Alkamides and Piperamides as Potential Antivirals against the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

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ABSTRACT: The pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has quickly spread globally, infecting millions and killing hundreds of thousands of people. Herein, to identify potential antiviral agents, 97 natural amide-like compounds known as alkamides and piperamides were tested against SARS-CoV-2 main protease (Mpro) and RNA-dependent RNA polymerase (RdRp), and the human angiotensin-converting enzyme 2 (ACE2) using molecular docking and molecular dynamics simulations. The docking results showed that alkamides and dimeric piperamides from *Piper* species have a high binding affinity and potential antiviral activity against SARS-CoV-2. The absorption, distribution, metabolism, and excretion (ADME) profile and Lipinski’s rule of five showed that dimeric piperamides have druglikeness potential. The molecular dynamics results showed that pipercyclobutanamide B forms a complex with Mpro at a similar level of stability than N3-I. Our overall results indicate that alkamides and piperamides, and specifically pipercyclobutanamide B, should be further studied as compounds with SARS-CoV-2 antiviral properties.

Coronaviruses are a type of single-stranded positive-sense RNA virus ((+)
ssRNA) and are classified in four groups: alpha, beta, delta, and gamma coronaviruses. Three new beta coronaviruses have been identified in the last two decades: the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2003, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, and the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in 2019. SARS-CoV-2 rapidly propagated and was declared a pandemic by the World Health Organization (WHO). MERS-CoV and SARS-CoV induce a mortality rate of 35 and 10% in humans, respectively. The mortality rate by SARS-CoV-2 in humans ranges from 2 to 10%, depending on the country. Coronavirus disease (COVID-19) is the infectious disease caused by SARS-CoV-2 and around 1/5 of infected people become seriously ill and have difficult breathing. People with diabetes, heart disease, high blood pressure, and cancer as well as older people are considered high risk.

The SARS-CoV and MERS-CoV outbreaks were contained, and the development of vaccines or antiviral drugs for coronaviruses was relegated. As of August 11, 2020, there are more than 20 000 000 confirmed COVID-19 cases and more than 738 000 deaths worldwide. With the magnitude of the pandemic and its consequences, there is a dire need for treatments. Some research groups are focusing on developing vaccines and repurposing approved antivirals, while others are searching for novel antivirals. A target for these antivirals is the nonstructural main coronavirus protease 3-chymotrypsin-like-protease (3CLpro, Nsp5 or Mpro), which is essential for the maturation of proteins during the viral cycle. Other viral proteins marked as targets include the RNA-dependent RNA polymerase (RdRp, Nsp12), crucial for the SARS-CoV-2 development cycle, and the angiotensin-converting enzyme 2 (ACE2), a human integral membrane glycoprotein highly expressed in the kidneys, heart, and pulmonary endothelium. SARS-CoV-2 and other coronaviruses use ACE2 as a cellular entry receptor; specifically, the union of the spike protein S1 of SARS-CoV-2 to the enzymatic domain of ACE2 in the extracellular surface induces endocytosis and translocation of the virus-ACE2 protein complex. Therefore, inhibiting the active sites of Mpro, RdRp, and/or ACE2 is a potential approach for antiviral development.

Compounds containing amide and aromatic groups, including the approved COVID-19 drug Remdesivir, are potential inhibitors of Mpro, RdRp, and/or ACE2. In this regard, we evaluated the possible antiviral activity of natural, plant-derived, amide-like compounds known as alkamides and piperamides. Alkamides and piperamides are compounds structurally diverse comprised of aromatic, polysubstituted, polyunsaturated and dimeric compounds,
Table 1. Docking Scores, Chemical Species, and Plant Distribution of Selected Alkamides and Piperamides Docked against SARS-CoV-2 Mpro and RdRp and to Human ACE2 Protein

| Compound ID | Plant Genus | Alkamide/Piperamide Type | Alkyl/moiety | Alkyl/moiety | Compound name | Chemical structure | MW (g/mol) | Docking score (Kcal/mol) | Nmr | RMSB | ACEI |
|-------------|-------------|--------------------------|--------------|--------------|---------------|------------------|-------------|-------------------------|-----|------|------|
| 2           | Acenea      | IB PLN                   | N-alkylated (2Z,5Z)-octanal|             | Amine moiety: IB = isobutyl, MB = 2-methylbutyl, PHE = phenylethyl. Acyl moiety: ACT = acetylenic, PLN = polyunsaturated, NST = monounsaturated. * = Not docked. # = Not tested. TPP = Triphosphate. |
| 4           | Acenea      | PHE Other                | 3-(2-phenylethyl) 3-phenoxy-2,6-pentanedione |             |               | 211.3 | -5.966 | -3.866 | -5.822 |
| 5           | Acenea      | PHE Other                | 5-(3-cyano-2-phenyl)-3-phenyloxy-2,6-pentanedione |             |               | 267.3 | -7.983 | -5.166 | -8.325 |
| 45          | Nicotiana   | Cinchonoytethyl | Pericyclobutane |             |               | 313.3 | *      | -4.014 | *    |
| 58          | Piper       | Dimero Piperamide A      | N-alkylated (2Z,5Z)-octanal |             |               | 188.2 | -5.696 | -4.316 | -6.997 |
| 59          | Piper       | Dimero Piperamide B      | 3-(2-phenylethyl) 3-phenoxy-2,6-pentanedione |             |               | 196.7 | -5.827 | -4.019 | -7.54   |
| 62          | Piper       | Pipercyclobutanamide C   | 3-(2-phenylethyl) 3-phenoxy-2,6-pentanedione |             |               | 318.4 | *      | -5.452 | *    |
| 64          | Piper       | Pipercyclobutanamide D   | 3-(2-phenylethyl) 3-phenoxy-2,6-pentanedione |             |               | 317.3 | *      | -5.475 | *    |

Figure 1. Molecular docking for SARS-CoV-2 Mpro against N3-I (A–C) and pipercyclobutanamide B (D–F). Interaction representations of the complexes N3-I-Mpro (A) and pipercyclobutanamide B-Mpro (D) showing the main residues that interact through Hbonds (purple arrows), π–π stacking (green dotted line), polar attractions (light-blue residues and contour), and hydrophobic interactions (light-green residues and contour). Protein ribbons representations showing the binding region of N3-I (B) and pipercyclobutanamide B (E), close to the Mpro β-barrel motif. Protein surface representations showing the 3D configuration of the SARS-CoV-2 Mpro pocket and N3-I (C) and pipercyclobutanamide B (F).
generally synthesized from the enzymatic reaction between acyl chains and amino acid-derived compounds.21−23 These compounds have shown bioactivity in viruses, bacteria, fungi, and animals including humans.22,24−27 Thus, we explored the interaction between 97 alkamides and piperamides and Mpro, RdRp, and ACE2 using molecular docking simulations. Additionally, we study the interaction of the best-docked compound against Mpro using molecular dynamics (MD) simulations.

The docking score energy values (DS) generated from the binding of Mpro and the compounds with the best DS are listed in Table 1 (see Table S1). The compounds with at least 70% of the Mpro native inhibitor (N3-I) DS values were considered as potential Mpro inhibitors. The best-docked compound against SARS-CoV-2 Mpro was piperyclobutanamide B (DS = −7.827 kcal/mol) which is comparable to N3-I (DS = −7.348 kcal/mol). Other structures with considerable DS were piperyclobutanamide A (DS = −7.244 kcal/mol), nigramid R (DS = −6.979 kcal/mol), chabamide K (DS = −6.381 kcal/mol), nigramid Q (DS = −5.968 kcal/mol), N-(2-phenylethyl)-3-phenyl-2E-propenamide (DS = −5.963 kcal/mol), chabamide J (DS = −5.713 kcal/mol), and chabamide I (DS = −5.346 kcal/mol). Figure 1 and Table 2 show the molecular interactions of N3-I and piperyclobutanamide B with Mpro. N3-I fits into the pocket of the active site of Mpro (Figure 1B,C). As well, piperyclobutanamide B fits into the Mpro pocket (Figure 1E,F), but on the opposite side of N3-I (Figure 1B,C). The N3-I binding is mainly via polar interactions, whereas piperyclobutanamide B binds mainly through hydrophobic ones. Polar interactions are influenced mainly by amino and carbonyl groups (Figure 1A). The hydrophobic interactions are influenced mainly by aromatic rings and double bonds (Figure 1D).

### Table 2. Interacting Residues of Mpro, RdRp, and Human ACE2 with Their Native Inhibitors and Best-Docked Alkamides and Piperamides

| Protein | Ligand       | Residues in contact                                | Residues in contact through a Hbond       |
|---------|--------------|---------------------------------------------------|------------------------------------------|
| Mpro    | N3-I         | Gln-189, Gly-143, His-41, Glu-166, Arg-188, Asp-187, Asp-48, Glu-47, Ser-46, Thr-45, Cys-145 and Tyr-118 | Gln-189 and Gly-143                       |
|         | piperyclobutanamide B | Gln-189, Arg-188, Thr-190, His-41, Cys-44, Val-42, Met-165, Phe-140 and Leu-141 |                                             |
| RdRp    | GS-441524-TPP | Arg-553, Cys-622, Lys-621, Mg-1004, Asn-691, Asp-452, Thr-556, Arg-555, Lys-551, Arg-624 and Asp-623 | Arg-189 and Gln-622 and Lys-621            |
|         | 8,9-dihydropiptline | Mg-1004, Cys-622 and Ala-688                      |                                            |
| ACE2    | DCBICA       | Arg-273, His-505, His-345, Gln-375, Tyr-515, Arg-518, His-374, Zn-803, Glu-375, Arg-273, Arg-514, Phe-512 and Tyr-510 | Arg-273, His-505, His-345, Gln-375, and Tyr-515 |
|         | piperyclobutanamide B | Ala-348, Thr-371, Zn-803, Arg-518, Glu-145, His-345, Thr-445, His-378, Thr-365, Lys-363, Thr-362, Cys-344, Phe-274, Cys-361 and Met-360 | Ala-348 and Thr-371                       |

Figure 2. Molecular docking for SARS-CoV-2 RdRp against GS-441524-TPP (A−C) and 8,9-dihydropiptline (D−F). Interaction representations of the complexes GS-441524-TPP-RdRp (A) and 8,9-dihydropiptline-RdRp (D) showing the main residues that interact through Hbonds (purple arrows), π−π stacking (green dotted line), polar attractions (light-blue residues and contour), and hydrophobic interactions (light-green residues and contour). Protein ribbon representations showing the binding region of GS-441524-TPP (B) and 8,9-dihydropiptline (E), in the active site across the RdRp synthetic channel. Protein surface representations showing the 3D configuration of the SARS-CoV-2 RdRp pocket and GS-441524-TPP (C) and 8,9-dihydropiptline (F).
The dimeric piperamides in the *Piper* genus,\(^2^8\),\(^2^9\) had DS similarities to N3-I. Particularly, pipercyclobutanamides A and B had the highest DS and therefore the highest potential to interfere with Mpro. Both, N3-I and pipercyclobutanamide B dock through an Hbond to Gln-189 and polar interactions to Arg-188. Pipercyclobutanamide B stabilizes in the same pocket that N3-I, but with some differences due to polarity. The amino acids involved in the stabilization of N3-I and pipercyclobutanamide B are also involved in the interaction with other Mpro inhibitors such as rutin, ritonavir, emetine, and hesperidin.\(^3^0\) As expected, N3-I binds to the crucial catalytic residues His-41 and Cys-145.\(^1^2\) However, pipercyclobutanamide B interacts only with His-41 through polar-hydrophobic interactions between the imidazole group of His-41 and the piperidine-carbonyl-enyl moiety of pipercyclobutanamide B. As shown in Figure 1 and Table 2, N3-I and pipercyclobutanamide B also interact with other important residues of the active site such as Met-49, Gly-143, His-163, His-164, Glu-166, and Pro-168.\(^1^3\),\(^1^8\)

The higher DS value of pipercyclobutanamide B over N3-I could be due to the interactions with the protein residues or the 3D configuration. Pipercyclobutanamide B has an X-form conformation that docks in an X-form protein pocket, (Figure 1D–F). Pipercyclobutanamides A and B are considered trace constituents of black peppercorns (*Piper nigrum*) with less than 0.12% and 0.006% by dry weight, respectively.\(^2^8\) However, these compounds can be fully synthesized.\(^3^1\),\(^3^2\) To our knowledge, this is the first report of dimeric-piperamides, specifically pipercyclobutanamides, as potential antivirals via inhibition of SARS-CoV-2 Mpro.

The best-docked ligand against SARS-CoV-2 RdRp was the triphosphate form of GS-441524 (DS = −8.495 kcal/mol), the RdRp native inhibitor, that is metabolically derived from Remdesivir. None of the analyzed compounds reached 70% of the inhibitor DS value (Table 1). However, some compounds with considerable DS (60% of GS-441524-TPP DS) were 8,9-dihydropiplartine (DS = −5.432 kcal/mol), pipercyclobutanamide A (DS = −5.417 kcal/mol) and cis-piplartine (DS = −5.073 kcal/mol). TPP-GS-441524 assembles into the pocket of the active site (Figure 2A–C) whereas the 8,9-dihydropiplartine molecule docks deeper into the pocket of the active site of SARS-CoV-2 RdRp (Figures 2D–F). The interaction between the residues of RdRp and TPP-GS-441524 and 8,9-dihydropiplartine is reported in Table 2.

The molecular docking results showed that alkamides and piperamides are less effective against SARS-CoV-2 RdRp compared to SARS-CoV-2 Mpro. The alkamides/piperamides DS values did not reach that of the SARS-CoV-2 RdRp native inhibitor because of the triphosphate moiety in the GS-441524-TPP and its mimicry with the nucleotide triphosphates that are the RdRp native ligands. Also, the crystallized structure of RdRp shows that the Hbonds to Arg-553, Thr-687, and Asp-760 are important for its catalytic activity.\(^3^3\) In the crystallized structure, the triphosphate moiety is hydrolyzed into 2-pyrophosphate and the monophosphate-inhibitor form. Arg-553 forms two Hbonds to the \(\beta\)-phosphate of the free 2-pyrophosphate, Asp-760 forms two Hbonds to the bonded \(\alpha\)-monophosphate, and Thr-687 forms one more Hbonds to the hydroxy group at the cyano-oxolane moiety.\(^3^3\) After redocking the triphosphate form of the inhibitor, the phosphate groups remained as the docking points. The anchor residues change in the interaction importance since only Arg-553 remains to form an Hbond and Cys-622 along with Lys-621 binds via Hbonds to the beta-phosphate group. As expected, interactions with...
Mg-1004 are present in both crystallized and redocked inhibitor-protein complexes.33

The tested compounds failed to reach a comparable DS to the SARS-CoV-2 RdRp inhibitor. However, some piperamides (present in *Piper tuberculatum*34) had sufficient DS to warrant future research. 8,9-Dihydropiplartine and pipercyclobutanamide A were the best-docked compounds with similar DS. Both compounds coordinate with Mg-1004 through the

| ID | MW    | DonorHB/AcceptHB | Human oral absorption | QPlogow | QPlogS  | QPlogBB | QPlogKhsa | CNS   | RO5/RO3 |
|----|-------|------------------|-----------------------|----------|---------|---------|-----------|-------|---------|
| 4  | 251.3 | 1/1              | high                  | 4.25     | −4.75   | −0.34   | 0.44      | 0     | 0/0     |
| 48 | 532.6 | 1/1              | high                  | 3.93     | −4.79   | 0.00    | 0.21      | 0     | 1/0     |
| 49 | 446.7 | 2/2              | low                   | 6.38     | −8.26   | −0.76   | 0.94      | −1    | −1/1    |
| 50 | 446.7 | 2/2              | low                   | 6.49     | −7.00   | −1.11   | 1.16      | −2    | −1/1    |
| 56 | 544.6 | 0/0              | low                   | 4.58     | −6.30   | −0.13   | 0.39      | −1    | −1/1    |
| 57 | 544.6 | 0/0              | high                  | 3.89     | −4.56   | −0.04   | −0.05     | −1    | −1/0    |
| 58 | 570.7 | 0/0              | high                  | 4.87     | −5.36   | −0.13   | 0.38      | −1    | −1/0    |
| 59 | 596.7 | 0/0              | low                   | 5.83     | −6.89   | −0.26   | 0.72      | −1    | 2/1     |
| 62 | 319.4 | 0/5.25           | high                  | 3.32     | −4.54   | −0.81   | 0.13      | −1    | −1/0    |
| 64 | 317.3 | 0/5.25           | high                  | 2.92     | −3.97   | −0.43   | −0.13     | 0     | 0/0     |
| 98 | 680.8 | 2.75/13.75       | low                   | 2.84     | −6.03   | −4.03   | −0.40     | −2    | 2/3     |
| 99 | 531.2 | 4/19.65          | low                   | −1.82    | −1.76   | −5.25   | −2.53     | −2    | 3/1     |
| 100| 428.3 | 3/7              | low                   | 1.91     | −4.58   | −1.28   | −0.15     | −2    | 0/1     |

*ID = Identification number according to Table 1. ADME parameters: MW = molecular weight, DonorHB = number of Hbond donors, AcptHB = number of Hbond acceptors, QPlogow = octanol/water partition coefficient, QPlogS = predicted aqueous solubility, QPlogBB = brain/blood partition coefficient, QPlogKhsa = prediction of binding to human serum albumin, CNS = predicted central nervous system activity on a −2 (inactive) to +2 (active) scale, RO5 = number of violations to rule of five, RO3 = number of violations to rule of three.*

Figure 4. Molecular dynamics for Mpro−N3-I and Mpro-pipercyclobutanamide B complex. Heatmap plots of RMSD changes for binding pocket amino acid residues in the Mpro−N3-I (A) and Mpro-pipercyclobutanamide B (B) during 5.5 ns of simulation. The color key ranges from the smallest movements in blue to the largest movements in dark red. Ligands movement RMSD after complexing with Mpro during 5.5 ns of MD simulation (C). Mpro protein backbone movement RMSD after complexing with the respective ligand during 5.5 ns of MD simulation (D). RMSD values were calculated as the deviation from the initial structure models at 0 ns. Representation of the differences in structural conformation of N3-I (E) and pipercyclobutanamide B (F) from the beginning to the end of the simulation. Average protein−ligand contact analysis during MD simulations for N3-I and pipercyclobutanamide B (G).
carbonyl moiety of the amide groups. No important interactions are formed to the catalytic Arg-533, Thr-687 and Asp-760, but the proximity of these residues to π-electrons could be contributing to the DS of 8,9-dihydropiplartine and pipercyclobutanamide A. The lower DS of the alkamides is due to the different size and configuration compared to the docked SARS-CoV-2 RdRp inhibitor and the native ligands, however, 8,9-dihydropiplartine and pipercyclobutanamide A docked in the active site. The phosphate groups are important in the search for effective inhibitors of RdRp polymerase, as previously documented. Therefore, future research on non-nucleoside inhibitors should include the development of alkamide-phosphate like compounds with piperamide moieties. The use of *P. tuberculatum* or 8,9-dihydropiplartine as an antiviral has not been previously reported.

As expected, the best ligand against human ACE2 was the native inhibitor DCBICA (DS = −10.558 kcal/mol). Some of the tested compounds docked with considerable DS (65% of DCBICA): pipercyclobutanamide B (DS = −7.34 kcal/mol), pipercyclobutanamide A (DS = −6.997 kcal/mol), and nigramid Q (DS = −6.855 kcal/mol). Carboxylic groups of DCBICA are involved in most of these interactions as seen in Figure 3A. The DCBICA molecule docks into the pocket of the active site duct (Figure 3B,C). Pipercyclobutanamide B fits in the pocket along internal ducts around the active site (Figure 3D–F). The interactions between the residues of ACE2 and DCBICA and pipercyclobutanamide B are reported in Table 2.

The DS value of the DCBICA-human ACE2 complex was the highest overall, showing that DCBICA is a strong inhibitor of human ACE2. Similar to the SARS-CoV-2 Mpro and RdRp results, some piperamides could also be potential inhibitors of ACE2. No comparable residue interactions between the DCBICA and the piperamide ACE2 complexes were identified, reflecting their low DS. The considerable DS of piperamides could be due to the attachment between the 3D configuration of the docked ligands and the 3D configuration of the human ACE2 pocket. The protein pocket entrance-size also needs to be considered. Although the best-docked piperamides bound with regular DS, they may be too large to enter the human ACE2 pocket. In that case, other nondimeric alkamides or piperamides with lower DS and smaller size could be investigated (Table 1; i.e., N-isobutyl-(2E,4Z)-octadienamide and N-(2-phenethyl)-cis-2,3-epoxynona-6,8-diyamine).

The absorption, distribution, metabolism, and excretion (ADME) properties of the native inhibitors and the tested compounds with the highest DS for each protein are listed in Table 3. According to their molecular properties, Lipinski’s Rule of five (ROS) was used to evaluate the potential of these alkamides as orally active drugs in humans. Additionally, the Jorgensen Rule of three (RO3) was used to evaluate the oral availability of the compounds. The violations to the RO5 and RO3 are listed in Table 3. The dimeric piperamides violate the rules of maximum molecular weight and/or permeability. However, they performed better than N3-I and GS-441524-TPP. Therefore, these piperamides have considerable drug potential, especially considering their favorable traits such as high oral absorption, brain/blood partition coefficient, predicted binding to human serum albumin, and activity in the central nervous system (Table 3).

We used MD simulations to explore the evolution of the complex ligand-Mpro through time. MD analysis is a very computationally demanding process, hence we selected pipercyclcobutanamide B, the piperamide with the best docking results, and the native inhibitor (N3-I) for the analysis. The MD simulations results show that N3-I binds stably to the Mpro active site residues (Figure 4A, Tables 4 and S2).

| Mpro residue | RMSD change for Mpro–N3-I complex | RMSD change for Mpro–pipercyclobutanamide B complex |
|--------------|-----------------------------------|-----------------------------------------------|
| 41           | 1.040 1.953 0.411                 | 0.968 2.987 0.297                              |
| 145          | 1.423 1.907 0.170                 | 1.352 1.915 0.241                              |

"RMSD values are shown in Å.

Similarly, the same Mpro residues in contact with the pipercyclobutanamide B maintained low RMSD changes, except for the 188 and 189 residues that increased their RMSD movement at the last nanosecond of the MD simulation (Figure 4B, Tables 4 and S3). During the MD simulation, Mpro–ligand complexes were able to maintain low ligand movement below 3.5 Å and stabilize at an average of 2.5 Å after 1 ns. This indicates proximity and stronger binding for both compounds to the Mpro active site (Figure 4C). Protein movement RMSD stabilizes around 1.5 Å after 2 ns for both ligands, following similar trajectories (Figure 4D). Additionally, small conformational changes were observed for protein and ligands during MD simulation (Figure 4E,F). The active site residues contact number for Mpro–N3-I and Mpro–pipercyclobutanamide B complexes show a similar level of protein–ligand interaction during the simulation for both ligands (Figure 4G).

A contact between two molecules is defined when the heavy atom of one molecule is within a cutoff distance from the heavy atom of another molecule. The contact fingerprint of Mpro with pipercyclobutanamide B shows that, during the 5.5 ns of the MD simulation, the ligand had contacts with all the heavy atoms from the protein backbone with most of the amino acids that form the binding pocket (Figure 4G). On average, pipercyclobutanamide B contacts increased with Glu-166, His-163, and Asn-142 compared to N3-I (Figure 4G), amino acids that belong to the binding pocket subunit S1. Ligand–protein interactions with amino acids in the subunit S1 such as Glu-166 and His-163 are important interactions with inhibitors like cinacérin, nellinavir, pralmorelin, and N3. Also, the residue His-41 that belongs to the Mpro catalytic dyad increased the number of contacts as well as Met-49, Met-165, and Asp-187 that belong to the binding pocket subunit S2, while Cyst-145 contacts remain similar as N3-I (Figure 4G).

The molecular docking results for SARS-CoV-2 Mpro and RdRp, and human ACE2 indicate that some alkamides and piperamides have high antiviral potential against SARS-CoV-2, especially, the dimeric piperamides of *P. nigrum* and *Piper chaba*. The phenethyl-alkamides found in the *Capsicum* genus and major piperamides of *P. nigrum* (piperine and trichostachine) affect RdRp in particular. The potential anti-SARS-CoV-2 activity of the tested compounds is linked mainly to interference with the
Mpro function. Therefore, the effect of piperine-enriched essential oils of *Piper* species and piperamide-like purified compounds should be examined in vitro and in vivo. The ADME studies further support the anti-SARS-CoV-2 potential of the dimeric piperamides from *Piper* species, primarily against the main protease (Mpro) of SARS-CoV-2, but also considerably against SARS-CoV-2 RdRp and the human ACE2. The MD simulations showed that piperyclobutanamide B forms a complex with Mpro with similar stability to N3-I, indicating a promising performance for future in vitro and in vivo experiments. Then, piperamides and related compounds should be considered for a possible alkamide/piperamide-based treatment. Many of the examined compounds have common culinary uses, including the piperamides and capsaicinoids found in the common pepper (*P. nigrum*) and chilli pepper (*Capsicum annuum*), respectively. Possible mitigating effects of an alkamide/piperamide-rich diet on coronavirus susceptibility should be studied. Based on past and herein presented data, amide-aromatic-like natural products could potentially act as antivirals against SARS-CoV-2 and related coronaviruses. These results show the potential of dimeric piperamides, specifically piperyclobutanamide B, as antivirals against SARS-CoV-2.

**Experimental Section**

The SARS-CoV-2 Mpro (PDB ID: 6LU7; resolution 2.14 Å), the SARS-CoV-2 RdRp (PDB ID: 7BV2; resolution 2.5 Å), and the human ACE2 (PDB ID: 1R4L; resolution 3 Å) protein crystal structures were retrieved from the Protein Data Bank ([www.rcsb.org/](http://www.rcsb.org/)). The Mpro chain A (306 amino acids) of the structure was prepared using the Protein Preparation Wizard and the Virtual Screening Workflow tools of Maestro Schrödinger software. The protein states were generated at pH of 7.0 ± 0.5 and HBonds were optimized using “sample water orientations” at option pH 7.0 after deleting the ligated inhibitor N3-I (N-[((5-methyl-1,2-oxazol-3-yl)carbonyl]-l-alan-yl-l-valyl-N-{(2S,3E)-5-(benzoxlyo)-5-oxo-l-[(3S)-2-oxo-3-pyrroldiny]-3-penten-2-yl]-l-leucinamide). States were minimized converging heavy atoms to RMSD of 0.3 Å and an OPLS3e option force field. All other parameters were set to default values. The RdRp and ACE2 proteins were prepared following the same steps as for 6LU7 but with the following considerations. For 7BV2, only the chain A (888 amino acids) was used and the RNA molecule was deleted to avoid incorrect interactions during docking simulations. For 1R4L, the chain A (782 amino acids) was used. Each protein was prepared on a separate project to facilitate the results inspection.

Based on published literature and to cover a variety of chemical structures, 97 alkamides and piperamides were selected and retrieved from PubChem. The structures of the Mpro, RdRp, and ACE2 inhibitors were retrieved from PubChem. All structures are listed in Supporting Information Tables S1 and SMILES, including their respective PubChem ID. All ligands were prepared by generating states at a pH of 7.0 with desalt and generating tautomers options. There were generated for each ligand at most 32 possible states using the OPLS3e force field option. The compounds that did not have interaction with Mpro, RdRp, and ACE2 were not included in the results in Table 1. However, they were included in Supporting Information Table S1, since negative results have a scientific value.

The molecular docking was performed between each protein and ligand using the Grid-Based Ligand Docking with Energetics (GLIDE) module of Maestro Schrödinger software. The grids for each protein was generated using the Grid Generation tool. The grid box of processed-6LU7 was centered at the same coordinates of the crystallized ligand (x: -1074, y:10.36, z:68.95), previously deleted. For processed-7BV2, the grid box was centered by picking on the ligated inhibitor GS-441524 triphosphate ((2R,3R,4S,5R)-2-(4-amino- pyrrolo)[2,1-f][1,2,4]triazin-7-yl)-3,4-dihydroxy-5-(hydroxymethyl)oxalane-2-carbonitrile-TPP) at x:91.74, y:92.43, z:103.75. For processed-1R4L, the grid box was centered by picking on the ligated inhibitor DCBICA ((S,S)-2-((1-carboxy-2-[(3S,3,5-dichloro-benzyl)-3H-imidazol-4-yl]-ethylamino)-4-methyl-pentanoic acid) at x:40.61, y:5.82, z:27.84. Each ligand was docked into each protein, based on the respective grid, using a standard precision (SP) docking algorithm with a flexible ligand sampling option. The Qik-Prop module of Maestro Schrödinger was used to determine the ADME profile of the alkamides with the highest docking score for the respective protein.

Molecular dynamics simulations were run using the Large-scale Atomic/Molecular Massively Parallel Simulator code (LAMMPS) and using CHARMM36 additive force field for protein as well as protein–ligand complexes. The system was minimized with the Polak–Ribiere version of the conjugate gradient (CG) algorithm and then equilibrated applying bond and angle constraints to specified bonds and angles in the simulation with the SHAKE algorithm and the canonical NVT ensemble. The production of MD simulations was performed at a constant temperature of 310.15 K and a constant pressure of 1 bar using the isothermal–isobaric ensemble (constant temperature and constant pressure ensemble). All the simulations input scripts were generated with the CHARMM-GUI Solution Builder. To generate the input files for simulations and prepare the solvent system, we used the interactive web-based platform CHARMM-GUI. The protein–ligand complexes were placed in an octahedral waterbox with a 5.0 Å of edge distance, 0.1 M KCl ions, and the Monte Carlo ion placing method. The simulations were conducted for 5.55 ns and performed on a workstation with Windows 10 Pro 64 bits, AMD Threadripper 1950X, 16 cores, 64 GB RAM. The stability of the protein and protein–ligand complex system was analyzed by calculating the root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) using the Bio3d package in R-Studio. A contact analysis between the residues in the binding pocket (residues 40–190) and the corresponding ligand was made with the Timeline plugin (V.2.3) in the Visual Molecular Dynamics (VMD) software and the heatmaps were constructed with the data matrix extracted from the RMSD Visualizer Tool plugin in VMD. The representations in structural conformation differences showed in Figure 4 were made with PyMol. The RcolorBrewer pallet was used to assign color blind-friendly colors to the graphs.

**Associated Content**

**Supporting Information**

The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acs.jpclett.0c01685](https://pubs.acs.org/doi/10.1021/acs.jpclett.0c01685).

Table with docking scores, PubChem accession number, and other chemical properties of the alkamides (PDF)

RMSD changes (XLSX)

Optimized structures (ZIP)
Molecular formula strings (XLSX)

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Notes
The authors declare no competing financial interest.

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ABBREVIATIONS
SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, middle east respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, associated syndrome of SARS-CoV-2; Mpro, SARS-CoV-2 main protease; NsP5, nonstructural protein 5; 3CLpro, 3C-like protease; RdRp, RNA-dependent RNA polymerase; Nsp12, nonstructural protein 12; ACE2, angiotensin-converting enzyme 2; DS, docking score; Hb, hydrogen bond; N3-1, N-[(5-methyl-1,2-oxazol-3-yl)-carbonyl]-l-alanyl-l-valyl-N-(2S,3E)-5-[(benzoyloxy)-5-oxo-1-{(3S,2-oxo-3-pyrrolidinyl)-3-penten-2-yl]}-i-leucinamide); GS-441524-TPP, (2R,3R,4S,5S)-2-[(4-Aminopyrrolo[2,1-f]-[1,2,4]triazin-7-yl]-3,4-dihydroxy-5-[(hydroxymethyl)oxolane-2-carbonitrile-triphosphate; DCBICA, (S,S)-2-[(1-carboxy-2-[3-(3,5-dichloro-benzyl]-3H-imidazol-4-yl)-ethylamino]-4-methyl-pentanoic acid); ADME, favorable absorption, distribution, metabolism, and excretion.

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