NATURAL DRUGS

THE EFFICIENCY OF SOME ACTIVE INGREDIENTS OF *ARUM PALAESTINUM* AS INHIBITORS TO 3CL^{PRO} AND NSP15 PROTEINS

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Abstract: Two active coronaviral proteins (3CL^{PRO} and Nsp15) have been studied using both the GC-MS and docking methods. These coronaviral proteins have been examined with the methanol extract generated from leaves of the *Arum palaestinum*. According to the GC-MS findings, 19 major natural compounds are present in the plant's methanolic extract. The lowest Binding Energy (LBE) and the inhibition constant (K_i) have been used to identify and classify the potential of these lead drugs with their pharmacological properties. The affinity of these compounds with coronaviral proteins has been evaluated to reveal the usage of these compounds at the active sites of the receptors, 3CL^{PRO} (PDB ID: 6LU7) and Nsp15 (PDB ID: 6VWW). The results of β -Sitosterol, Androstan-3-one, Phenobarbital, Maltose, and α -Tocopherol show more affinity to Nsp15 and 3CL^{PRO} than to the supporting control drugs. Furthermore, an evaluation of the interactions of these components with the amino acids of 3CL^{PRO} and Nsp15 revealed that β -Sitosterol has the best LBE score and K_i value as compared with those of the approved medication and all other compounds under investigation. Consequently, these potential compounds may be modern inhibitors of coronavirus. Further *in vitro* and *in vivo* studies are needed for such computational findings.

Keywords: *Arum palaestinum*, GC-MS, Computer-Aided Drug Design, Nsp15, 3CL^{PRO}, β -Sitosterol

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The economic burden and reported serious side effects of conventional medicines cause the researchers to seek alternative treatment sources against various diseases (1). For instance, several phytochemicals have been recently reported to have a powerful antiviral efficacy, which allows them to inhibit the high rate of coronavirus replication (2-5). Those phytochemicals give a ray of hope for the detection of new COVID-19 inhibitors in nature (3-6). In general, natural substances are highly chemically diverse, have lower production costs than biopharmaceutical compounds, and have either milder or non-existent adverse consequences as opposed to synthetic compounds (7).

In Jordanian folk medicine, herbal drugs are widely fashioned in the treatment of various ailments because they are rich sources of bioactive constituents (8). For instance, the *A. palaestinum* Boiss. *A. palaestinum* is an invaluable herb because it is commonly prescribed by numerous traditional Arabic herbalists for the treatment of bones diseases, jaundice, diabetes, renal infections, skin problems, syphilis, rheumatoid arthritis, cancer, and worms' infection (9). To date, the available analytical data have elaborated the acceptance of the *A. palaestinum* Boiss. *A. palaestinum* due to the presence of herbal constituents, such as *viz.* isovanillin, linolenic acid, β -sitosterol, isovitexin, and vitexin (10). These herbal constituents are prevalent in a crude methanolic extract of the *A. palaestinum* (11). The embedded phytoconstituents, chemical exploration, and therapeutic activity of the *A. palaestinum* have been reported (12).

The day-to-day rise in industrialization leads to uprooting, low production, less cultivation, and destruction (13). Consequently, preservation and conservation of the remaining flora and fauna of the *A. palaestinum* are required. Therefore, phytochemical analysis is needed not only for the uncovering of the analytical markers but also for the development of a validated, precise, appropriate, and efficient technique needed for the characterization of the biography of the *A. palaestinum*. Detailed chemoprofiling of the *A. palaestinum* leaves by using liquid chromatography requires a long run time, tedious analytical procedure, and compulsory high volume of organic solvents for sample preparation (14).

The aim of this study was to assess the phytochemical architecture by GC-MS analysis and to investigate a mechanism-based study, which may be accountable for the pharmacological activity of the *A. palaestinum*. This work demonstrates a GCMS-based phytochemical analysis and uncovers

a significant bioactive mechanism that is responsible for the positive attitude of the *A. palaestinum*. In addition, the current research attempts to screen and assess the probable inhibitory activity of *A. palaestinum*-derived compounds against SARS-CoV-2 (3CL^{pro} and Nsp15), possibly presenting new recognized compounds against the novel pandemic coronavirus disease (COVID-19). Moreover, our proposed method is simple, short, precise, accurate, economical, and requires a low volume of organic solvent for sample preparation.

MATERIALS AND METHODS

Collection of plant materials

Wild-growing leaves of fresh Jordanian *A. palaestinum* plant were obtained from the Karak governorate of Jordan at the start of the spring term of 2019. The method of obtaining these leaves was described by Al-Eisawi (13). The plant's leaves were dried in the shade, isolated at room temperature to a consistent weight, crushed, and stored in a dark area.

Extraction process

One gram of the cleaned and dried Jordanian *A. palaestinum* leaves was recovered with 10 mL of methanol and stirred at room temperature for 5 days. The supernatant was centrifuged at 4500 rpm for 10 minutes. At room temperature, 3.0 mL of the supernatant was transferred to a 10 mL test tube then was evaporated. The residues were reformed in 100 μ L of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) solution before injection into the GC-MS.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS investigation was conducted out using an Agilent technology model 6890 GC that was fitted with a Split-splitless injector and HP-5MS capillary column type that coated with a film of 5 percent phenylmethylpolysiloxane (30 m $\times$ 0.25 mm, 0.25 μ m film thickness). The agile amount 6890 GC was fitted with 5973C mass spectrometer style with Inert MSD (Mass Spectrometer with mass Selective Detector and GC-MS). The temperature applied on the column oven was programmed as follows: The starting temperature was 60°C, then was raised up to 300°C with a ramp of 15°C/min, and finally was remained at 300°C for 7 min before all elution was done. The split valves were opened after 15 seconds to purge the injector for 3 minutes. Both injections (1 μ L) were produced using a syringe of

10 μ L. Helium gas was used with a purity of 99.999% as a carrier gas with a flow rate of 1.0 mL/min (15).

Molecular modeling process

The structures of the targeted protein receptor included pplab from SARS-CoV-2 (chain A of PDB ID: 6LU7 (16)) and (chain B of PDB ID: 6VWW (17)). UCSF Chimera 1.15 (18) was used for modeling and analysis processes. Ligands and solvent molecules were removed from the protein structures. Hydrogen atoms were added, Dunbrack 2010 rotamer library (19) was used for building incomplete side chains, and the AMBER14SB force field (20) was used for standard residue parameterization. The default AMBER force field parametrization is to fulfill the physiological pH condition (i.e., pH = 7.4) in each receptor. That is, Asp and Glu residues were deprotonated, Arg and Lys residues were protonated, and His residues kept neutral. Receptor file format (pdb) was generated using UCSF Chimera 1.15 (18), whereas receptor file format (pdbqt) was generated using AutoDockTools (21, 22) in MGLTools 1.5.7.

An in-house ligand library of potential binding compounds was generated from the PubChem database (23) or built using Avogadro molecule editor (24). Missing hydrogen atoms were added, and the Gasteiger method (25) was used for assigning charges using Antechamber (26). Ligand mol2 file format was generated using UCSF Chimera 1.15 (18), whereas ligand pdbqt file format was generated using open babel 2.4.1 (27).

Molecular docking process

Molecular docking was performed using Autodock Vina 1.1.2 (28) in a virtual screening study. Binding sites for each receptor were predicted using P2Rank 2.2 (29) and were searched in a grid box of $30 \times 30 \times 30 \text{ \AA}^3$ with exhaustiveness 24 to ensure comprehensive sampling.

Molecular docking analysis

The formation of the ligand-receptor complex is represented by the following basic thermodynamic equilibrium equation, where K_{eq} is the equilibrium constant.

$$K_{eq} = \frac{[\text{Ligand-Receptor}]}{[\text{Ligand}][\text{Receptor}]}$$

Figure 1. GC-MS chromatogram of the *A. palaestinum* methanolic extract.

The Gibbs free energy (ΔG), which is also called the binding affinity, is related to the equilibrium constant by $\Delta G = -RT \ln K_{eq}$, where R is the gas constant in a unit of kcal/(mol·K) and T is the temperature in a unit of kelvin. The inhibition constant (K_i) is related to the equilibrium constant by $K_i = 1/K_{eq}$; therefore, the free energy of binding in terms of K_i can be written as $\Delta G = RT \ln K_i$. The 2D ligand–protein interaction diagrams were generated using LigPlot⁺ 2.2.4 (30).

RESULTS

GC-MS Analysis of the Extract

The extracted constituents of the *A. palaestinum* have been investigated using GC–MS. Each ingredient in the methanolic extract has been measured and identified by comparing mass fragmentation patterns to standards such as Wiley 9 library spectral data and NIST standards (Figure 1). The detailed information of 19 peaks is shown in Table 1; one component has an area percent of 18.4%, 2 components have area percent of around 5.6%, 11 components have area percent between 1 and 5%, and 5 components have area percent less than 1%.

Molecular docking

The *A. palaestinum* contains a variety of natural phytochemical compounds. These natural phytochemical compounds have been studied with in

Table 1. GC-MS Results of Phytonutrients of the *A. palaestinum*.

Components	Retention Time (min)	Composition Percentage (%)
Ethylamine	5.53	0.17
Cyanuric acid	6.06	0.03
1,3- Propanediol	9.49	1.15
Androstan-3-one	11.45	0.03
Malic acid	11.46	2.01
N-Butylglycine	12.55	0.08
3-Hydroxydodecanoic acid	14.14	18.40
L-Ascorbic acid	14.27	5.61
3-Hydroxytetradecanedioic acid	14.64	4.48
3-Hydroxysebacic acid	14.64	4.48
Undecanedioic acid	14.65	3.19
Palmitic acid	15.45	1.35
Glucuronic acid	15.95	2.49
Isobutyric acid	16.53	1.51
Phenobarbital	18.89	1.53
α -D-Glucopyranose	19.03	4.80
Maltose	20.92	0.02
α -Tocopherol	22.26	5.63
β -Sitosterol	24.67	2.66

silico technique to examine the alleged anti-corona behavior. The utilized AutoDock Vina algorithm can reveal the potential binding associations of 19 known compounds found in the methanolic extract of the *A. palaestinum*. Docking simulation employs a grid-based energy evaluation method, in which pre-calculated interaction energies are being used as lookup tables to allow rapid assessment of ligand-protein interactions. Even so, unless

complex side chains are treated beyond the grid, the execution of this grid-based method will entail strict processing of the target molecule. The grid box parameters for the most probable binding pockets in the proteins and their residues are shown in Table 2.

The association of 19 *A. palaestinum* phyto-constituents with the two target SARS-CoV-2 proteins has been evaluated using molecular docking

Table 2. Docking grid box parameters.

Receptor	PDB ID	Pocket No.	Grid Center			List of Adjacent Residues
			<i>x</i>	<i>y</i>	<i>z</i>	
pplab from SARS-CoV-2	6LU7 (3CL ^{pro})	1	−11.0778	16.9853	67.6274	Thr3288, Thr3289, Leu3290, His3304, Met3312, Phe3403, Leu3404, Asn3405, Gly3406, Ser3407, Cys3408, His3426, His3427, Met3428, Glu3429, Asp3450, Arg3451, Gln3452
pplab from SARS-CoV-2	6VWW (Nsp15)	1	−46.0066	32.7991	27.9534	His6686, Gln6696, Leu6697, Gly6698, His6701, Lys6741, Val6743, Ser6745, Met6782, Trp6784, Thr6792, Tyr6794, Leu6797
		2	−64.3941	31.1839	27.5128	Lys6522, Lys6541, Ser6649, Arg6650, Asn6651, Leu6652, Leu6703, Leu6717, Asp6719, Met6723, Asp6724, Ser6725, Thr6726, Lys6728, Tyr6730, Val6746, Ile6747, Asp6748

Table 3. Lowest Binding Energy (LBE) scores and the inhibition constant (K_i) values for the 19 *A. palaestinum* phytoconstituents and two FDA approved HIV inhibitors (Hydroxychloroquine and Chloroquine) with 3CL^{pro} (6LU7) and Nsp15 (6VWW).

Group	No.	Compounds	6LU7 (3CL ^{pro})		6VWW (Nsp15)			
			LBE (kcal/mol)	K_i (μ M)	1 st pocket		2 nd pocket	
					LBE (kcal/mol)	K_i (μ M)	LBE (kcal/mol)	K_i (μ M)
Phytoconstituents of <i>A. palaestinum</i>	1	1,3-Propanediol	−3.4	3217.55	−3.5	2717.8	−3.8	1637.92
	2	3-Hydroxydodecanoic acid	−4.9	255.803	−4.6	424.45	−6.4	20.337
	3	3-Hydroxysebacic acid	−5.4	109.993	−4.9	255.80	−5.9	47.296
	4	3-Hydroxytetradecanedioic acid	−5.2	154.164	−4.6	424.45	−5.9	47.296
	5	α -D-Glucopyranose	−4.8	302.840	−4.4	594.90	−5.9	47.296
	6	α -Tocopherol	−6.5	20.337	−5.7	66.289	−7.4	3.760
	7	β -Sitosterol	−6.7	12.256	−7.8	1.914	−8.5	0.587
	8	Androstan-3-one	−6.4	20.337	−7.1	6.239	−7.6	2.683
	9	Cyanuric acid	−5.1	182.511	−4.1	987.12	−4.9	255.80
	10	D-Glucuronic acid	−5.3	130.219	−4.6	424.45	−5.9	47.296
	11	Ethylamine	−2.4	17402.3	−2.7	10487	−2.7	10487
	12	Isobutyric acid	−3.7	1939.11	−3.7	1939.1	−4.3	704.294
	13	L-Ascorbic acid	−5.1	182.511	−4.7	358.52	−6.1	33.745
	14	Malic acid	−4.8	302.840	−4.8	302.84	−5.3	130.219
	15	Maltose	−6.2	28.504	−6.0	39.950	−7.5	3.176
	16	N-Butylglycine	−4.1	987.121	−4.3	704.29	−4.7	358.527
	17	Palmitic acid	−4.5	502.502	−5.0	216.07	−5.5	92.909
	18	Phenobarbital	−6.6	14.510	−5.7	66.289	−7.7	2.266
	19	Undecanedioic acid	−5.1	182.511	−5.1	182.51	−5.6	78.478
Positive Controls	1	Chloroquine	−5.9	47.296	−5.5	92.909	−6.2	28.504
	2	Hydroxychloroquine	−6.3	24.076	−5.9	47.296	−6.5	17.178

on 3CL^{pro} and Nsp15 proteins. The best binding ability of the interacting molecules to 3CL^{pro} and Nsp15 active site residues has been determined. As a positive control, the analysis has included FDA-approved HIV inhibitors, namely, hydroxychloroquine and chloroquine (31). Table 3 displays the Lowest Binding Energy (LBE) scores and K_i values for 19 *A. palaestina* with target proteins. An extended version of Table 3 is available in the supplementary material (Virtual Screening Results.xlsx).

DISCUSSION

GC-MS chromatogram (Figure 1) shows the methanolic crude extract of *A. palaestinum* has 19 peaks with retention times between 5.5 and 24.6 min (Table 1). The analysis has revealed that

the extract is composed of oxygenated hydrocarbons, carboxylic acids, fatty acids (saturated and unsaturated), and aromatic compounds. Ten major identified compounds have been found in the methanolic crude extract of the *A. palestina* with an area percent more than 2%. These phytoconstituents are 3-Hydroxydodecanoic acid (18.4%), α -Tocopherol (5.63%), L-Ascorbic acid (5.61%), α -D-Glucopyranos (4.80%), 3-Hydroxytetradecenedioic acid (4.48%), 3-Hydroxysebacic acid (4.48%), Undecanedioic acid (3.19%), β -Sitosterol (2.66%), D-Glucuronic acid (2.49%), and Malic acid (2.01%). Figure 2 shows the structure of the main compounds that have area percent more than 2% and Phenobarbital as a potential inhibitor based on LBE and K_i results.

All the known compounds have been docked to 3CL^{pro} (6LU7) pocket as well as to the first and second pockets of Nsp15 (6VWW). The phytoconstituent

Figure 2. Structure of the major identified compounds in methanolic extract of *A. palestinum*.

compounds have varying scores with the enclosed amino acids (Table 3). Surprisingly, β -Sitosterol, Phenobarbital, Maltose, and α -Tocopherol outperform the other phytoconstituent compounds and the optimistic controls as shown in Table 3 by creating a powerful association with the protease of both enzymes as revealed by the LBE and K_i values. Clearly, β -Sitosterol has a lower LBE score and lower K_i value than that of the control drugs and all chosen compounds with 3CL^{pro} and both pockets of Nsp15. Phenobarbital could inhibit both proteins despite its area percent is less than 2% in the methanolic extract of the *A. palaestinum*. Figure 3 and Figure 4 show the analysis of the best score of these potential inhibitors by LigPlot+.

Even though Phenobarbital and Androstan-3-one have small composition percentages in the extract, specifically, 1.53% and 0.03%, respectively, they are expected to have significant

Figure 3. 2D interaction diagram of β -Sitosterol with 3CL^{pro} (6LU7).

Figure 4. 2D interaction diagrams of β -Sitosterol with Nsp15 (6VWW). (a) first binding pocket, (b) second binding pocket.

effect on the coronavirus. In Table 3, four compounds are found to inhibit 3CL^{pro} (6LU7) with LBE scores and K_i values less than that of the positive controls, Hydroxychloroquine (-6.3 kcal/mol, 24.076 μ M) and Chloroquine (-5.9 kcal/mol, 47.296 μ M). These potential inhibitors are arranged in the following order: β -Sitosterol (-6.7 kcal/mol, 12.256 μ M) > Phenobarbital (-6.6 kcal/mol, 14.510 μ M) > α -Tocopherol (-6.5 kcal/mol, 20.337 μ M) > Androstan-3-one (-6.4 kcal/mol, 20.337 μ M). Furthermore, three potential inhibitors are found to inhibit first pocket of Nsp15 (6VWW) with LBE scores and K_i values less than that of the positive controls, Hydroxychloroquine (-5.9 kcal/mol, 47.296 μ M) and Chloroquine (-5.5 kcal/mol, 92.909 μ M). These potential inhibitors are arranged in the following order: β -Sitosterol (-7.8 kcal/mol, 1.914 μ M) > Androstan-3-one (-7.1 kcal/mol, 6.239 μ M) > Maltose (-6.0 kcal/mol, 39.950 μ M). In addition, five potential inhibitors are found to inhibit second pocket of Nsp15 (6VWW) with LBE scores and K_i values less than that of the positive controls, Hydroxychloroquine (-6.5 kcal/mol, 17.178 μ M) and Chloroquine (-6.2 kcal/mol, 28.504 μ M). These potential inhibitors are

arranged in the following order: β -Sitosterol (-8.5 kcal/mol, 587 μ M) > Phenobarbital (-7.7 kcal/mol, 2.266 μ M) > Androstan-3-one (-7.6 kcal/mol, 2.683 μ M) > Maltose (-7.5 kcal/mol, 3.176 μ M) > α -Tocopherol (-7.4 kcal/mol, 3.760 μ M).

Interestingly, the most potent natural compounds with LBE scores and K_i values in contrast to the normal regulation for both enzymes are discovered to be in the following order: β -Sitosterol > Androstan-3-one > Phenobarbital > Maltose > α -Tocopherol. This finding suggests that these compounds may be effective medications for inhibiting coronavirus viral replication by inhibiting the action of the two basic proteins.

Surprisingly, the putative pocket protein binding site in 6LU7 has shown a greater binding affinity to β -Sitosterol than to Chloroquine and Hydroxychloroquine due to the presence of two hydrogen bonding between β -Sitosterol and Leu3404 and Gly3406 amino acids. Furthermore, the binding of β -Sitosterol to the first binding pocket of 6VWW demonstrates two hydrogen bonding with Ser6745 and Val6743. In addition, the second binding pocket of 6VWW shows three hydrogen bonding between β -Sitosterol and Lys6522,

Ser6725, and Thr6726. According to our findings, the docking scores of the β -Sitosterol with all three pockets are the best and have the lowest binding energy. When compared to reference medicines, these results show that β -Sitosterol has a high affinity to all pockets.

CONCLUSIONS

The methanolic extract extracted from the leaves of the *A. palaestinum* has been examined, addressed for the first time for its bioactive constituents, and analyzed using a GC-MS instrument and docking method on two active coronavirus proteins. According to the results of GC-MS, the plant's methanolic extract contains 19 main natural compounds. Ten of the 19 compounds have a composition percentage greater than 2%. Docking results have shown that β -Sitosterol, Androstan-3-one, Phenobarbital, Maltose, and α -Tocopherol have higher LBE scores and K_i values than supportive control drugs for Nsp15 and 3CL^{pro}. Furthermore, the study of the interactions of these compounds with the amino acids in 3CL^{pro} and Nsp15 has revealed that β -Sitosterol has the best LBE score and K_i value than the licensed drugs and all other studied compounds, owing to its ability to form tight hydrogen bonds with proteins. Consequently, we have possible compounds that may be novel coronavirus inhibitors. Such computational results need further *in vitro* and *in vivo* research.

Acknowledgments

We acknowledge the Center for Computational Sciences at the University of Kentucky (Lexington, KY, USA) for the allocation of computing time on the high-performance computing facility (Lipscomb Cluster). Molecular graphics and analyses performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311.

Funding

The authors did not receive support from any organization for the submitted work.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

REFERENCES

1. Al-samman A.M.M.A., Imran M., Bakht M.A., Kahkashan, Siddique N.A.: J. J. Biol. Sci. 13, 659 (2020).
2. Attia Y.A., Alagawany M.M., Farag M.R., Alkhatib F.M., Khafaga A.F., et al.: Front Vet. Sci. 7, 573159 (2020).
3. Wang Z., Yang L.: Front Pharmacol. 11, 1013 (2020).
4. Benarba B., Pandiella A.: Front Pharmacol. 11, 1189 (2020).
5. Verma S., Twilley D., Esmear T., Oosthuizen C.B., Reid A.M., et al.: Front Pharmacol. 11, 561334 (2020).
6. Joshi R.S., Jagdale S.S., Bansode S.B., Shankar S.S., Tellis M.B., et al.: J. Biomol. Struct. Dyn. 39, 3099 (2021).
7. Elfiky A.A.: J. Biomol. Struct. Dyn. 39, 3204 (2020).
8. Saad B., Said O.: Greco-Arab and Islamic Herbal Medicine: Traditional System, Ethics, Safety, Efficacy, and Regulatory Issues, 1st ed., John Wiley & Sons 2011.
9. Husein A.I., Ali-Shtayeh M.S., Jondi W.J., Zatar N.A., Abu-Reidah I.M., Jamous R.M.: Pharm. Biol. 52, 1249 (2014).
10. Farid M.M., Hussein S.R., Trendafilova A., Marzouk M.M., El Oqlah A.A., Saker M.M.: J. Mater. Environ. Sci. 8, 2585 (2017).
11. Ali-Shtayeh M.S., Jamous R.M., Salameh N.M., Jamous R.M., Hamadeh A.M.: J. Ethnopharmacol. 187, 104 (2016).
12. Zakariyyah M., Mohamad A., Mahomoodally F.: S. Afr. J. Bot. 138, 424 (2021).
13. Al-Eisawi D.M.: Field Guide to Wild Flowers of Jordan and Neighbouring Countries, Jordan Press-Foundation "Al-Rai", Amman 1998.
14. Abu-Reidah I.M., Ali-Shtayeh M.S., Jamous R.M., Arráez-Román D., Segura-Carretero A.: Food Res. Int. 70, 74 (2015).
15. Krafczyk N., Glomb M.A.: J. Agric. Food Chem. 56, 3368 (2008).
16. Jin Z., Du X., Xu Y., Deng Y., Liu M., et al.: Nature 582, 289 (2020).
17. Kim Y., Jedrzejczak R., Maltseva N.I., Wilamowski M., Endres M., et al.: Protein Sci. 29, 1596 (2020).
18. Pettersen E.F., Goddard T.D., Huang C.C., Couch G.S., Greenblatt D.M., et al.: J. Comput. Chem. 25, 1605 (2004).
19. Shapovalov M.V., Dunbrack Jr. R.L.: Structure 19, 844 (2011).

20. Maier J.A., Martinez C., Kasavajhala K., Wickstrom L., Hauser K.E., Simmerling C.: *J. Chem. Theory Comput.* 11, 3696 (2015).
21. Sanner M.F.: *J. Mol. Graph. Model* 17, 57 (1999).
22. Morris G.M., Huey R., Lindstrom, Sanner W.M.F., Belew R.K., et al.: *J. Comput. Chem.* 30, 2785 (2009).
23. Kim S., Chen J., Cheng T., Gindulyte A., He J., et al.: *Nucleic Acids Res.* 49, D1388 (2021).
24. Hanwell M.D., Curtis D. E., Lonie D. C., Vandermeersch T., Zurek E., Hutchison G. R.: *J. Cheminform.* 4, 17 (2012).
25. Gasteiger J., Marsili M.: *Tetrahedron* 36, 3219 (1980).
26. Wang J., Wang W., Kollman P.A., Case D.A.: *J. Mol. Graph. Model* 25, 247 (2006).
27. O'Boyle N.M., Banck M., James C.A., Morley C., Vandermeersch T., Hutchison G. R.: *J. Cheminform.* 3, 33 (2011).
28. Trott O., Olson A.J.: *J. Comput. Chem.* 31, 455 (2010).
29. Krivak R., Hoksza D.: *J. Cheminform.* 10, 39 (2018).
30. Laskowski R.A., Swindells M.B.: *J. Chem. Inf. Model* 51, 2778 (2011).
31. Pastick K.A., Okafor E.C., Wang F., Lofgren S.M., Skipper C.P., et al.: *Open Forum Infect. Dis.* 7, ofaa130 (2020).