Binding repurposed drugs and aminothiourea derivatives to SARS-CoV-2 enzymes—a docking perspective

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Abstract
Binding of over 20 approved drugs proposed for the repurposing for COVID-19 treatment and over 160 aminothioureas derivatives to SARS-CoV-2 enzymes which structures became available very recently have been evaluated using a few docking algorithms. These studies support potential effectiveness of homobarringtonine, chloroquine, rimcazole, and benserazine. From among studied aminothioureas thia diazoles with pyrrol-derived substituents at the carbon atom, and ortho-hydroxyphenyl at the nitrogen atom are potential lead compounds for future drugs development.

Highlights
Based on docking results to SARS-CoV-2 enzymes homobarringtonine, chloroquine, rimcazole, and benserazine are approved drugs with potential use in treatment of COVID-19 infection Pyrrol substituted thia diazoles with ortho or meta substituted phenyl group attached to the nitrogen atom show strongest binding from among the studied aminothioureas

Introduction
With the outbreak of the SARS-CoV-21 devastating pandemic that claimed already lives of over 50000 people worldwide the need for therapeutics is needed urgently. For the future prevention a few vaccine strategies are being tested. At the same time medicines that can be used in fighting the infection are being sought.

The family of coronaviruses is relatively well known due to the previous severe for humans epidemies of SARS3 and MERS.4 In haste search for medicines instantly available, repurposing of already approved drugs is carried out as the first line of defenses. This approach has been attempted during the MERS epidemic,5 and is also being tried currently.6 In the first of these studies an NIH library of over 700 compounds has been screened experimentally, identifying around 80 with the anti-coronavirus effect. The recent study concentrated on the interaction of the spike protein (S-protein) of the virus with the human ACE2 receptor, which is thought to be responsible for the viral recognition of host cells. By combining virtual high-throughput screening with ensemble docking of over 9000 compounds from the SWEETLAND library,7 and using COVID-19 S-protein (NCBI: YP_009724390.1) and human ACE2 receptor (PDB: 2AJF) as a template to generate the SARS-CoV-2 S-protein and ACE2 receptor complex model, around 80 compounds already in clinical use that should exhibit anti-coronavirus activity have identified.

While repurposing of compounds currently in medical use is the necessity for quick response to public
health threat, in the long run drugs specific to this particular virus will be needed. With this aim in mind we have used the SARS-CoV-2 S-protein - ACE2 receptor complex model and its components to evaluate binding properties of thiosemicarbazides, thiadiazoles, and triazoles that we have studied for their anti-Toxoplasma gondii,8,9,10,11 antiviral,12 antibacterial,13,14,15,16,17,18 anticancer,17,19,20,21 anticonvulsant,22,23,24 analgesic,25,26 activities. Additionally, we have carried out docking to phosphatase (PDB: 6W02),27 hydrolase (PDB: 6W9C),28 protease (PDB: 5RE5),29 and endoribonuclease (PDB: 6W01)30 SARS-CoV-2 enzymes that have been released recently at the Protein Data Bank.31 Our results point to three already identified in the previous studies currently used drugs and add as lead compounds for the future studies two compounds from thiosemicarbazides and their dehydrocyclic derivatives groups.

Material And Methods

Studied compounds

Approved drugs, selected as the best candidates in studies previously reported in literature,5,6 and compounds containing N-N-C(S)-N skeleton have been studied. The considered drugs are listed in Table 1 and referred to as series L1 (best from reference 6) or L2 (best from reference 5).

Additionally, we have studied three drugs (labeled A-1 - A-3). Chloroquine (A-1) was originally used as antiparasitic drug for treatment of malaria, but it has been shown to have also antiviral activity32 and has been currently recommended recently for use in SARS CoV-2 infection.33,34 Thus we have considered the other two which also are potent antiparasitic.

Among the compounds comprising the N-N-C(S)-N skeleton we have evaluated representatives of three classes of linear or cyclic molecules that have been studied in our laboratory in recent years for their inhibitory activity against a few enzymes. Their structures contain three main cores; linear carbonylthiosemicarbazide and its two cyclic derivatives: 1,3,4-thiadiazole and 1,2,4-triazole each decorated with two substituents. As the C-substituent one of the four five-member rings was used while the N-substituent was a benzene ring or its ortho, meta, or para substituted derivatives. All these components are collected in Table 2 where also partial codes for the fragments are provided (in bold). Thus, for example, compound code ICpNO2 corresponds to 1-(4-methylimidazol-5-oyl)-4-(4-
nitrophenyl)thiosemicarbazide, illustrated in Fig. 1. This compound (together with CCpNO₂, CCmI, other IC) has been shown to have no cytotoxicity in our recent studies on anti-Toxoplasma gondii activity (unpublished results).

In total, 191 have been studied; 167 of the above compounds and 24 drugs.

**Table 1.** List of the approved drugs considered in current studies

| Drug Name                  | Code   |
|----------------------------|--------|
| eriodictyol (L1-1)         |        |
| dimethyl coclaurine (L1-2) |        |
| vidarabine (L1-3)          |        |
| tazebactum (L1-4)          |        |
| benserazine (L1-5)         |        |
| quercetol quercitin (L1-6) |        |
| nitrofurantoin (L1-7)      |        |
| protirelin (L1-8)          |        |
| pyruvic acid isoniazid (L1-9) |    |
| pemirolast (L1-10)         |        |
| phenformin (L1-11)         |        |
| sapropterin (L1-12)        |        |
| luteolin monoarabinose (L1-13) |    |
| vildagliptin (L1-14)       |        |
| carbazochrome (L1-15)      |        |
| rimcazole (L2-1)           |        |
| triptolide (L2-2)          |        |
| fludarabine (L2-3)         |        |
| benz bromarone (L2-4)      |        |
| hexachlorophene (L2-5)     |        |
| homobarringtonine (L2-6)   |        |
| chloroquine (A-1)          |        |
| sulfadazine (A-2)          |        |
| trimetoprym (A-3)          |        |

**In silico evaluations**

We have used the published model6 of the interface between the viral S-protein and the human ACE2 receptor that was constructed from COVID-19 S-protein (NCBI: YP_009724390.1) and human ACE2 receptor (PDB: 2AJF) as a template. The structure of this model has been recently confirmed experimentally.35 For the selected compounds we have performed additional docking to other SARS-CoV-2 enzymes, which structures have become available very recently.27-30

A few docking algorithms have been tested. Initially, following literature data, Vina36 docking program has been used. However, the scores obtained for strongest inhibitors were very close and not discriminating. We have, therefore switch to FlexX algorithm,37 as implemented in the LeadIT platform.38 The scoring function of this program did better job in differentiation of binding affinities of studied compounds but showed some constrains, the most serious being the limit on the size of the acceptor, which precluded in some cases searches of the whole enzyme. SwissDock39 at our hands also turned out not practical as only a single ligand per submission to the server was possible. We have therefore finally decided to continue with the ChemPLP algorithm40 as implemented in the Gold program.41 It is also noteworthy that this algorithm has been considered as one of the best in most recent benchmark studies.42
Table 2. List of structural fragments of compounds used in current studies

| R¹ | core | R² |
|----|------|----|
| ![2-methylfuran-3-yl (F)](image) | ![carbonylthiosemicarbazide (C)](image) | ![R³ = H, ortho, meta, or para: F, Cl, Br, I, OH, OMe, Me, NO₂](image) |
| ![4-methyl-imidazol-5-yl (I)](image) | ![1,3,4-thiadiazole (S)](image) |
| ![pyrrol-2-yl (P)](image) | ![1,2,4-triazole-3-thione (T)](image) |
| ![cyclopentyl (C)](image) |

For the proteins and ligands preparation and visualization apart from those embedded in the docking programs, Hyperchem,43 Gaussview,44 Chimera,45 and Mercury46 were used.

Results And Discussion
The focus of the present studies was on the binding to virus S-protein, ACE2 human receptor, and their interface. The best 10 scores for each target, obtained using ChemPLP docking algorithm, are collected in the first 23 rows of Table 3. As can be seen four drugs already in clinical use; L2-1, L2-6, A-1, and L1-5 exhibit the strongest binding. Among these rimcazole (L2-1) is the top one in binding to S-protein of the virus and second in binding to ACE2 receptor and their interface, although its
strongest affinity is to the inside of the ACE2 receptor (with score of 64.37). Also, outstandingly strong binding to the interface is exhibited by chloroquine (A-1) most probably because of interactions with both proteins at the same time, as illustrated in Fig. 2. The source of the antiviral activity of in this case is, however, not clear because A-1 binds even better to the complex than to individual proteins, acting rather like a binder than an inhibitor.

The analysis of structures containing the N-N-C(S)-N motif from the chemical point of view indicates that thia diazoles with pyrrol substituent are among the top binding compounds, especially to the virus S-protein. The substituent attached to the nitrogen atom most frequently contains hydroxyl group in the ortho position.

**Table 3.** ChemPLP docking scores of studied compounds to S-protein, ACE2 receptor and their interface

| compound | virus S-protein | human ACE2 receptor | interface |
|----------|----------------|---------------------|-----------|
| L2-1     | 59.78          | 55.60               | 56.76     |
| L2-6     | 58.94          | 48.61               | 55.93     |
| A1       | 51.94          | 51.48               | 66.29     |
| PSOMe    | 49.05          | 47.45               | 52.09     |
| FCpF     | 49.01          | 45.56               | 46.48     |
| PSOF     | 49.01          | 47.68               | 51.83     |
| PSmMe    | 48.98          | 49.64               | 50.57     |
| PSmCl    | 48.73          | 48.87               | 50.63     |
| PSoCl    | 48.46          | 47.48               | 50.24     |
| PSmBr    | 48.05          | 49.56               | 50.69     |
| L1-5     | 44.44          | 57.86               | 51.50     |
| FSooH    | 39.89          | 53.16               | 54.71     |
| FSoOH    | 45.62          | 52.72               | 54.38     |
| CCCpNO2  | 41.24          | 52.6                | 53.25     |
| FCmF     | 43.07          | 52.41               | 52.27     |
| FCoOH    | 41.71          | 51.80               | 47.11     |
| PCoNO2   | 44.41          | 51.73               | 48.48     |
| FCoOH    | 46.07          | 51.68               | 50.24     |
| PSpOH    | 42.04          | 48.04               | 53.16     |
| PCoOH    | 45.80          | 52.02               | 53.09     |
| FSoMe    | 41.29          | 49.44               | 52.66     |
| FSpOMe   | 40.70          | 47.29               | 52.61     |
| PSOnO2   | 44.16          | 48.76               | 52.61     |
| L2-3     | 39.72          | 47.36               | 39.33     |
| PToNo2   | 42.56          | 47.76               | 42.08     |
| PToi     | 44.58          | 47.85               | 43.61     |
| L1-14    | 45.62          | 49.08               | 44.89     |
| ICoNO2   | 44.57          | 45.51               | 41.78     |
| FCoOH    | 43.88          | 44.08               | 40.61     |
| PToOme   | 42.08          | 44.78               | 41.31     |
| FSH      | 47.85          | 47.73               | 47.34     |
| PTmNO2   | 44.46          | 45.55               | 43.07     |
| L1-13    | 47.85          | 48.87               | 47.35     |
| L2-2     | 39.34          | 38.78               | 37.31     |

In the bottom part of Table 3 we have ranked (by the maximum score difference) compounds which binding to the interface is weaker than to individual proteins, what would be expected for a typical competitive inhibitor. On the top of this list is again approved drug - fludarabine.
These compounds, and the top 3 from the best binding list, have been docked to other SARS-CoV-2 proteins, which crystal structures have been solved recently. There are; ADP ribose phosphatase (PDB: 6W02), papain-like protease (PDB: 6W9C) and main protease (PDB: 5RE5), and endoribonuclease (PDB: 6W01). The results are collected in Table 4.

| compound  | 6W01 | 6W02 | 6W9C | 5RE5 |
|-----------|------|------|------|------|
| L2-3      | 39.93| 62.89| 55.01| 32.54|
| PToNO2    | 49.27| 53.16| 50.92| 37.11|
| L1-14     | 48.55| 54.28| 51.84| 36.14|
| ICNO2     | 43.3 | 70.47| 62.78| 34.61|
| FCPoH     | 35.4 | 63.86| 51.36| 26.37|
| PToOMe    | 34.14| 67.59| 54.28| 27.21|
| FSH       | 41.92| 45.4 | 45.77| 33.89|
| PToNO2    | 49.6 | 54.97| 54.79| 32.42|
| L1-13     | 50.71| 56.96| 60.76| 28.05|
| L2-2      | 40.44| 42.37| 37.46| 25.95|
| L2-1      | 52.89| 62.65| 64.09| 38.56|
| L2-6      | 43.47| 65.55| 62.96| 29.04|
| A1        | 48.97| 56.33| 57.44| 51.79|
| A447      | 49.52| 73.2 | 61.78| 34.82|

As can be seen again the currently used drugs, L2-1 and L1-5 exhibit the largest scores. Also, A1 recently recommended for inclusion in the anticoronavirus treatment shows very high affinity to the phosphatase although this class of enzymes is generally considered undruggable.48

Conclusions
Presented computational studies support recent observations that repurposing some drugs seems a promising way for COVID-19 treatment. Out of the studied drugs the most promising is homobarringtonine (L2-6) in agreement with recently suggested49 its potential effectiveness of this drug in treating SARS-CoV-2 infection. Similar arguments can be claimed for chloroquine (A1).

Furthermore, our studies point to rimcazole (L2-1), and benzerazine (L1-5) as equally potential antivirus drugs especially because they seem to be nonspecific and strongly binding to a few virus enzymes.

From among studied compounds that are not approved for clinical use, it seems that thia diazoles with pyrrol or its derivatives substituents at the carbon atom, and ortho- hydroxyphenyl at the nitrogen atom might provide reasonable lead compounds for future drugs.

Declarations
Authors contributions statement

Both authors contributed to calculations, writing and reviewing the manuscript.

Competing interests

The authors declare no competing interests.

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Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Figures

![Figure 1](image-url)

Chemical structure of compound ICpNO2
Figure 2

Chloroquine (A-1) bound to virus S-protein – human ACE2 receptor interface