Potential inhibitors interacting in Neuropilin-1 to develop an adjuvant drug against COVID-19, by molecular docking

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ABSTRACT

The COVID-19 pandemic continues without specific treatment. In this study it is proposed compounds that can be developed as adjuvant / complementary drugs against COVID-19. Through a search for molecular docking, for the development of a new drug using pharmacological compounds targeting the b1 region in neuropilin-1 (NRP1), which is important for the interaction with the S1 region of the S-Protein of SARS-CoV-2, to slow down the infection process of this virus.

A molecular docking was performed using almost 500,000 compounds targeted to interact in the region between amino acids (Thr316, Asp320, Ser346, Thr349, and Tyr353) in NRP1 to determine compounds able to hinder the interaction with the S1 region in the S-Protein.

In this study, ten compounds are proposed as potential inhibitors between S1 region in the S-Protein of SARS-CoV-2 with the b1 region in NRP1, to develop a new adjuvant / complementary drug against COVID-19, and to hinder the interaction between SARS-CoV-2 and human cells, with a high probability to be safe in humans, validated by web servers for prediction of ADME and toxicity (PreADMET).

1. Introduction

The COVID-19 pandemic continues today without a specific treatment, infections and deaths continue.[1–4] In this pandemic, different treatments have been proposed, the development of new antivirals with different therapeutic targets, studies with therapeutic targets in RNA-Dependent RNA Polymerase (RdRp), Polyproteins (3CLpro and PLpro), Spike Protein (S-Protein)[5,6] and membrane fusion inhibitors[7–10] from SARS-CoV-2, as well as there are works that use as therapeutic targets the interaction regions between RBD in the S-Protein and the ACE2.[11,12]

Studies aimed at describing the role of angiotensin-converting enzyme 2 (ACE2) as an entry receptor for SARS-CoV-2 have gone into this pandemic. There is current evidence that describes that there is a low expression of ACE2 at the pulmonary level, with a higher expression in the kidney and intestines, so there are proposals that there must be other mechanisms in the interaction process between the virus and the cell; co-receptors / binding factors have been identified, such as neuropilins.[13–16] S-Protein of SARS-CoV-2 has been proposed to contain a furin cleavage site that has the potential to generate a C-terminus (CendR) which, according to predictions from molecular models, is capable of binding to the b1 domain in the neuropilin-1 (NRP1).[13,17] This could describe another component in the infectious process in

COVID-19 and that there are variables in the population that cause a tropism in SARS-CoV-2, due to the presence in the cell membrane of the ACE2 and NRP1 proteins that they interact with the S-Protein. Therefore, neuropilin-1 has taken a more important role in COVID-19 to develop studies that identify the impact on the infectious process of this disease.

Neuropilin-1 (NRP1) is a transmembrane glycoprotein expressed on the cell surface, multifunctional; NRP1 is present in various physiological processes, has been identified in various signaling and interaction functions with different ligands (as pleiotropic coreceptors), with various diseases including leukemia / adult *T*-cell lymphoma (ATL),[18] as well as NRP1 works in several steps of the angiogenic cascade (with VEGF ligand binding)[19,20]; to study the above, there are studies that demonstrate the role of NRP1 as a receptor and risk factor for developing viral diseases, such as those caused by the Epstein Barr Virus (EBV) and the Human *T*-lymphotropic Virus Type-1 (HTLV-1); in which it has been shown how NRP1 interacts directly with EBV,[21] and the NRP1 is a risk factor that favors the entry of EBV into nasopharyngeal epithelial cells (in which this type of cells has more expression of NRP1), as well as the relationship with a CendR interaction region of HTLV-1, which facilitates interaction with NRP1 and favors penetration into cells,[18,22,23] as well as S1 of SARS-CoV-2 Protein-S, showing that NRP1 can potentiate

infection in the presence of other host factors.[16]

Therefore, NRP1 is a therapeutic target that can have an effect in different diseases, there are reports of NRP1 inhibitors since the year 2000, using peptides, such as ATWLPPR,[24] in which favorable effects of its use in retinopathies, since it is related to an anti-angiogenesis effect,[20,25] but most of these peptides do not have important characteristics for the development of a drug, such as the Lipinski's rule,[26] since most of the peptides reported are above of 500 Da MW (example Ala-Thr-Trp-Lys-Pro-Pro-Arg: 855 MW), however the residues that are important for the interaction between these peptides with NRP1, as well as the concentrations for the IC$_{50}$ of these peptides in a range between 5.86 and 10.22 μM,[27] also antibodies against NRP1 have been proposed,[20,28] which shows that the development of molecules that impede the function of NRP1 is possible. Subsequently, the use of small molecules as inhibitors of NRP1 (EG00299),[29] in which the concentrations and probable sites of interaction have been reported, which are also taken into account to carry out this study, for which the compound EG00299 has shown a selective inhibitory effect on NRP1 with some of its ligands,[20,29] in addition evaluating its effects in *in vivo* tests with cancer cells[30] and as a molecule to perform derivatives[31] (such as EG01377), in which they have reported the important residues to achieve the inhibitory effect and a range of concentrations used between 10 and 30 μM. Therefore, we use data from the references that use the compound EG00299 in NRP1 and its interaction with VEGF-A, there is an antagonist effect on NRP1 that inhibits the binding of VEGF-A, as well as, it was determined that it inhibited the interaction between the b1 domain with the S1 region of SARS-CoV-2, showing that the infectious process is hindered by the virus,[14,15,17,32] since this study seeks to develop an inhibitor (small molecule), which it can accomplish the Lipinski's rule,[26] because these molecules have better pharmacokinetic properties in comparison with peptide-based molecules.

It is reported the crystallographic structure of the interaction between NRP1 with EG00229 (PDB:3I97); which is demonstrating that the main amino acids important are: Thr316, Asp320, Ser346, Thr349 and Tyr353 in NRP1 to interact with EG00229, in which the same corresponding amino acids are identified when NRP1 interacts with VEGF-A.[33] Therefore, we used the crystallographic structure of NRP1 (PDB:2QQI) to carried out a docking directed to the region between amino acids: Thr316, Asp320, Ser346, Thr349 and Tyr353, using a library of compounds (EXPRESS-pick Collection from Chembridge Corp.) to select the compounds with the best binding average, to propose compounds that can be tested as adjuvants in the treatment against COVID-19.

2. Material and methods

2.1. Preparation of receptor protein and definition of binding sites

Atomic coordinates of the NRP1 (Crystal Structure of the b1b2, domains from Human Neuropilin-1) were obtained from the Protein Data Bank (PDB: 2QQI). The structure was used as protein targets for docking procedures. The protonation and energy minimization of PDB file was performed using Molecular Operating Environment (MOE) software with the default parameters and the CHARMM27 force field.[34,35] We select one region to interact in NRP1 (T316, D320, S346, T349 and Y353).[14,15,17]

2.2. Screening library

The EXPRESS-pick Collection Stock screening library from Chembridge Corp. was used for docking.[36] This collection of compounds contains over 500,000 chemical compounds that fulfill the druggable properties of Lipinski's rules[26,37] and cover a broad area of chemical space, as well as, the structure of EG00229 to evaluate the interaction with NRP1.[14]

2.3. Molecular docking

For docking, the receptors were kept rigid, while the ligand atoms were released to move to a maximal number of rotatable bonds. All crystallographic water molecules were deleted from the initial structures. High-throughput virtual molecular docking was carried out by means of the software AutoDock and MOE,[36,38] using default parameters (Placement: Triangle Matcher, Rescoring 1: London ΔG, Refinement: Forcefield, Rescoring 2: London ΔG, for each compound up to 100 conformations were generated).

2.4. Calculation of the free binding energy (ΔG$_{binding}$)

The binding affinity of each complex (Ligand-protein) was estimated by the ratio of General Born *vs* Volume Integral (GB/VI), using parameters in MOE.[39,40] General Born or non-bonded interaction energies comprise Van der Waals, Coulomb electrostatic interactions and implied solvent interaction energies.[40]

2.5. Selection of compounds

The results of up to 30 conformers of each compound were used to select the best compounds, determining the best average ΔG$_{binding}$ value between NRP1 with each compound, as well as the standard deviation for each one, using the Excel software (Microsoft-365), the description of chemical properties by PhysChem - ACD/Labs,[41] the theoretical toxicity,[42] carcinogenicity and mutagenicity were considered.[42–44] The calculated interactions between NRP1 with each compound were visualized with Ligand-interaction interactions implemented in MOE.

3. Results

3.1. Selection of compounds by docking

For docking, we used 502,530 compounds, and up to 100 conformers of each compound, interacting in the NRP1 (the region between amino acids: Thr316, Asp320, Ser346, Thr349 and Tyr353, Fig. 1), the selection criteria of the best compounds was based on the calculation of the ΔG$_{binding}$ average of each compound, using the values of conformers (24–29 conformers), determining an average range from −7.72 to −8.11 kcal/mol^{-1} for the best compounds (Table 1, and details on the supplementary material Table S1). We selected ten compounds depicted here as N1 to N10 from the Express-pick Collection Stock from Chembridge library (ChemBridge Corp.) and the analysis of the interaction of each compound with NRP1 was carried out with the interaction report (Table 2 and details in Table S1–S11). In addition, it was determined the average interaction for compound EG00229 and EG01377 (with reports of inhibitory effect between NRP1 with VEGF-A[29,31] and S-Protein of SARS-CoV-2[32]), with an average value of −4.95 kcal/mol^{-1} and −4.86 kcal/mol^{-1} respectively (interaction details in Tables S1 and S12). Afterwards, the theoretical toxicity for the ten compounds was evaluated with two websites (Prediction of Toxicity and PreADMET web server).

The description of the theoretical toxicity (Table S13), ADME characteristics (Table S14) and chemical properties of each compound (N1–N10, Table S15), are presented in the supplemental material.

3.2. Interaction of compounds N1 – N10, EG00229 and EG01377 with NRP1

To describe the probable interaction sites between each compound (N1 – N10, EG00229 and EG01377) with NRP1, we analyzed up to 30 conformers of each compound with the better ΔG$_{binding}$ average values of interaction in the region between amino acids: Thr316, Asp320, Ser346, Thr349 and Tyr353 (Fig. 1). From dockings result (Tables S2–S12), we determined the main amino acids in NRP1 to interact with the ten compounds, these are Tyr297, Asn300, Trp301,
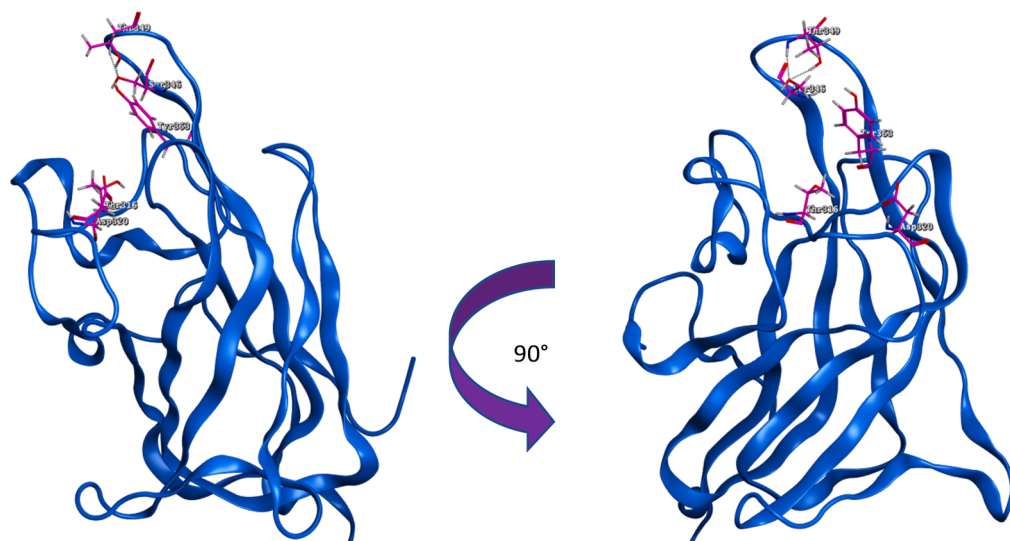
Fig. 1. NRP1 (Blue) shows amino acids Thr316, Asp320, Ser346, Thr349 and Tyr353 (Pink), as region chosen for docking.

Thr316, Gly318, Glu319, Asp320, Ser321, Arg323, Glu348, Lys351, Tyr353, Trp411, Thr413 and Gly414 for N1 – N10 compounds, and for EG00229 and EG01377 the amino acids: Tyr297, Trp301, Gly318, Asp320, Glu348, Thr349, Lys351, Thr413 and Ile415 (Table 2). It is reported that Asp320 is very important for NRP1 to interact with different ligands and VEGF-A,[29,33] as well as for the CendR region in HTLV-1[18,22,23] and the SARS-CoV-2,[17] which is present in the analyzed interactions.

From the docking results, it can be proposed that the conformers of the compounds of compound N1 and N2 generate more ionic bonds (Tables S2 and S3), as well as in the ten selected compounds, all the conformers demonstrate interactions of hydrogen bridge bonds with amino acids of the proposed potential site (average range from -7.72 to -8.11 kcal/mol^{-1}, Tables S2–S11), and these interactions together are better than the determined by EG00299 and EG01377 compounds (average of -4.95 kcal/mol^{-1} and -4.86 kcal/mol^{-1} respectively, Table S12). The details of the interaction between NRP1 with conformers of each compound are shown in the supplementary material (Figs. S1–S11).

4. Discussion

Today, the development of vaccines and drugs to attend the pandemic caused by SARS-CoV-2 is booming around the world,[8–11,45-47] almost 1 year after the first work against COVD-19 began, there is still no treatment that demonstrates a therapeutic advantage, which shows the need for the development of drugs directed at a selective target that can alter the evolution of this disease.

As it was mentioned already, the amino acid Asp320 is very important for NRP1 to interact with different ligands and VEGF-A,[29,33] as well as for the CendR region in the SARS-CoV-2,[17,32] therefore, the region between the amino acids Thr316, Asp320, Ser346, Thr349 and Tyr353 has a very important role for the S1 region of SARS-CoV-2 to interact with NRP1; in this study was carried out a docking directed to amino acids in the b1 region reported in the NRP1 (Thr316, Asp320, Ser346, Thr349 and Tyr353),[17] that is important to interact with the S-Protein (S1 region) of SARS-CoV-2,[16] since it has been shown that by preventing this interaction, the infectious process caused by this virus could be reduced.[14,15,17]

It was determined that the amino acids Tyr297, Asn300, Trp301, Thr316, Gly318, Glu319, Asp320, Ser321, Arg323, Glu348, Lys351, Tyr353, Trp411, Thr413 and Gly414 are important for the majority of the ten compounds to interact with the NRP1 (Table 2) and the chosen

compounds have a better $\Delta G_{binding}$ average value than the reference compounds (EG00229 -4.95 kcal/mol^{-1} and EG01377 -4.86 kcal/mol^{-1}, Table S1), although this difference obtained theoretically, it does not guarantee that a better $\Delta G_{binding}$ value of the chosen compounds causes a greater inhibition when comparing them with EG00229.[30,32] To justify the selection of these ten compounds, it is necessary to show that N1 - N10 compounds have a higher probability of interaction with NRP1, according to the results of the docking, the EG00229 and EG01377 compounds, are less specific, since the 30 conformers and the 29 conformers respectively are interacting in a bigger region (Fig. 2, Table 2 and Figs. S11 and S12), and if this is compared with the results of the conformers of the N1 – N10 compounds, these are interacting in smaller regions (as example N1 and N2 compound, Fig. 2); therefore, the ten compounds proposed should have a better interaction, more specific, and it achieves a better $\Delta G_{binding}$ value to each conformer, which is demonstrated in the $\Delta G_{binding}$ averages of them (Table 2 and Figs. S1–S10).

The development of new drugs requires a high investment of time and financial resources, so it is necessary to be able to offer potential drugs to development them, even if it is to start in a theoretical way, since this is already developing in the world.[20,32] On the other hand, there is still much to know about COVID-19, since the tropism that SARS-CoV-2 has, it is a challenge to understand it. NRP1 has become important to determine the evolution of the infectious process, since it has been identified that this protein can increase the degree of infection when it is present.[14–17] Therefore, evaluating the effect and control of NRP1 could generate new theories about the tropism of SARS-CoV-2.

Proposing NRP1 as a therapeutic target, and being able to develop a compound that has an interaction in the b1 region of NRP1, could help to develop drugs that can be complementary or independent to reduce the infectious process,[14,15,17,32] since the compound EG00229 does not have a sufficient inhibitory effect,[32] the opportunity is open to develop more efficient drugs with greater selectivity.

For the selection of the compounds (taking into account the results of up to 30 conformers of each compound), these were also validated by two toxicity prediction web servers (Table 2 and Tables S13 and S14), since in previous works,[48–50] in our team work, we have correlated these theoretical results with experimental toxicity tests, in this way, it is proposed that these ten compounds have acceptable potential values, as well as a very low probability of toxicity. From theoretical toxicity results (Table S13),[43] the compounds EG00299 and EG01377 are positive in the Ames-TA1535_NA test, conversely, the ten proposed compounds are negative in the same test, in addition the LD$_{50}$ determined
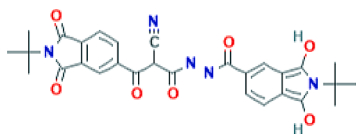
Table 1

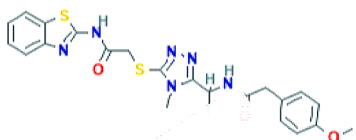PubChem CID, ID Chembridge Corp. and Structure of the top ten compounds with the best binding energies, N1 to N10.
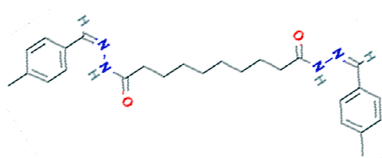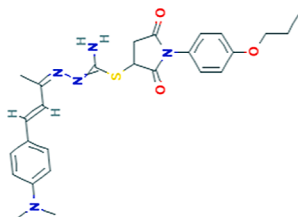
N1.- 2861991, 5687320.
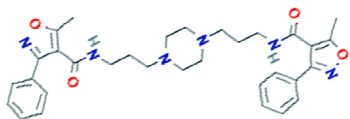
N2.- 2866528, 5749798.

N3.- 2977914, 7970685.

N4.- 5572721, 5468351.

N5.- 2977934, 7970751.

N6.- 2233306, 7970059.

N7.- 5721801, 5466862.
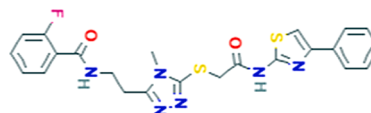
N8.- 2978142, 7971408.

N9.- 132463079, 5665553.
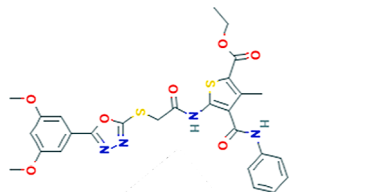
N10.- 5336047, 5667359.

EG00229.- 44631827[29]
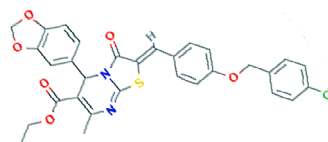
EG01377.- 133,081,972[31]
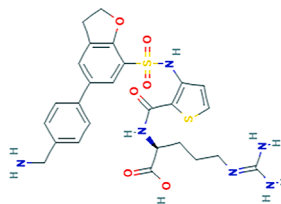
(Table 2)[42] for all compounds it is above 500 mg/kg, these results are important to propose that the compounds are probably safe for use in humans.

In the dosage of drugs, a synergy could be sought between compounds that are directed towards the regions that are important for the S-Protein of SARS-CoV-2 to interact with the cell, such as the ACE2 and NRP1 proteins, this could increase the effect therapeutic and reduce the infectious process of SARS-CoV-2. Using these ten compounds in combination with some of the compounds that are already proposed against ACE2.[11,12]

The compounds proposed do not have any specific registered use, nor a scientific article or registered patent, all the compounds are available at many laboratories to acquire them, to perform *in vitro* assays and to determine the effect on the interaction between NRP1- with S-Protein of SARS-CoV-2.

Table 2

ID compound, Canonical SMILES, Interaction with residues in NRP1, Number of conformers used, $\Delta G_{binding}$ average (kcal/mol^{-1}) with standard deviation (SD), Ames test and strain used (positive or negative) and LD$_{50}$.[42]

Compound ID Chembridge Corp.	Canonical SMILES	Interaction with residues in NRP1 (Tables S2 –S12)	Number of conformers	Average of $\Delta G_{binding}$ and SD	PreADMET Ames test and LD$_{50}$ -TA100_10RL -TA100_NA -TA1535_10R -TA1535_NA +500 mg/kg
N1.- 5687320	CC1 = C(C(=NO1)C2 = CC = CC = C2)C(=O)NCCCN3CCN(CC3)CCCNC(=O)C4 = C(ON = C4C5 = CC = CC = C5)C	Tyr297, Trp301, Asp320, Ser321, Glu348, Lys351, Tyr353, Trp411	29	−8.11 ± 0.64	Mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N2.- 5749798	CCOC1 = CC = CC(=C1)N2C(=O)CC(C2 = O)N3CCN(CC3)C4CC(=O)N(C4 = O)C5 = CC(=CC = C5)OCC	Trp301, Gly318, Asp320, Ser321, Trp411	28	−7.89 ± 0.79	Mutagen -Positive -Negative -Negative -Negative +500 mg/kg
N3.- 7970685	CC(C)(C)N1C(=C2C = CC(=CC2 = C1O)C(=O)N = NC(=O)C(C#N)C(=O)C3 = CC4 = C(C = C3)C(=O)N(C4 = O)C(C)(C)C)O	Tyr297, Trp301, Glu319, Asp320, Arg323, Lys351	24	−7.86 ± 0.53	Non mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N4.- 5468351	CCOC(=O)C(=NNC1 = CC = C(C = C1)CC2 = CC = C(C = C2)NN = C(C#N)C(=O)OCC)C#N	Asn300, Trp301, Gly318, Asp320, Arg323, Glu348, Gly414	28	−7.83 ± 0.73	Non mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N5.- 7970751	CC(C1 = NN = C(N1C)SCC(=O)NC2 = NC3 = CC = CC = C3S2)NC(=O)CC4 = CC = C(C = C4)OC	Tyr297, Gly318, Glu319, Asp320, Tyr353, Trp411, Thr413	26	−7.80 ± 0.74	Mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N6.- 7970059	CN1C(=NN = C1SCC(=O)NC2 = NC(=CS2)C3 = CC = CC = C3)CCNC(=O)C4 = CC = CC = C4F	Tyr297, Trp301, Asp320, Glu348, Tyr353, Thr413	27	−7.79 ± 0.73	Non mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N7.- 5466862	CC1 = CC = C(C = C1)C = NNC(=O)CCCCCCCC(=O)NN = CC2 = CC = C(C = C2)C	Tyr297, Glu319, Asp320, Ser321, Arg323, Lys351	24	−7.77 ± 0.81	Mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N8.- 7971408	CCOC(=O)C1 = C(C(=C(S1)NC(=O)CSC2 = NN = C(O2)C3 = CC(=CC(=C3)OC)OC)C(=O)NC4 = CC = CC = C4)C	Tyr297, Trp301, Thr316, Glu319, Asp320, Glu348, Thr349, Thr413	26	−7.74 ± 0.59	Mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N9.- 5665553	CCCOC1 = CC = C(C = C1)N2C(=O)CC(C2 = O)SC(=NN = C(C)C = CC3 = CC = C(C = C3)N(C)C)N	Trp301, Asp320, Arg323, Glu348, Lys351, Thr413	27	−7.72 ± 0.80	Non mutagen -Negative -Negative -Negative -Negative +500 mg/kg
N10.- 5667359		Asp320, Ser321, Arg323, Lys351, Trp411	27	−7.72 ± 0.80	Mutagen

Table 2 (*continued*)

| Compound ID Chembridge Corp.	Canonical SMILES	Interaction with residues in NRP1 (Tables S2 –S12)	Number of conformers	Average of $\Delta G_{binding}$ and SD	PreADMET Ames test and LD_{50}
					-TA100_10RL -TA100_NA -TA1535_10R -TA1535_NA +500 mg/kg
	CCOC(=O)C1 = C(N = C2N(C1C3 = CC4 = C(C = C3)OCO4)C(=O)C(=CC5 = CC = C(C = C5)OCC6 = CC = C(C = C6)Cl)S2)C				-Positive -Negative -Negative -Negative +500 mg/kg
EG-00229 44631827	C1 = CC2 = NSN = C2C(=C1)S(=O)(=O)NC3 = C(SC = C3)C(=O)NC(CCCN = C(N)N)C(=O)O	Tyr297, Trp301, Gly318, Asp320, Glu348, Thr349, Lys351, Thr413, Ile415	30	−4.95 ± 0.53	Mutagen
					-Negative -Negative -Negative -Positive +500 mg/kg
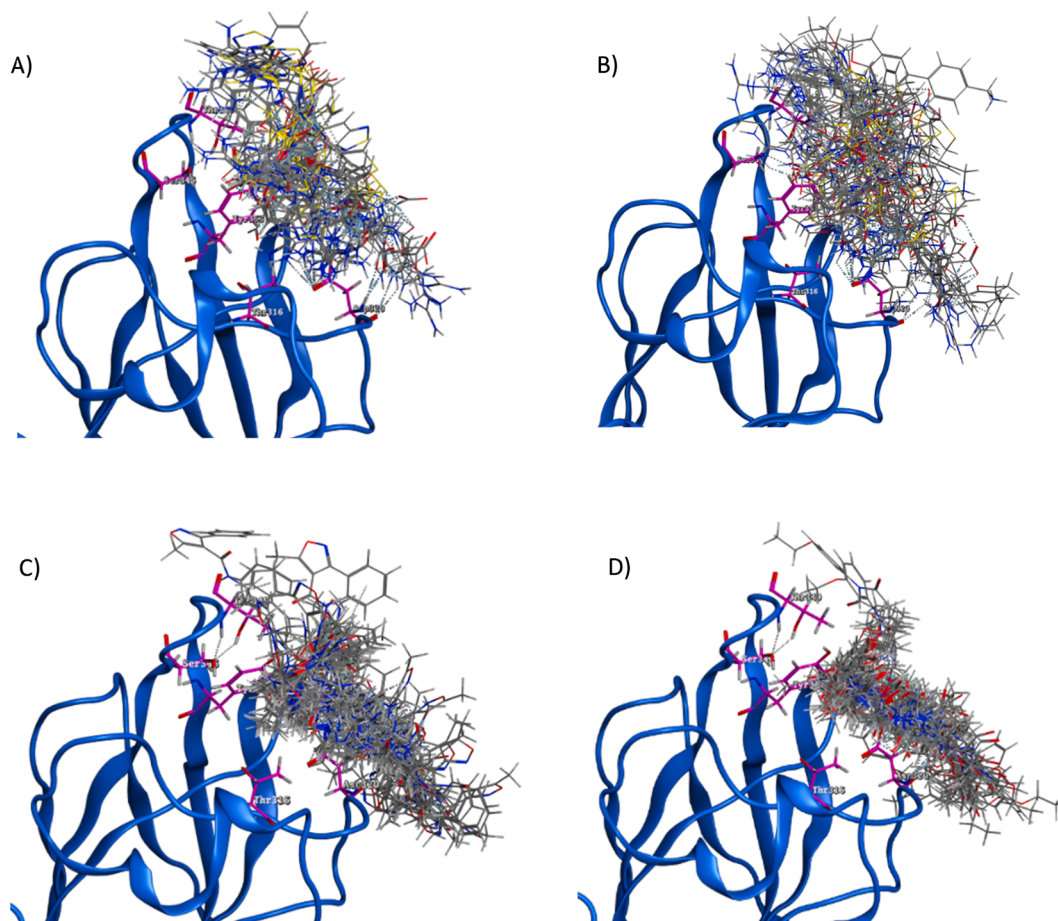
Fig. 2. NRP1 (Blue) shows amino acids Thr316, Asp320, Ser346, Thr349 and Tyr353 (Pink), with the conformers of each compound (Gray) interacting in a bigger or smaller region. A) NRP1 with 30 conformers of EG00229 (Gray), B) NRP1 with 29 conformers of EG01377 (Gray), C) NRP1 with 29 conformers of N1 compound (Gray) and D) NRP1 with 28 conformers of N2 compound (Gray); in N1 and N2 the conformers are interacting in a smaller region.

5. Conclusions

The neuropilin-1 (NRP1) is a multifunctional protein on the cell membrane, with an impact on physiological functions and diseases in the human organism,[16,18,19,20,21,22,23] for which for more than 20 years specific molecules have been developed that can inhibit/regulate some of its functions,[20,24,25,27,28,29,30,31] but they are still developments today and the NRP1 continues to demonstrate new signals/functions, which need further investigation. Therefore, the role of NRP1 in the infectious process of COVID-19 has taken on greater relevance,[16,32] since by being able to limit these functions on COVID-19, they could generate a disease with less impact on the human organism and this could help the immune system and being able to alter as it is known today, the natural history of the COVID-19 disease.

In this study are propose ten compounds with a high probability of interacting in the specific region in the NRP1 (Thr316, Asp320, Ser346, Thr349 and Tyr353), in order to develop a drug that could be complementary to COVID-19 treatments or as a drug that can limit the infectious process of SARS-CoV-2. Furthermore, these ten compounds have a high probability of being safe in humans, as they were validated by the PreADMET server (ADME and Toxicity Predictor).

Declaration of Competing Interest

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.

Acknowledgments

Appendix A. Supplementary material

Supporting information includes figures and tables of interactions for compounds with NRP1, as well as details of the interaction of each compound with NRP1 per amino acid, toxicity theoretical results, ADME characteristics and physical chemistry which support the information given in the results and discussion. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116040.

References

1 Zhao J, Li X, Gao Y, Huang W. Risk factors for the exacerbation of patients with 2019 Novel Coronavirus: a meta-analysis. *Int J Med Sci.* 2020;17(12):1744–1750. https://doi.org/10.7150/ijms.47052.

2 de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol.* 2016;14(8):523–534. https://doi.org/10.1038/nrmicro.2016.81.

3 Guan W, Ni Z, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* 2020. https://doi.org/10.1056/NEJMoa2002032. February 2020:NEJMoa2002032.

4 Hu Y, Sun J, Dai Z, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): a systematic review and meta-analysis. *J Clin Virol.* 2020;127, 104371. https://doi.org/10.1016/j.jcv.2020.104371.

5 Calligari P, Bobone S, Ricci G, Bocedi A. Molecular investigation of SARS–CoV-2 proteins and their interactions with antiviral drugs. *Viruses.* 2020;12(4):445. https://doi.org/10.3390/v12040445.

6 Huang J, Song W, Huang H, Sun Q. Pharmacological therapeutics targeting RNA-dependent RNA polymerase, proteinase and spike protein: from mechanistic studies to clinical trials for COVID-19. *J Clin Med.* 2020;9(4):1131. https://doi.org/10.3390/jcm9041131.

7 Iftikhar H, Ali HN, Farooq S, Naveed H, Shahzad-ul-Hussan S. Identification of potential inhibitors of three key enzymes of SARS-CoV2 using computational approach. *Comput Biol Med.* 2020;122, 103848. https://doi.org/10.1016/j.compbiomed.2020.103848.

8 Wu C, Liu Y, Yang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharm Sin B.* 2020;10 (5):766–788. https://doi.org/10.1016/j.apsb.2020.02.008.

9 Xia S, Yan L, Xu W, et al. A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci Adv.* 2019;5(4):eaav4580. https://doi.org/10.1126/sciadv.aav4580.

10 Xia S, Liu M, Wang C, et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Res.* 2020;30 (4):343–355. https://doi.org/10.1038/s41422-020-0305-x.

11 Benítez-Cardoza CG, Vique-Sánchez JL. Potential inhibitors of the interaction between ACE2 and SARS-CoV-2 (RBD), to develop a drug. *Life Sci.* 2020;256, 117970. https://doi.org/10.1016/j.lfs.2020.117970.

12 Khelfaoui H, Harkati D, Saleh BA. Molecular docking, molecular dynamics simulations and reactivity, studies on approved drugs library targeting ACE2 and SARS-CoV-2 binding with ACE2. *J Biomol Struct Dyn.* 2020:1–17. https://doi.org/10.1080/07391102.2020.1803967.

13 Zamorano Cuervo N, Grandvaux N. ACE2: evidence of role as entry receptor for SARS-CoV-2 and implications in comorbidities. *Elife.* 2020;9. https://doi.org/10.7554/eLife.61390.

14 Kielian M. Enhancing host cell infection by SARS-CoV-2. *Science (80-).* 2020;370 (6518):765–766. https://doi.org/10.1126/science.abf0732.

15 Moutal A, Martin LF, Boinon L, et al. SARS-CoV-2 Spike protein co-opts VEGF-A/Neuropilin-1 receptor signaling to induce analgesia. *bioRxiv Prepr Serv Biol.* 2020. https://doi.org/10.1101/2020.07.17.209288.

16 Cantuti-Castelvetri L, Ojha R, Pedro LD, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science (80-).* 2020;370(6518):856–860. https://doi.org/10.1126/science.abd2985.

17 Daly JL, Simonetti B, Klein K, et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science (80-).* 2020;370(6518):861–865. https://doi.org/10.1126/science.abd3072.

18 Tezuka K, Fuchi N, Okuma K, et al. HTLV-1 targets human placental trophoblasts in seropositive pregnant women. *J Clin Invest.* 2020;130(11):6171–6186. https://doi.org/10.1172/JCI135525.

19 Guo H-F, Vander Kooi CW. Neuropilin functions as an essential cell surface receptor. *J Biol Chem.* 2015;290(49):29120–29126. https://doi.org/10.1074/jbc.R115.687327.

20 Raimondi C, Brash JT, Fantin A, Ruhrberg C. NRP1 function and targeting in neurovascular development and eye disease. *Prog Retin Eye Res.* 2016;52:64–83. https://doi.org/10.1016/j.preteyeres.2016.02.003.

21 Gu Y-Y, Luo B, Li C-Y, et al. Expression and clinical significance of neuropilin-1 in Epstein-Barr virus-associated lymphomas. *Cancer Biomarkers.* 2019;25(3):259–273. https://doi.org/10.3233/CBM-192437.

22 Wang H-B, Zhang H, Zhang J-P, et al. Neuropilin 1 is an entry factor that promotes EBV infection of nasopharyngeal epithelial cells. *Nat Commun.* 2015;6(1):6240. https://doi.org/10.1038/ncomms7240.

23 Harhaj EW, Shembade N. Lymphotropic viruses: chronic inflammation and induction of cancers. *Biology (Basel).* 2020;9(11):390. https://doi.org/10.3390/biology9110390.

24 Binetruy-Tournaire R. Identification of a peptide blocking vascular endothelial growth factor (VEGF)-mediated angiogenesis. *EMBO J.* 2000;19(7):1525–1533. https://doi.org/10.1093/emboj/19.7.1525.

25 Wang J, Wang S, Li M et al. The Neuropilin-1 Inhibitor, ATWLPPR peptide, prevents experimental diabetes-induced retinal injury by preserving vascular integrity and decreasing oxidative stress. Lewis AS, ed. PLoS One. 2015;10(11):e0142571. 10.1371/journal.pone.0142571.

26 Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev.* 2001;46(1–3):3–26. http://www.ncbi.nlm.nih.gov/pubmed/11259830.

27 Fedorczyk B, Lipiński PFJ, Puszko AK, et al. Triazolopeptides inhibiting the interaction between neuropilin-1 and vascular endothelial growth factor-165. *Molecules.* 2019;24(9):1756. https://doi.org/10.3390/molecules24091756.

28 Kim Y-J, Baek D-S, Lee S, et al. Dual-targeting of EGFR and Neuropilin-1 attenuates resistance to EGFR-targeted antibody therapy in KRAS-mutant non-small cell lung cancer. *Cancer Lett.* 2019;466:23–34. https://doi.org/10.1016/j.canlet.2019.09.005.

29 Jarvis A, Allerston CK, Jia H, et al. Small molecule inhibitors of the neuropilin-1 vascular endothelial growth factor A (VEGF-A) interaction. *J Med Chem.* 2010;53(5):2215–2226. https://doi.org/10.1021/jm901755g.

30 Pal K, Madamsetty VS, Dutta SK, Wang E, Angom RS, Mukhopadhyay D. Synchronous inhibition of mTOR and VEGF/NRP1 axis impedes tumor growth and metastasis in renal cancer. *npj Precis Oncol.* 2019;3(1):31. https://doi.org/10.1038/s41698-019-0105-2.

31 Powell J, Mota F, Steadman D, et al. Small molecule neuropilin-1 antagonists combine antiangiogenic and antitumor activity with immune modulation through reduction of transforming growth factor beta (TGFβ) production in regulatory T-cells. *J Med Chem.* 2018;61(9):4135–4154. https://doi.org/10.1021/acs.jmedchem.8b00210.

32 Perez-Miller S, Patek M, Moutal A, et al. In silico identification and validation of inhibitors of the interaction between neuropilin receptor 1 and SARS-CoV-2 Spike protein. *bioRxiv Prepr Serv Biol.* 2020. https://doi.org/10.1101/2020.09.22.308783.

33 Parker MW, Xu P, Li X, Vander Kooi CW. Structural basis for selective vascular endothelial growth factor-A (VEGF-A) binding to neuropilin-1. *J Biol Chem.* 2012;287 (14):11082–11089. https://doi.org/10.1074/jbc.M111.331140.

34 Brooks BR, Brooks CL, Mackerell AD, et al. CHARMM: The biomolecular simulation program. *J Comput Chem.* 2009;30(10):1545–1614. https://doi.org/10.1002/jcc.21287.

35 Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J Comput Chem.* 1996;17(5–6):490–519. https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6<490::AID-JCC1>3.0.CO;2-P.

36 Corporation, ChemBridge. http://www.chembridge.com/screening_libraries/.

37 Thangapandian S, John S, Lee Y, Kim S, Lee KW. Dynamic structure-based pharmacophore model development: a new and effective addition in the histone deacetylase 8 (HDAC8) inhibitor discovery. *Int J Mol Sci.* 2011;12(12):9440–9462. https://doi.org/10.3390/ijms12129440.

38 Soga S, Shirai H, Kobori M, Hirayama N. Use of amino acid composition to predict ligand-binding sites. *J Chem Inf Model.* 2007;47(2):400–406. https://doi.org/10.1021/ci6002202.

39 Labute P. The generalized Born/volume integral implicit solvent model: estimation of the free energy of hydration using London dispersion instead of atomic surface area. *J Comput Chem.* 2008;29(10):1693–1698. https://doi.org/10.1002/jcc.20933.

40 Wadood A, Ghufran M, Hassan SF, Khan H, Azam SS, Rashid U. In silico identification of promiscuous scaffolds as potential inhibitors of 1-deoxy- d -xylulose 5-phosphate reductoisomerase for treatment of Falciparum malaria. *Pharm Biol.* 2017;55(1):19–32. https://doi.org/10.1080/13880209.2016.1225778.

41 www.acdlabs.com/. https://www.acdlabs.com/products/percepta/index.php.

42 ADMETlab. http://admet.scbdd.com/calcpre/index_sys/.

43 PreADMET. https://preadmet.bmdrc.kr/toxicity/.

44 ProTox-II - Prediction of TOXicity. http://tox.charite.de/protox_II/index.php?site=compound_input.

45 Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. *Cell Host Microbe*. 2020. https://doi.org/10.1016/j.chom.2020.03.002.

46 Ton A-T, Gentile F, Hsing M, Ban F, Cherkasov A. Rapid identification of potential inhibitors of SARS-CoV-2 main protease by deep docking of 1.3 billion compounds. *Mol Inform*. 2020. https://doi.org/10.1002/minf.202000028. minf.202000028.

47 Zhang L, Lin D, Sun X, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science*. 2020. https://doi.org/10.1126/science.abb3405. eabb3405.

48 Vique-Sánchez JL, Caro-Gómez LA, Brieba LG, Benítez-Cardoza CG. Developing a new drug against trichomoniasis, new inhibitory compounds of the protein triosephosphate isomerase. *Parasitol Int*. 2020. https://doi.org/10.1016/j.parint.2020.102086.

49 Benítez-Cardoza CG, Vique-Sánchez JL. Potential inhibitors of the interaction between ACE2 and SARS-CoV-2 (RBD), to develop a drug. *Life Sci*. 2020:117970. https://doi.org/10.1016/j.lfs.2020.117970.

50 Vique-Sánchez JL, Jiménez-Pineda A, Benítez-Cardoza CG. Amoebicidal effect of 5,5′-[(4-nitrophenyl)methylene]bis-6-hydroxy-2-mercapto-3-methyl-4(3H)-pyrimidinone), a new drug against Entamoeba histolytica. *Arch Pharm (Weinheim)*. 2020. https://doi.org/10.1002/ardp.202000263.