. The effective repurposing of Taroxaz-104 molecule was attained after the satisfactorily interesting results of the anti-COVID-19 bioassay were secured, since these data demonstrated that Taroxaz-104 showed very efficient anti-COVID-19 actions (anti-SARS-CoV-2 $EC_{50} = 0.42\ \mu M$) with specific promising efficacy against the new SARS-CoV-2 strains. Additional research studies for the progress of Taroxaz-104 and other related polyphenolic 2,5-disubstituted-1,3,4-oxadiazole analogs as successful anti-SARS-CoV-2 medications, *via*, e.g., preclinical/clinical trials, are pressingly required.

1. Introduction

A year and a half ago at the last days of 2019, a deadly novel coronavirus (2019-nCoV), which is called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), abruptly emerged in China [1]. With its continuous prevalence, the respiratory symptoms of the virus characteristic infection in humans (coronavirus disease 2019 "COVID-19") had been detected and the global pandemic appeared [1,2]. Currently, in July 2021, about 184 millions of COVID-19 cases have been globally affirmed, with more than 4 millions of them sadly died as a consequence of the untreated fatal effects and symptoms of the infection [3]. The predictions for emergence of more malicious new mutated strains in 2021 were already present in 2020, since then all the relevant scientists everywhere in the world are in a hurry to reach successful potent medications able to fight this viral enemy and cure the dangerous severe sequelae of the COVID-19 [2,4].

In July of 2020, the first three effective synthetic under-investigation anti-COVID-19 agents (Cyanorona-20, CoViTris2020, and ChloViD2020) have been successfully discovered [5,6] (Fig. 1). Drug repositioning is a strategy which employs a well-known molecule in a new chance to combat a challenging disease. This tactic could be considered as a substitutional drug development strategy that is characterized by a shorter time period and reduced costs when compared to the traditional methods of developing new therapies [7]. Furthermore, since the predominant inflammatory conditions and oxidative stress significantly contribute to almost all pathogeneses and molecular pathways of diseases, there are considerable chances in testing the probability of repurposing known antioxidant/antiinflammatory agents as therapeutic

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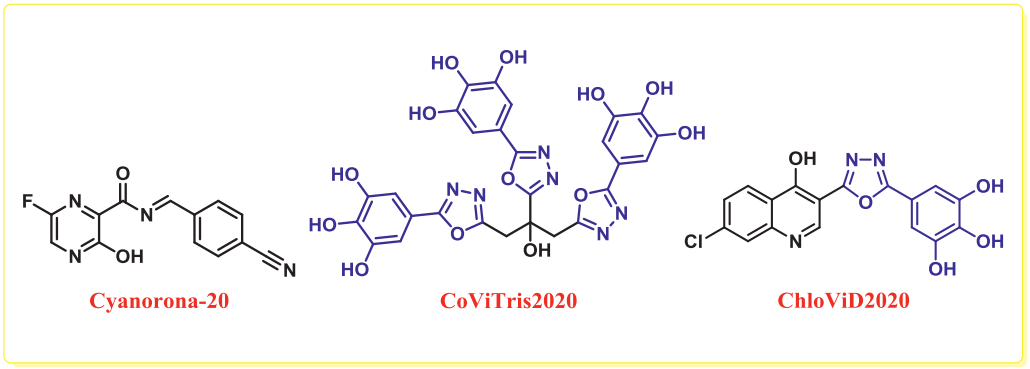


Fig. 1. Structural formulas of the recently-discovered under-investigation anti-SARS-CoV-2 agents (Cyanorona-20, CoViTris2020, and ChloViD2020).

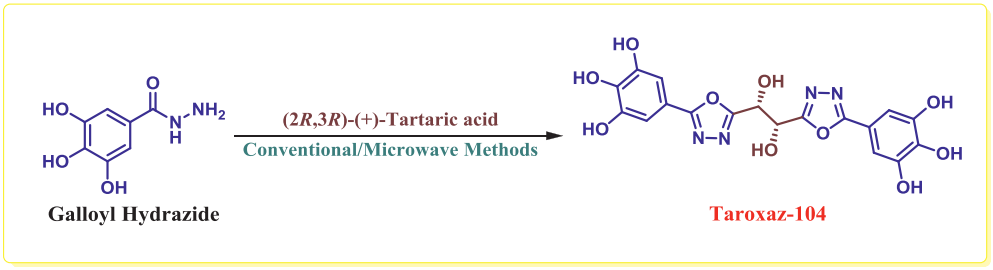


Fig. 2. Synthetic scheme and chemical structure of Taroxaz-104 compound.

options for COVID-19 [8,9]. The role of drug repurposing against SARS-CoV-2 for the COVID-19 pandemic is highly apparent [10], since many old drugs, e.g., hydroxychloroquine, remdesivir, favipiravir, ivermectin, and arbidol, were proposed to have significant efficacy and effectiveness against this virulent virus [11–22]. Making maximal use of this drug repositioning strategy, we have theoretically/computationally sifted and tested almost all the compounds of our own small libraries (with a focus on those agents that have antioxidant, antiinflammatory, and antiviral activities) [23,24] against the known target COVID-19 proteins. Up to June 2020, about 181 SARS-CoV-2 protein constructions were totally detected and released in the Protein Data Bank (PDB), which are mainly related to more than eleven diverse types of the SARS-CoV-2 proteins. Interestingly, this screening and reassessment gave rise to the development of mainly three potent anti-COVID-19 agents of the polyhydroxy-containing 2,5-disubstituted-1,3,4-oxadiazole type, ChloViD2020 [6] (Fig. 1), Taroxaz-104 (which is reported herein) (Fig. 2), and CoViTris2020 [6] (Fig. 1), which have one, two, and three 5-(3,4,5-trihydroxyphenyl)-1,3,4-oxadiazole moiety(moieties), respectively.

Chemically, the polyphenolic Taroxaz-104 compound is 5,5′-{5,5′-[(1*R*,2*R*)-1,2-dihydroxyethane-1,2-diyl]bis(1,3,4-oxadiazole-5,2-diyl)}dibenzene-1,2,3-triol that was precedingly synthesized from the dicarboxylic acid (2*R*,3*R*)-(+)-tartaric acid through either the conventional route or the microwave one, and evaluated as a successful antioxidant agent since about five years [23] (Fig. 2). Taroxaz-104 molecule can be considered as a dimer of the ideal 2-substituted-5-(3,4,5-trihydroxyphenyl)-1,3,4-oxadiazole monomer 5-[5-(hydroxymethyl)-1,3,4-oxadiazol-2-yl]benzene-1,2,3-triol (HMOBT; Fig. 3). Polyphenols are extremely bioactive natural phytochemical classes, since they have strong several pharmacological activities, e.g., antioxidant/anticancer, antiinflammatory/immunomodulatory, antimicrobial/antiviral, and recently potential anti-SARS-CoV-2 activities [23–31]. Polyphenols are expected to block most stages of the SARS-CoV-2 life cycle [25–31]. It is important to know that the antioxidant Taroxaz-104 compound has a considerable number of oxygen atoms (10 atoms) and nitrogen atoms (4 atoms) in its structure, making it a suitable prospective inhibitor of the viral proteins (e.g., coronaviral-2 protein). Taroxaz-104 chemical structure is obviously featured by a significant degree of structural bioflexibility; therefore the flexible backbone of this molecule is supposed to add further inhibitory/blocking binding capabilities to its net chemical structure when striking any coronaviral protein [6]. The considerable number of aromatic nuclei and hydrogen bond-donating/accepting entities (which afford the potent and hydrophobic interactions and hydrogen bonds needed for the perfect binding with the binding domains of most active pockets of the COVID-19 targets [6]) is also predicted to strengthen the inhibitory binding abilities of Taroxaz-104 molecule against the coronaviral-2 particles. It is worth noticing that Taroxaz-104 ligand structure has a considerable similarity with SARS-CoV-2 RNA nucleotides, making this polyphenolic compound able to act as an antimetabolite in COVID-19 therapy. In addition, the nitrogen/oxygen-containing structure of Taroxaz-104 is distinguished by a favorable predicted advantage of being a strong multiple zincophore (i.e., a potent Zn atom-chelating/sequestering agent), as it has more than fourteen active zincophoric moieties, making it an excellent model candi-

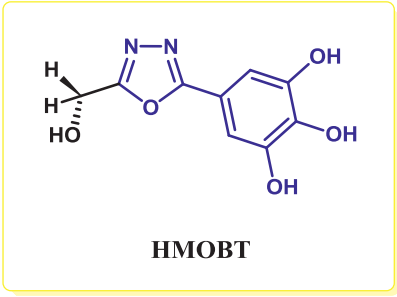


Fig. 3. Chemical structure of the monomeric molecule HMOBT.

date to act as a potent Zn-transporting agent. Zn^{2+} ions, in specific concentrations, were reported to highly inhibit SARS-CoV-2 RNA-dependent RNA polymerase (nCoV-RdRp) bioactivity, specially in the *in vitro* environment, therefore they have a critical role in ruling the performance of the coronaviral-2 RNA-synthesizing machinery and the replication processes of SARS-CoV-2 particles inside the cells [6,15,20,32]. Blocking the replication processes mediated by the polymerase is a recently-designed strategy to combat the COVID-19 pathogenesis through searching for zincophoric ligands that have very potent zinc-carrying properties, thus Taroxaz-104 is predicted to act as an anti-COVID-19, additionally, through serving as a powerful anti-SARS-CoV-2 zincophoric agent [6,15,20,32]. The strong resonance effect of Taroxaz-104 molecule structure may have a significant role in decreasing the binding energies and potentiating the inhibitory biostabilities of the molecular complexes of Taroxaz-104 with the allosteric/active residues of the targeted coronaviral-2 enzymes/proteins (such as nCoV-RdRp), and, as a result, elevating the net potential anti-SARS-CoV-2 and anti-COVID-19 activities [6,23]. Mechanistically, the favorable antioxidant activities of Taroxaz-104 may also uphold its expected clinical anti-COVID-19 activities through the potent reduction of the oxidizing parts of the targeted SARS-CoV-2 proteins, resulting in deactivation of these active enzymes/proteins (it is also worth mentioning that these reducing activities will have important secondary roles in diminishing the critical overall oxidative stress/inflammatory status of the SARS-CoV-2-infected patients [4,23]). The presence of the active antimicrobial heterocyclic 1,3,4-oxadiazole rings in the chemical structure of Taroxaz-104 compound is supposed to considerably aid and boost the anti-COVID-19 effects of Taroxaz-104 ligand [6,23]. The eligible balanced proportions of lipophilic/hydrophilic characteristics of Taroxaz-104 molecule are ideal to give the desired optimal pharmacokinetic properties and druglikeness parameters (Taroxaz-104 has an expected moderate log *P* value, which is smaller than 5) [33].

The prime computational docking studies of Taroxaz-104 ligand in all the known coronaviral-2 protein macromolecules detected the interestingly potent binding affinities and interactions with the coronaviral-2 protein structures [34], showing specific promising data with the pivotal enzyme nCoV-RdRp. The encouraging preliminary findings were far reassessed through another docking server to make sure of the previous data and extensively examine the nCoV-RdRp-Taroxaz-104 binding interactions in broader details. The computational molecular reevaluation affirmed the strong inhibitory interactions of the Taroxaz-104 ligand with several amino acids of the most pivotal interactive domains and motifs of the nCoV-RdRp macromolecule. Depending on the preceding encouraging strong computational multitarget/specific-target binding actions of Taroxaz-104 with SARS-CoV-2, the bioevaluation of the anti-COVID-19 effects of Taroxaz-104 compound was performed using a new *in vitro* anti-COVID-19 test/assay. Comparable to the predictive computational data, the anti-COVID-19 test values were also very significant, as these values even considerably surpassed those of the reference agent (potent positive control drug). All the previous encouraging results uphold the potential pharmacological effectiveness of Taroxaz-104 compound as a very potent anti-COVID-19 agent.

2. Methods

2.1. Computational molecular modeling protocols of Taroxaz-104

The fundamental computational screening and modeling (to speculatively expect and assess the anticoronaviral-2 features) of Taroxaz-104 were carried out utilizing the COVID-19 Docking Server, which is one of the newest molecular docking web servers programmed for testing and predicting the inhibitory binding mechanisms between important COVID-19 targets and diverse candidate ligands, using mainly the renowned AutoDock Vina and CoDockPP molecular docking engines [35]. Open Babel program was used to perform 3D coordinate generation and format transformation of all the uploaded files. The strong antiviral active metabolite of remdesivir, GS-443902, was employed as the positive control compound (reference) for comparator purposes [36]. Taroxaz-104 and GS-443902 chemical structures were better uploaded in the form of strict SDF file format. For simulative docking of Taroxaz-104 and GS-443902, computational type was put on the "Docking" mode. A balanced value of 12 (which is the default exhaustiveness value option in this server) was selected to get the best possible accurate docking results. JSmol software was used to visualize the top ten models outputs in 3D representations.

To affirm the data resulted from the COVID-19 Docking Server methodology and examine the best inhibitory binding interactions/modes in deeper details, a secondary web server, PatchDock, was utilized for molecular docking [37]. PatchDock web server is an adequate rigid molecular docking software which chiefly depends on the principles of shape complementarity [37,38]. The allowed inputs in this server are the chemical structures of any two molecules (e.g., compounds/drugs, enzymes/proteins/peptides, DNA/RNA, and antibodies/antigens), while the obtained outputs are full lists of predicted complexes sorted by the various standards of shape complementarity. To start the docking procedure for both molecules, Taroxaz-104 and GS-443902, the files of the optimized free chemical structures of the receptor (the enzyme nCoV-RdRp of the PDB code 7BV2; the protein which gives the strongest inhibitory binding interactions and best scores from the preceding COVID-19 Docking Server methodology) and the ligand (Taroxaz-104/GS-443902) molecules were uploaded in PDB format in their relevant positions in the docking web page of the server. A clustering RMSD default value of 4.0 along with an enzyme-inhibitor complex type were opted in this current case. Resulted Data were seen and downloaded in the form of solution tables (data were ordered in a downward order according to the definitive score) in the results web page. Furthermore, the best PatchDock solutions were additionally refined in another validated web server, FireDock Server, which is employed for rapid and conclusive refinement of the docking binding interactions [39].

After carrying out the molecular docking methodologies, the constructures of nCoV-RdRp-Taroxaz-104 complex and nCoV-RdRp-GS-443902 complex were further analyzed (3D) and accurately characterized through using the renowned automated Protein-Ligand Interaction Profiler (PLIP) web server [40,41]. A full speculative toxicological study was done using the ProTox-II "Prediction of Toxicity of Chemicals" web server, which is a virtual laboratory for the prediction of small molecules toxicities [42]. ProTox-II web server combines the principles of molecular similarity, fragment propensities, fragment similarity-based cluster cross-validation machine learning, and most frequent features of artificial intelligence (AI), forming a collection of more than 33 models for the prediction and analysis of diverse toxicity endpoints, e.g., cytotoxicity, general acute toxicity, organ toxicity "hepatotoxicity", carcinogenicity, immunotoxicity, mutagenicity, adverse outcomes (Tox21) pathways, and toxicity targets. Toxic doses in this virtual laboratory are given as LD50 (Lethal Dose 50%, i.e., the median lethal dose) values in mg/kg body weight (BWt) [42–44]. The LD50 can be defined as the dose at which 50% of test subjects die upon exposure to a compound [42–44]. This web server assorts chemicals into 6 gradual/descending toxicity classes (according to the Globally Harmonized System of Classification and Labeling of Chemicals "GHS"); which are class I "fatal if swallowed" ($LD50 \leq 5$ mg/kg BWt), class II "fatal if swallowed" (5 mg/kg BWt $<$ $LD50 \leq 50$ mg/kg BWt), class III "toxic if swallowed" (50

mg/kg BWt < LD50 ≤ 300 mg/kg BWt), class IV "harmful if swallowed" (300 mg/kg BWt < LD50 ≤ 2000 mg/kg BWt), class V "may be harmful if swallowed" (2000 mg/kg BWt < LD50 ≤ 5000 mg/kg BWt), and class VI "nontoxic" (LD50 > 5000 mg/kg BWt) [42–44].

2.2. In vitro anti-COVID-19 and cytotoxicity bioassays of Taroxaz-104

This *in vitro* anti-COVID-19 bioassay principally depends on the original protocol and methodologies of Chu and colleagues in the literature [11,45]. All the procedures and steps were done in a very specialized biosafety level 3 laboratory (BSL-3 Lab.). The tested new viral strain of SARS-CoV-2, the first Variant of Concern from 2020, December (VOC-202012/01), was carefully extracted from the fresh nasopharynx aspirate and throat swab of a confirmed 40-years-old COVID-19 male patient using Vero E6 cells (ATCC CRL-1586). As a first step, the stock virus ($10^{7.25}$ $TCID_{50}$/mL) was prepared after three serial passages in Vero E6 cells in infection media (DMEM supplemented with 4.5 g/L D-glucose, 100 mg/L sodium pyruvate, 2% FBS, 100,000 U/L Penicillin-Streptomycin, and 25 mM HEPES). Following the original procedures of Rabie and coworkers, Taroxaz-104 agent was perfectly synthesized (beginning with the intermediate material galloyl hydrazide), purified (≥ 96% purity), elucidated/characterized, and loaded in a middle-size dark brown glass container to be used in the procedures [23]. The pure reference drug, GS-443902, was bought from MedChemExpress (MCE®) to be used as the positive control agent in both the anti-COVID-19 and cytotoxicity assays. The standard stocks of the two assayed compounds were precisely prepared by dissolving each of them in dimethylsulfoxide "DMSO" (100 mM concentration). To assess the *in vitro* anti-SARS-CoV-2 effects of the targeted compound, Taroxaz-104, as compared to those of GS-443902, Vero E6 cells were pretreated with the two ligands diluted in infection media for 1 h prior to infection by the novel viral variant of the SARS-CoV-2 at MOI = 0.02. Both tested compounds were maintained with the virus inoculum during the 2-h incubation period. The inoculum was removed after incubation, and the cells were overlaid with infection media containing the diluted tested compounds. After 48 h of incubation at 37°C, supernatants were instantly collected to quantify viral loads by $TCID_{50}$ assay or quantitative real-time RT-PCR "it is also called qRT-PCR" (TaqMan™ Fast Virus 1-Step Master Mix) [11,45]. Viral loads in this assay were fitted in logarithm scale ($\log_{10}$ $TCID_{50}$/mL and $\log_{10}$ viral RNA copies/mL) [11,45], not in linear scale [46], under ascending concentrations/amounts of the tested compounds. Four-parameter logistic regression (GraphPad Prism) was employed to fit the dose-response curves and estimate the EC_{50} of the tested compounds that inhibit SARS-CoV-2 viral replication ($CPEIC_{100}$ was also calculated for each assayed compound). Cytotoxicities of the two tested agents were estimated in Vero E6 cells using the renowned CellTiter-Glo® Luminescent Cell Viability Assay (Promega) [11,47]. Final resulted data were represented as the mean (μ) ± the standard deviation (SD) from the bioassays (which were done in triplicates). Statistical analysis was carried out by employing SkanIt 4.0 Research Edition software (Thermo Fisher Scientific) and Prism V5 software (GraphPad). All reported data were significant at $p < 0.05$.

3. Results and discussion

3.1. Computational molecular modeling outputs of Taroxaz-104

The virtual molecular docking examination of Taroxaz-104 ligand showed that the most powerful inhibitory binding interactions, and consequently the maximum effectiveness, of the compound specifically appeared on striking the nCoV-RdRp; therefore, Table 1 concentrates on the obtained data for the nCoV-RdRp-Taroxaz-104 complex in comparison with the reference nCoV-RdRp-GS-443902 complex. The recognition and interpretation of the best nCoV-RdRp-Taroxaz-104 binding modes paves the ways to develop this compound as a potential anti-COVID-19 medication. It also aids in proving the proposed anti-SARS-CoV-2/anti-COVID-19 mechanism of action of Taroxaz-104 along with its expected spectrum of effectiveness.

Table 1
Score values (of the least binding free energies) of the predicted anti-nCoV-RdRp characteristics of the target polyphenolic compound, Taroxaz-104, and the potent reference drug, GS-443902, respectively, using the COVID-19 Docking Server methodology.

Target COVID-19 Polymerase	Top Pose Score Value of Inhibitory Binding Energies for Docking of nCoV-RdRp Protein Target (kcal/mol)	
	Taroxaz-104	GS-443902
RdRp-RNA (RTP site)	-10.60	-9.20
RdRp (RNA site)	-9.10	-7.90

Upon inspection of the hypothetically-estimated best pose score values (Table 1) of docking nCoV-RdRp (with and without RNA, respectively) using COVID-19 Docking Server, it is clearly observed that Taroxaz-104 ligand has a considerable superiority over GS-443902 ligand in the inhibitory binding affinities, with highly stable binding energies of -10.60 and -9.10 kcal/mol with RdRp-RNA (RTP site) and RdRp alone (RNA site), respectively. The preceding data reflect the significant detected nCoV-RdRp-inhibiting properties of the potent antioxidant target compound Taroxaz-104, as these values interestingly override those of the used reference GS-443902 (this reference agent has lower binding energies of -9.20 and -7.90 kcal/mol with RdRp-RNA and RdRp alone, respectively), through showing the potent interactions/binding with the SARS-CoV-2 polymerase enzyme with/without RNA (these strong interactions compose very stable two deactivated complexes). These promising computational results are very motivating as they support the high possibility of Taroxaz-104 to be a very potent potential inhibitor of nCoV-RdRp. Fig. 4**a-d** displays the respective docking output 3D images using COVID-19 Docking Server methodology (PDB code of the used nCoV-RdRp: *7BV2*).

To affirm the resulted data from the COVID-19 Docking Server, the complementary PatchDock Server methodology was done. The 2D representations in Fig. 5**a,b** support that Taroxaz-104 interacts and binds with at least one of the proposed allosteric/active sites (motifs and domains) of the nCoV-RdRp protein in similar mode(s) of action to that/those of GS-443902. Taroxaz-104 molecule approximately hits 50% of the same active amino acids that the reference GS-443902 binds with in the chain A of the complex structure of nCoV-RdRp, which are His256, Tyr265, Ile266, Lys267, Trp268, Pro322, and Pro323, respectively, disclosing another allosteric/active site(s) in this polymerase in addition to the previously-hypothesized one known for the potent antiviral favipiravir [48,49]. Taroxaz-104 interacts with the previously-mentioned 7 amino acid residues with stronger interactions than GS-443902 does, therefore it is expected that Taroxaz-104 displays better inhibitory binding interactions with nCoV-RdRp than GS-443902 does. It was also revealed that Taroxaz-104 forms higher number of strong hydrophobic interactions and hydrogen bonds with the nCoV-RdRp as compared to GS-443902 (this is mainly due to the relatively higher number of aromatic rings/hydroxyl groups present in Taroxaz-104 molecule). All these results largely uphold the preceding data resulted upon employing the methodology of COVID-19 Docking Server.

PLIP 3D visualization of the best poses of docking Taroxaz-104 and GS-443902 ligands in nCoV-RdRp protein (Fig. 6**a,b**) displays the considerably-balanced degrees of molecular flexibility of

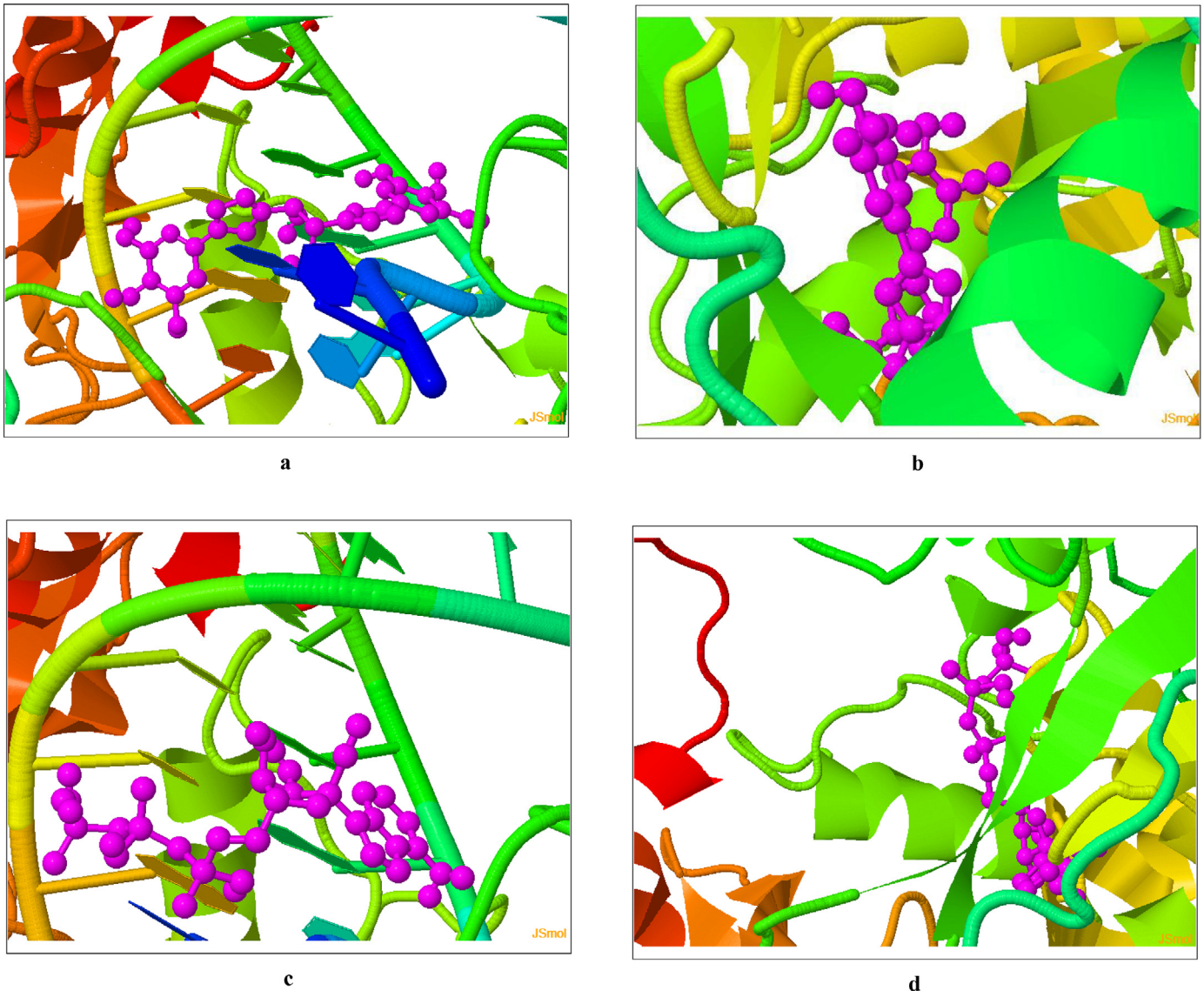


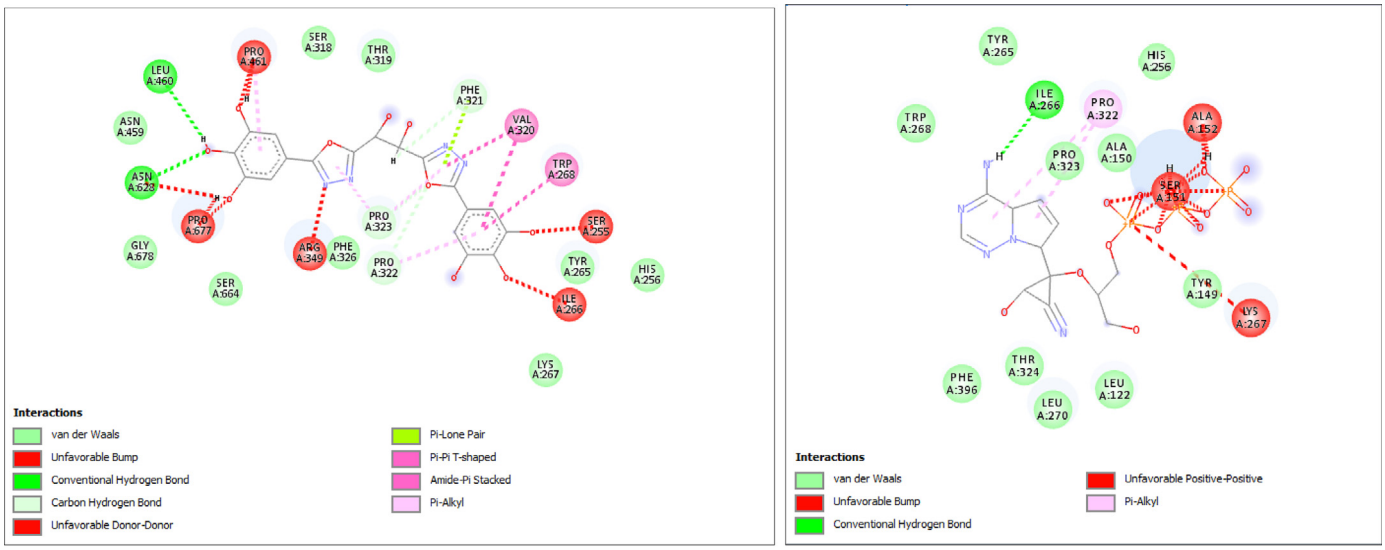
Fig. 4. 3D-represented COVID-19 Docking Server outputs of the top predicted binding model of docking of Taroxaz-104 molecule (colored pink) in: (**a**) nCoV-RdRp-RNA "RTP site" (colored with other various colors), (**b**) nCoV-RdRp "RNA site" (colored with other various colors); and GS-443902 molecule (colored pink) in: (**c**) nCoV-RdRp-RNA "RTP site" (colored with other various colors), (**d**) nCoV-RdRp "RNA site" (colored with other various colors).

Taroxaz-104 compound in comparison to GS-443902 agent which shows lower flexibility. Taroxaz-104 molecule is predicted to be superflexible inside the biosystems, as its chemical structure is a bulky disubstituted derivative of tartaric acid with larger topological polar surface area (TPSA) and higher number of atoms as compared to the chemical structure of GS-443902 molecule. This significant apparent flexibility is much needed for any hitter/ligand of the enzyme nCoV-RdRp to fit its structural backbone with the ideal posture and targeting of the main active binding pockets of the COVID-19 polymerase macromolecule, resulting in more efficient and strong inhibition/blockade of the coronaviral-2 replication processes which are carried out and controlled by the nCoV-RdRp enzyme.

The prediction of toxicities of any new or repurposed compound is a very important aspect of the drug design development journey. Interestingly, modern virtual toxicity estimations have many desired advantages over the actual classic toxic dose determinations in animals, since they, e.g., provide faster results, minimize research expenses, and reduce the number of animal experiments. The data resulted from utilizing the ProTox-II Virtual Laboratory revealed the high predicted biosafety of Taroxaz-104 compound (over the more toxic GS-443902 agent). The results showed that Taroxaz-104 lies in the safe toxicity class V with a predicted LD50 $\geq$ 2032 mg/kg BWt (with a prediction accuracy of about 55%), which is approaching the mean value of dataset (see Fig. 7). The resulted data also predicted that Taroxaz-104 is cytotoxicologically inactive (with a probability percentage of about 75%). Interestingly, analysis of all the diverse toxicity endpoints using the diverse ProTox-II toxicity model tests showed that Taroxaz-104 has very significant degrees of safety in respect to different organ toxicities and adverse outcomes pathways. Almost all the preceding computational/virtual predictions significantly uphold the primary theoretical speculation for Taroxaz-104 compound to effectively act as a very potent safe anti-COVID-19 agent (i.e., a successful SARS-CoV-2 inhibitor).

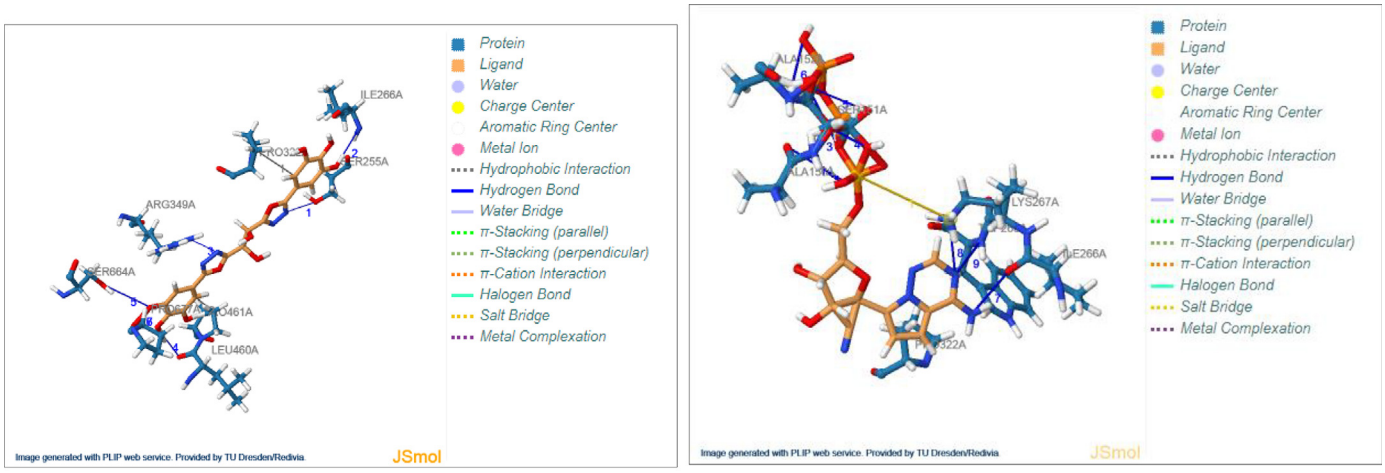
3.2. In vitro anti-COVID-19 and cytotoxic bioactivities of Taroxaz-104

The data obtained from the *in vitro* anti-COVID-19 and cytotoxicity assays (concentrations are in μM) are presented in



a b

Fig. 5. 2D-representated inhibitory binding interactions, of **a**) Taroxaz-104; **b**) GS-443902 (showing opening and rearrangement of the ribofuranosyl moiety into smaller cyclopropane ring during the predicted metabolic and striking procedures), with the amino acid residues of one or more of the allosteric/active sites of nCoV-RdRp.



a b

Fig. 6. 3D-represented inhibitory binding interactions, of **a**) Taroxaz-104; **b**) GS-443902, with the amino acid residues of one or more of the allosteric/active sites of nCoV-RdRp (using PLIP web service).

Table 2. Recently, highly resistant and virulent variants and lineages (i.e., more prevalent and infectious strains) of SARS-CoV-2 have been detected (e.g., VOC-202012/01, previously recognized in the U.K. as the first Variant Under Investigation in December 2020 "VUI-202012/01" and also as the lineage B.1.1.7 or 501Y.V1; and 501.V2, which was chiefly appeared and traced in South Africa) due to several genetic mutations, specially in the structure of the coronaviral-2 spike (resulting in considerable modifications and changes in the spike receptor binding site), and almost all of these new strains are still under accurate deep tracing (Fig. 8) [50–53]. The results of Table 2 interestingly disclosed the considerably higher SARS-CoV-2 inhibitory activity of Taroxaz-104 (specially against the new mutant strains of SARS-CoV-2) in comparison to that of the reference drug GS-443902. Taroxaz-104 was found to severely inhibit SARS-CoV-2 replication and transcription in Vero E6 cells with EC_{50} much smaller than 100 μM. Taroxaz-104, with EC_{50} = 0.42 μM, exhibits about 43 times the potency of GS-443902 (EC_{50} = 18.05 μM) in respect to the *in vitro* anti-VOC-202012/01/anti-SARS-CoV-2 bioactivity. The results of the cytotoxicity assay showed that the CC_{50} of Taroxaz-104 is much higher than 100 μM, therefore this compound is expected to have very significant clinical selectivity index "SI" ($SI_{Taroxaz-104}$ > 238.10; which is considerably broader than that of the positive control agent GS-443902, $SI_{GS-443902}$ > 5.54), reflecting the specific/selective anti-RNA activities of the molecule against the new coronaviral-2 variants/strains rather than the human genetic material. Taroxaz-104 shows also very small value of the concentration that causes 100% inhibition of the cytopathic effects of the coronaviral-2 VOC-202012/01 strain *in vitro* ($CPEIC_{100}$ = 1.20 μM), which is much smaller than that of the reference GS-443902 ($CPEIC_{100}$ = 20.50 μM). Furthermore, Taroxaz-104 displays very minute value of the concentration that causes 50% reduction in the RNA copies of SARS-CoV-2 (VOC-202012/01 strain) *in vitro* (0.46 μM), which is again much smaller than that of GS-443902 (18.67 μM).

Importantly, we cannot ignore the present possibility that Taroxaz-104 molecule may intracellularly undergo a biotransformative metabolism into more active forms (e.g., diverse phosphate

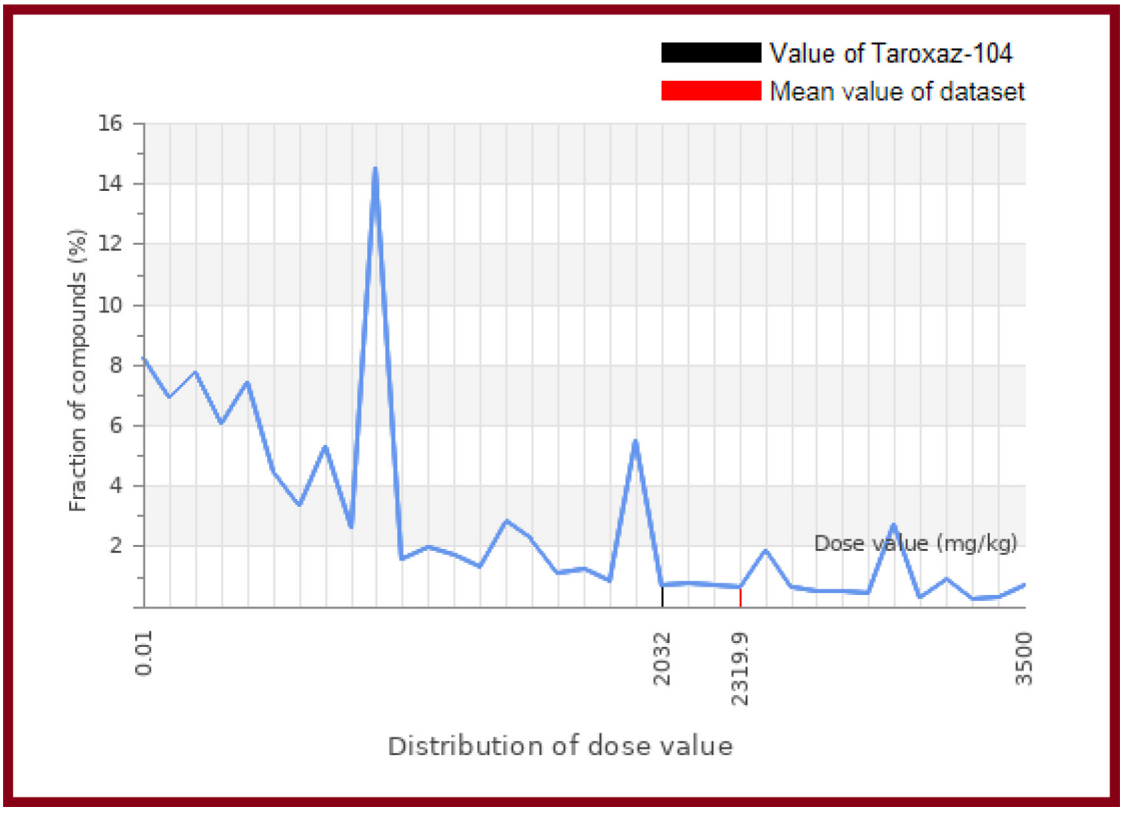


Fig. 7. Comparison of LD50 of Taroxaz-104 (input compound) with that of dataset compounds (using ProTox-II Virtual Laboratory methodology).

Table 2
Anti-SARS-CoV-2/anti-VOC-202012/01 (anti-COVID-19) bioactivities and *in vitro* cytotoxicities of the target compound, Taroxaz-104, and the reference agent, GS-443902, against SARS-CoV-2 (VOC-202012/01 strain) in Vero E6 cells.

			Inhibition of SARS-CoV-2 *in vitro* (Anti-VOC-202012/01 Bioactivities) (μM)		
Classification	Compound Name	CC_{50}[a] (μM)	100% CPE Inhibitory Concentration ($CPEIC_{100}$)[b]	50% Reduction in Infectious Virus (EC_{50})[c]	50% Reduction in Viral RNA Copy (EC_{50})[d]
Target Compound	Taroxaz-104	> 100	1.20 ± 0.08	0.42 ± 0.02	0.46 ± 0.03
Reference Compound	GS-443902	> 100	20.50 ± 0.93	18.05 ± 0.88	18.67 ± 0.90

[a] CC_{50} or 50% cytotoxic concentration is the concentration of the tested compound that kills half the cells in an uninfected cell culture. CC_{50} was determined with serially-diluted compounds in Vero E6 cells at 48 h postincubation using CellTiter-Glow Luminescent Cell Viability Assay (Promega).

[b] $CPEIC_{100}$ or 100% CPE inhibitory concentration is the lowest concentration of the tested compound that causes 100% inhibition of the cytopathic effects (CPE) of SARS-CoV-2 VOC-202012/01 virus in Vero E6 cells under increasing concentrations of the tested compound at 48 h postinfection. Compounds were serially diluted from the stock 100 mM concentration.

[c] EC_{50} or 50% effective concentration is the concentration of the tested compound that is required for 50% reduction in infectious SARS-CoV-2 VOC-202012/01 virus particles *in vitro*. EC_{50} is determined by infectious virus yield in culture supernatant at 48 h postinfection ($\log_{10}$ $TCID_{50}$/mL).

[d] EC_{50} or 50% effective concentration is the concentration of the tested compound that is required for 50% reduction in SARS-CoV-2 VOC-202012/01 viral RNA copies *in vitro*. EC_{50} is determined by viral RNA copies number in culture supernatant at 48 h postinfection ($\log_{10}$ RNA copies/mL).

forms), which may also differ from tissue to another according to its type (e.g., primary human airway epithelial cells and hepatocytes). The intracellular metabolic activation is supposed to attach very minute biocompatible and bioavailable moieties to the scaffold of Taroxaz-104 molecule, leading to increased total anti-COVID-19 bioactivities and clinical tolerance of Taroxaz-104. All experimental biological results observed here are very consistent with the preceding computational theoretical estimations. Interestingly, the leading superiority, in most anti-COVID-19 properties/activities and cytotoxicological determinations, of the target repurposed compound Taroxaz-104 over the potent reference antiviral agent GS-443902 makes Taroxaz-104 a potent and safe candidate SARS-CoV-2 antidote.

4. Conclusions

Evolution of the diverse new strains and variants of SARS-CoV-2 represents a pivotal challenging watershed point in the current struggle against this dangerous virus. In the second wave of the pandemic, the matter got worse with the appearance of the SARS-CoV-2 B.1.1.7 variant in the U.K. in December of the year 2020, followed by the prevalence of the South African and Brazilian variants, and continued with the emergence of the SARS-CoV-2 B.1.617 variant “the new double mutant variant” in India (the Indian new COVID-19 variant) officially in April 2021. It is worth mentioning that the Indian SARS-CoV-2 Variant of Interest (VOI) has the world sharpest spike in coronavirus infec-

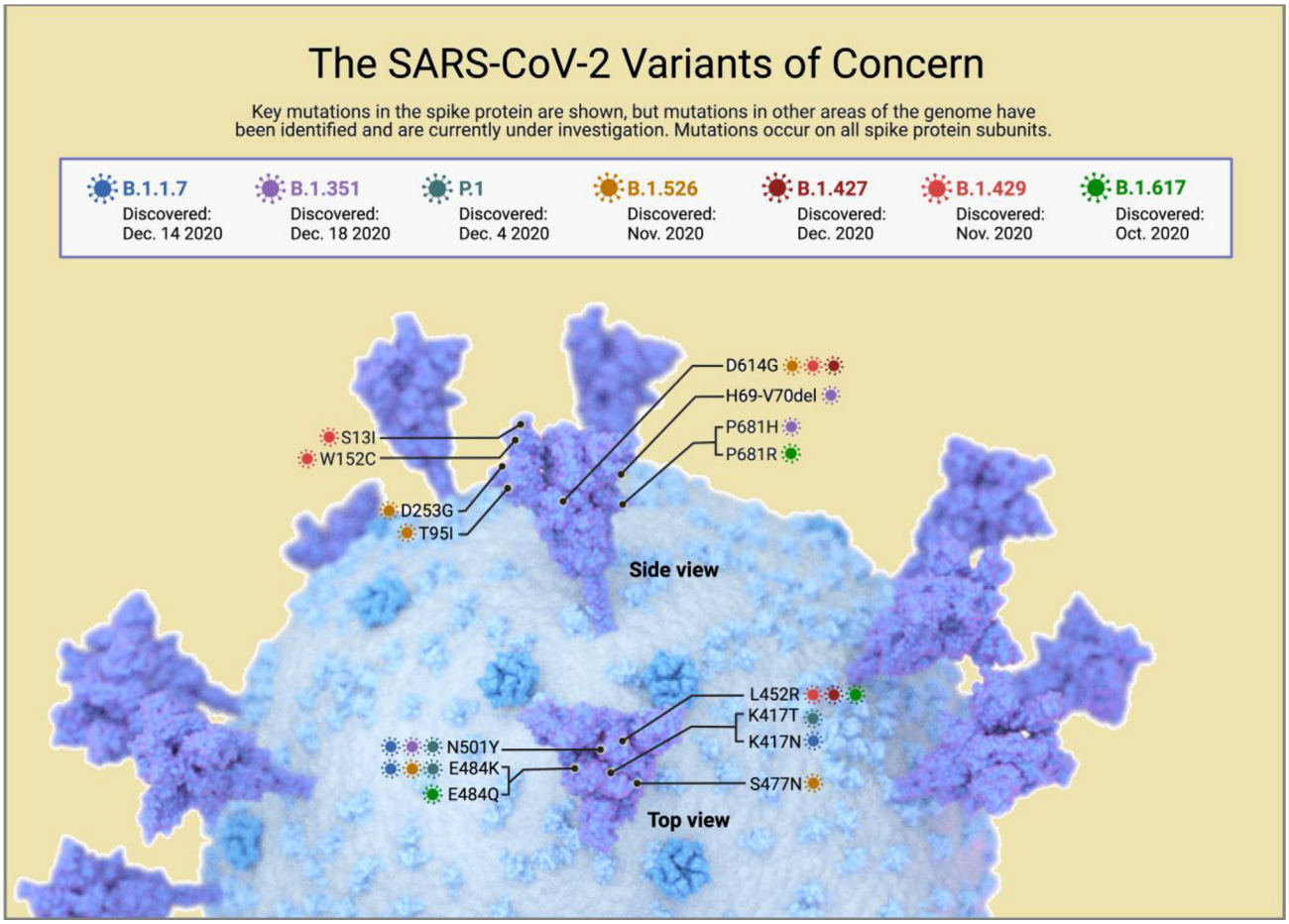


Fig. 8. Presentation of the different types of the SARS-CoV-2 Variants of Concern.

tions until now, since it contains two key mutations to the outer spike portion of the coronavirus that attaches to human cells, this would make the virus more transmissible, causing more severe disease, and/or evading all vaccines immunities [54]. A U.S. government interagency group established a Variant Classification scheme that recognizes three major classes of SARS-CoV-2 variants; they are the VOI class, the VOC class (Fig. 8), and the Variant of High Consequence (VOHC) class [55]. Most virologists and epidemiologists are pointing to the possibility of considerable further opportunities for coronaviral-2 development, specially in patients with significantly-compromised immune systems, since these individuals easily tend to suffer from long-term chronic infections, during which the coronavirus can linger for extended intervals of weeks and months. Strong inhibition/blockade of the most important protein targets of the coronaviral-2 particle, e.g., nCoV-RdRp, is considered as the most efficient and applicable strategy for successful design and development of anti-SARS-CoV-2/anti-COVID-19 drugs. Our extensive efforts and researching led to the promising repositioning and discovery of an interesting potent anti-nCoV-RdRp/anti-SARS-CoV-2 agent through the efficient anti-COVID-19 biological evaluation of the known antioxidant ligand Taroxaz-104 (5,5′-{5,5′-[(1*R*,2*R*)-1,2-dihydroxyethane-1,2-diyl]bis(1,3,4-oxadiazole-5,2-diyl)}dibenzene-1,2,3-triol), which was found to inhibit the most pivotal stage of the coronaviral-2 life cycle, the replication phase, with a very promising EC_{50} value of 0.42 μM. Taroxaz-104 shows about 43-fold anti-SARS-CoV-2 bioactivities (specially against the newly-appeared SAR-CoV-2 mutant, VOC-202012/01) in comparison to the reference agent GS-443902. At the same time, the discovery of the very potent anti-COVID-19 compound Taroxaz-104 makes us receiving and accepting this compound as a third member (beside the two preceding members, CoViTris2020 and ChloViD2020 compounds) of the first discovered therapeutic class of anti-COVID-19 agents (the polyphenolic 1,3,4-oxadiazoles or 2,5-disubstituted-1,3,4-oxadiazoles class) [6]. The preceding inclusive computational/virtual molecular modeling studies disclosed the ideal pharmacokinetic and druglikeness parameters values that Taroxaz-104 compound has. Molecular docking investigation and analysis of the best inhibitory binding models of Taroxaz-104 molecule with the various SARS-CoV-2 enzymatic/protein targets revealed that the polyhydroxyphenyl/1,3,4-oxadiazole species considerably boost the inhibitory interactions at the active site(s) of the COVID-19 polymerase (binding energies reach -10.60 kcal/mol) in comparison to GS-443902 molecule (which lacks both potent antioxidant and electron-rich species). Successful repurposing of Taroxaz-104 ligand offers a very important development in the anti-COVID-19 therapeutic discoveries, as it is the first and pioneer agent that shows very significant inhibiting activities against the more fatal new coronaviral-2 lineages and variants (i.e., Taroxaz-104 is specifically featured by its extra inhibitory anti-COVID-19 activities against the newest and most resistant strains of SARS-CoV-2, and this gives this compound a specific unique property in comparison to the other general under-investigation anti-COVID-19 agents). To get it short, in this new discovery research paper, the antioxidant Taroxaz-104 compound was reported to be successfully repurposed to act as a very promising anti-SARS-CoV-2/anti-COVID-19 agent and, in addition, as the first reported anti-VOC-202012/01 agent.

Declaration of Competing Interest

I hereby declare that I totally have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this new paper.

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