



Novel octapeptide containing the RGD sequence as a potential anti-SARS-CoV-2 agent: design, synthesis, and theoretical studies

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Abstract

The design of peptide-based inhibitors targeting cell receptors represents a promising strategy in the development of antiviral agents. In this study, a novel octapeptide containing the RGD sequence was rationally designed to explore its potential interaction with integrins. The peptide was functionalized with a malonic moiety to enhance its binding capabilities and potential bioactivity. Conformational and physicochemical properties were evaluated using DFT-PBEh-3c calculations. Molecular docking studies revealed favorable interactions with the integrin $\alpha_5\beta_1$, including coordination with the Mg^{2+} ion at the active site. The peptide was successfully synthesized via Fmoc-based solid-phase peptide synthesis (SPPS) and fully characterized by NMR, IR, MS, and RP-HPLC.

Keywords RGD motif · Integrin $\alpha_5\beta_1$ · DFT calculations · Solid-phase peptide synthesis

Introduction

Arginine-glycine-aspartic (RGD) (Fig. 1) is a cell adhesion motif displayed on many extracellular matrix and plasma proteins (Colombo and Bianchi 2010). Since RGD was first identified as specific binding sites for fibronectin (FN) and the FN receptor, (Pierschbacher and Ruoslahti 1984) it has attracted widespread attention and research.

It has been reported that membrane proteins associated with extracellular matrix glycoprotein receptors on the cell surface were called integrins, which were members of the adhesion receptors (Hynes 1987). RGD plays a crucial role in cell recognition and adhesion, and has been utilized in tumor therapy and tissue engineering through recombinant and chemical methods. RGD-based ligands for integrins are studied in pathology and pharmacology (Colombo and Bianchi 2010). Furthermore, the RGD-integrin system is exploited to target cell recognition and internalization, which is applied to man-made constructs by mimicking pathogens. This system enables the study of various aspects, including diagnostics, therapeutics, and the regeneration of transplanted tissue. RGD-modified drugs and imaging agents have been investigated and developed by conjugation of the RGD peptides with a carrier device. This one has been equipped with drug molecules or reporter molecules. RGD-peptides and RGD-mimetics have also been applied

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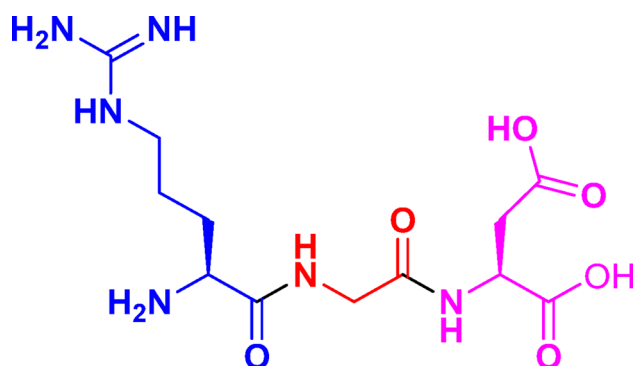


Fig. 1 Structure of RGD sequence. Arg(R) and Asp(D) are in the L-configuration, and glycine is achiral

to modify liposomes, polymers, and peptides by chemical means to improve the biological effects of therapeutic agents (Wang et al. 2013). Additionally, RGD-peptides were utilized in gene delivery by viral and non-viral vectors (Temming et al. 2005). The surface modification technology with fixed RGD peptides has promoted the application of integrin-mediated cell adhesion to develop tissue engineering, especially for biomaterials.

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), caused by a new type of coronavirus (2019-nCoV), has emerged from China and has led to thousands of deaths in the world (Zhu et al. 2020). The viruses often bind to receptor proteins on the surface of cells to enter human cells. In the case of the SARS virus, the spike glycoprotein mediates receptor recognition and membrane fusion. The receptor binding domain (RBD), contained in the spike glycoprotein, binds directly to the peptidase domain of the angiotensin-converting enzyme 2 (ACE2) (Chen et al. 2020; Han et al. 2006).

Dakal 2021 suggests an additional mechanism of virus recognition through integrins. The pair-wise sequence alignment of SARS-CoV-2 spike protein RBD contains an RGD sequence, and SARS-CoV-2 may be attached to integrins via the RGD motif. The development of high-affinity RGD-based ligands for integrin receptors has been extensively advanced by Kessler and co-workers, whose pioneering contributions in cyclic peptide design and structure–activity relationships have set the foundation for targeting integrin $\alpha_5\beta_1$ and related receptors (Kapp et al. 2016; Ludwig et al. 2021; D’Amore et al. 2023). The interaction of the RGD motif with the integrins may be involved in facilitating virus entry into host cells (Dakal 2021). The integrin $\alpha_5\beta_1$ is the major cellular receptor for the extracellular matrix protein fibronectin, and it functions as a fibronectin receptor. This integrin has an RGD motif binding site located at the interface between the α_5 and β_1 chain, which potentially affects the binding of $\alpha_5\beta_1$ with proteins or peptides (Nagae et al. 2012). Previous work has reported the antiviral properties

of some peptides, with potent broad-spectrum antiviral activities (Lee et al. 2022). Interestingly, there are antiviral peptides demonstrated to exert prophylactic and therapeutic effects against coronaviruses (Mahendran et al. 2022).

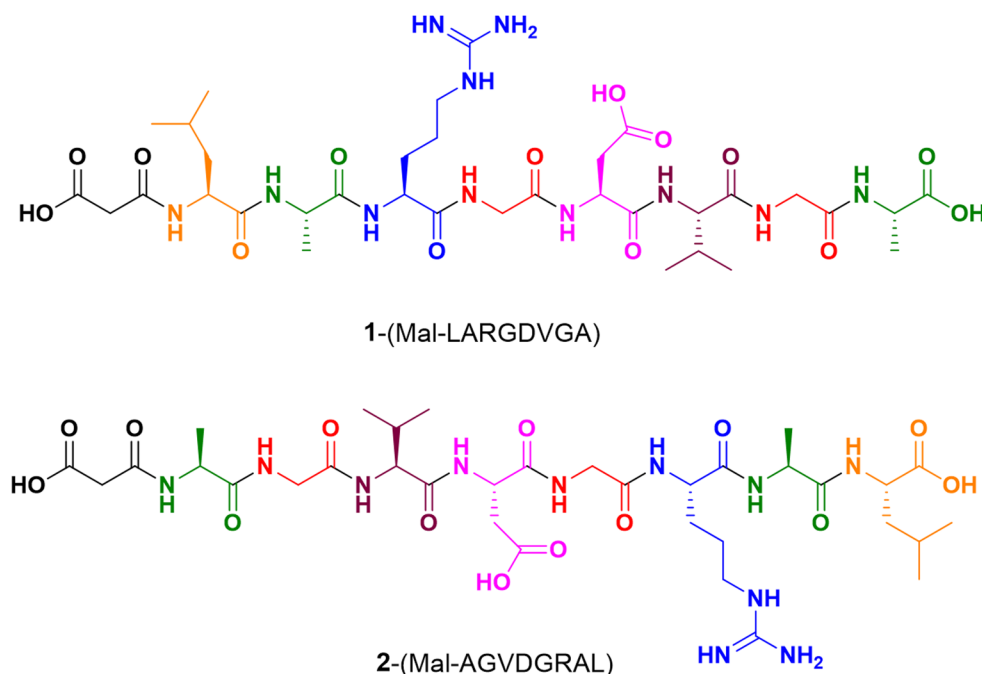
Recent studies have suggested that, in addition to angiotensin-converting enzyme 2 (ACE2), certain integrins, including $\alpha_5\beta_1$, may act as co-receptors that facilitate SARS-CoV-2 cell entry by recognizing the RGD motif present in the receptor-binding domain (RBD) of the spike protein (Liu et al. 2022; Cai et al. 2024). This alternative binding pathway can enhance viral adhesion to host cells, potentially increasing infectivity (Sigrist et al. 2020; Simons et al. 2021). Therefore, designing synthetic peptides that selectively bind to $\alpha_5\beta_1$ could competitively block this interaction, limiting the number of integrin receptors available for spike protein engagement (Park et al. 2021). Such an approach represents a targeted strategy to disrupt a secondary viral entry mechanism and may complement therapies aimed at the ACE2–RBD interface (Simons et al. 2021; Calver et al. 2021).

Nowadays, numerous research groups have engaged in the study of therapies for this new virus, based on the understanding of the COVID-19 target–ligand interactions. Many efforts have been made to design and screen therapeutics for the current SARS-CoV-2. Therefore, this work focuses on the design and theoretical evaluation of two small peptides containing the RBD sequence, and a rational synthesis based on molecular docking results as potential anti-SARS-CoV-2 agents. To clarify the rationale behind peptide design, both synthesized peptides were engineered around the RGD motif, which is key for integrin $\alpha_5\beta_1$ recognition. The surrounding amino acids, including Ala, Gly, Val, Asp, Leu, and Arg, were chosen to optimize solubility, hydrogen bonding potential, and compact conformation favorable for receptor binding. The malonic acid moiety was strategically added to promote interaction with the divalent Mg^{2+} ion at the MIDAS site of the integrin, enhancing binding affinity and specificity.

Results and discussion

In the search for new compounds as antivirals, a study was conducted on the synthesis and theoretical calculations of peptides capable of interacting with $\alpha_5\beta_1$ integrin receptors. Two octapeptides were designed containing the RGD sequence functionalized with a malonic moiety to evaluate their interactions with the integrin $\alpha_5\beta_1$, see Fig. 2.

Fig. 2 Structure of octapeptides **1** and **2** containing the RGD sequence. All amino acids are in the L-configuration, except glycine, which is achiral



The presence of the malonate (Mal) moiety in a peptide would increase the number of interactions with biological receptors (Shi et al. 2024; Wilhelm et al. 2012). In addition to its ability to form stable complexes with metals and promote metal chelation of calcium and magnesium, it has potential applications in medicinal chemistry (Ojha et al. 2010). Similar strategies have been reported to enhance integrin ligand affinity (Kapp et al. 2016; Wu et al. 2019; Ludwig et al. 2021).

The sequence design also intended to explore conformational differences and interaction patterns by altering the order of residues around the RGD motif, but keeping the L-conformation of all used amino acids. The flanking residues were selected seeking to enhance solubility, conformational stability, and interaction with the integrin receptor. Specifically, the peptide includes residues such as Ala, Gly, Val, Leu, and Arg to support structural compactness and foster internal hydrogen bonding, while the malonate moiety increases the interaction potential at the MIDAS site, as was mentioned before. This method is widely used in the design of peptides to modulate recognition by biological receptors (Atzori et al. 2013).

For a better understanding of the structural properties of peptides **1** and **2**, a theoretical conformational analysis was performed. In the first place, the molecule was pre-optimized with the low-cost B97-3c (Brandenburg et al. 2018) generalized gradient approximation method. The obtained

structure was further optimized using a hybrid PBEh-3c (Grimme et al. 2015) composite scheme, which presents excellent results close to the MP2 method and outperforms the popular B3LYP/6-31G approach at lower computational cost, see Fig. 3. The Cartesian atom coordinates are given in Table S1 of the Supporting Information.

Modified octapeptide **1** exhibits a highly compact structure characterized by strong interactions between residues, together with the extra interactions due to the presence of the malonic moiety. The malonic acid's hydroxyl group is located close to arginine's backbone carbonyl and forms a strong hydrogen bond $\text{OH} \cdots \text{O}=\text{C}$, with a 2.05 Å distance. Instead, compound **2** shows a cyclic-like conformation promoted by two hydrogen bonds between the Mal fragment and the arginine residue. The carbonyl in the malonic moiety presents a synclinal relative position to each other with a torsion angle of 65.7° (**1**) and 64.0° (**2**), which permits the previously mentioned interaction with Arg residue.

Asp residue produces several strong interactions with vicinal residues such as glycine ($\text{OH} \cdots \text{O}=\text{C}$, 1.71 Å) and valine and alanine residues ($\text{C}=\text{O} \cdots \text{HN}$, 2.25 Å and 2.52 Å respectively) for **1**. For peptide **2**, only the aspartate residue formed hydrogen bonds with the glycine ($\text{OH} \cdots \text{HN}$, 2.03 Å).

The analysis of non-covalent interactions (NCI) confirms the strong intramolecular interactions observed in the DFT calculations for both peptides, as shown in Fig. 4.

The green isosurfaces represent weak van der Waals interactions, and the blue ones represent strong attractive interactions. Strong intramolecular hydrogen bonds are observed between the malonate group and the guanidinium

Fig. 4 NCI representation (up) and electrostatic potential maps (down) of **1** and **2**. For better visualization, the regions with strong interactions are amplified

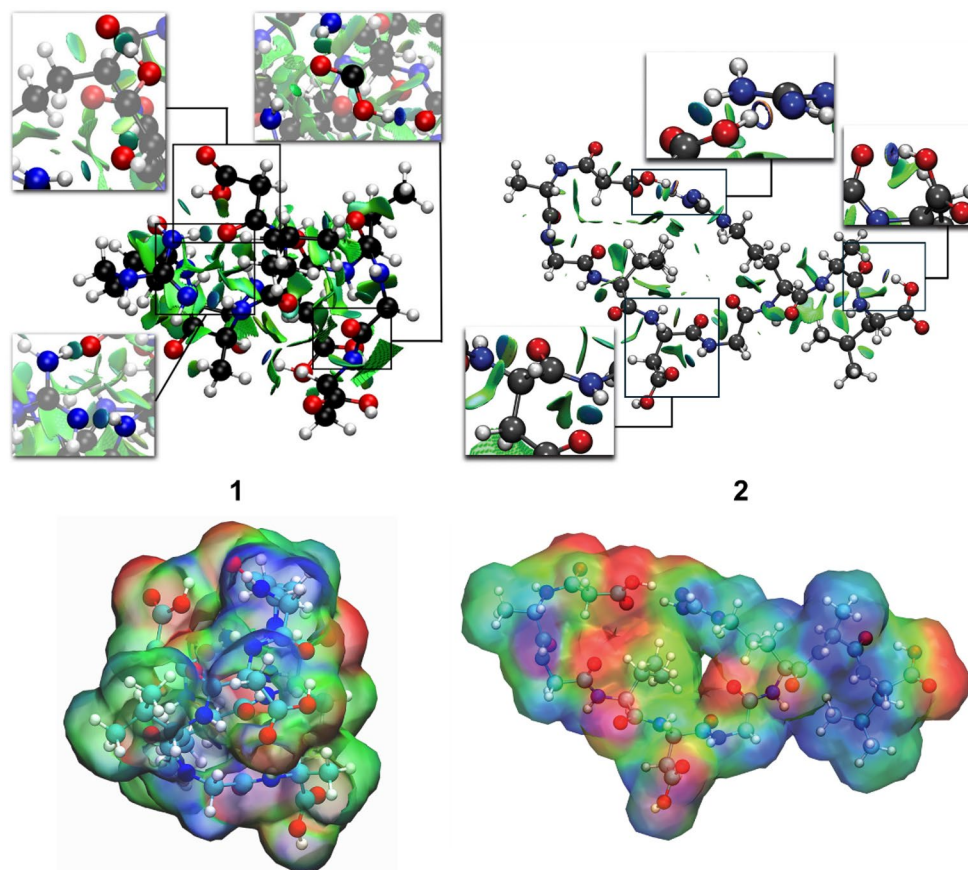


Table 1 Molecular predicted descriptors for peptides 1 and 2

Parameters (Schrödinger, Release 2021)	1	2
Volume (Å ³) ^a	2031.93	2280.73
SASA (Å ²) ^b	850.65	1086.291
TPSA (Å ²) ^c	406.60	406.60
PSA (Å ²) ^d	381.92	438.840
Glob ^e	0.91	0.77
QPlogPo/w ^f	-2.49	-2.82

^aTotal volume calculated by QikProp

^bSolvent-accessible surface area calculated by QikProp

^cTopological polar surface area calculated by the BioTriangle web server

^dVan der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms predicted by QikProp

^eGlobularity descriptor predicted by QikProp. (Globularity is 1.0 for a spherical molecule)

^fPredicted octanol/water partition coefficient by QikProp

al. 2023). These results provide additional support for the predictive capability of our computational approach.

Interestingly, peptide **2** shows an H-bond with Asp227 (α5), a residue involved in the recognition of the RGD motif in the RBD protein. On the other hand, the mechanism of integrin–ligand recognition involves a direct coordinated carbonyl or carboxylate oxygen from the

ligand with a divalent metal. The Mg²⁺ is coordinated by two carboxylic groups of the Mal-AGVDGRAL (**2**) peptide, while for one carbonyl group of Mal-LARGDVGA (**1**). The peptide **1** leaves a coordination site free on the metal and could be coordinated by an amino acid residue of the integrin. This ligand-Mg-protein interaction (metal ion–dependent adhesion site, MIDAS) is crucial for protein–protein inhibition as it intervenes in protein recognition. Complete Mg coordination could be more effective, but it is less selective in an inhibitor design (Gerencer and McGuffin 2023). In this type of coordination, the interaction with the metal is independent of the protein type; furthermore, this could affect other physiological functions where the metal is involved in other biological processes (Dakal 2021). Both peptides form the same number of H-bonds with the active residues.

Our results were evaluated by comparison with published crystallographic and modeling studies of fibronectin-derived RGD ligands bound to integrin α₅β₁. Previous work has reported conserved interactions involving the Asp residue of the RGD motif and coordination with the MIDAS metal ion, along with hydrogen bonding to residues such as Asp227 and Tyr178

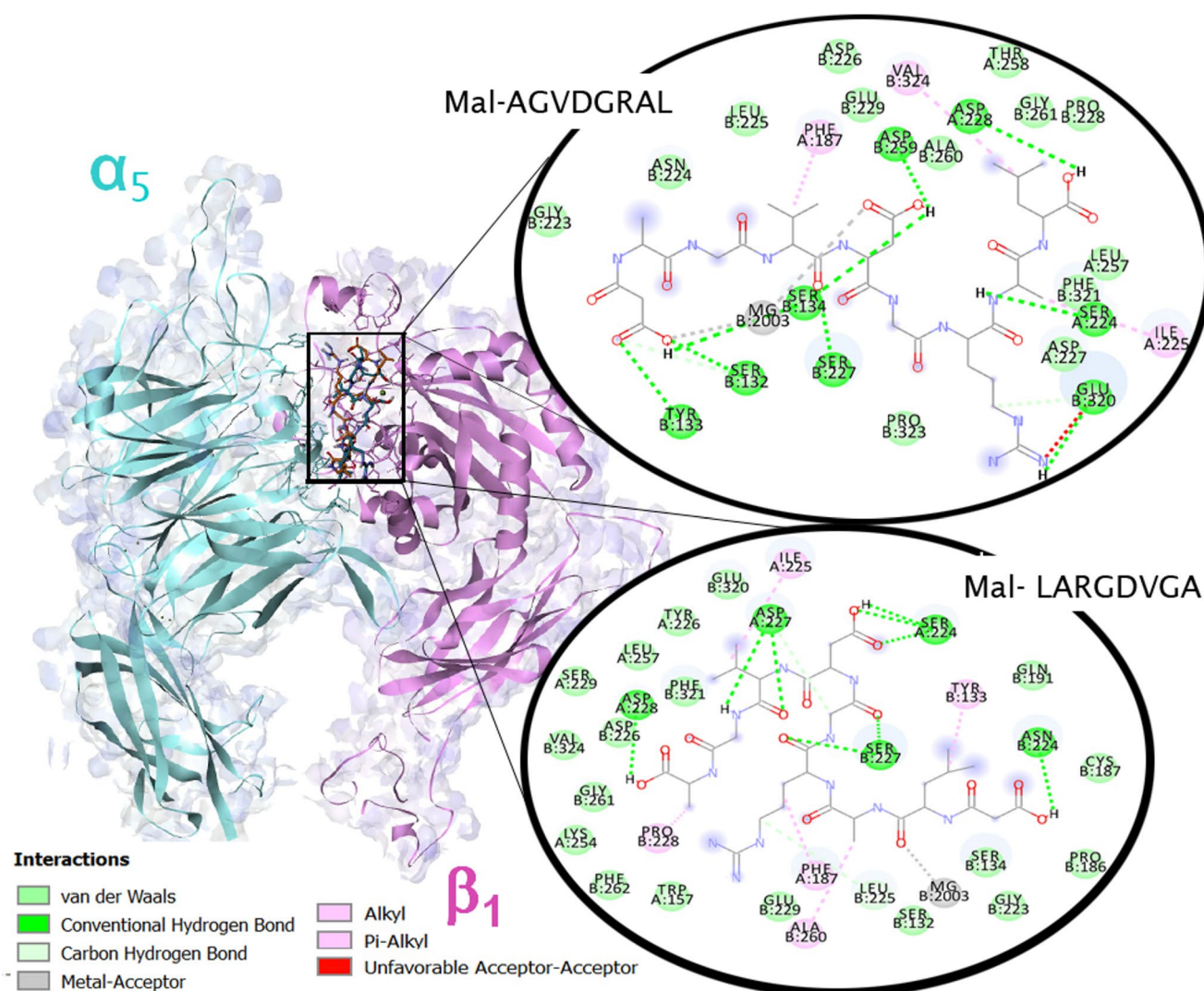


Fig. 5 Molecular model of octapeptides **1** and **2** interactions with $\alpha_5\beta_1$. 2D interacting residues are represented

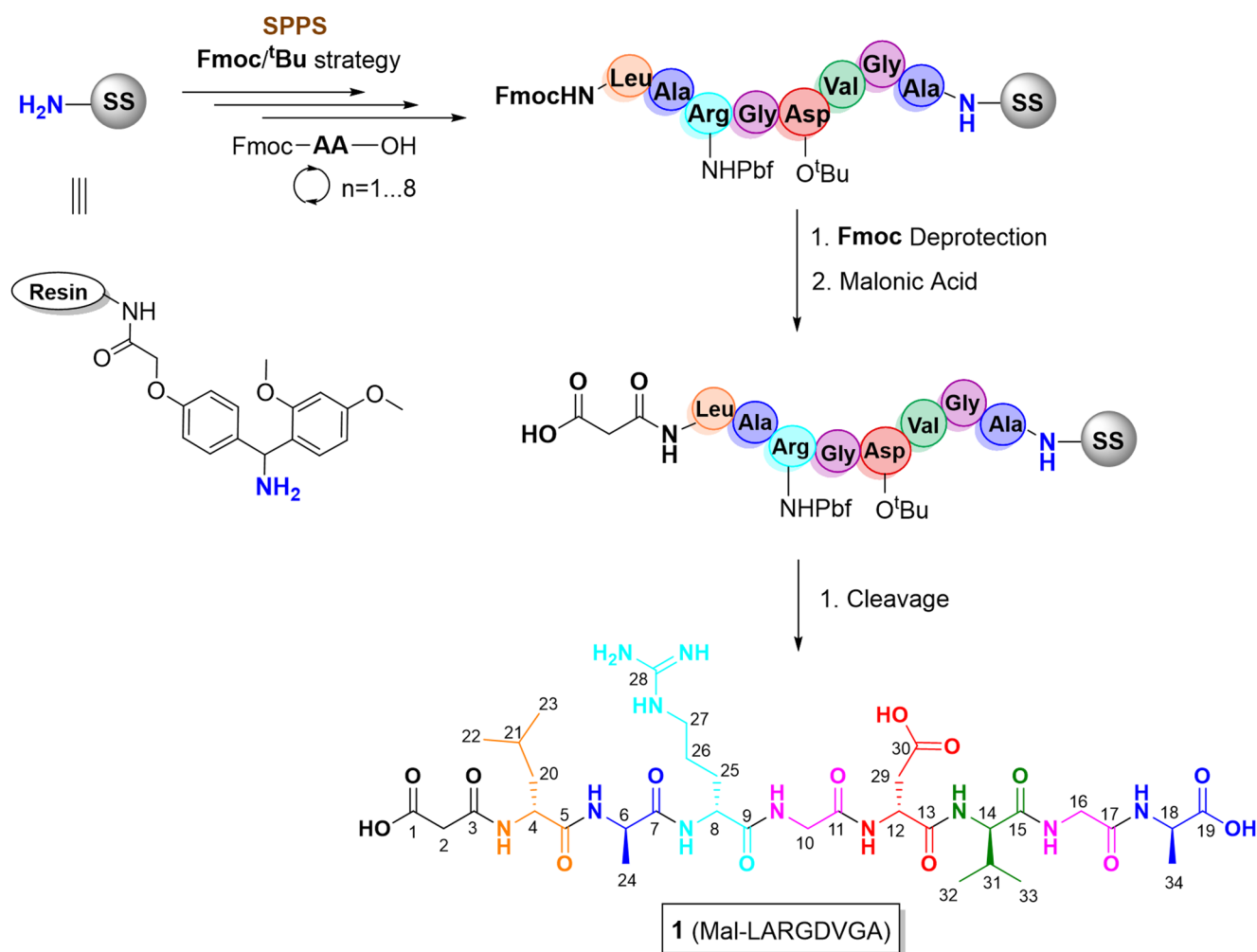
(Xiong et al. 2001; Xia and Springer 2014; Schumacher et al. 2021). The docking models obtained for Peptides **1** and **2** in this study reproduce these key structural features, particularly the orientation of the RGD motif and its engagement with the MIDAS site. This agreement with experimentally determined and computationally validated integrin–ligand complexes reinforces the plausibility of our predicted binding modes.

Theoretical studies suggest that the Mal-LARGDVGA peptide (**1**) will be a better potential inhibitor due to the number of specific interactions observed and the interaction with the magnesium atom. Taking this into account, a synthesis strategy was developed to obtain compound **1**. Octapeptide **1** was synthesized on

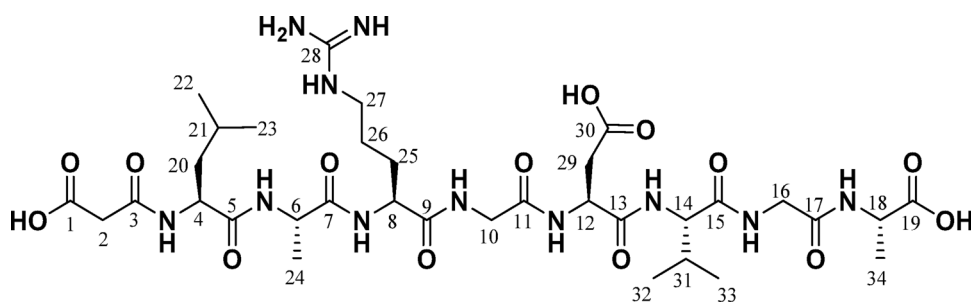
Fmoc-Ring-Amide MBHA resin by a solid-phase procedure, using the Fmoc/tBu strategy (See Scheme 1).

The coupling of each amino acid was achieved using the activating mixture of diisopropylcarbodiimide/1-hydroxybenzotriazole (DIC/HOBt), and the completion of the reaction was verified by the Ninhydrin test. After loading the Fmoc-Ala-OH, the subsequent elongation of the peptide was achieved by stepwise coupling of the Fmoc-amino acids, such as Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(tBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ala-OH, and Fmoc-Leu-OH in this order. The deprotection of the Fmoc group was carried out using a solution of piperidine (20%) in DMF (*N,N*-dimethylformamide), before the next amide coupling process.

In the final stage of the reaction, a malonate fragment is added, which increases polarity and the number of strong



Scheme 1 Fmoc Rink Amide MBHA resin of Mal-octapeptide **1**. Fmoc Deprotection: piperidine (20%) in DMF; Cleaved: TFA/TIS/H₂O (95:2.5:2.5)



interactions with the receptor. It also enables the generation of heterocycles and alkylated derivatives (Brosge et al. 2021) and cyclopropanations with appropriate substrates such as fullerenes (Lemos et al. 2025). The covalent binding of the malonyl group to the *N*-terminus of the peptide was realized using malonic acid and the mixture of TBTU/DIEA in DMF at room temperature. The resulting malonyl-peptide was cleaved from the resin employing a mixture of TFA/TIS/H₂O (95:2.5:2.5), and it was precipitated with diethyl

ether and centrifuged. Finally, the product was dissolved in a mixture of acetonitrile/H₂O (1:2) and then lyophilized. Peptide **1** was obtained as a white solid powder, with an 82% yield. For details, see Experimental Section.

Structural characterization of **1** was carried out by a combination of different spectroscopic and analytical techniques, see the Experimental Section and Supporting Information. Using a combination of 1D and 2D NMR techniques enabled the signals in the ¹H and ¹³C NMR spectra

to be assigned. ^1H -NMR spectrum shows groups of signals corresponding to the protons present in the molecule. The methylene protons of the malonate fragment appear as a singlet at $\delta=3.87$ ppm. Between 7.4 and 8.3 ppm are signals assignable to the protons attached to the NH protons, and in the zone 4.8–0.89 ppm, the signals corresponding to the aliphatic hydrogen are observed. Because we had achieved unambiguous assignments for the ^1H -NMR resonances, the ^{13}C -NMR resonances were assigned straightforwardly by analysis of the HSQC spectra for the protonated carbon atoms based on their chemical shift, substituent effects, and DEPT data. Quaternary carbon atoms were assigned by analysis of the HMBC spectra.

The ^{13}C NMR spectrum shows the presence of the signals of three carboxyl groups at 177.8, 175.8, and 175.8 ppm, the first one assignable to the carboxyl group of the malonate fragment. The other eight signals between 174.7 and 170.6 ppm are assigned to the C=O of the amide groups present. The carbon of the C=NH group appears at 158.7 ppm, and the signal of the carbon of the methylene group of the malonate fragment is located at 43.8 ppm. The other 21 signals appearing in the spectrum were unambiguously assigned to the remaining carbon atoms in the molecule. In the ^{15}N - ^1H correlation HMBC spectrum, the signals corresponding to the nitrogen atoms present in the molecule can be observed at 127.21, 121.65, 119.69, 116.97, 116.71, 115.82, 108.31, 105.54, 101.22, 101.13 ppm (see Figure S7), verifying the assignment of the proton bonded to the nitrogen atoms.

The malonyl-octapeptide **1** was analyzed through reverse phase-high performance liquid chromatography (RP-HPLC), determining that the peptide has high purity (See Figure S9) and was characterized by electrospray ionization time of flight mass spectrometry (TOF-MS ESI), which supported the proposed structure. The ESI spectrum shows a peak at $m/z=843.51$, which corresponds to its molecular weight, see Figure S10 in the Supporting Information. The IR spectrum shows the broad feature the 3425 cm^{-1} characteristic of the O–H stretching band of the acid, which is known to be in dimeric form due to hydrogen bonding. The CH_2 and CH_3 symmetric and asymmetric stretching vibrations are detected from 2960 to 2855 cm^{-1} , the carbonyl bands appear at 1740 cm^{-1} , see Figure S8 in the Supporting Information.

Also, the thermal stability of octapeptide **1** was evaluated by thermogravimetric analysis (Figure S11, Supporting Information). The thermograms exhibit three main weight losses. The first one, between 100 and 180°C , is presumably due to the loss of solvents like water and the possible decarboxylation of the malonic fragment. (Caires et al. 2010) The range from 180 to 250°C could be considered the remainder of the decomposition of terminal carboxyl groups and the elimination of ammonia from amine residues. From

250°C onwards, a continuous loss related to the breaking of peptide bonds is observed, which is a common behavior of small peptides. In summary, the peptide can be considered stable for biomedical applications, as it is stable up to 100°C , a temperature at which it begins to decompose depending on its structure.

The use of *in silico* calculations in the design of a novel octapeptide containing an RGD sequence and potential inhibitory activity against SARS-CoV-2 has proven to be an efficient and cost-effective strategy. This computational approach allowed us to focus subsequent efforts on the most promising structures, peptide **1**, based on favorable binding affinities and interaction profiles. Nevertheless, while these *in silico* results provide a potential activity of the designed peptide, further experimental validation through *in vitro* and *in vivo* tests will be necessary to validate its biological efficacy and potential therapeutic relevance.

Experimental section

General Methods. Solvents were dried by standard procedures. All reagents were of commercial quality and were used as supplied unless otherwise specified. The analysis of the purity of the malonyl-peptide synthesized was carried out by RP-HPLC in a Shimadzu equipment I-760-287, column C18 (Vydac, $4.6 \times 150\text{ mm}$, $5\text{ }\mu\text{m}$), and the injection volume of $100\text{ }\mu\text{L}$. The mobile phases used were 0.1 (v/v) of TFA in water (A) and $0.05\%\text{ (v/v)}$ of TFA in acetonitrile (B). A linear gradient of 5 to 60% of B during 35 min. , with a flux of 0.8 mL/min , was used to elute the analyte. The retention time (R_t) and the peak area (P_{as}) were determined at a wavelength of 226 nm . FTIR spectra were carried out using ATR of the solid compounds. The ESI-MS spectra were obtained in orthogonal hybrid configuration spectrometers Q-ToF 1 or Q-ToF 2 (Micromass, England) with a nanospray ionization source. The voltages of the borosilicate capillary and the inlet cone were set at 900 and 35 V , respectively. A solution of sodium and cesium iodide was used as a reference for the calibration of the spectrometer. The Masslynx version 4.1 program (Micromass, England) was used for the processing of mass spectra. The accepted error for the determination of the experimental MM was 0.01% of the theoretical MM. ^1H NMR spectra were recorded in methanol- d_4 (CD_3OD) at 700 MHz , and ^{13}C NMR at 175 MHz with a Bruker Avance 700 instrument. The one-bond heteronuclear correlation (HSQC), the long-range ^1H - ^{13}C correlation (HMBC), and ^1H - ^{15}N correlation (HSQC) spectra were obtained by use of the *inv4gs* and the *inv4gslplrnd* programs with the Bruker software. TGA analyses were carried out under air and nitrogen in a TATGA-Q500 apparatus. The sample ($\sim 0.5\text{ mg}$) was introduced inside a platinum

crucible and equilibrated at 90 °C, followed by a 10 °C/min ramp between 90 and 1000 °C.

Computational methods

All molecules were built with Avogadro (Hanwell et al. 2012), and DFT calculations were performed with ORCA 4.2.1 (Neese et al. 2020). All structures were pre-calculated using the B97-3c (Brandenburg et al. 2018) composite scheme as a fast DFT approach. Then structures were optimized using DFT with a hybrid functional PBEh-3c composite scheme, which includes a polarized double- ζ basis set and London dispersion correction with Becke–Johnson damping (D3BJ) method (Grimme et al. 2015). This method presents very good accuracy at a reasonable computational cost. Very tight convergence as keywords were employed, improving the numerical precision as Orca implantation permits. The Lebedev302 grid (Grid4) during the SCF iterations and Lebedev434 (Grid 5) as a final grid for the final energy evaluation after SCF convergence were set for integration precision. No imaginary frequencies were found after calculations of frequencies on optimized structures using the same level of theory. Optimized structures were used for the calculation of properties and visualization of the electrostatic potential. Most properties were predicted by QikProp from Schrödinger (Schrödinger Release 2024-4: Maestro, Schrödinger 2024) packaged using DFT optimized structures. For the molecular electrostatic map, first the mep.py Python script written by Marius Retegan (Retegan 2019; Mep.Py. mep.py (source code), github repository, <https://gist.github.com/mretegan/5501553>. n.d.) was used for cube file preparation, and then visualized using VMD 1.9.3 (Humphrey et al. 1996) Non-covalent interactions were generated by Multiwfn 3.7 (Lu and Chen 2012) program, and then NCIPLOT 4.0 (Boto et al. 2020) software was used. The VMD program was used together with vmd script generated by NCIPLOT, the visualization of NCI.

Molecular modeling

The PDB file of the integrin $\alpha_5\beta_1$ (PDB code: 3VI3) was retrieved from the Protein Data Bank (<http://rcsb.org>). Ligand-optimized structures were obtained from the DFT method. The PDB files of proteins and ligands were converted to PDBQT format with AutoDockTools. The simulation box with size $40 \times 50 \times 72 \text{ \AA}^3$ was positioned around the active site, matching the center of the ligands with the center of the simulation box. Docking simulations were performed using AutoDock 4.2. (Morris et al. 2009) Figures graphically representing the mode of interaction were prepared using Discovery Studio Visualizer 2020.

Synthesis and characterization

Solid phase peptide synthesis of the malonyl-octapeptide (1)

General resin preparation

Fmoc Rink amide MBHA resin (0.015 mmol) was placed in a peptide synthesis vessel, swollen in DMF, and deprotected with 5 ml of 20% piperidine/DMF for 4 min. Washings between the first deprotection, coupling, and subsequent deprotection steps were carried out with DMF (5×0.5 min) and DCM (5×0.5 min) using 10 ml of solvent/g of resin each time.

The peptide was synthesized manually on MBHA[®] resin, functionalized with the spacer Am (4-(2,4-methoxy benzhydryl)phenol acetic acid), by a stepwise solid-phase procedure (Fmoc-Am-MBHA, 0.50 g, 0.48 mmol/g, 0.35 mmol) using the Fmoc/tBu (fluoren-9-ylmethoxycarbonyl/tert-buthyl) strategy. The coupling of each amino acid was achieved using the activating mixture of DIC (218 μ L, 1.38 mmol)/HOBt (diisopropylcarbodiimide/1-hydroxybenzotriazole). The completion of the reaction was verified by the ninhydrin test. All amino acids are L-conformation, except Gly, which is achiral. The order of addition of each amino acid was: Fmoc-Ala-OH $\times$ H₂O (0.46 g, 1.40 mmol), Fmoc-Gly-OH (0.42 g, 1.41 mmol), Fmoc-Val-OH (0.48 g, 1.41 mmol), Fmoc-Asp(tBu)-OH (0.58 g, 1.41 mmol), Fmoc-Gly-OH (0.42 g, 1.41 mmol), Fmoc-Arg(Pbf)-OH (0.91 g, 1.40 mmol), Fmoc-Ala-OH $\times$ H₂O (0.46 g, 1.40 mmol), Fmoc-Leu-OH (0.49 g, 1.40 mmol). The successive deprotection of the Fmoc group was carried out using a solution of piperidine (20%) in DMF (*N,N*-dimethylformamide) for 20 min. Afterward, the peptide was purified in order to get rid of the excess of piperidine, through four washes of 1 min for each one with DMF. The covalent binding of the malonyl group to the *N*-terminus of the peptide was carried out using malonic acid (0.15 g, 1.44 mmol) and the mixture of *O*-(Benzotriazol-1-yl)-*N,N,N'*-tetramethyluronium tetrafluoroborate, TBTU (0.45 g, mmol/g), and *N,N*-Diisopropylethylamine, DIEA (356 μ L) in DMF at room temperature. The resulting malonyl-peptide was cleaved from the resin using the mixture trifluoroacetic acid/triisopropylsilane/water (TFA/TIS/H₂O, 95:2.5:2.5) for 2 h. It was then precipitated over diethyl ether at -80°C and centrifuged. Finally, the product was dissolved in a mixture of acetonitrile/H₂O (1:2) and then lyophilized. The product was isolated as a white solid. Yield: 82% (330 mg, 0.82 mmol).

ATR-FTIR: ν 2423, 2959, 2925, 2855, 1740 (C=O), 1462, 1492, 1462, 1186, 1061, 969 cm^{-1} . ¹H NMR (700 MHz, CD₃OD) δ 8.74 (d, $J=5.9$ Hz, 1H, NH), 8.28 (m, 2 H, HA-NH₂, NH), 8.26 (d, $J=5.6$ Hz, 1H, NH), 8.22

(d, $J=7.4$ Hz, 1H, NH), 8.18 (t, $J=5.8$ Hz, 1H, NH), 7.97 (d, $J=7.2$ Hz, 2 H, HB-NH₂, NH), 7.85 (d, $J=6.9$ Hz, 1H, NH), 7.46 (s, 1H, NH), 7.40 (t, $J=5.3$ Hz, 1H, NH), 7.08 (s, 1H, NH), 4.75 (q, $J=7.1$ Hz, 1H, H12), 4.32 (m, 1H, H18), 4.29–4.24 (m, 3 H, H4, H6, H8), 4.08 (m, 1H, H14), 3.90–3.81 (m, 4 H, H2, H16), 3.48 (d, $J=16.2$ Hz, 1H, H10a), 3.32 (d, $J=16.0$ Hz, 1H, H10b), 3.20 (q, $J=6.7$ Hz, 2 H, H27), 2.92 (dd, $J=17.0$, 6.5 Hz, 1H, H29a), 2.78 (dd, $J=17.0$, 7.1 Hz, 1H, H29b), 2.20 (m, 1H, H31), 1.91 (m, 1H, H25a), 1.81–1.73 (m, 2 H, H21, H25b), 1.70–1.57 (m, 4 H, H26, H20), 1.41 (d, $J=7.2$ Hz, 3 H, H24), 1.38 (d, $J=7.4$ Hz, 3 H, H34), 0.99 (m, 9 H, H23, H32, H33), 0.93 (d, $J=6.6$ Hz, 3 H, H22).

¹³C{¹H} NMR (176 MHz, CD₃OD) δ 177.9 (COOH), 175.8 (COOH), 175.8 (COOH), 174.8 (C=O), 174.5 (C=O), 174.1 (C=O), 173.6 (C=O), 172.8 (C=O), 171.9 (C=O), 171.4 (C=O), 170.6 (C=O), 158.7 (C28), 61.4 (C14), 55.0 (C4), 54.9 (C8), 51.6 (C12), 51.5 (C6), 50.4 (C18), 43.9 (C16), 43.8 (C2), 43.1 (C10), 42.0 (C27), 41.0 (C20), 36.3 (C29), 31.0 (C31), 29.3 (C25), 26.1 (C26), 25.8 (C21), 23.3 (C23), 21.6 (C22), 19.6 (C32), 18.72 (C33), 18.0 (C34), 17.00 (C24). ¹⁵N{¹H} NMR (71 MHz, CD₃OD) δ 127.21, 121.65, 119.69, 116.97, 116.71, 115.82, 108.31, 105.54, 101.22, 101.13. MS ESI-TOF: m/z : M⁺ Calcd for C₃₄H₅₇N₁₁O₁₄: 843.48; Found: 843.51. Anal. Calcd for C₃₄H₅₇N₁₁O₁₄: C, 48.39; H, 6.81; N, 18.26; Found: C, 48.42; H, 6.83; N, 18.30.

Conclusions

Two octapeptides containing the RGD sequence and a malonate moiety were designed and theoretically evaluated as potential inhibitors of the SARS-CoV-2 infection. Computational analysis demonstrated that peptide **1** exhibits favorable intramolecular interactions and enhanced binding to integrin $\alpha_5\beta_1$ through coordination with the Mg²⁺ ion at the MIDAS site. Peptide **1** was successfully synthesized by solid-phase peptide synthesis and fully characterized, confirming its chemical structure and purity. While in silico studies suggest that peptide **1** may serve as a potential integrin-targeting antiviral agent, further in vitro and in vivo studies are needed to confirm its therapeutic potential.

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Data availability All data are included within this paper and its Supplementary Information files. Further information can be requested to the authors.

Declarations

Conflict of interest The authors declare no competing interests.

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