Modified Intelligent Magnetic Nano particles as A Treatment for SARS Corona Virus Type 2 In Silico

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Abstract

The pandemic situation of the new corona virus (SARS-COV-2) forces drug designers to formulate a new intelligent drug for this disease effective to treat all mutations of the virus. One way to control all mutations of virus is inhibition of spike protein (binding with Angiotensin converting enzyme 2 (ACE-2)) duo to inhibit the viral entry. Viral entry is the first step for virus to start infection. In this work the interactions of SARS-COV-2 spike protein and ACE-2 are evaluated Insilico by docking process and four different Ligands are estimated to simulate those interactions, so as to avoid bindings with ACE-2 needed for viral entry in reality. All Ligand – receptor interactions are considered. Results approves the suggested Ligands in this work, have definite inhibitory effect on SARS-COV-2 spike protein based on the interactions which they make with receptor binding domain (RBD). Docking process are done repeatedly to assure conclusions.

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1.Introduction

In biology and other experimental sciences, an Insilico experiment is one performed on computer or via computer simulation. The phrase is pseudo-Latin for 'in silicon' (in Latin it would be in silicio), referring to silicon in computer chips. Actually In silico study helps us to estimate effectiveness of hypothesizes before doing any animal and clinical trials, so it can reduce the risk of animals and people death.[2] By estimating the bond energies, scientists can assure the possibility of protein – ligand interactions and drug activity. As an example, this technique could be utilized to study antiviral effects of candidate drugs to cure coronavirus disease (COVID-19) caused by SARS-COV-2. COVID - 19 is an infectious disease caused by a novel SARS-CoV-2 pandemic which initially started in Wuhan province in China and has now affected > 200 countries worldwide and declared a pandemic. [1,3,4] As per the statistics available, mortality is high in older age group individuals (> 60 years of age) and people with other morbid conditions. In addition to acute respiratory distress syndrome and respiratory failure, COVID-19 is now known to manifest as systemic inflammation, leading to sepsis, acute cardiac injury, and heart failure and multi-organ dysfunction in patients at high risk.[3]Metal oxide nanoparticles with their novel properties have had increasing interest

for these kinds of biomedical applications. In recent years, monodispersed super paramagnetic iron oxide nanoparticles were developed for various biological applications such as antiviral activity , drug delivery, protein purification, MRI and hyperthermia treatment.[5,6] Magnetic nanoparticles with a size range of less than 1000 nm have been used to treat proteins (5–50 nm), genes (10– 100 nm), viruses (20–450 nm) and cells (10–100 mm) without any difficulty. The advantages associated with super paramagnetic nano particles are easy preparation, active surface functionality, chemical stability, fast response under an external magnetic field, low toxicity and cost effectiveness. However, it is difficult to use pure Fe_3O_4 nanoparticles for these applications due to their high surface to volume ratio, strong dipole–dipole interaction between the particles and agglomeration.[7]All these problems were solved by encapsulating the iron oxide nanoparticles in surface active agents. The encapsulation provides improved chemical, mechanical, solubility and biological stability to the environment, so based on this information, modified magnetic nanoparticles were used as a treatment for COVID – 19 in this work. In the first step the interactions (ligand – receptor = drug – target protein) were confirmed by aid of Insilco assay.[8] The Insilico assay results are described in this paper in details. Not any available ligand structure in databanks was used to design the drug and all information about suggested drug are noble.

2.Methods

2.1. Proteins

Macromolecules especially proteins are mostly the targets of bindings with other molecules. It is important to know how a special molecule would make a complex with proteins. In order to assure these protein – drug bindings, the procedure was followed:

2.1.1. Selection of protein

Crystal structure of SARS-COV-2 spike protein receptor binding domain bound with ACE2(PDB ID = 6M0J) was selected from protein data bank to estimate ligand– receptor bindings. [9]

2.1.2. Separation of target protein

Discover studio version (4.2) was used to separate receptor binding domain of spike protein from mentioned crystal structure by neglecting ACE2 receptor and water molecules.

2.1.3. Optimization of protein

We optimized the separated structure by MOE molecular viewer 2013. a quick receptor optimization was used and probable defects were discovered and amino acids were localized on active sites of this protein by stereochemical estimations. Finally, the last structure was the most stable conformer with least energy level. [10]The results of optimization (physicochemical properties, changes in physiological environments and effects of different parameters) are described in conclusions with details.

2.1.4. Estimation of interactions with water molecules

The interactions were studied again to assure optimization in the presence of water molecules.

It helped to have more detailed information about estimated ligand – receptor interactions.

2.1.5. Finding the active sites

The active sites of optimized structures were found by MOE virtual docker 2013 by analyzing the amino acid sequences as a result of site finder item in MOE application.

2.2. Ligand

Ligand is any kind of molecule which can have certain interactions with receptor. In this work the receptor is the optimized structure of SARS - COV – 2 spike protein receptor binding domain and ligand is modified magnetic nanoparticles (Fe_3O_4)

2.2.1. 2D Structure of the ligand

First a cluster of Fe_3O_4 magnetic nanoparticles were drawn in 2D Chem draw professional 16. In next step the cluster was modified in 4 different methods to consider the best candidate for final structure based on stereochemical parameters.

2.2.2. 3D design

After drawing a 2D structure of estimated ligand, structure was optimized by Chem 3D 16 to have optimized 3D structure of the ligands.

2.2.3. Complete optimization

The 3D structure was optimized completely by Gaussian version (09). The application optimized the structure based on Huckel principle and molecular dynamics.

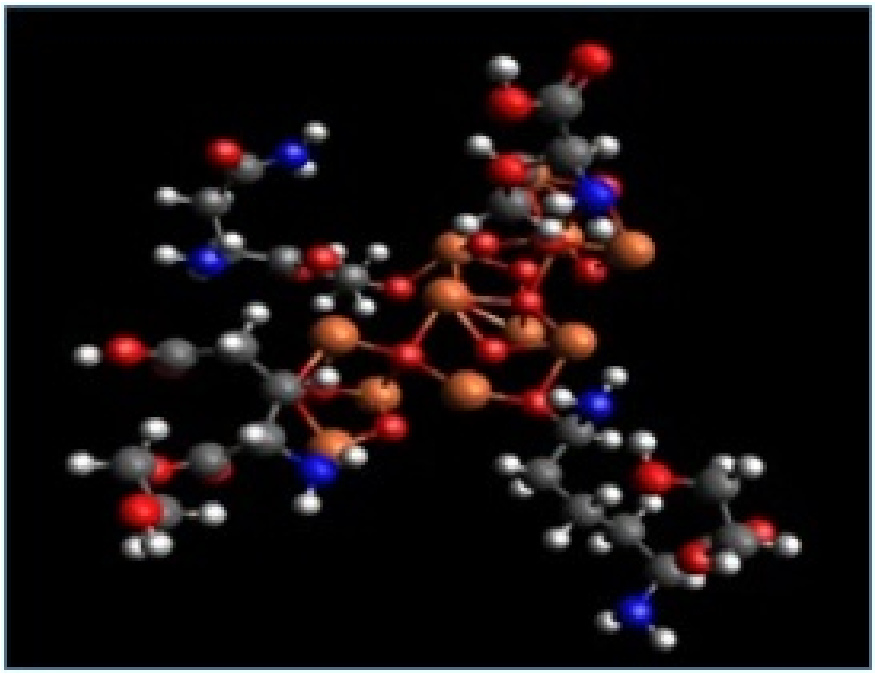
2.3. Molecular docking

Molecular docking simulates the ligand – receptor interactions. It is the most important procedure which helps to assure all estimations are reliable. The docking was repeated several times to prove results are not affected by random. Auto dock 4.2, MOE 2019 and Molegro virtual docker 2013 were used to do the dock. The optimized structure of SARS – COV – 2 spike protein receptor binding domain unit was selected as receptor and the candidate drug was selected as a ligand and all interactions were considered.[11] The results are discussed in results and discussion section.

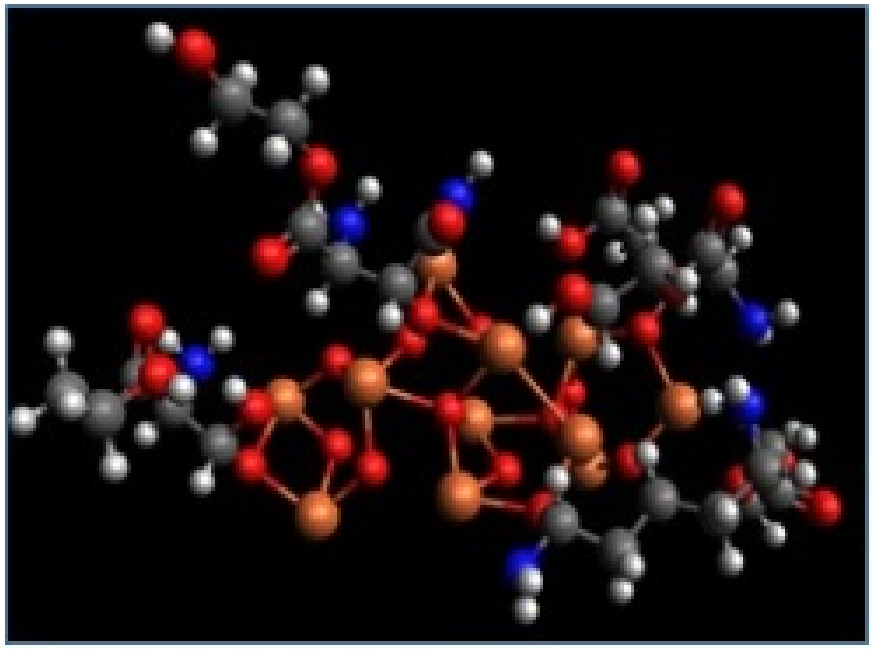
3. Conclusions

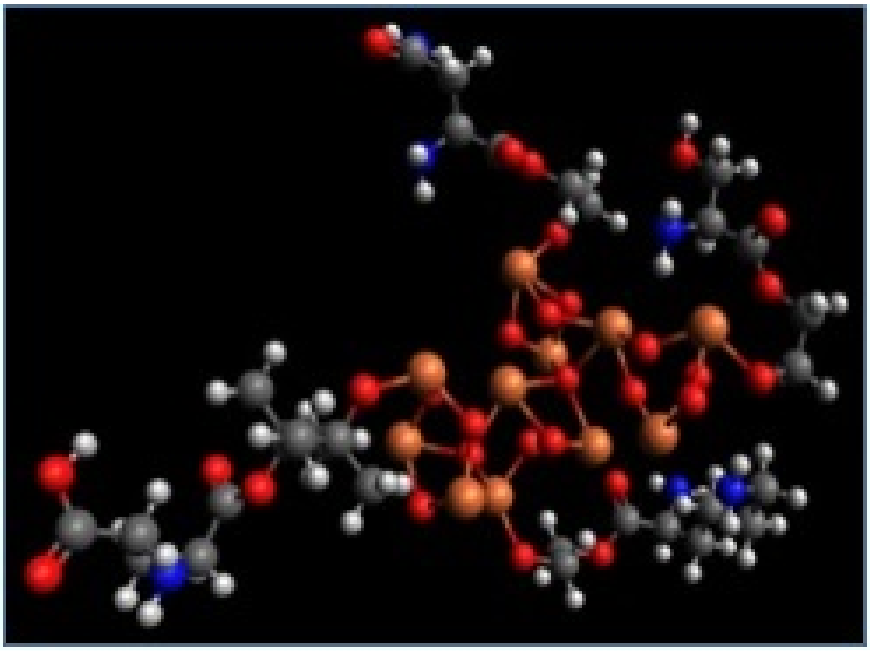
3.1. Structures and properties of ligands

In the first step, ligands were designed in 4 different structures. you can see the ligands in details in figure 1:



Ligand 1 ligand 2





Ligand 3 ligand 4

Figure 1. **Structures of ligands**

Different bond (stretch, bend and total) energies of 4 ligands were calculated to compare the stability of ligands by MOE molecular viewer 2013 and choosing the nest ligand to bond with the target. results are shown in table 1 in details below:

Table 1. Properties of ligands

Bond Energies (Kcal/mol)	Ligand 1	Ligand 2	Ligand 3	Ligand 4
Stretch	23.3293	50.9228	502.9228	13.6654
Bend	316.2747	124.4518	1247.4518	239.4375
Total energies	-147.180	-212.12	-212.12	-277.74

3.2. Physicochemical properties of ligands

The physicochemical properties (pK_a, Log S, Log P) of ligands were calculated by ALOGPS online server to assume solubility, stability and other properties of ligands which help to have better selection among 4 created ligands. The results are shown in table 2 below:

Table 2. Physicochemical properties of ligands

Properties	Ligand 1	Ligand 2	Ligand 3	Ligand 4
M_w (g/mol)	1365.43	1527.52	1541.54	1597.6
Log p	-2.42	-1.46	-1.64	0.35
Log S	-2.74	-2.76	-2.53	-3.84
pk_a	-41.153	4.772	13.06	4.772

3.3. Selection of protein

The structure of SARS-CoV-2 RBD bound to ACE2

with Protein data bank (PDB) ID of 6M0J was selected in order to estimate the Ligand – protein interactions by separation of the structure of SARS-COV-2 spike protein and ACE2 RBD. You can see the structure in figure 2 and the structure exists in PDB website. More explanations about the structure could be found in mentioned website too.

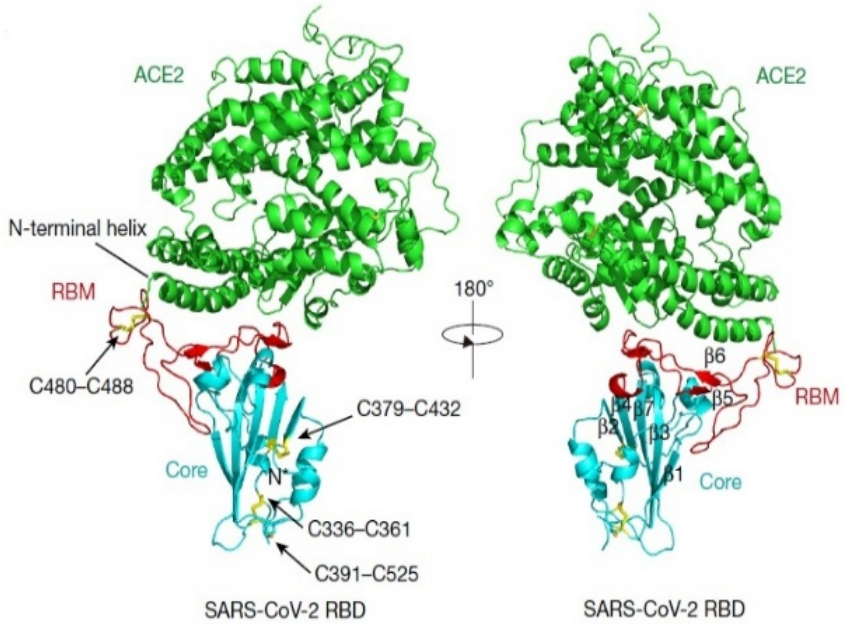


Figure 2. Overall structure of SARS-CoV-2 RBD bound to ACE2 (6M0J)[12]

ACE2 is shown in green. The SARS-CoV-2 RBD core is shown in cyan and RBM in red. Disulfide bonds in the SARS-CoV-2 RBD are shown as sticks and indicated by arrows. The N-terminal helix of ACE2 responsible for binding is labelled.

The structure of SARS-CoV-2 spike protein in prefusion state (flexibility analysis, 1-up open conformation) (PDB ID = 6ZP7) is shown in figure 3 to complete information. This structure was used as receptor for docking with ligands to consider interactions.

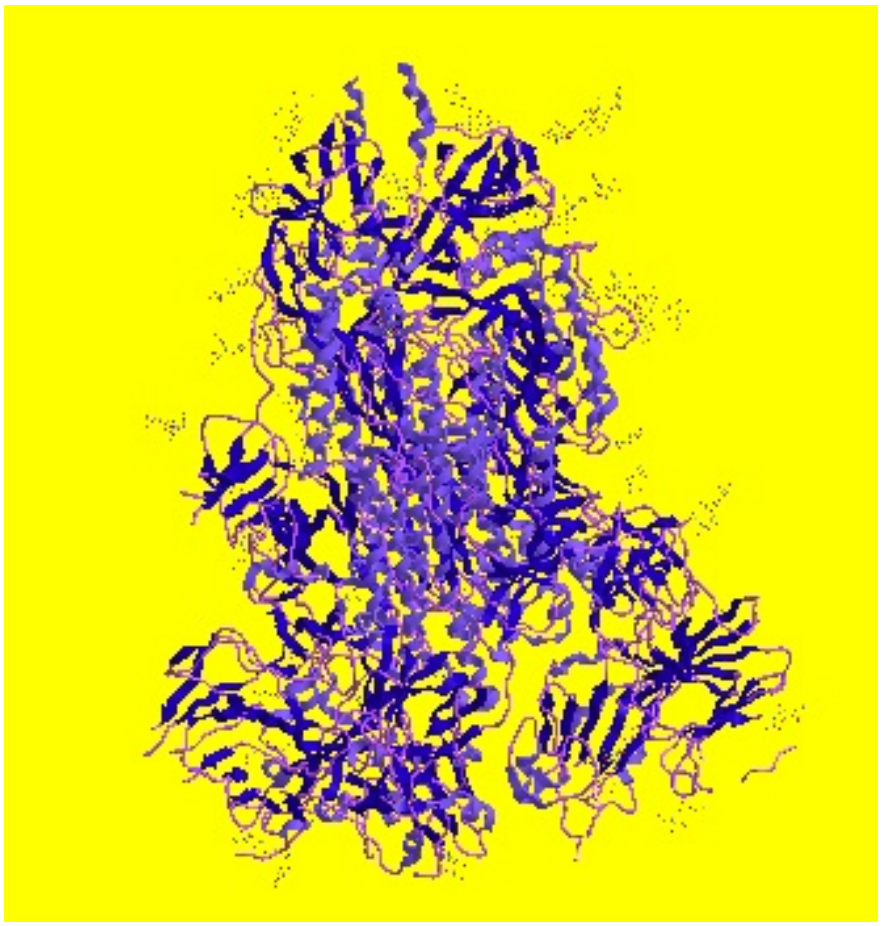


Figure 3. The structure of SARS-CoV-2 spike protein in prefusion state (flexibility analysis, 1-up open conformation) (PDB ID = 6ZP7)

3.4. Ligand – receptor interactions

Finally, all interactions were considered by docking of the receptor and ligands. All kinds of interactions contain Hydrogen bonds, steric bonds and etc approved possibility of ligands to bind the receptor. These interactions approve the antiviral effects of ligands as you can see in figures 4-7:

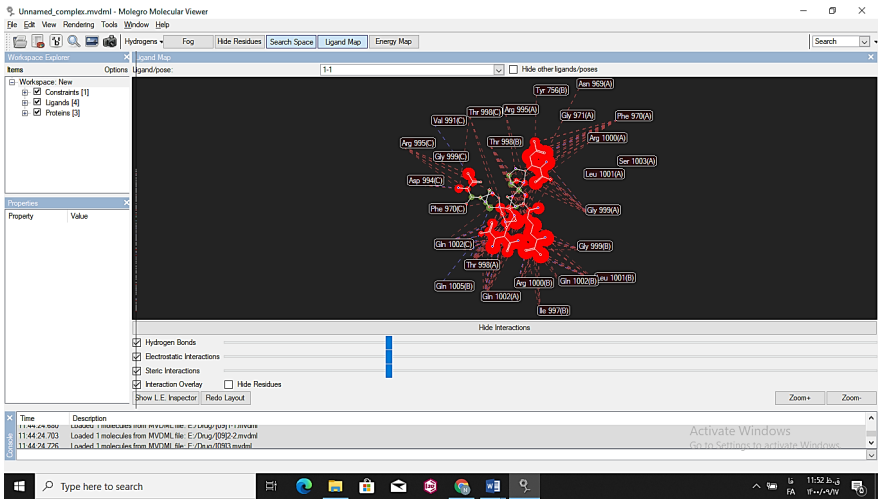


Figure (4) interactions of Ligand 1

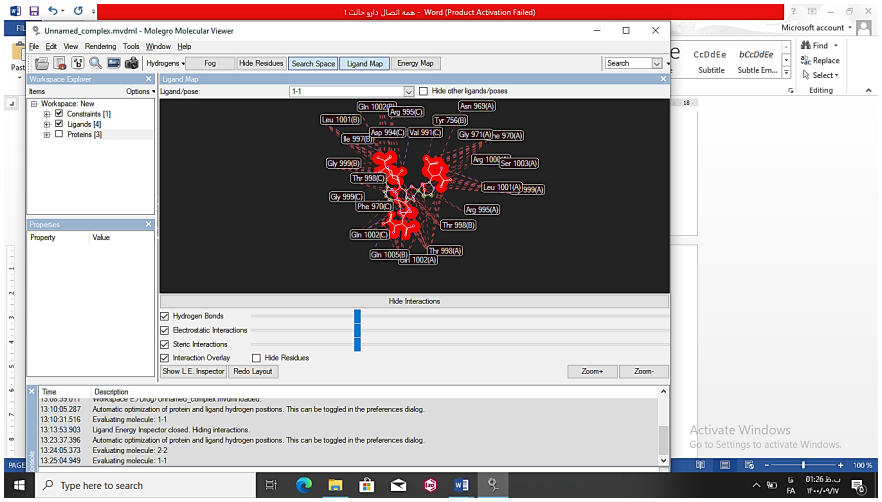


Figure (5) interactions of Ligand 2

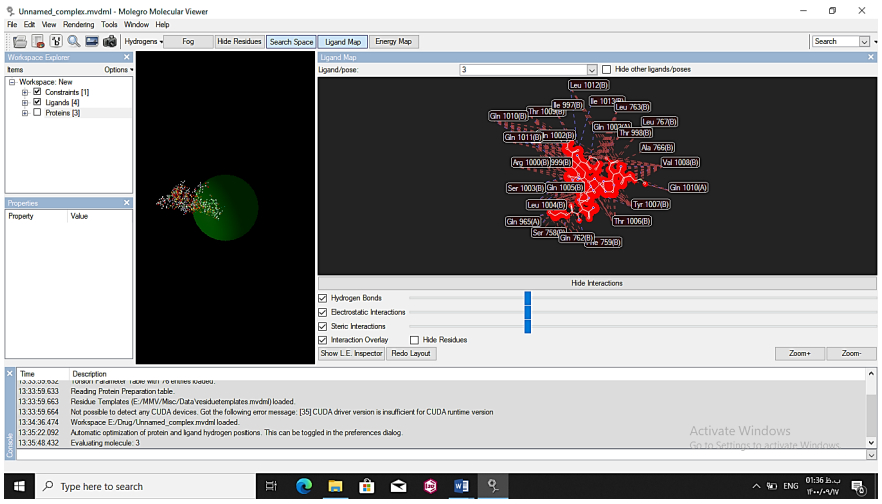


Figure (6) interactions of Ligand 3

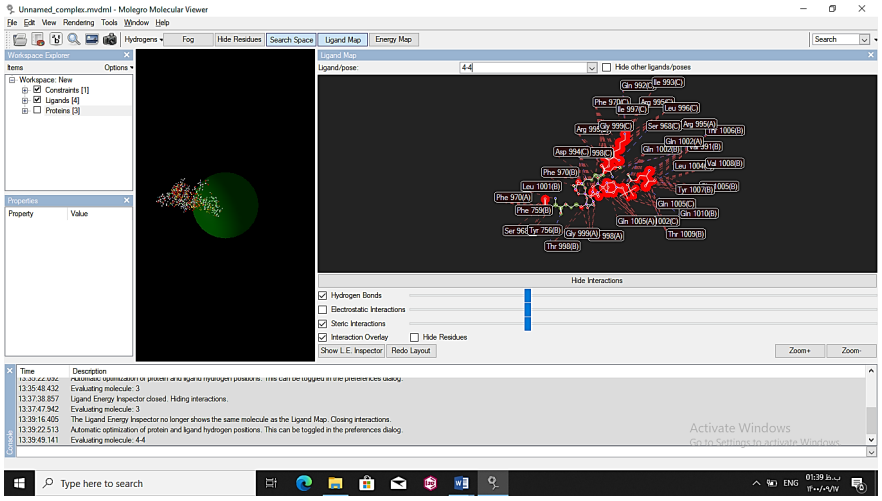


Figure (7) interactions of Ligand 4

Information about ligand –protein bond energies (Hydrogen bonds – steric bonds – total energies) and affinity of ligand to protein as the receptor is summarized in table 3. This information approves the antiviral effects of ligands. Totally Docking, as a method to estimate possible ligand- receptor interactions, was done by different applications such as Auto dock 4.2, MOE 2019, Molegro virtual docker 2013 and online servers to approve designed ligands have antiviral effect by making interactions with SARS -COV -2 spike glycoprotein and interfering viral entry.

Table 3. ligand – protein bond energies

Bond energies (kj/mol)	Ligand 1	Ligand 2	Ligand 3	Ligand 4
Hydrogen	-10	-11	-2.62	-2.52
Steric	-42	-40	-20	-2.73
Total	-66	-63	-20	-18
Affinity	-8.34	-7.1	-5.43	-2.73

As the result, ligands approved to have antiviral effect on SARS -COV -2. These results were obtained by ligand energy inspector in Molegro 2013. It shows ligand 1 and 2 have higher affinity than other ligands which is completely compatible with results invitro done by this paper authors. [13]

Acknowledgments

We thank Dr. Maryam Rahimi, Marzieh Vanayi, Zahra Farahani, Mohammad Javad Hallaji, and Dr. Reza Gheshlaghi for their cooperation in carrying out the project. University of Malayer for their helps, Garsha Pajooh Scientific-Based Company and Kian Asa Pars Center for Preventive Medicine and Health Promotion have provided financial support for the project.

Keywords: Nanoparticles, Virus, Docking, Ligand, Receptor, Protein

References and Notes

1. KUMAR, S. Rajesh, et al. An in vitro analysis of H1N1 viral inhibition using polymer coated superparamagnetic Fe3O4 nanoparticles. *RSC Advances* , **2014** , 4.26: 13409-13418.
2. HAMEROFF, Stuart R. *Ultimate computing: biomolecular consciousness and nanotechnology* . Elsevier, **2014** .
3. WANG, Dawei, et al. Clinical characteristics of 138 hospitalized patients with **2019** novel coronavirus–infected pneumonia in Wuhan, China. *Jama* , **2020** , 323.11: 1061-1069.
4. LIN, Guozhen, et al. Community evidence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission through air. *Atmospheric Environment* , **2021** , 246: 118083.
5. REDDY, L. Harivardhan, et al. Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications. *Chemical reviews* , **2012** , 112.11: 5818-5878.
6. AHMADI, Shideh, et al. Synthesis of Fe3O4 nanocrystals using hydrothermal approach. *Journal of Magnetism and Magnetic Materials* , **2012** , 324.24: 4147-4150.
7. LIAO, Yetong, et al. Magnetite nanoparticle-supported coordination polymer nanofibers: synthesis and catalytic application in Suzuki-Miyaura coupling. *ACS Applied Materials & Interfaces* ,**2010** , 2.8: 2333-2338.
8. RAHMAN, Md Mahbubor, et al. Highly temperature responsive core–shell magnetic particles: synthesis, characterization and colloidal properties. *Journal of colloid and interface science* ,**2011** , 360.2: 556-564.
9. BHARATHALA, Subhashini, et al. In silico and experimental studies of bovine serum albumin-encapsulated carbenoxolone nanoparticles with reduced cytotoxicity. *Colloids and Surfaces B: Biointerfaces* ,**2021** , 202: 111670.
10. GRONT, Dominik, et al. Optimization of protein models. *Wiley Interdisciplinary Reviews: Computational Molecular Science* ,**2012** , 2.3: 479-493.
11. FAROUQ, Mohammed AH, et al. Biomolecular interactions with nanoparticles: applications for coronavirus disease 2019. *Current opinion in colloid & interface science* ,**2021** , 54: 101461.
12. LAN, Jun, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* , **2020** , 581.7807: 215-220.
13. SHIRMOHAMMADI, Nima, et al. Formulation of new intelligent nanoparticle inhibited H1N1 influenza subtype and SARS coronavirus type 2 (COVID-19) in vitro. *Biomedical and Biotechnology Research Journal (BBRJ)* , **2021** , 5.4: 389.**GRAPHICAL ABSTRACT**

Arefeh Khodaee, Nima Shirmohammadi, Reza Aghanouri

A 3D view of ACE-2 bound with candidate drugs

Ligand – receptor interactions are the most important interactions for drug effectiveness. In order to estimate the effectiveness of designed drugs, scientist can use a computational process called docking .in this work, interactions between designed drugs and the receptors in body (ACE2) is estimated by docking with different docking servers. You can see a 3D picture of ACE-2 (as the receptor) bound with candidate drugs (as the

Ligand) in graphical abstract. The ligands are bound with their receptor as a complex.