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Investigation of the factors effecting inhibition by changing substitute groups of 6LU7 protein-favipiravir molecules used in the treatment of COVID 19

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Abstract

In this study, favipiravir molecules with different substituent groups are designed, and it is aimed to investigate the change of binding energies to 6LU7 protein depending on the electronic distribution, dipole moment and entropy changes in the molecule. 38 favipiravir derivatives connected to different groups are modeled with Gaussian program and optimized and the interactions of these molecules with 6LU7 protein are investigated with the help of Autodock / vina program. Then, the values obtained from the Autodock / vina program are analyzed statistically (anova, correlation and regression analysis) with the help of IBM SPSS Statistics 23 program. As a result of the analysis, it is determined that only entropy has an effect on the binding energy. It has been determined that there is no statistically significant relationship between dipole moment and electronegativity and binding energy.

Keywords: favirapiravir, covid-19, gaussian, autodock / vina, statistical analysis.

1. Introduction

Coronovirus (Covid-19) disease is caused by coronovirus 2 (Sars-Cov-2), which causes acute respiratory syndrome. The coronavirus comes from the corona, which means "crown" in Latin. The coronavirus is a virus that is about 125 nanometers in size and consists of 30 genes. The virus can only be seen under an electron microscope. The virus emerges very quickly and spreads very quickly. Scientists estimate that the virus could cause 40 million deaths worldwide if not brought under control [1].

For this reason, very intensive studies have been going on in the last few months. In addition to the search for an effective vaccine, some approved and experimental antiviral small molecule drugs are also being tested for efficacy against the virus [2]. The most popular of these drugs is Favipiravir (T-705, 6-fluoro-3-hydroxy-2 pyrazinecarboxamide), which is a drug developed by the Japanese and used in treatments in our country. Favipiravir, the general structure of which is given in Figure 1, was designed by Toyama (Fujifilm group) company for influenza treatment in 2014 [3]. After obtaining permission for use as an experimental treatment in covid-19 infections that we were exposed to very quickly, it was tested in 340 people in Wuhan and Shenzhen and promising results were obtained [4]. Covid-19 tests were negative for those who were given drugs in Shenzhen, on average four days after they were exposed to the infection, and 11 days after the infection for those who did not. In addition, improvement was observed in the lungs of 91% of the patients who were given medication. This rate was 62% in patients who could not be treated at the beginning [5].

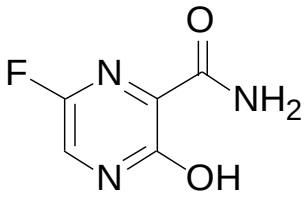

Figure 1: Structure of the favirapiravir molecule.

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The entry of the Covid-19 virus into the human body occurs primarily by interacting with the Ace-2 enzyme on the surface of the living cell and taking it into the cell and by replication in the RNA polymerase enzyme. Therefore, two effective enzyme structures can be mentioned for Covid-19, and studies can be performed using these two enzymes in modelling studies. The main protease of Covid-19 was isolated by Jin et al for active drug design (PDB number: 6LU7). The enzyme whose structure is given in Figure 2 was used in the new drug design [7].

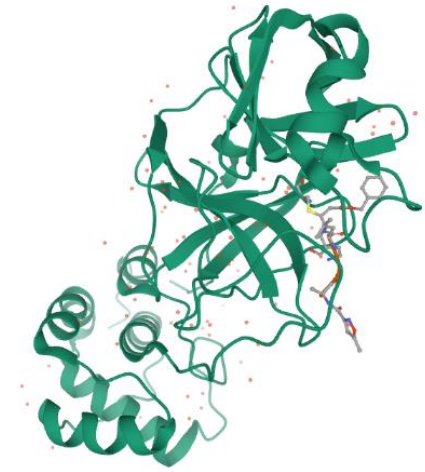

Figure 2: Isolated 6LU7 enzyme for covid-19 virus.

On the other hand, especially the cost of drug trials is very high and the synthesis steps take a lot of time. For this reason, it is important to model molecules beforehand, to examine their properties and to focus on syntheses whose properties are above the expected value. In addition, the development of programs such as Density Function Theory, autodock vina and the rapid advancement of computer science in recent years have increased the interest in this field. Therefore, some substituted favirapiravir molecules with different electronegative groups were modelled in this study. Optimized structures of the modelled molecules were calculated in the B3LYP / 6-311 ++ G (d, p) method and base set, and their optimal geometries were determined. Docking studies of these molecules with the appropriate protein molecule (PDB no = 6LU7) were performed with Autodock vina program and their binding energies were determined. The binding energies of the molecules were listed according to physicochemical parameters such as dipole moment, electronegativity values of the added groups and entropy, and it was aimed to determine the statistical relationship between them. It is expected that this study will guide scientists working on drug design in determining the physicochemical properties to be considered in the groups to be added.

2. Material and method

In this study, 38 molecules of favirapiravir derivative whose general structure is given in Figure 3 and listed in Table 1 according to R and R 'groups were used. Density function theory (YFK / B3LYP) [7] and 6-311 ++ G (d, p) base sets were used in optimization and frequency calculations of molecules. The lack of negative frequency of molecules supports that molecules are optimized in the most accurate geometry. Optimized molecular parameters are used in molecular docking studies. .Pdbqt files were obtained from optimized molecular structures and molecular docking calculations were made with the autodock vina program. The crystal structure of the DNA molecule used has been downloaded from the protein data bank (PDB) (PDB no: 1BNA). For each molecule, the binding types in 9 different conformers were examined and the most suitable binding type was determined (RMSD <2 Å).

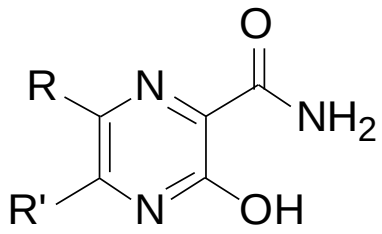

Figure 3: General structure of Favirapiravir derivative molecules

Table 1. List of groups added to the favirapiravir molecule used in the study

Molecule	R	R′	Molecule	R	R′
1	-F	-H	**20**	-Cl	-H
2	-F	-C6H5	**21**	-Cl	-C6H5
3	-F	-C6H4-p-Br	**22**	-Cl	-C6H4-p-Br
4	-F	-C6H4-p-Cl	**23**	-Cl	-C6H4-p-Cl
5	-F	-C6H4-p-F	**24**	-Cl	-C6H4-p-F
6	-F	-C6H4-m-Br	**25**	-Cl	-C6H4-m-Br
7	-F	-C6H4-m-Cl	**26**	-Cl	-C6H4-m-Cl
8	-F	-C6H4-m-F	**27**	-Cl	-C6H4-m-F
9	-F	-Br	**28**	-Cl	-Br
10	-F	-Cl	**29**	-Cl	-Cl
11	-F	-F	**30**	-Cl	-F
12	-F	-C4H3S	**31**	-Cl	-C4H3S
13	-F	-C4H4N	**32**	-Cl	-C4H4N
14	-F	-CH3	**33**	-Cl	-CH3
15	-F	-NH2	**34**	-Cl	-NH2
16	-F	-NO2	**35**	-Cl	-NO2
17	-F	-OH	**36**	-Cl	-OH
18	-F	-C5H9	**37**	-Cl	-C5H9
19	-F	-SO3H	**38**	-Cl	-SO3H

3. Findings and discussion

The most probable geometries of the determined favirapiravir and derivative molecules were optimized using the Density Functional Theory (YFK) / B3LYP method and 6-311 ++ G (d, p) base set and frequency calculations were made. Dipole moment and entropy values obtained from the output files are listed in Table 2.

In addition, the electronegativity values of each molecule added are also given in the table. Optimized molecular geometries were used in molecular docking studies and suitable files for docking studies were obtained by Autodock program. The binding energies of each molecule were calculated with the Autudock / vina program and listed in Table 2.

When Table 2 is examined, it is determined that the molecule with the highest binding energy is the number 5 molecule. It is the favirapiravir molecule containing fluoro phenyl group in its molecular structure, and its binding energy has been calculated as -29.29 kJ / mol.**Statistical analysis:** Correlation, ANOVA and regression analysis were performed to determine the relationships between the bond energies and the physicochemical parameters such as the dipole moment of the molecules, the electronegativity values of the added groups and entropy.

Table 2: Dipole moment, electronegativity, entropy and DNA binding energies of favirapiravir and derivative molecules

Molecule	Input		Output	
	Dipole moment	**Electronegativity**	**Entropy (S, J/molK)**	**Binding Energy (kJ/mol)**
1	5,9280	2,20	404,14	-20.92
2	6,5666	0,00	504,69	-27.61
3	5,7345	2,96	523,80	-28.03
4	5,7307	3,16	536,32	-28.45
5	5,7666	3,98	548,64	-29.29
6	4,7777	2,96	537,64	-28.45
7	4,7248	3,16	548,48	-28.87
8	4,7799	3,98	525,55	-28.45
9	5,7684	2,96	446,72	-20.50
10	5,7520	3,16	434,40	-21.34
11	5,7672	3,98	421,33	-22.59
12	7,0831	0,51	505,64	-25.52
13	6,0989	1,88	498,27	-25.10
14	6,2553	2,55	441,49	-21.34
15	7,3331	3,25	432,90	-23.01
16	6,2563	4,19	470,00	-22.59
17	7,4787	1,40	424,83	-23.01
18	6,6605	0,02	538,64	-25.94
19	8,4128	4,06	512,01	-24.69
20	4,1589	2,20	392,00	-20.50
21	4,4485	0,00	446,01	-26.36
22	3,7025	2,96	485,18	-27.20
23	3,7029	3,16	473,67	-27.20
24	3,7470	3,98	462,96	-27.61
25	2,5331	2,96	486,77	-28.87
26	2,4685	3,16	475,51	-28.45
27	2,5565	3,98	464,51	-28.03
28	3,9061	2,96	410,66	-21.34
29	3,8962	3,16	426,60	-20.92
30	3,9188	3,98	409,20	-21.76
31	4,8758	0,51	440,41	-24.27
32	3,8808	1,88	450,57	-23.85
33	4,5930	2,55	420,87	-21.34
34	5,5174	3,25	414,72	-21.34
35	4,9751	4,19	438,40	-22.18
36	5,5778	1,40	416,43	-22.59
37	4,9584	0,02	463,42	-24.27
38	6,9386	4,06	456,60	-24.27

Table 3: Correlation analysis

		Binding energy	**Dipole moment**	**Electronegativity**	**Entropy**
Binding energy	Pearson Correlation	1	-,238	,012	,828**
	Sig. (2-tailed)		,150	,945	,000
Dipole moment	Pearson Correlation	-,238	1	-,168	,140
	Sig. (2-tailed)	,150		,314	,402
Electronegativity	Pearson Correlation	,012	-,168	1	-,001
	Sig. (2-tailed)	,945	,314		,994
Entropy	Pearson Correlation	,828**	,140	-,001	1
	Sig. (2-tailed)	,000	,402	,994	
Correlation is significant at the 0.01 level (2-tailed).					

According to the correlation analysis given in Table 3; It was determined that there is a strong positive (0.828) and significant relationship between binding energies and entropy. Accordingly, it is concluded that when entropy increases, the binding energies also increase. However, it was found that there was

no significant relationship between binding energies and dipole moment and electronegativity. In the same analysis, it was found that there was no significant relationship between physicochemical parameters.

Table 4: Anova analysis

		Sum of Squares	df	Mean Square	F	Sig.
Dipole moment	Between Groups	46,890	19	2,468	1,638	,150
	Within Groups	27,113	18	1,506		
	Total	74,003	37			
Electronegativity	Between Groups	37,418	19	1,969	1,335	,272
	Within Groups	26,546	18	1,475		
	Total	63,964	37			
Entropy	Between Groups	64558,120	19	3397,796	5,235	,000
	Within Groups	11682,605	18	649,034		
	Total	76240,725	37			

When the anova analysis in Table 4 is examined, it has been found that there is a statistically significant relationship between the binding energy and only the entropy parameter. This result coincides with the result obtained from the correlation analysis

Table 5: Regression analysis-model summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	,903a	,815	,798	1,32949	2,043

a. Predictors: (Constant), Dipole moment, Electronegativity, Entropy
b. Dependent Variable: Binding energy

When the multiple regression analysis model summary given in Table 5 is examined, it was obtained as R Sguare = 0,815. According to this result, 81,5% change in binding energies is explained by the variables Dipole moment, Electronegativity, Entropy in the model. The remaining part of 18,5% shows the effect of other variables not included in the model. The multiple regression mathematical model obtained as a result of the regression analysis given in Table 6 was obtained as follows;

Binding energy= 2,294+Dipole moment(-0.773)+ Electronegativity(-0,111)+Entropy(0,057)

Table 6: Regression analysis-coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	2,294	2,366		,970	,339
Dipole moment	-,773	,158	-,369	-4,882	,000
Electronegativity	-,111	,169	-,049	-,657	,516
Entropy	,057	,005	,879	11,791	,000

a. Dependent Variable: Binding energy

Using this model for the dipole moment, electronegativity and entropy values of the compound number 5 with the highest binding energies (-29.29 kJ / mol), the bond energy of the compound was obtained as -28.66 kJ / mol. This value shows that there is a 2% margin of error between the binding energy calculated with the Autudock / vina program.

It contains electronegative atoms such as fluorine atom in the structure of the molecule no.5 with the highest binding energy. While this group is not reflected in the dipole moment of the molecule, it increases the entropy value. Molecular structures, possible weak interactions and statistical input parameters (entropy, dipole moment and electronegativity) obtained as a result of molecular docking calculations with the Autodock vina program and 6LU7 protein, optimized in the Gaussian program, are illustrated in Figure 4. As a result of molecular docking studies, the amino acids

LYS137, ARG131, THR199, LEU272, LEU286 and ASP289 were determined as the molecule numbered 5 interacted with the 6LU7 protein (interaction length <3.5 Å). Hydrogen bonds are the weak interactions that make the greatest contribution to the interaction of the ligand molecule with the protein, and it is the most important parameter that determines the activity of the ligand molecule. According to these results, it is thought that this molecule, which contains an electronegative fluorine atom, interacts like a hydrogen bond. For this reason, although the binding energy of this molecule is expected to be high, the effective parameters on the binding energy have been determined by statistical approaches.

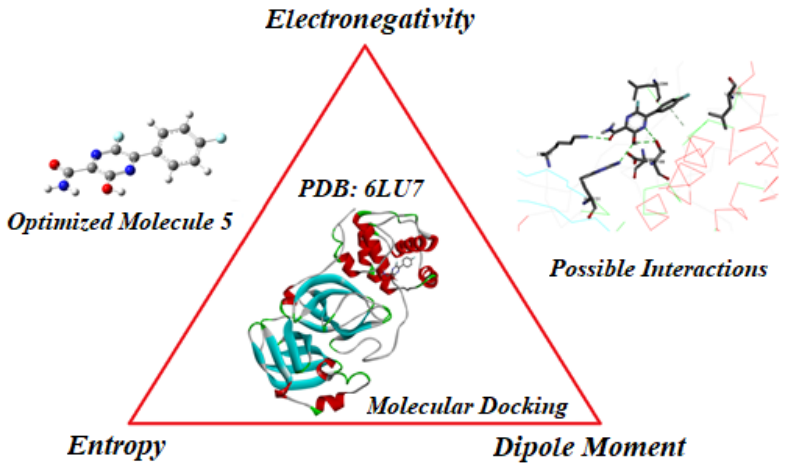

Figure 4. Optimized structure of molecule 5, molecular docking structure with 6lu7 enzyme, possible interactions and physicochemical parameters affecting molecular docking

4. Conclusion and evaluation

The fact that it has not been taken under control since the day it first came to the present and the drug active substance that treats COVID-19 disease has not been found, further increases the importance of studies in this field. The results of this study, which has a different point of view today, when the focus is on drug synthesis, will attract the attention of scientists working in this field.

The binding energies of 38 different favirapiravir derivative molecules with different groups were calculated according to physicochemical parameters (electronegativity, dipole moment, entropy) and the relationships between these parameters and the binding energies were statistically analyzed. As a result of the examinations, the following results were obtained;

- Only entropy has an effect on binding energy,
- There is no statistical relationship between electronegativity and dipole moment and binding energy,
- In the regression analysis, the 5th molecule can have the highest binding energy.

These results are thought to be able to be modeled according to the entropy of possible new drug active substances that may be formed by the addition of different organic groups to the favirapiravir molecule used for treatment against this virus with the covid-19 outbreak and will guide scientists working in this field.

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