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Synthesis of Novel Acylhydrazone-Oxazole Hybrids and Docking Studies of SARS-CoV-2 Main Protease †

Verónica G. García-Ramírez [1], Abel Suarez-Castro [1,*], Ma. Guadalupe Villa-Lopez [1], Erik Díaz-Cervantes [2], Luis Chacón-García [1] and Carlos J. Cortes-García [1,*]

[1] Laboratorio de Diseño Molecular, Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, C.P. 58033 Morelia, Michoacán, Mexico; veronicagrmz@gmail.com (V.G.G.-R.); magpevl.3@gmail.com (M.G.V.-L.); lchacon@umich.mx (L.C.-G.)

[2] Departamento de Alimentos, División de Ciencias de la Vida, Campus Irapuato-Salamanca, Universidad de Guanajuato, C.P. 37975 Tierra Blanca, Guanajuato, Mexico; e.diaz@ugto.mx

* Correspondence: abel.suarez@umich.mx (A.S.-C.); ccortes@umich.mx (C.J.C.-G.)

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Abstract: A novel synthetic strategy to obtain acylhydrazone-oxazole hybrids in three-step reactions in moderate to good yields is reported. The key step reaction consists in a Van Leusen reaction using a bifunctional component of both an aldehyde and a functional group. The target molecules were evaluated via in-silico by molecular docking with the main protease enzyme of SARS-Cov-2, where two acyl hydralazine-oxazoles yielded good predicted free energy values in comparison to the co-crystalized ligand.

Keywords: oxazoles; acylhydrazones; Van Leusen reaction; docking studies; SARS-CoV-2

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1. Introduction

Both 1,3-oxazoles and acylhydrazones based molecules are of biological and pharmacological relevance as they are considered pharmacophoric subunits [1–4]. Some examples of drugs or drugs candidates containing these pharmacophoric fragments are shown in Figure 1. In addition, the biological diversity of acylhydrazones is due to the nitrogen atom of the azomethine group having the lone pair of electrons in its sp^2 hybridized orbital and to the presence of geometrical isomers E and Z of azomethine group [5–7].

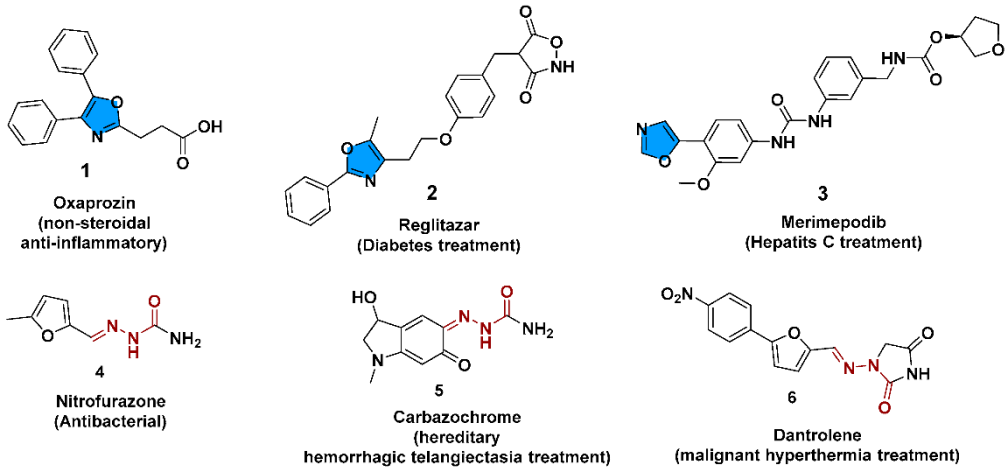


Figure 1. Drugs and drugs candidates containing 1,3-oxazole and acylhydrazone scaffolds.

On the other hand, molecular hybridization is a novel strategy within medicinal chemistry that has as its main objective the search and efficient development of new candidate drug molecules. It is based on the combination of two of more pharmacophores of different bioactive substances to produce a hybrid compound with better biological activity and pharmacokinetic profile when compared to parent drugs [8–10]. Hybrid molecules can be prepared by linking the pharmacophoric fragments directly or with spacer agents [11]. Herein, we report a novel synthetic strategy to obtain the acyl hydrazone-oxazole hybrids 7a–h by using the molecular hybridization strategy and based on the pharmacological relevance of 1,3-oxazoles and acylhydrazones derivates (Figure 2).

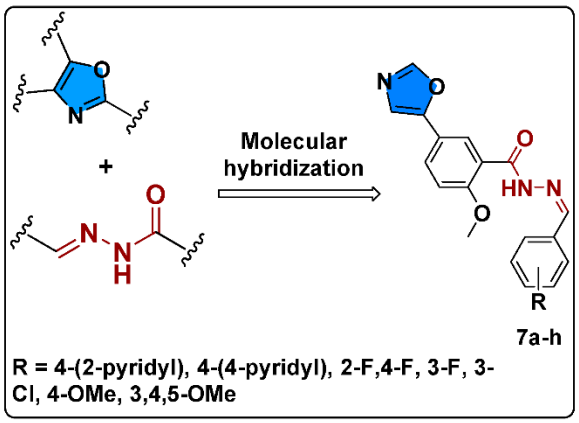


Figure 2. Molecular hybridization strategy to obtain our target molecules by combining the 1,3-oxazoles and acylhydrazones scaffolds.

In addition, with the global health problem caused by SARS-CoV-2 that generates COVID-19 and that has infected and killed millions of people worldwide, medicinal and synthetic chemists are challenged to find new molecules that can act as possible antiviral agents for the inhibition of this virus [12–18]. Thus, we decided to evaluate the target molecules via in-silico by molecular docking with the main protease enzyme of SARS-Cov-2.

2. Materials and Methods

2.1. Experimental Section and Computational Details

All reagents, reactants and solvents were purchased from Merck (Darmstad, Germany)) without further purification. Melting points (uncorrected) were determined on a Fischer apparatus. Thin-layer chromatography (TLC) was performed with silica gel plates from Merck (silica gel 60 F_{254}) and by using as eluent a mixture of heptane-AcOEt. NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Varian Mercury 400 spectrometer, using DMSO-d_6 as the solvent and TMS as the internal standard. Chemical Shift (δ) are reported in ppm and *J* values are given in Hertz. IR spectra were recorded on a Thermo Scientific NICOLET iS10 by ATR method using neat compounds. The wavelengths are reported in reciprocal centimeters (ν/cm^{-1}).

2.1.1. Ligand Preparation

All the structures of compounds 7a–h were modeled as 2D structures with the software ChemBio Draw Ultra 12.0 (Perkin Elmer, San Bernardino, USA) [19] and were converted into 3D structures in MDL format. Their protonated states were then computed using the online tool Chemicalize [20]. The geometries of the compounds and co-crystalized ligand were calculated at the semiempirical AM1 level in the Gaussian 16 software package [21]. Finally, using Autodock Tools [22], the ligands were prepared by adding polar hydrogens and Gasteiger charges and rotatable (i.e., single) bonds were assigned by default and a pdbqt file was generated.

2.1.2. Receptor Preparation

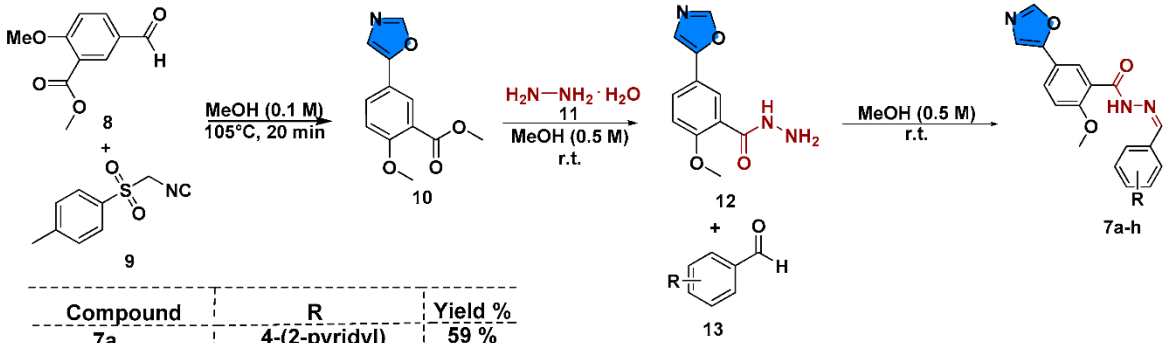
The X-ray coordinates of the M^{pro} protease receptor of SARS-CoV-2 were retrieved from the Protein Data Bank [23] (PDB code: 6LU7). Molecular water was removed from the crystallographic structure and the final preparation and minimization of the receptor structure was carried out using the Dock Prep module of Chimera software [24] using the AMBER-ff14SB force field. Lastly, Kollman charges were added using Autodock Tools and a pdbqt file was generated.

2.1.3. Docking Calculations

Rigid receptor molecular docking was carried out in Autodock4 using the Lamarckian genetic algorithm [25]. We used grid maps with 70 × 70 × 70 points in the active site of the receptor with the coordinates x = −12.202, y = 11.499, z = 69.669 and a grid-point spacing of 0.375 Å. AD4.dat parameters were applied to all the ligands. The parameters used were 10 runs, a population size of 100 and a run-termination criterion of a maximum of 27,000 generations or a maximum of 10,000,000 energy evaluations. The visualization and analysis of the nonbonded interactions as hydrogen bonds of the best poses were carried out using Discovery Studio Visualizer software [26].

3. Results and Discussion

The novel acylhydrazones-1,3-oxazoles hybrids were synthetized via a three-step reaction and is shown in Scheme 1. The first step reaction is the global key step due to the formation of the first pharmacophoric fragment (1,3-oxazole moiety) via a Van Leusen reaction by the previously described method by research team [27]. The reacting TOSMIC and the bifunctional aldehyde component 8 that contain a methyl ester group provided the intermediate methyl ester-1,3-oxazole 10 in good yield. The second step was the hydrazinolysis reaction of the methyl ester group by using hydrazine hydrate to give the intermediate hydrazide-1,3-oxazole 12 in moderate yield. Finally, the third step was the Schiff condensation of hydrazide-1,3-oxazole 12 with different functionalized aldehydes 13 to give the novel acyl hydrazone-oxazole hybrids 7a–h in moderate to good yields. In addition, fluorinated aromatic aldehydes were used given the biological and pharmacological relevance of fluorine in medicinal chemistry.



Compound	R	Yield %
7a	4-(2-pyridyl)	59 %
7b	4-(4-pyridyl)	36 %
7c	2-F	86 %
7d	4-F	73 %
7e	4-OMe	72 %
7f	3-F	65 %
7g	3-Cl	81 %
7h	3,4,5-tri-OMe	85 %

Scheme 1. General synthetic route for the synthesis acylhydrazones-1,3-oxazoles hybrids 7a–h.

Moreover, to explore the possible mode or modes of interaction of compounds 7a–h against the M^{pro} of SARS-CoV-2, molecular docking studies were carried out. N3 was the reference compound as a co-crystalized ligand in the receptor (PDB: 6LU7), which has the

active site in position His41 and Cys145, as some key residues for the M^{pro} protease inhibition [28]. The predicted free energy and affinity constant values are listed in Table 1.

Table 1. Results of docking studies of the tested acylhydrazone-oxazole hybrids.

Compound	ΔG (kcal/mol)	ki (μM)	pki
7a	−7.72	2.19	5.66
7b	−7.76	2.05	5.69
7c	−7.53	3.04	5.52
7d	−7.21	5.14	5.29
7e	−7.28	4.6	5.34
7f	−7.58	2.79	5.55
7g	−8.22	0.94	6.03
7h	−7.5	3.17	5.50
N3	−7.7	1.70	5.77

Almost all the predicted free energy for all the acylhydrazone-oxazole hybrids are comparable N3. Nevertheless, only 7a, 7b and 7g (Figure 3), showed the lowest free energy with some interesting ligand-receptor interactions such as π-sulfur interaction between Cys145 and Met161 residues in the active site with the 1,3-oxazole moiety (Table 2).

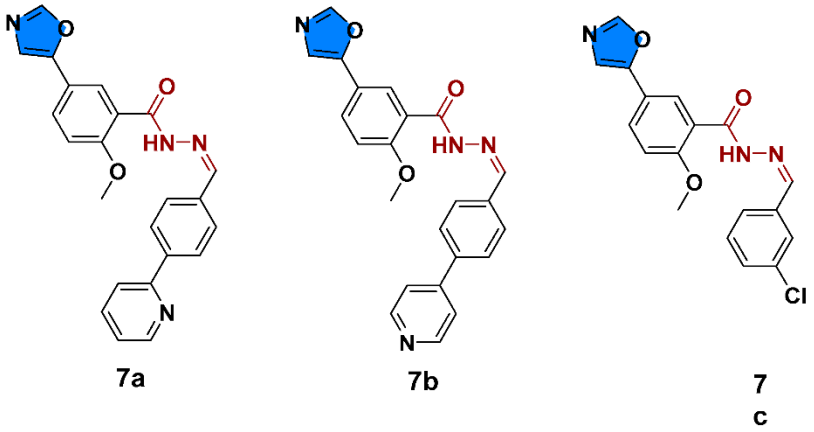


Figure 3. Acylhydrazone-oxazole hybrids with the best predicted free energy in docking studies.

Current efforts to inhibit the M^{pro} of SARS-CoV-2 take into account the presence of Cys145 as a site of inhibition [28]. Notably, this residue is capable of interacting with aromatic systems like the 1,3-oxazole moiety (Figure 4, which is present in the synthesized compounds in our research group.

Table 2. Results of docking studies of acylhydrazone-oxazole hybrids.

Compound	Residues of Interaction	Type of Interaction
7a	Cys145	π-sulfur
	Met165	π-sulfur
	Thr190	π-amide
7b	Cys145	Hydrogen bond
	Met165	π-sulfur
	Thr190	π-amide
7g	Cys145	π-alkyl
	Met165	π-sulfur

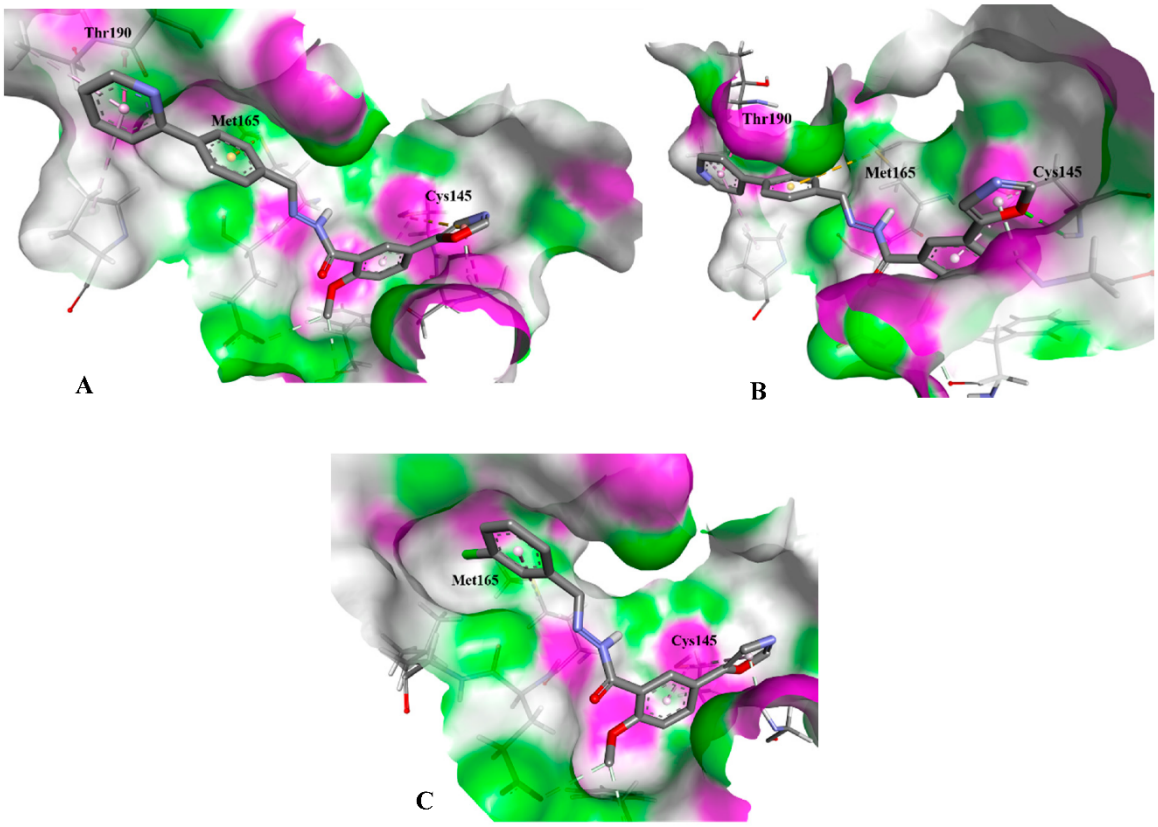


Figure 4. Poses of the best predicted acylhydrazone-oxazole hybrids ligands into the active site of M^{pro} protease of SARS-CoV-2. (A): 7a; (B): 7b and (C): 7g.

4. Conclusions

A novel synthetic strategy to obtain acylhydrazone-oxazole hybrids in three-step reactions in moderate to good yields (36–86%) is reported. The developed synthetic methodology offers operational simplicity and allows obtaining the target molecules in a fast and efficient way and with a diverse electronic environment. The prediction of π-sulfur interactions opens a possibility to investigate the chemical characteristics that these ligands may present in diverse environments including the active site of M^{pro} of SARS-CoV-2.

Author Contributions: Conceptualization, C.J.C.-G. and L.C.-G.; methodology, V.G.G-R. and M.G.V.-L.; software, A.S.-C. and E.D.-C.; validation, A.S.-C. and E.D.-C.; formal analysis, L.C.-G.; investigation, C.J.C.-G. and A.S.-C.; resources, C.J.C.-G.; data curation, V.G.G.-R. and E.D.-C.; writing-original draft preparation, C.J.C.-G. and A.S.-C.; writing-review and editing C.J.C.-G., A.S.-C. and L.C.-G.; visualization, E.D.-C and M.G.V.-L.; supervision, C.J.C.-G.; project administration, C.J.C.-G. and L.C.-G.; funding acquisition, C.J.C.-G. All authors have read and agreed to the published version of the manuscript.

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References

1. Zhang, A.H.; Zhao, Z.; Zhou, C. Recent advance in oxazole-based medicinal chemistry. *Eur. J. Med. Chem*. **2018**, *144*, 444–492, doi:10.1016/j.ejmech.2017.12.044.
2. Joshi, S.; Bisht, A.S.; Juyal, D. Systematic scientific study of 1, 3-oxazole derivatives as a useful lead for pharmaceuticals: A review. *Pharma Inov. J*. **2017**, *6*, 109–117.

3. Thota, S.; Reodrigues, D.A.; Pinheiro, P.M.; Lima, L.M.; Fraga, C.A.M.; Barreiro, E.J. N-Acylhydrazones as drugs. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2797–2806, doi:10.1016/j.bmcl.2018.07.015.
4. Bala, S.; Uppal, G.; Kajal, A.; Kamboj, S.; Sharma, V. Hydrazones as Promising Lead with Diversity in Bioactivity-therapeutic Potential in Present Scenario. *Int. J. Pharm. Sci. Rev. Res.* **2013**, *18*, 65–74.
5. Saini, D.; Gupta, M. Hydrazones as potential anticancer agents: An update. *Asian J. Pharm. Pharmacol.* **2018**, *4*, 116–122, doi:10.31024/ajpp.2018.4.2.4.
6. Singh, N.; Ranjana, R.; Kumari, M.; Kumar, B. A Review on Biological Activities of Hydrazone Derivatives. *Int. J. Pharm. Clin. Res.* **2016**, *8*, 162–166.
7. Kumar, P.; Narasimhan, B. Hydrazides/Hydrazones as Antimicrobial and Anticancer Agents in the New Millennium. *Mini-Rev. Med. Chem.* **2013**, *13*, 971–897, doi:10.2174/1389557511313070003.
8. Kumar, H.M.S.; Herrmann, L.; Tsogoeva, S.B. Structural hybridization as a facile approach to new drug candidates. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127514, doi:10.1016/j.bmcl.2020.127514.
9. Ivasiv, V.; Albertini, C.; Goncalves, A.E.; Rossi, M.; Bolognesi, M.L. Molecular Hybridization as a Tool for Designing Multitarget Drug Candidates for Complex Diseases. *Curr. Top. Med. Chem.* **2019**, *19*, 1694–1711, doi:10.2174/1568026619666190619115735.
10. Mishra, S.S.; Sing, P. Hybrid molecules: The privileged scaffolds for various pharmaceuticals. *Eur. J. Med. Chem.* **2016**, *124*, 500–536, doi:10.1016/j.ejmech.2016.08.039.
11. Bosquesi, P.L.; Melo, T.R.F.; Vizioli, E.O.; dos Santos, J.L.; Ching, M.C. Anti-Inflammatory Drug Design Using a Molecular Hybridization Approach. *Pharmaceuticals* **2011**, *4*, 1450–1474, doi:10.3390/ph4111450.
12. World Health Organization. Available online: https://www.who.int/hiv/pub/drugresistance/hivdr-report-2019/en/ (accessed on 1 November 2019).
13. Khan, Z.; Karatas, Y.; Rahman, H. Anti COVID-19 Drugs: Need for More Clinical Evidence and Global Action. *Adv. Ther.* **2020**, *37*, 2575–2579, doi:10.1007/s12325-020-01351-9.
14. Harapan, H.; Itoh, N.; Yufika, A.; Winardi, W.; Keam, S.; Te, H.; Megawati, D.; Hayati, Z.; Wagner, A.L.; Mudatsir, M. Coronavirus disease 2019 (COVID-19): A literature review. *J. Infect. Public Health* **2020**, *13*, 667–673, doi:10.1016/j.jiph.2020.03.019.
15. Elfiky, A.A. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. *Life Sci.* **2020**, *253*, 117592, doi:10.1016/j.lfs.2020.117592.
16. Elfiky, A. Anti-HCV, nucleotide inhibitors, repurposing against COVID-19. *Life Sci.* **2020**, *248*, 117477, doi:10.1016/j.lfs.2020.117477.
17. Li, G.; De Clercq, E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat. Rev. Drug Discov.* **2020**, *19*, 149–150, doi:10.1038/d41573-020-00016-0.
18. Cortés-García, C.J.; Chacón-García, L.; Mejía-Benavides, J.E.; Díaz-Cervantes. E. Tackling the SARS-CoV-2 main protease using hybrid derivatives of 1,5-disubstituted tetrazole-1,2,3-triazoles: An in silico assay. *Peer J. Phys. Chem.* **2020**, *2*, e10, doi:10.7717/peerj-pchem.10.
19. Cousins, K.R. Computer Review of ChemDraw Ultra 12.0. *J. Am. Chem. Soc.* **2011**, *133*, 8388, doi:0.1021/ja204075s.
20. Swain, M. chemicalize.org. *J. Chem. Inf. Mod.* **2012**, *52*, 613–615, doi:10.1021/ci300046g.
21. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Petersson, G.A.; Nakatsuji, H.; et al. (Eds.) *Gaussian 16*; Revision, C.01; Gaussian, Inc.: Wallingford, CT, USA, 2016.
22. Sanner, M.F. A Programming Language for Software Integration and Development. *J. Mol. Graph. Model*. **1999**, *17*, 57–61, doi:10.1016/S1093-3263(99)99999-0.
23. Rose, P.W.; Prlić, A.; Bi, C.; Bluhm, W.F.; Christie, C.H.; Dutta, S.; Green, R.K.; Goodsell, D.S.; Westbrook, J.D.; Woo, J.; et al. The RCSB Protein Data Bank: Views of structural biology for basic and applied research and education. *Nucleic Acid. Res.* **2015**, 43, D345–D356, doi:10.1093/nar/gku1214.
24. Pettersen, E.F.; Goddard, T.D.; Huang. C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera—A visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *13*, 1605–1612, doi:10.1002/jcc.20084.
25. Morris, G.M.; Goodsell, D.S.; Halliday, R.S.; Huey, R.; Hart, W.E.; Belew, R.K.; Olson, A.J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639–1662, doi:10.1002/(SICI)1096-987X(19981115)19:14<1639:AID-JCC10>3.0.CO;2-B.
26. BIOVIA; Dassault Systèmes. *Discovery Studio 2019*; Dassault Systèmes: San Diego, CA, USA, 2019.
27. García-Ramírez, V.G.; Contreras-Celedón, C.; Rodriguez-García, G.; Chacón-García, L.; Cortés-García, C.J. Synthesis of 1,3-Oxazoles via Van Leusen Reaction in a Pressure Reactor and Preliminary Studies of Cations Recognition. *Proceedings* **2020**, *41*, 1–6, doi:10.3390/ecsoc-23-06463.
28. Jin, Z.; Du, X.; Xu, Y.; Liu, M.; Zhao, Y.; Zhang, B.; Li, X.; Zhang, L.; Jiang, H.; Rao, Z.; et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature* **2020**, *582*, 1–5, doi:10.1038/s41586-020-2223-y.