

EXPERIMENTAL STUDY

Could boron compounds be effective against SARS-CoV-2?

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ABSTRACT

BACKGROUND: Seven dioxaborole compounds are investigated in this study. Structural and spectral characterization is done at M062X/6-31+G(d,p) level in the water. Active sites of these compounds are determined using molecular electrostatic potential (MEP) maps. Electrophilic and nucleophilic attack regions are determined.

AIM: We aimed to determine whether Boron-Containing Compounds (BCCs) inhibitor used in the treatment of COVID-19 are effective against SARS Cov-2 in silico.

RESULTS AND CONCLUSION: Since SARS-CoV-2 is a worldwide health problem, anti-viral properties of studied boron-containing compounds were investigated by molecular docking calculations. In addition to these calculations, MM/PSBA calculations were performed. It was found that boron compounds can be good drug candidate against SARS-CoV-2 and the best compound is ((R)-1-((S)-3-(4-(aminomethyl)phenyl)-2-benzamidopropanamido)-4-guanidinobutyl)boronic acid (C26) (Tab. 2, Fig. 6, Ref. 29). Text in PDF www.elis.sk

KEY WORDS: boronate ester, dioxaborole, in silico, SARS-CoV-2, MD calculations.

Introduction

In December 2019, the novel coronavirus that first appeared in bats in Wuhan, Hubei province of China and is thought to be transmitted, with unknown intermediary animals, was named as novel coronavirus 2019 (2019-nCoV) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and appeared as a pandemic threatening the world. The disease caused by SARS-CoV-2 was named coronavirus disease-19 (COVID-19) (1). It has 7 strains in humans, including coronaviruses, 229E, NL63, OC43, HKU1, Middle East respiratory syndrome (MERS) -CoV, severe acute respiratory syndrome (SARS)-CoV, and 2019-novel coronavirus (nCoV) (2). Approximately 28 million 680 thousand COVID-19 cases and 917 thousand 500 deaths due to COVID-19 have been reported by the World Health Organization (WHO) to date (13/09/2020) (3). The incubation period in COVID-19 varies between 2 and 14 days, and it is transmitted by contact or respiration with infected droplets. Symptoms are usually fever, cough, fatigue, weakness and shortness of breath. While the disease is asymptomatic in most people, it is mild in some people and progresses to pneumonia, acute respiratory distress syndrome (ARDS) and multi-organ dysfunction in the elderly and patients with comor-

bid diseases. The mortality rate is thought to vary between 2 % and 3 % (4–6). The most important structural proteins of coronaviruses are trimeric spike (S) protein, nucleocapsid (N) protein, membrane (M) protein and envelope (E) protein. Beta-Coronaviruses contain the hemagglutinin esterase (HE) glycoprotein, just like the influenza virus. This hemagglutinin esterase enzyme is found especially in the envelope of beta coronaviruses. Hemagglutinin esterase mediates reversible binding to O-acetylated sialic acids and acts as enzyme that destroy both lectin and the receptor (7). Scientists are conducting many drug studies for the treatment of COVID-19 disease. It has been stated in the studies conducted to date that many drugs such as interferon-alpha, ribavirin, chloroquine phosphate, arbidol and favipiravir can be used for the treatment of COVID-19. However, no effective drug can be used to treat COVID-19 (8, 9). It has been shown in previous studies that compounds containing boronic acid and boron have anti-viral effects against various viruses. It has been stated in many studies that boron is a potent anti-viral agent. Nocentini A. et al. have shown in their studies that benzoxaborols are effective against Hepatitis C (HCV) virus. Dijana Saftić et al demonstrated the high anti-viral effects of orthocarborane conjugates against Human cytomegalovirus (HCMV), herpes simplex virus type 1 (HSV-1), encephalomyocarditis virus (EMCV), human parainfluenza virus type 3 (HPIV-3), vesicular stomatitis virus. They showed that. In another study, Zeynep Ustaoglu et al. showed that sodium pentaborate pentahydrate in textiles reduced the growth of adenovirus type 5 and polyvirus type 1 by 60 % (10–12). Two molecular structures are thought to be essential for boron compounds' anti-viral activity, as the synthesis of boronic acid modifications and structures similar to nucleotide structures. Boronic acid modifications have a molecular structure that is of interest to glycoprotein structures. Viruses en-

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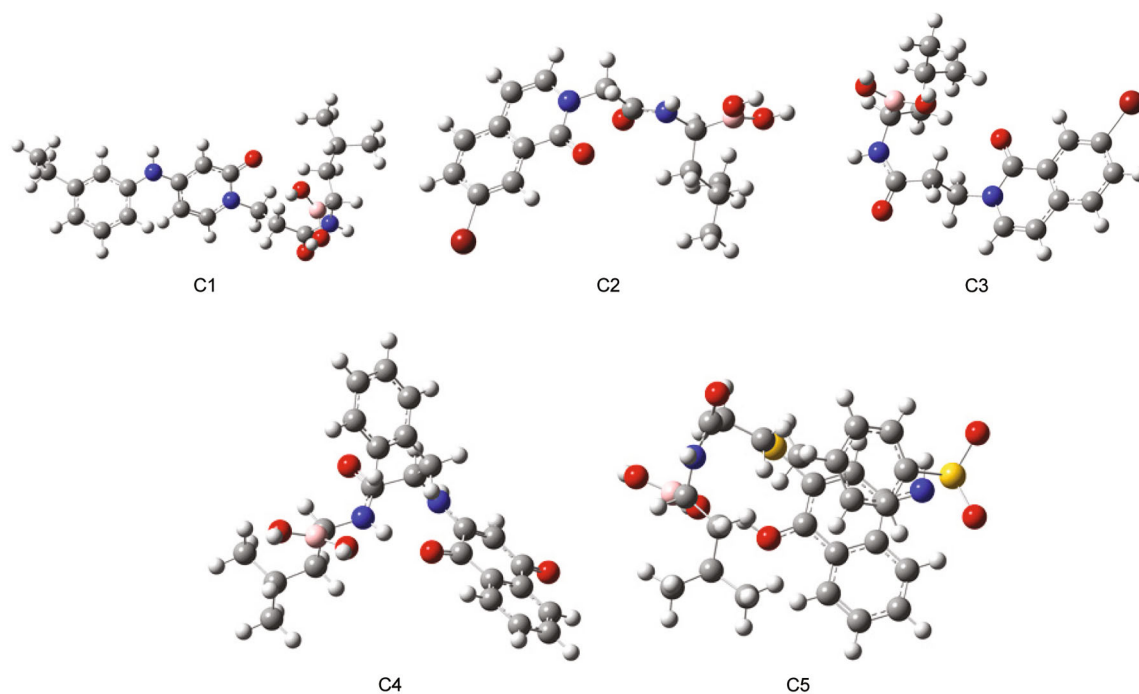


Fig. 1. The optimized structures of boron compounds C1 – C5.

ter the cell by binding to the cell membrane. And this they make through the glycoproteins it carries on itself or the receptors on the host cell's membrane. If we block these glycoproteins or receptors with boronic acid, it cannot enter the host cell, it cannot reproduce. It is thought that boron molecules formed by the synthesis of similar nucleotide structures replace purines and pyrimidines used in genetic coding and inhibit reproduction genetically (13, 14). Boron containing compounds (BCCs) have broad application area such as optic, biological, anticancer, anti-viral, etc. It has been reported that medical drugs containing boron have toxicity. In the late 20th century, it has been precisely reported that elemental boron and many BCC have low toxicity for human and animals. Researches on this subject have shown that the toxicity is not directly caused by boron (15). Many researches and preclinic test are still being performed for this aim (15). In this study, sixty-three boron-containing compounds are analyzed as an anti-viral agent against SARS-CoV-2 by in silico technique. Furthermore, molecular mechanics energies combined with the Poisson-Boltzmann surface area (MM/PBSA) analysis is performed.

Initially, the whole compounds are optimized at B3LYP-D3/6-31+G(d,p) level in the water. Then, these compounds are minimized by OPLS_3e method for the ligand preparation stage. In the second stage

of molecular docking analyses, target proteins which are 6M0J (16), 5RF1, and 7BV2 (17) are minimized with the same method. 6X6P, 5RF1, and 7BV2 proteins are spike glycoprotein, main protease, and RNA dependent RNA polymerase of SARS-CoV-2 virus, respectively. After minimizations, site map or ligand-binding domains (LBDs) of target proteins are calculated. Especially, receptor-binding domains (RBD) are selected from LBDs and defined for calculations. Molecular docking calculations are performed, and compounds that could be effective in the treatment of

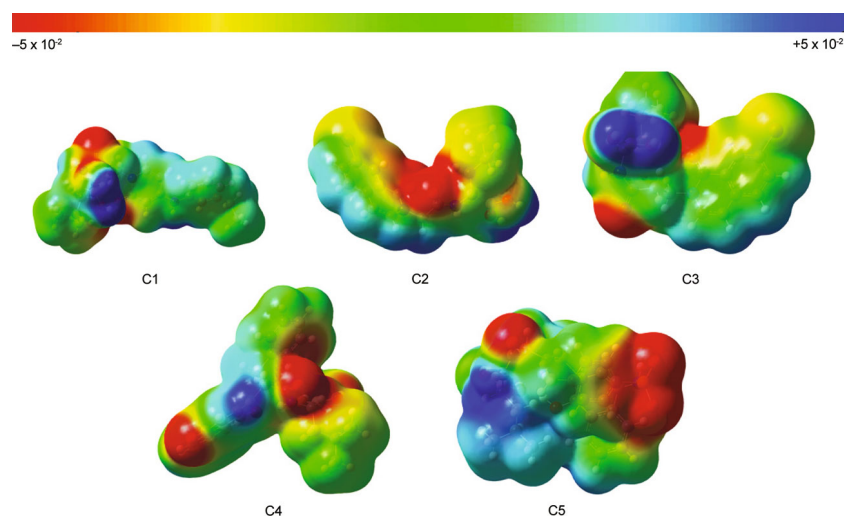


Fig. 2. The MEP maps of studied BCCs, C1 – C5.

Tab. 1. Molecular docking results of selected BCCs against target proteins.

Compounds	DS ^a	LE ^a	E _{vdw} ^a	E _{Coul} ^a	E _{Int} ^a	E _{HBond} ^a
5RF1						
C59	-8.87	-0.22	-38.30	-26.20	-64.51	-2.74
C26	-7.42	-0.22	-22.49	-21.71	-44.20	-3.06
C11	-7.04	-0.17	-34.53	-13.39	-47.91	-2.52
C48	-6.42	-0.26	-30.45	-8.03	-38.48	-1.71
6M0J						
C26	-6.71	-0.20	-24.49	-11.94	-36.43	-3.20
C59	-6.32	-0.15	-27.74	-15.61	-43.36	-2.50
C51	-6.09	-0.29	-22.25	-11.73	-33.99	-2.43
7BV2						
C26	-8.34	-0.25	-36.67	-33.85	-70.52	-3.05
C27	-7.22	-0.18	-43.20	-27.43	-70.63	-2.43
C50	-7.06	-0.31	-27.90	-9.61	-37.51	-4.44
C57	-6.64	-0.13	-50.71	-18.38	-69.09	-2.14
C12	-6.61	-0.15	-46.97	-17.28	-64.25	-2.58
C17	-6.37	-0.46	-15.54	-23.32	-38.86	-3.48
C56	-6.15	-0.26	-32.05	-14.76	-46.81	-2.60
C25	-6.10	-0.22	-39.57	-8.72	-48.29	-2.64

^a in kcal/mol

COVID19 are predicted. Finally, the MM/PBSA calculations are performed, and the most stable drug candidate in the interaction with target protein is determined in detail.

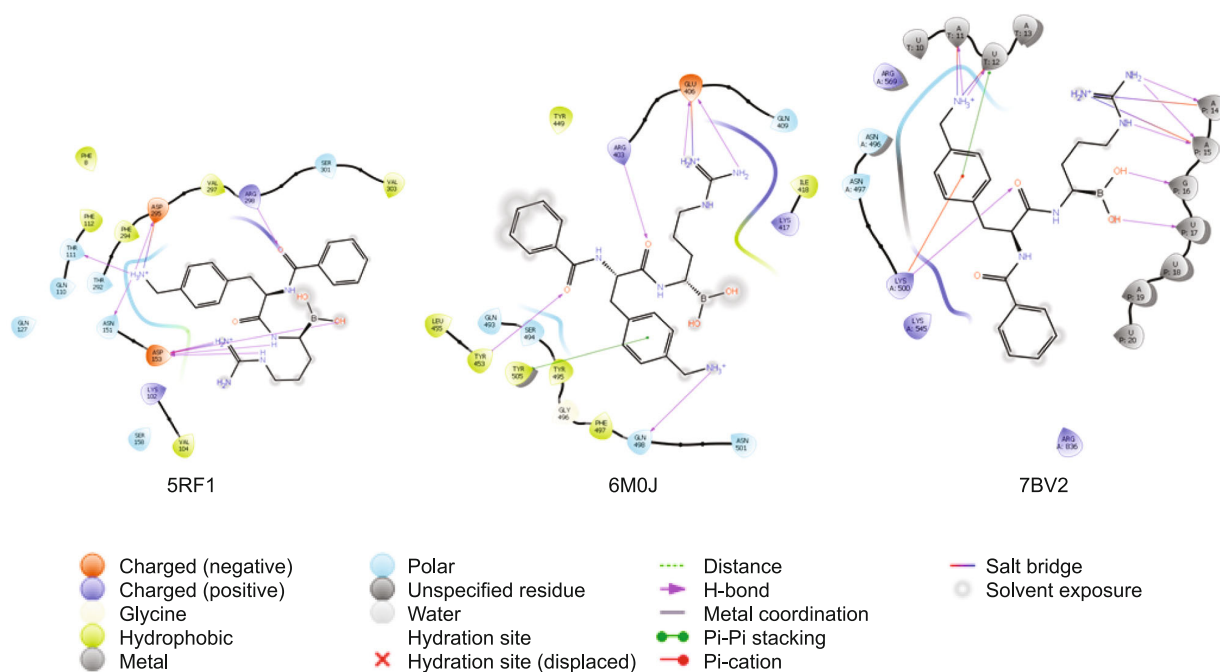
Methods

In silico analyses of BCCs compounds were done using four softwares, Gaussian, Maestro, Visual Molecular Dynamics and Nanoscale Molecular Dynamics (18–25). Initially, selected com-

pounds are optimized at B3LYP-D3 method with 6-31+G(d,p) level in the water. To take into account the solute-solvent interactions, the polarizable continuum model (PCM) using the integral equation formalism variant (IEF-PCM) was used. These optimization calculations were done only to obtain ground-state structures. These calculations were performed using the Gaussian program.

In silico studies, molecular docking calculations were done using the Maestro 19.4 program. In this step, LigPrep, Protein Preparation, SiteMap, Receptor Grid Generation, Ligand Docking and Ligand Interaction modules were used. Studied compounds were re-minimized by OPLS3e method at $\text{pH} = 7 \pm 2$. 6M0J (16), 5RF1, and 7BV2 (17) were prepared, and site maps were calculated. LBDs were calculated by using SiteMap module. Receptor binding domains (RBD) were defined using the Receptor Grid Generation module. Molecular docking calculations between studied boron compounds and target proteins were performed.

In the last step, molecular mechanics energies combined with the Poisson-Boltzmann or generalized Born and surface area (MM/PBSA) were done for each ligand-protein interaction by NAMD and VMD software (24, 25). In every five ns, Gibbs binding energy, van der Walls energy, kinetic energy and potential energy were calculated.

**Fig. 3. The interaction schematic structures of C26 with the target proteins.**

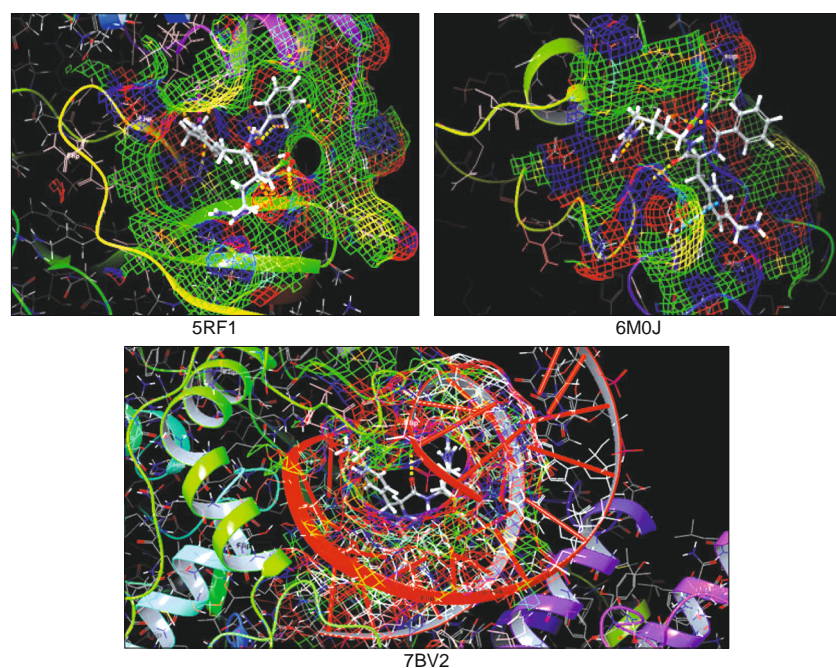


Fig. 4. The SED diagram of target proteins.

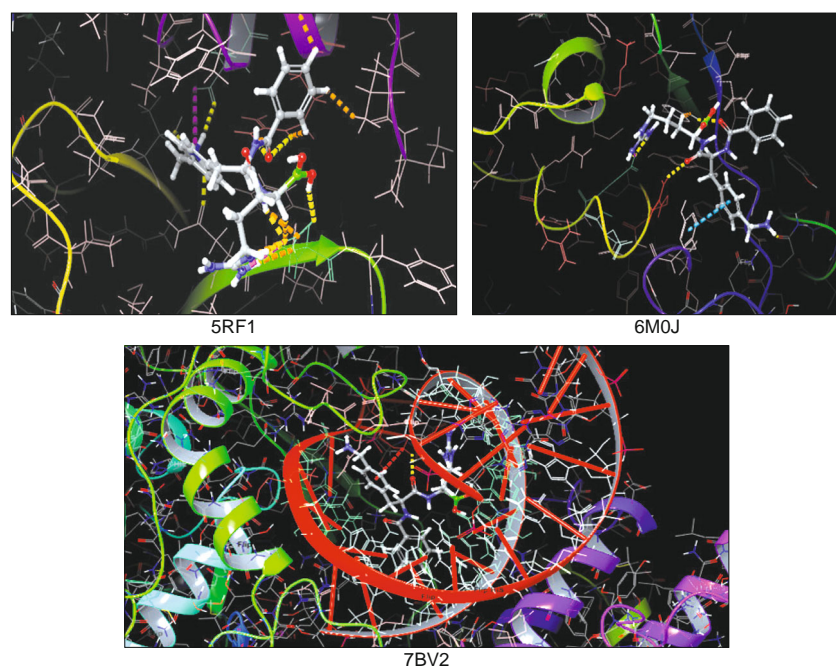


Fig. 5. Molecular docking structure between C26 and the target protein.

Results and discussions

Sixty-three boron compounds were optimized at B3LYP-D3/6-31+G(d,p) in water, and optimized structures of C1-C5 are represented in Figure 1. Additionally, optimized structures of the remaining compounds are represented in the supplemental material.

The optimization results show that the whole compounds are at the ground state, and no imaginary frequency is obtained from the calculations. The electronic geometry of the environment of boron atom is mainly found as trigonal and tetrahedral structures. The most important points are the determination of biological activity or drug-likeness properties. Some papers claimed that BCCs are effective in the prevention of SARS-CoV-2. How these compounds affect SARS-CoV-2 is still a matter of problem. There is only one paper about this problem published by Çetiner et al. 2021 (26). Before the *in silico* investigations, the determination of active sites is significant for the prediction of effectivity. For this aim, a molecular electrostatic potential (MEP) map is calculated and examined in detail. Initially, MEP maps of C1 – C5 are represented in Figure 2. MEP maps of the other ones are represented in the supplemental material.

According to Figure 2, there is a colour scale above the MEP maps. The red colour implies the highest electron density region while blue one implies the lowest electron density region (27). In MEP map, red colour is mainly localized on the environment of the heteroatoms. Additionally, green colour is mainly localized on the nitrogen atoms due to the steric hurdle. These diagrams give an important clue which is about how the compounds interact with the target protein (28). Finally, oxygen may play an important role in the inhibitor-protein interaction. However, it is certain that it will contribute to the interaction with other atoms.

As for the other analyses, *in silico* analyses are done in detail. Molecular docking and MM-PBSA calculations are done. Initially, all ligands are prepared at $\text{pH} = 7 \pm 2$ using OPLS3e method. Then, three proteins which are spike glycoprotein (PDB ID: 6M0J), RNA dependent RNA polymerase (PDB ID: 7BV2) and main protease (PDB ID: 5RF1) are minimized at the same level of theory. These three proteins are important for the SARS-CoV-2 virus. The effectivity of studied BCCs is investigated against

these proteins. Molecular docking calculations were performed. Nearly the whole studied compounds are inhibiting the target protein. Molecular docking results of studied BCCs docking scores of which are lower than -6 kcal/mol are given in Table 1. Docking score (DS), ligand efficiency (LE), van der Waals energy (E_{vdw}), coulomb energy (E_{Coul}), interaction energy (E_{int}) and H bond en-

Tab. 2. The MM-PBSA results for compound C26.

Time (ns)	5RF1		6MJ0		7BV2	
	E_{Binding}^a	SD	E_{Binding}^a	SD	E_{Binding}^a	SD
0	-2782.0	–	-2732.2	–	-3097.0	–
5	-1859.6	±237.6	-2615.4	±114.9	-3056.0	±232.4
10	-2169.1	±254.1	-2515.1	±89.2	-3028.8	±329.8
15	-1928.2	±119.4	-2439.1	±95.3	-3225.3	±266.1
20	-2432.4	±198.4	-2162.5	±161.8	-2995.9	±201.5
25	-1699.7	±318.0	-2361.2	±80.2	-3146.8	±290.0
30	-1973.2	±238.0	-2803.1	±289.3	-2875.3	±229.5
35	-1927.0	±145.1	-2413.4	±169.4	-2990.6	±344.4
40	-2340.5	±154.3	-2416.0	±107.6	-2438.1	±246.0
45	-2296.4	±147.6	-2348.9	±152.4	-3098.5	±413.2
50	-1979.5	±201.7	-2705.6	±116.6	-2274.8	±328.5
55	-2075.1	±273.1	-2790.4	±172.6	-2607.7	±344.6
60	-2108.8	±89.2	-2760.2	±119.4	-3924.2	±397.7
65	-1751.8	±203.0	-2321.4	±195.7	-3258.9	±363.3
70	-2373.6	±207.3	-2626.2	±245.4	-3431.4	±201.4
75	-2322.6	±173.6	-2616.2	±103.6	-2899.9	±299.8
80	-1978.0	±101.0	-2538.0	±91.1	-3168.9	±330.6
85	-2430.7	±159.4	-2750.7	±112.3	-3903.8	±370.6
90	-2342.2	±198.9	-3063.1	±213.2	-3569.4	±251.6
95	-2252.6	±255.4	-2605.6	±125.1	-2871.1	±234.8
100	-1817.7	±146.3	-2976.0	±189.9	-3132.1	±313.3

^a in kcal/mol

ergy (E_{HBond}) are reported in the same table. Molecular docking results of All compounds are given in Supp. Table S1 for 5RF1, 6M0J and 7BV2, respectively.

According to the docking results, nearly all studied BCCs interacted with target proteins. However, inhibitors to be focused on are selected according to their docking scores. Compounds with a docking score of less than -6 kcal/mol are taken into consideration. According to Table 1, studied BCCs are active against three main targets of SARS-CoV-2. However, it can be said that the compounds studied are effective on RdRp and main protease but not on spike glycoprotein, according to others. The first parameter is docking score which is related with key-lock compatibility (29). This is the first wanted property due to the fact that ligand should be inserted in the ligand-binding domain of target protein. As for the ligand efficiency, this parameter is related with docking score and atom with nonhydrogen. The other three parameters are van der Walls, Coulomb and interaction energy. They are related with

binding energy of ligand (8). The van der Walls energy is related with chemical interaction while Coulomb energy is related with physical interactions. The interaction energy is a sum of van der Walls and coulomb energy. This parameter is as important as docking score is, because, interaction energy shows that ligand can inhibit the protein or not. The last parameters is H Bond energy which occurred in ligand-protein interaction. The two studied compounds which stand out in this project are C26 and C59. However, C59 inhibits the main protease and spike glycoproteins while C26 inhibits the all target proteins in this study. The interaction schematic structures of C26 are represented in Figure 3.

According to Figure 3, the dominant interaction types are hydrogen bond, polar, hydrophobic, charged (negative), pi-cation and solvent exposure (29,30). However, the most striking one is hydrogen bond due to the fact that H-bonding potential of C26 is

high. The surface electron distribution (SED) of the active site of proteins interacting with the ligand is shown in Figure 4.

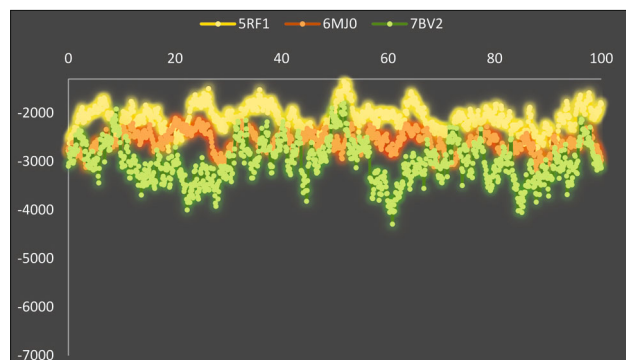
According to Figure 4, there are three main colours, red, green and blue. The red one implies the highest electron density region, while the blue one shows the poorest electron density sites. Generally, H-bond occurs between inhibitor and red region of receptor binding site. Finally, the docking structures are represented in Figure 5 for C26.

The final investigation is Molecular Mechanics-Poisson-Boltzmann Surface Area (MM-PSBA) calculation. Whether the target protein can be inhibited by ligand or not can be predicted from molecular docking calculations. These calculations cannot give the stability of interactions. However, this stability can be analyzed by MM-PSBA calculations. In this study, the stability is investigated in each 5 nanosecond (ns) in the range of 0–100 ns. The binding energy is calculated in each 5 ns for the all protein-ligand complexes. Additionally, standard deviation is calculated, too. The results of MM-PSBA which are binding energy (E_{Binding}) and standard deviation (SD) analyses, are given in Table 2.

According to Table 2 and Figure 6, it can be said that, studied BCCs are effective against SARS-CoV-2. It can be said that C26 is effective against spike glycoprotein, RNA dependent RNA polymerase and main protease of SARS-CoV-2.

Conclusions

Boron containing compounds attract the attention of researcher. Especially, many of papers indicate that the BCCs have biological activity. In this study, the 63 compounds are examined against SARS-CoV-2. Initially, these compounds are optimized at B3LYP-D3/6-31+G(d,p) level in the water. Then, molecular docking analyses are performed against spike glycoprotein, main protease and

**Fig. 6.** The Gibbs binding energy in the range of 0 – 100 ns.

RNA dependent RNA polymerase of SARS-CoV-2. Additionally, molecular mechanics-poisson-boltzmann surface area calculation is performed for selected interaction. In the view of all results, ((R)-1-((S)-3-(4-(aminomethyl)phenyl)-2-benzamidopropanamido)-4-guanidinobutyl)boronic acid is found as the best drug candidate for the treatment of COVID19.

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