Design and Synthesis of Five Cyclobuta-1,3-Dien-1-yl-Steroid Derivatives to Evaluate Their Theoretical Activity Against COVID-19

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Abstract: Several drugs have been prepared to evaluate their interaction with coronavirus disease (COVID-19) using some docking models. The aim of this research was to prepare five cyclobuta-1,3-dien-1-yl-steroid derivatives from 3-ethylinaniline using some chemical reactions. In addition, the interaction of compounds 3 to 7 with COVID-19 (6LU7 protein) was evaluated using chloroquine, remdesivir, and favipiravir as controls in a theoretical model. The results showed that compounds 6 have a higher affinity by 6LU7-protein surface compared with either chloroquine, remdesivir, and favipiravir. In addition, remdesivir have lower affinity by 6LU7-protein in comparison with compound 5. In conclusion, this phenomenon suggests that either compounds 5 or 6 could exert some changes in the biological activity of COVID-19.

Keywords: Steroid; derivative; 3-ethylinaniline; COVID-19.

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1. Introduction

Respiratory diseases are one of the main health problems worldwide [1-6]. Some of these clinical pathologies have been related to severe acute respiratory syndrome coronavirus 2 (SARS-COV) [7-12]. It is noteworthy that some antiviral drugs have been used for the treatment of SARS-COV; unfortunately, at present, there is no drug to decrease respiratory diseases caused by SARS-COV. In search of some therapeutic alternatives, several compounds have been synthesized to the treatment of SARS-COV: For example, the synthesis of compound an oxohexanoic-acid analog from hydroxyhexanoic-acid derivative [13]. Furthermore, a report showed the synthesis of an oxo-pyrrolidin-3-yl]butanamide analog by the reaction of a α-hydroxyamide with periodinane [14]. Other study shown the preparation of an indole derivative from 2,3-Dioxo-2,3-dihydro-1H-indole-5-carboxylic acid and 2-(bromomethyl)naphthalene [15]. Additionally, a report indicated the synthesis of a carboxamide via reaction of pyridine-3-carboxaldehyde and 4-tert-butylaniline [16]. Other
data, showed the synthesis of piperidine from of Naphthyl)ethyl]-4-methoxycarbonylpiperidine and with dimethylformamide [17]. All these data have shown the synthesis of several drugs with activity against some SARS-CoV strains. However, the binding of these compounds with several SARS-CoV strains is very confusing; perhaps this phenomenon could be due to the different chemical structure of each compound. Analyzing these data, the objective of this investigation was to synthesize five cyclobuta-1,3-dien-1-yl-steroid derivatives to evaluate their theoretical activity against COVID-19 using the 6LU7-protein as a theoretical model on the DockingServer.

2. Materials and Methods

2.1. General methods.

The steroid derivatives were prepared using a previous method reported [18, 19]. In addition, all reagents used in this investigation were acquired from Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were evaluated with a Thermo Scientific iSOFT-IR spectrometer. $^1$H and $^{13}$C NMR spectra were recorded using a Varian VXR300/5 FT NMR spectrometer at 300 MHz in CDCl$_3$ using TMS as an internal standard. EIMS spectra were obtained with a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/02400 elemental analyzer.

2.2. Synthesis of (3-Ethynyl-phenyl)-methyl-amine (2).

In a round bottom flask (10 ml), 3-ethynilaniline (100 µl, 0.90 mmol), potassium hydroxide (50 mg, 0.89 mmol), palladium on carbon (100 mg, 0.93 mmol) and 10 ml of methanol were stirred at reflux for 12 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:hexane:water (4:2:1) system; yielding 60% of product; m.p. 70-72 ºC; IR (V$_\text{max}$, cm$^{-1}$) 3310 and 1602: $^1$H NMR (300 MHz, CDCl$_3$-d$_2$) $\delta$H: 2.82 (s, 1H), 2.88 (s, 3H), 4.82 (broad, 1H), 6.54-7.12 (m, 4H) ppm. $^{13}$C NMR (300 Hz, CDCl$_3$) $\delta$C: 30.32, 78.22, 84.00, 114.50, 122.12, 122.35, 126.22, 129.70, 151.30 ppm. EIMS m/z: 131.07. Anal. Calcd. for C$_9$H$_9$N. C, 82.41; H, 6.92; N, 10.68. Found: C, 82.40; H, 6.90.

(13S,17S)-13-methyl-17-[4-[(methylamino)phenyl]cyclobuta-1,3-dien-1-yl]-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (3).

In a round bottom flask (10 ml), compound 2 [100 mg, 0.76 mmol], 17α-ethynilestradiol (fragment A) ([225 mg, 0.76 mmol], Copper(II) chloride [105 mg, 0.78 mmol] and 5 ml of methanol were stirred at room temperature for 72 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:water (4:1) system; yielding 55% of product; m.p. 165-167 ºC; IR (V$_\text{max}$, cm$^{-1}$) 3400 and 3312: $^1$H NMR (300 MHz, CDCl$_3$-d$_2$) $\delta$H: 0.72 (s, 3H), 1.12-2.50 (m, 13H), 2.74 (s, 3H), 2.77-2.80 (m, 2H), 6.12 (d, 1H, J = -0.96 Hz), 6.26 (broad, 3H), 6.28 (d, 1H, J = -0.96 Hz), 6.60 (m, 1H), 6.72-6.76 (m, 2H), 6.80-7.16 (m, 2H), 7.20 (m, 1H), 7.26 (m, 1H) ppm. $^{13}$C NMR (300 Hz, CDCl$_3$) $\delta$C: 15.12, 23.76, 26.25, 27.96, 30.26, 30.28, 33.04, 33.12, 40.70, 42.60, 44.30, 50.88, 79.56, 112.54, 113.64, 115.96, 116.00, 119.55, 126.57, 126.76, 128.44, 128.60, 131.92, 135.66, 138.20, 138.50, 142.00, 146.06, 156.02 ppm. EIMS m/z: 427.25. Anal. Calcd. for C$_{29}$H$_{33}$NO$_2$. C, 81.46; H, 7.78; N, 3.28; O, 7.48. Found: C, 81.44; H, 7.73.
In a round bottom flask (10 ml), compound 2 [100 mg, 0.76 mmol], 2-nitro-17αethynilestradiol (fragment B) [260 mg, 0.76 mmol], Copper(II) chloride [105 mg, 0.78 mmol] and 5 ml of methanol were stirred at room temperature for 72 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:hexane:water (4:2:1) system; yielding 52% of product; m.p. 144-146 °C; IR (\(V_{\text{max}}\), cm\(^{-1}\)) 3040, 3310, 1622 and 1222. \(^1\)H NMR (300 MHz, CDCl\(_3\); \(\delta_H\)) 0.72 (s, 3H), 1.12-2.58 (m, 13H), 2.74 (s, 3H), 2.82-2.86 (m, 2H), 5.96 (broad, 1H), 6.06-6.08 (m, 2H), 6.60-6.80 (m, 2H), 7.16 (m, 1H), 7.18-7.40 (m, 2H), 7.48-7.60 (m, 2H), 10.02 (s, 1H), 10.06 (s, 1H) ppm. \(^{13}\)C NMR (300 Hz, CDCl\(_3\); \(\delta_C\)) 17.80, 24.24, 24.55, 27.26, 27.76, 29.62, 30.26, 35.74, 36.34, 40.90, 44.60, 52.78, 61.78, 111.92, 116.00, 118.90, 124.75, 125.40, 126.10, 128.44, 129.85, 132.13, 135.16, 135.66, 135.90, 136.70, 143.50, 147.44, 150.92, 192.12, 195.32 ppm. EI-MS m/z: 472.23. Anal. Calcd. for C\(_{29}\)H\(_{32}\)N\(_2\)O\(_4\). C, 73.70; H, 6.83; N, 5.93; O, 13.54. Found: C, 73.68; H, 6.80.

(15S,16S)-16-methyl-15-[4-(3-methylamino)phenyl]cyclobuta-1,3-dien-1-yl]-5-oxapentacyclo[7.7.0.0.4.8.2.0.8.4.6.0.12,16]octadeca-2(8),3,6-trien-15-ol (5).

In a round bottom flask (10 ml), compound 2 [100 mg, 0.76 mmol], oxirenol-steroid derivative (fragment C) [225 mg, 0.76 mmol], Copper(II) chloride [105 mg, 0.78 mmol] and 5 ml of methanol were stirred at room temperature for 72 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:water (4:1) system; yielding 66% of product; m.p. 170-172 °C; IR (\(V_{\text{max}}\), cm\(^{-1}\)) 3310, 1622 and 1222. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta_H\) 7.32. \(^{13}\)C NMR (300 Hz, CDCl\(_3\)) \(\delta_C\) 15.30, 23.76, 26.25, 27.86, 29.82, 30.26, 33.04, 33.14, 40.70, 42.60, 45.40, 50.88, 79.56, 108.84, 108.90, 112.56, 116.00, 119.55, 126.57, 128.44, 128.60, 132.33, 135.66, 138.50, 142.00, 145.08, 146.06, 148.46 ppm. EI-MS m/z: 425.23. Anal. Calcd. for C\(_{29}\)H\(_{32}\)N\(_2\)O\(_2\). C, 81.85; H, 7.34; N, 3.29; O, 7.52. Found: C, 81.82; H, 7.32.

(13S,17S)-13-methyl-17-[4-(3-methylamino)phenyl]cyclobuta-1,3-dien-1-yl]-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-dicarbaldehyde (6).

In a round bottom flask (10 ml), compound 2 [100 mg, 0.76 mmol], 17α-ethyl-steroid-3,17-dicarbaldehyde (fragment D) [245 mg, 0.76 mmol], Copper(II) chloride [105 mg, 0.78 mmol] and 5 ml of methanol were stirred at room temperature for 72 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:water (3:1) system; yielding 65% of product; m.p. 144-146 °C; IR (\(V_{\text{max}}\), cm\(^{-1}\)) 3312, 1720 and 1602. \(^1\)H NMR (300 MHz, CDCl\(_3\)); \(\delta_H\) 1.10 (s, 3H), 1.12-2.58 (m, 13H), 2.74 (s, 3H), 2.82-2.86 (m, 2H), 5.96 (broad, 1H), 6.06-6.08 (m, 2H), 6.60-6.80 (m, 2H), 7.16 (m, 1H), 7.18-7.40 (m, 2H), 7.48-7.60 (m, 2H), 10.02 (s, 1H), 10.06 (s, 1H) ppm. \(^{13}\)C NMR (300 Hz, CDCl\(_3\)); \(\delta_C\) 17.80, 24.24, 24.55, 27.26, 27.76, 29.62, 30.26, 35.74, 36.34, 40.90, 44.60, 52.78, 61.78, 111.92, 116.00, 118.90, 124.75, 125.40, 126.10, 128.44, 129.85, 132.13, 135.16, 135.66, 135.90, 136.70, 143.50, 147.44, 150.92, 192.12, 195.32 ppm. EI-MS m/z: 451.25. Anal. Calcd. for C\(_{31}\)H\(_{33}\)NO\(_2\). C, 82.45; H, 7.37; N, 3.10; O, 7.09. Found: C, 82.42; H, 7.34.
(13S,17S)-13-methyl-17-[4-[(3-methylamino)phenyl]cyclobuta-1,3-dien-1-yl]-2-nitro-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-dicarbaldehyde (7).

In a round bottom flask (10 ml), compound 2 [100 mg, 0.76 mmol], 17-ethynyl-2-nitrostereoid-3,17-dicarbaldehyde (fragment E) [265 mg, 0.76 mmol], Copper(II) chloride [105 mg, 0.78 mmol] and 5 ml of methanol were stirred at room temperature for 72 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:water (4:1) system; yielding 56% of product; m.p. 188-190 °C; IR (νmax, cm⁻¹) 3312, 1722, 1600 and 1540; ¹H NMR (300 MHz, CDCl₃-d) δH: 1.10 (s, 3H), 1.12-2.58 (m, 12H), 2.74 (s, 3H), 2.82-2.92 (m, 3H), 5.96 (broad, 1H), 6.06-6.08 (m, 2H), 6.60-7.40 (m, 4H), 7.80-7.92 (m, 2H), 10.06 (s, 1H), 10.80 (s, 1H) ppm. ¹³C NMR (300 Hz, CDCl₃) δC: 17.80, 24.24, 24.55, 27.26, 27.76, 29.62, 30.26, 35.74, 36.34, 40.90, 44.98, 52.78, 61.78, 111.92, 116.00, 118.90, 122.72, 126.22, 126.50, 128.44, 129.85, 132.13, 135.16, 135.66, 143.50, 145.52, 147.44, 151.74, 152.74, 194.22, 195.32 ppm. EI-MS m/z: 496.23. Anal. Calcd. for C₃₁H₃₂N₂O₄. C, 74.98; H, 6.50; N, 5.64; O, 12.89. Found: C, 74.96; H, 5.63.

2.3. Ligand-protein interaction.

The interaction of the steroid derivatives with the COVID-19 surface was evaluated using 6LU7 protein as a theoretical model [20]. Furthermore, to calculate binding energy involved in the interaction of the steroid derivative and 6LU7-protein surface, either chloroquine, remdesivir, and favipiravir as controls were used on a docking Server software [21].

2.4. Pharmacokinetics Parameter.

To evaluate some pharmacokinetic factors involved in the chemical structure of the steroid derivatives, the SwissADME software was used [22].

3. Results and Discussion

Several drugs have been developed for the treatment of coronavirus disease (COVID-19) using some reagents which are dangerous and require special conditions [13-17]. Furthermore, the interaction with COVID-19 is very confusing; perhaps, this phenomenon could be to different structure chemical of each compound. Analyzing these data, in this investigation, five cyclobuta-1,3-dien-1-yl-steroid derivatives were synthesized to evaluate their interaction with COVID-19 using several strategies as follows.

3.1. Methylation reaction.

There are studies for methylation of some compounds using several reagents such as Copper [23], Ruthenium [24], Iridium chloride [25], Cobalt [26], Manganese chloride [27], Palladium [28], Nikel [29] and others. In this research, the compound 3-Ethynyl-phenyl-methyl-amine (2) was prepared from 3-ethynylaniline and methanol in the presence of palladium on carbon in middle conditions (Figure 1).

The ¹H NMR spectrum of 2 showed several signals at 2.82 ppm for alkyne group; at 2.82 ppm for methyl group; at 4.82 for the amino group; at 6.54-7.12 ppm for phenyl group. The ¹³C NMR spectra display chemical shifts at 30.32 ppm for methyl group; at 78.22 and
84.00 for alkyne group; at 114.50-151.30 ppm for phenyl group. Additionally, the mass spectrum from 2 showed a molecular ion (m/z) 13.07.

![Figure 1](https://nanobioletters.com/) Synthesis of (3-Ethynyl-phenyl)-methyl-amine (2). Reagents and conditions: i = 3-ethynylaniline, palladium on carbon, methanol, potassium hydroxide, reflux, 12 h.

3.2. Synthesis of cyclobuta-1,3-dien-1-yl-steroid derivatives.

Several cyclobutadiene derivatives have been prepared using some reagents such as PdCl₂, [30], cobalt chloride [31], 1,2-dibromoethane [32], Cupric chloride [33], ruthenium chloride [34], tetrakis(trimethylsilyl)cyclobutadienylcyclopentadienyl cobalt complex [35] and others. In this study, five cyclobutadiene-steroid derivatives were prepared (compound 3 to 7) from some steroid-analogs (A to E fragments) and compound 2 in the presence of Copper(II) chloride (Figure 2). The 1H NMR spectrum of 3 showed several signals at 0.72 ppm for methyl groups bound to steroid nucleus; at 1.12-2.50, 2.77-2.80, 6.72-6.76 and 7.20 ppm for steroid moiety; at 2.74 for methyl bound to the amino group; at 6.26 ppm for both hydroxyl and amino groups; at 6.12 and 6.28 ppm for cyclobutadiene ring; at 6.60, 6.80-7.16 and 7.26 ppm for phenyl group. The 13C NMR spectra display chemical shifts at 15.12 for methyl group linked to steroid nucleus; at 30.28 ppm for methyl bound to the amino group; at 23.76-30.26, 33.04-79.56, 113.64-115.96, 126.76, 131.92, 138.20 and 156.02 ppm for steroid moiety; at 112.54, 116.00-119.55, 128.44, 135.66 and 146.06 ppm for phenyl group; at 126.57, 128.60 and 138.50-142.00 for cyclobutadiene ring. Finally, the mass spectrum from 3 showed a molecular ion (m/z) 427.25.

On the other hand, other data display several signals involved in the 1H NMR spectrum for 4 at 0.72 ppm for methyl group bound to steroid nucleus; at 2.74 ppm for methyl group bound to the amino group; at 1.2-1.90, 2.77-2.90, 6.66 and 7.82 ppm for steroid moiety; at 6.12-6.26 ppm for cyclobutadiene ring; at 6.48 for both amino and hydroxyl groups; at 6.60 and 6.80-7.26 ppm for phenyl group. In addition, the 13C NMR spectra display chemical shifts at 12.42-15.30 ppm for methyl groups linked to steroid nucleus; at 30.26 ppm for methyl bound to the amino group; at 23.76-30.26, 33.04-79.56, 114.04, 123.54, 132.33-132.90, 145.08 and 148.46 ppm for steroid moiety; at 112.54, 116.00-119.55, 128.44, 135.66 and 146.06 ppm for phenyl group; at 126.57, 128.60 and 138.50-142.00 ppm for cyclobutadiene ring. In addition, the mass spectrum from 4 showed a molecular ion (m/z) 472.23.

In addition, the 1H NMR spectrum of 5 showed several signals at 0.72 ppm for methyl group bound to steroid nucleus; at 2.74 ppm for methyl group bound to the amino group; at 1.22-2.44, 2.77-2.80 and 6.30-6.32 ppm for steroid moiety; at 6.80-7.26 ppm for phenyl group; at 4.92 ppm for both hydroxyl and amino groups; at 6.12-6.26 ppm for cyclobutadiene ring. The 13C NMR spectra display chemical shifts at 15.30 ppm for methyl group linked to steroid nucleus; at 30.26 ppm for methyl bound to the amino group; at 23.76-29.82, 33.04-79.56, 114.04, 123.54, 132.33-132.90, 145.08 and 148.46 ppm for steroid moiety; at 112.54, 116.00-119.55, 128.44, 135.66 and 146.06 ppm for phenyl group; at 126.57, 128.60 and 138.50-142.00 ppm for cyclobutadiene ring. Additionally, the mass spectrum from 5 showed a molecular ion (m/z) 425.23.
Figure 2. Preparation of cyclobutadiene-steroid derivatives (3-7). reagents and conditions: ii = Copper(II) chloride, methanol, room temperature, 12 h. Fragments; A = 17α-ethynilestradiol; B = 2-nitro-17αethynilestradiol; C = oxirenol-steroid derivative; D = 17α-ethynyl-steroid-3,17-dicarbaldehyde; E = 17-ethynyl-2-nitro-steroid-3,17-dicarbaldehyde.

Other data involved in the $^1$H NMR spectrum of 6 showed several signals at 1.10 ppm for methyl group bound to steroid nucleus; at 2.74 ppm for methyl group bound to the amino group; at 1.22-2.58, 2.82-2.86, 7.16 and 7.48-7.60 ppm for steroid moiety; at 5.96 ppm for the amino group; at 6.06-6.08 ppm for cyclobutadiene ring; at 6.60-6.80 and 7.18-7.40 ppm for phenyl group; at 10.02 and 10.06 ppm for aldehyde groups. The $^{13}$C NMR spectra display chemical shifts at 17.80 ppm for methyl group linked to steroid nucleus; at 30.26 ppm for methyl bound to the amino group; at 24.24-29.62, 35.74-61.78, 124.75-128.10, 135.90-136.70 and 150.92 ppm for steroid moiety; at 11.92-118.90, 129.85, 135.66 and 147.44 ppm for phenyl group; at 128.44, 132.13-135.16 and 143.50 ppm for cyclobutadiene ring; at 192.12-195.32 ppm for aldehyde groups. Additionally, the mass spectrum from 6 showed a molecular ion (m/z) 451.25.

Finally, the $^1$H NMR spectrum for 7 display several signals at 1.10 ppm for methyl group bound to steroid nucleus; at 2.74 ppm for methyl group bound to the amino group; at 1.22-2.50, 2.82-2.86 and 7.80-7.92 ppm for steroid moiety; at 5.96 ppm for the amino group; at 6.06-6.08 ppm for cyclobutadiene ring; at 6.60-7.40 ppm for phenyl group; at 10.02 and 10.80 ppm for aldehyde groups. The $^{13}$C NMR spectra display chemical shifts at 17.80 ppm for methyl group linked to steroid nucleus; at 30.26 ppm for methyl bound to the amino group; at 24.24-29.62, 35.74-61.78, 122.72-126.50, 145.52 and 151.74-152.74 ppm for steroid moiety; at 11.92-118.90, 129.85 and 135.66 and 144.44 ppm for phenyl group; at 128.44, 132.13-135.16, and143.50 ppm for cyclobutadiene ring; at 194.22-195.32 ppm for aldehyde groups. Besides, the mass spectrum from 7 showed a molecular ion (m/z) 491.23.

3.3. Ligand-protein interaction.

For several years, some techniques were used to analyze the coupling of biomolecules with some drugs; These techniques involve the flexible coupling of ligands on the surface of some either protein or enzyme. Furthermore, these studies involve the evaluation of the free binding energies and the solvation energies involved between the ligand-biomolecule interaction [36-41]. In this way, in this investigation a theoretical ass was carried out to analyze the interaction of steroid derivatives with the 6LU7-protein surface.
The results (Figures 3-7; Table 1) showed that there are different amino acid residues involved in the binding of the steroid derivatives with 6LU7 protein surface; however, the inhibition constant (Ki) for compound 6 was lower compared with either compounds 3-5, 7.
and the controls (favipiravir, chloroquine, and remdesivir). Furthermore, compound 5 also showed a good theoretical interaction with 6LU7-protein surface (Ki, 4.73; Table 2).

Table 1. Interaction of either steroid-derivatives and controls (chloroquine, remdesivir, and favipiravir) with 6LU7-protein surface.

| Compound | Favipiravir | Chloroquine | Remdesivir | 1 | 2 | 3 | 4 | 5 |
|----------|------------|-------------|------------|---|---|---|---|---|
| Met17    | His164     | Glu166      | Asn142     | Leu80 | Asn133 | Met165 |
| Gln19    | Met165     | Leu167      | Met165     | Pro9  | Met165 | Phe134 |
| Gln69    | Glu166     | Pro168      | Glu166     | Ile152 | Pro168 | Pro184 |
| Asn119   | Pro168     | Gln189      | Pro168     | Tyr154 | Gln189 | Gln189 |
| Gly120   | Gln189     | Ala191      | Gln189     | Phe294 | Gln192 | Thr198 |
| Ser121   | Ala193     |             |            |       |       | Met235 |

Table 2. Thermodynamic parameters involved in the binding of either steroid-derivatives and controls (chloroquine, remdesivir and favipiravir) with 6LU7-protein surface.

| Compound | Est. Free Energy of Binding | Est. Inhibition Constant (Ki) | vDW + H-bond Energy | Electrostatic Energy | Total Interm. Energy | Interact Surface |
|----------|---------------------------|-----------------------------|---------------------|---------------------|---------------------|-------------------|
| Chloroquine | -3.99                     | 1.20                        | -4.54               | -2.03               | -6.56               | 495.65            |
| Remdesivir | -2.45                     | 15.90                      | -6.09               | -0.38               | -6.48               | 643.33            |
| Fivipiravir | -3.73                     | 1.83                        | -3.97               | -0.06               | -4.03               | 338.65            |
| 3         | -6.56                     | 15.43                      | -8.04               | -0.04               | -8.09               | 716.06            |
| 4         | -5.11                     | 93.11                      | -6.42               | 0.10                | -6.32               | 611.75            |
| 5         | -7.27                     | 4.73                       | -7.80               | 0.00                | -7.79               | 726.52            |
| 6         | -4.08                     | 1.03                       | -4.81               | -0.11               | -4.92               | 636.16            |
| 7         | -5.58                     | 81.02                      | -5.98               | -0.04               | -6.02               | 595.67            |

3.4. Pharmacokinetic evaluation.

Several studies have reported the evaluation of some pharmacokinetic parameters of different drugs using theoretical models such as PKQuest [40], PharmPK [41] Gitub [42], SwissADME [43]. In this way, in this research, some pharmacokinetic parameters possibly involved in the steroid derivatives were evaluated using the SwissADME software. The results (Table 3) indicate that these compounds could have different gastrointestinal absorption and, consequently, their metabolism could involve different types of CYP enzymes (Cytochrome P450 system).

Table 3. The pharmacokinetics properties of the Fluoro-2,4dioxaspiro[bicyclo[3.3.1]indene derivative. The values determine using the SwissADME software.

| Parameter       | 3 | 4 | 5 | 6 | 7 |
|-----------------|---|---|---|---|---|
| GI absorption   | high | Low | high | high | Low |
| BBB permenat    | No | No | No | No | No |
| P-gp substrate  | No | Yes | No | No | Yes |
| CYP1A2 inhibitor | No | No | No | Yes | No |
| CYP2C19 inhibitor | No | No | No | No | No |
| CYP2C9 inhibitor | No | Yes | No | Yes | Yes |
| CYP2D6 inhibitor | No | No | No | Yes | No |
| CYP3A4 inhibitor | No | Yes | Yes | Yes | Yes |
| LogKp (cm/s)    | -5.06 | -4.97 | -5.69 | -4.77 | -5.17 |

4. Conclusions

In this investigation, the synthesis of five cyclobuta-1,3-dien-1-yl-steroid derivatives using several chemical strategies is reported. Furthermore, theoretical analysis of the interaction between steroid derivatives showed a higher affinity of compounds 5 and 6 by the
6LU7-protein surface. All these data suggest that both compounds 5 and 6 could be good candidates as COVID-19 inhibitors.

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Conflicts of Interest

The authors declare no conflict of interest.

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