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Review

The Crucial Role of Arginine Residues and the Arginine–Tetrazole Paradigm in Viral Infection: Therapeutic Opportunities for Tetrazole–Containing Angiotensin Receptor Blockers and Bisartans

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Abstract

Arginine residues are important determinants of molecular recognition because their guanidinium groups are strongly basic and predominantly protonated at physiological pH. This enables arginine to form electrostatic interactions and multiple hydrogen bonds with anionic or polar molecular surfaces, making arginine-rich regions particularly relevant to protein–protein interactions, proteolytic processing, receptor engagement, and viral entry. The severe acute respiratory syndrome coronavirus 2019 spike glycoprotein provides a notable example, in which basic residues within the polybasic S1/S2 cleavage region (PRRARS) and the S2′ region (KPSKRS) contribute to recognition by host proteases and activation of the viral fusion machinery. Mutations or modifications to arginine residues can alter the local electrostatic environment and influence viral phenotypes. A complementary pharmacophore is the tetrazole group, which, when deprotonated, provides an anionic tetrazolate capable of engaging positively charged residues through electrostatic and hydrogen-bonding interactions. Therefore, tetrazole-containing angiotensin receptor blockers represent an intriguing chemical platform for interactions with arginine-rich viral and host proteins. This concept is extended to novel bisartans, which incorporate two anionic tetrazole-containing pharmacophores and may consequently support multivalent or cooperative electrostatic recognition. Computational, enzymatic, and biophysical investigations have proposed that sartans and bisartans can interact with several

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viral and host-associated targets. These include angiotensin-converting enzyme 2, S-associated regions, and furin, supporting further investigation of physicochemical recognition as a potential basis for broader antiviral activity.

Keywords: ACE2; antiviral agents; arginine; arginine blockers; ARBs; sartans; bisartans; broad-spectrum antivirals; SARS-CoV-2; sartans; tetrazole

1. Introduction

Among the amino acid substitutions associated with enhanced molecular binding, arginine is particularly notable. This is because of its distinctive physicochemical properties, including high polarity and hydrophilicity, and a strongly basic guanidinium side chain, which enables robust electrostatic and hydrogen-bonding interactions with complementary molecular groups. Thus, targeting the molecular interactions mediated by positively charged arginine residues may represent a potential strategy for interfering with critical stages of viral infection. In the context of severe acute respiratory syndrome coronavirus 2019 (SARS-CoV-2), pharmacological disruption of interactions involving the spike (S) protein receptor-binding domain (RBD) and angiotensin-converting enzyme 2 (ACE2), together with inhibition of proteolytic processing at functionally key cleavage sites, represents a potential strategy to reduce viral entry and subsequent infection. Conventional angiotensin receptor blockers (ARBs) containing anionic pharmacophores, including tetrazole and carboxyl groups, tetrazole or carboxylate groups, provide an intriguing chemical framework for investigating whether these interactions can be targeted therapeutically [1]. Viral infection is fundamentally dependent upon a series of highly specific molecular interactions between viral proteins and host molecules [2–4]. Receptor recognition, proteolytic activation, membrane fusion, and intracellular replication rely on specific molecular interactions mediated by viral and host protein surfaces, whose structural and physicochemical properties govern molecular recognition and binding [4–7].

Among the proteinogenic amino acids, arginine is particularly important in molecular recognition because of its positively charged guanidinium group, which enables a broad range of electrostatic and hydrogen-bonding interactions [8–10]. At physiological pH, the arginine side chain is predominantly protonated [11], allowing it to form strong electrostatic interactions with negatively charged functional groups, including carboxylates, phosphates, and anionic heterocycles [12–14]. The planar guanidinium moiety can additionally engage in multiple hydrogen bonds and cation-π interactions, contributing to the formation and stabilization of protein–protein and protein–ligand complexes [10,15–17]. These distinctive physicochemical properties make arginine an important mediator of molecular recognition, and arginine-rich regions are frequently involved in interactions with nucleic acids, carbohydrates, phosphate-containing molecules, and proteins [18–20].

These physicochemical properties make arginine especially effective at mediating molecular recognition at protein-protein interfaces and within protease cleavage motifs. When multiple arginine residues occur in clusters, they can generate highly cationic molecular environments that may be recognized by host proteins and proteases [17].

SARS-CoV-2 provides a particularly relevant example of this principle. The viral S glycoprotein contains a polybasic S1/S2 cleavage motif, including the PRRAR sequence, which is recognized and cleaved by furin and other host proteases [21,22]. An additional basic sequence is present near the S2′ cleavage site (KPSKR). Proteolytic processing at these sites contributes to the conformational activation of the S protein and is an important step in facilitating viral entry into host cells [7,23–28].

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This observation raises an important question: can the positive charge of functionally important arginine residues in viral proteins be pharmacologically targeted through complementary anionic groups? This question provides the conceptual basis for the present review.

Importantly, this concept does not imply that all arginine residues are inherently amenable to pharmacological targeting. Rather, arginine residues that are functionally important, accessible, and appropriately positioned within protein interaction interfaces may represent potential molecular recognition sites for complementary anionic pharmacophores [10,12,16]. In this context, the tetrazole group, established in medicinal chemistry and a prominent structural feature of several ARBs, provides an intriguing potential chemical counterpart [29–31].

The subsequent development of bisartans, sartan-related compounds incorporating two anionic biphenyl-tetrazole pharmacophores, provides an additional framework for investigating whether multivalent anionic recognition can be exploited to target arginine-rich or otherwise cationic molecular environments in viral and host proteins [32–35]. This structural concept shifts the focus from conventional sequence-specific recognition towards physicochemical complementarity and provides a mechanistic basis for exploring whether appropriately designed anionic pharmacophores can modulate molecular interactions involved in viral infection.

2. Discussion

2.1. Arginine as a Molecular Determinant of Viral Recognition

The guanidinium group of arginine constitutes a highly unique and versatile molecular recognition motif. Unlike the terminal ammonium group of lysine, which carries a localized positive charge, the planar guanidinium group of arginine possesses a delocalized positive charge and multiple hydrogen-bond donor sites [9,12,36,37]. This distinctive electronic and structural configuration enables strong electrostatic and hydrogen-bonding interactions with carboxylates, phosphates, and other anionic groups [38–40]. Consequently, arginine plays a prominent role in biomolecular recognition, contributing to protein–protein, protein–deoxyribonucleic acid (DNA), and protein–ribonucleic acid (RNA) interactions (Figure 1) [18,41].

In viral proteins, arginine residues can contribute to receptor recognition, substrate recognition, protein–protein interactions, nucleic-acid binding and conformational stabilization [42–45]. Arginine-rich sequences are particularly relevant to proteolytic activation, as several host proteases preferentially recognize basic amino-acid motifs [46–48].

The biological significance of arginine is further evident by the molecular evolution of SARS-CoV-2. Molecular epidemiological analyses have identified recurrent arginine-associated substitutions and reported potential associations between specific arginine mutations and viral infectivity [49,50]. These observations should not be interpreted as evidence that arginine alone determines infectivity; rather, they emphasize how alterations in local charge distribution and molecular recognition can influence viral protein function.

Thus, the functional role that an arginine residue has can extend beyond its contribution to protein structure. Its distinctive physicochemical properties can directly influence interactions between viral proteins and host receptors, proteases, nucleic acids, or other molecular ligands. This molecular recognition capability provides a conceptual chemical basis for the arginine–tetrazole paradigm: when an exposed arginine residue contributes substantially to a biologically important interaction, a complementary anionic ligand may potentially modulate or disrupt that interaction.

Nevertheless, tetrazolate–guanidinium complementarity provides a plausible chemical basis for molecular recognition but does not, by itself, establish selective targeting of a particular arginine residue. Under physiological conditions, binding depends on the

broader molecular environment, including desolvation, ionic strength, ligand protonation, residue accessibility, competing charged groups, conformational dynamics, aggregation, and nonspecific electrostatic association. Accordingly, three distinct mechanisms should be differentiated: (i) nonspecific electrostatic association with positively charged molecular surfaces; (ii) site-specific guanidinium–tetrazolate recognition within a defined binding environment; and (iii) bivalent engagement, whereby a bisartan may interact simultaneously with multiple arginine residues or arginine-containing sites [1,32]. These mechanisms are not interchangeable and require different experimental controls for their discrimination. Ionic-strength dependence and competition experiments can help assess electrostatic contributions, whereas residue-directed mutagenesis and complementary structural or biophysical methods can provide evidence for site-specific recognition or multivalent engagement. Thus, the arginine–tetrazole paradigm represents a mechanistically testable hypothesis whose specificity and biological relevance must be established through convergent experimental evidence [50].

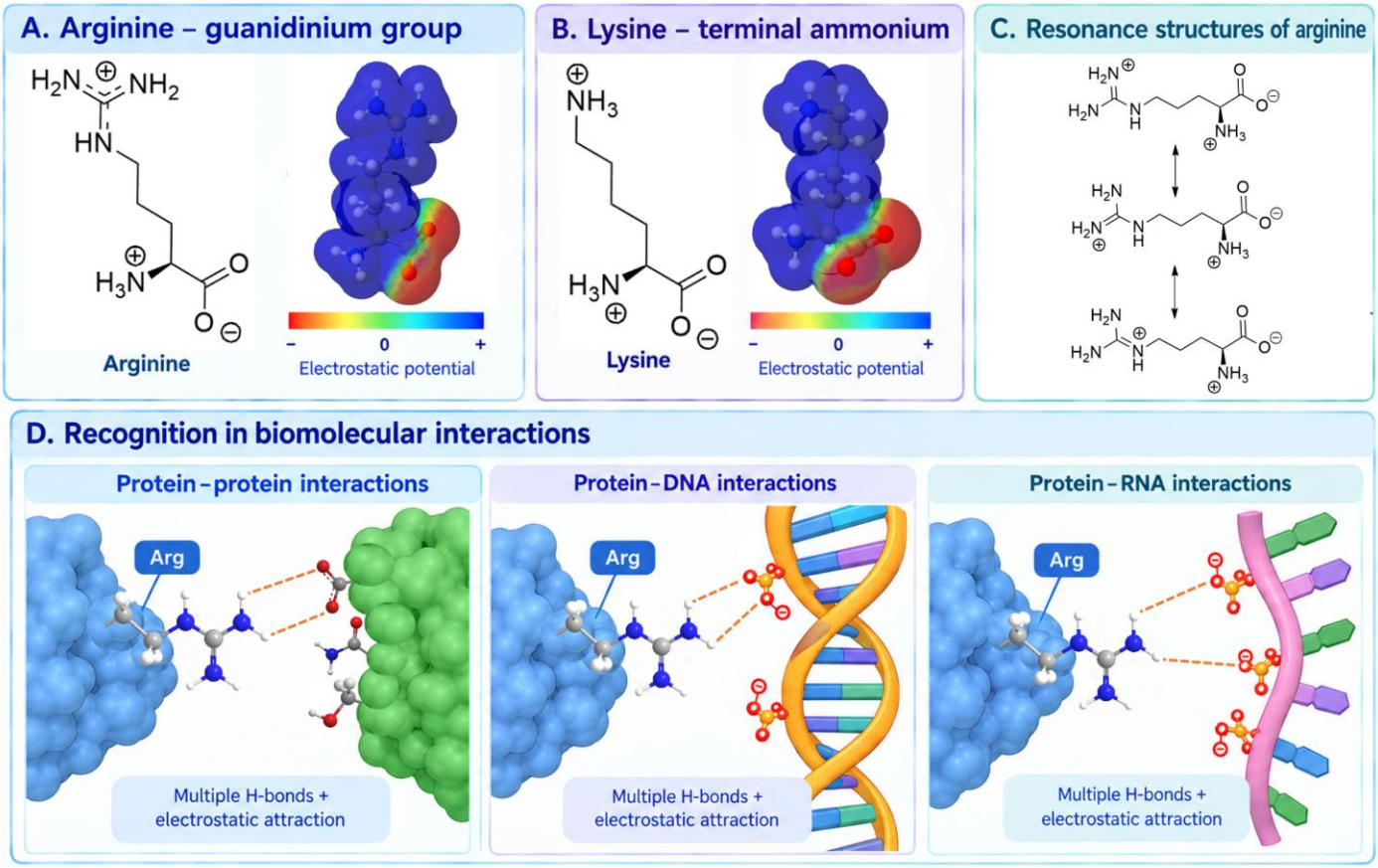


Figure 1. Structural comparison of (**A**) arginine and (**B**) lysine, shown in their protonated forms at physiological pH (pH = 7.4). Corresponding electrostatic potential maps are also presented. (**C**) Resonance structures of arginine. (**D**) Representative interactions between the positively charged guanidinium group of arginine and negatively charged functional groups in proteins, and phosphate groups of DNA and RNA.

2.2. Arginine-Rich Cleavage Sites in SARS-CoV-2

The SARS-CoV-2 S protein contains a polybasic furin cleavage site, with the cleavage occurring between R685 and S686, at the S1/S2 boundary (residues 681-686, sequence PRRAR-S) [7,21,51]. This sequence contains the distinctive PRRAR motif, generating a basic region that is efficiently recognized by furin and other proteases [24,25,27,51,52]. The acquisition of this polybasic cleavage site distinguishes SARS-CoV-2 from many other

closely related sarbecoviruses and has been associated with enhanced S protein processing, viral entry into host cells, and viral transmission [25–28]. Experimental studies have reported that the furin cleavage site contributes substantially to SARS-CoV-2 replication and pathogenesis, although its effects are context-dependent and involve the broader S protein structure and host–cell protease environment [27,53–55].

The S2′ cleavage site (residues 811-816, sequence KPSKR-S), with the cleavage occurring between R815 and S816, provides another proteolytic activation step. Following S1/S2 processing, cleavage near S2′ exposes the fusion peptide and facilitates membrane fusion. SARS-CoV-2 can use several host proteolytic pathways, including transmembrane protease, serine 2 (TMPRSS2)-mediated surface activation and endosomal cathepsins [7,56–58].

The presence of arginine and lysine residues creates localized basic environments at sites that are critical for viral entry [7,56]. This observation suggests two complementary therapeutic strategies. The first involves direct inhibition of the host protease, for example, through targeting furin or TMPRSS2 [59,60]. The second, and central to this review, involves interfering with the interaction between the viral substrate and its cognate protease by targeting the basic molecular environment of the viral cleavage region [61,62]. In principle, this approach could theoretically reduce proteolytic activation without requiring complete inhibition of the host protease. This distinction is important because host proteases perform diverse physiological functions. Ligands designed to selectively recognize basic regions within viral substrates, rather than the catalytic machinery of host proteases, may offer a distinct selectivity profile [61,63]. Although this hypothesis remains to be experimentally validated, the molecular architecture of the SARS-CoV-2 S cleavage region provides a compelling model for investigating whether anionic tetrazole-containing ligands can interfere with arginine-rich viral sequences.

2.3. Arginine and Viral Evolution

The importance of positively charged residues is also reflected in the evolution of SARS-CoV-2. Several variants have acquired mutations involving arginine residues in functionally relevant regions, such as L452R in the S protein RBD and P681R adjacent to the furin-cleavage region [7,64]. P681R is of particular interest in the context of the present hypothesis because it introduces an additional positively charged side chain immediately adjacent to the polybasic cleavage region. L452R occurs in the receptor-binding region and can influence local electrostatic and structural properties [64–68]. The biological consequences of these mutations are complex and cannot be attributed solely to charge. S protein conformation, receptor affinity, proteolytic processing, antibody escape and other factors all contribute to viral fitness [64,69]. Nevertheless, introduction or removal of arginine can alter local electrostatic and hydrogen-bonding environments. Molecular epidemiological analyses have consequently highlighted arginine-associated mutations as a potentially informative feature of SARS-CoV-2 evolution [50,70].

This observation supports a broader principle that positively charged residues located at functional interfaces can be subject to strong selective constraints because substitutions may alter molecular recognition [50,71,72]. This is potentially important for drug discovery. A viral target that depends on a conserved electrostatic environment may be less susceptible to escape through individual mutations than a highly variable surface epitope, although this must be demonstrated experimentally for each target.

The arginine–tetrazole paradigm therefore places emphasis not on a particular viral sequence but on a recurring physicochemical feature: an accessible, functionally important cationic environment.

2.4. Furin and Recognition of Arginine-Rich Basic Viral Sequences

Furin is a host proprotein convertase that is responsible for endoproteolytic processing of protein precursors and has a strong preference for basic substrate sequences [73,74]. Furin recognizes an extended substrate region rather than a simple four-residue motif, with multiple positions contributing to substrate binding and cleavage [46,75–77]. While the canonical furin recognition motif is commonly represented as R-X-X-R, the actual substrate-recognition determinants are more extensive and context-dependent [23,77].

In SARS-CoV-2, the spike S1/S2 region is recognized within a broader structural environment. The polybasic sequence containing arginines is important, but neighboring residues, accessibility, conformation and glycosylation can also influence processing. Consequently, molecules capable of interfering with the interaction between furin and an arginine-rich viral sequence could theoretically reduce viral protein processing. This provides a mechanistic rationale for investigating compounds that interact with arginine residues or otherwise alter the electrostatic environment surrounding a cleavage site.

Computational studies have also provided evidence that ligand recognition by furin can involve conformationally adaptable regions. In particular, W254 in the furin catalytic cleft has been proposed to function as a molecular gate that can influence access to a cryptic pocket and ligand binding [78]. This gating mechanism can be conceptually likened to a molecular switch, evoking the principle underlying optogenetics, the field recognized by the 2026 Nobel Prize in Physiology or Medicine, awarded to Karl Deisseroth, Peter Hegemann, and Georg Nagel for their discoveries concerning light-gated ion channels and optogenetics. Whereas optogenetics enables the controlled activation or inhibition of neuronal activity using light, the proposed role of W254 illustrates how conformational changes within a protein may regulate molecular access and recognition. This finding is relevant to the present concept because it illustrates that ligand binding cannot always be understood by considering only static structures. Protein dynamics and induced-fit processes may determine whether an antiviral ligand can reach and stabilize a functional binding state. Accordingly, docking, molecular dynamics and quantum-mechanical approaches should be regarded as complementary tools rather than definitive evidence of antiviral efficacy.

2.5. The Warhead Tetrazole Pharmacophore

Tetrazole is a well-established medicinal-chemistry pharmacophore and an important bioisostere of the carboxylic acid group [29,30,79,80]. When deprotonated, tetrazole provides an anionic tetrazolate group. Its electronic properties permit electrostatic and hydrogen-bonding interactions with positively charged residues, while the aromatic ring contributes additional hydrophobic and π-interactions. The physicochemical complementarity between tetrazolate and guanidinium is therefore particularly attractive [26,27].

The guanidinium group of arginine provides a positively charged, hydrogen-bond-rich recognition surface, whereas tetrazolate provides a negatively charged, delocalized recognition element. Guanidinium-anion recognition is a well-established principle in supramolecular and biological chemistry [18–20]. Tetrazole is therefore not simply a structural component of sartans; it can contribute directly to molecular recognition.

This distinction is important for the antiviral hypothesis. The antiviral potential of a tetrazole-containing ARB should not automatically be attributed to its classical angiotensin II (AngII) type 1 receptor (AT_1R) pharmacology. Instead, the tetrazole group can be considered a transferable molecular-recognition element capable of engaging cationic residues in other proteins.

Several clinically prescribed sartans contain tetrazole groups, including losartan, valsartan, irbesartan, candesartan and olmesartan. Other ARBs, such as azilsartan, eprosartan and telmisartan, differ in their acidic pharmacophores [81–83]. This structural diversity provides a natural medicinal-chemistry experiment: compounds with similar ARB pharmacology but different anionic groups can be compared to determine whether tetrazolate chemistry contributes specifically to antiviral target recognition.

2.6. Sartans, ARBS and the ACE2 Connection: The Role of Arginines

ARBs were originally developed to overcome the deficiencies of angiotensin-converting enzyme inhibitors [84]. ARBs counteract the effects of Ang II by selectively antagonizing AT_1R, thereby inhibiting the principal receptor-mediated actions of Ang II within the renin–angiotensin–aldosterone system (RAAS) [31,85,86]. The identification of ACE2 as the principal cellular receptor for SARS-CoV-2 created an unexpected connection between the RAAS and antiviral research [87,88]. ACE2 recognizes the receptor-binding domain of the SARS-CoV-2 S protein, and the S protein-ACE2 interaction is central to viral entry. Structural studies have established the molecular architecture of the complex and identified multiple charged and polar contacts at the interface [88–90].

These findings promoted investigations to determine if ARBs could interact with ACE2, the SARS-CoV-2 S protein or the S protein–ACE2 interface. Our earlier computational work identified binding of sartans and novel bisartans to molecular targets relevant to SARS-CoV-2 [32–35]. Importantly, the 2023 study by Kelaidonis et al. investigated the role of the tetrazole group in sartan interactions with the SARS-CoV-2 S protein RBD-ACE2 complex and combined computational and enzymatic studies [34]. The study has shown that the tetrazole group can make an important contribution to the predicted interaction. This is conceptually significant because it supports the view that the tetrazole group can function as a molecular-recognition element rather than simply as a structural substituent associated with AT_1R pharmacology.

Our two subsequent studies strengthen this line of investigation. Ridgway et al. reported novel benzimidazole ARBs with anti-SARS-CoV-2 activity in computational and experimental studies, with activity reported as comparable to nirmatrelvir in the experimental studies conducted [91]. Apostolopoulos et al. subsequently examined eight compounds, including commercially available ARBs and newly synthesized tetrazole-containing compounds, against ACE2 using density functional theory (DFT) and enzyme activity assays [92]. Particularly notable was the predicted interaction of telmisartan and benzimidazole bis-N,N′-biphenyltetrazole (ACC519TT) with the ACE2 active site, including proton-transfer interactions involving the guanidinium group of Arg273 [92].

Nevertheless, these findings should be interpreted appropriately. DFT shows a favorable interaction and is not equivalent to proof of antiviral activity. Likewise, alteration of ACE2 enzymatic activity does not necessarily demonstrate inhibition of viral entry. These studies provide mechanistic evidence that can be integrated with surface plasmon resonance (SPR), mutagenesis, biochemical and virological experiments.

2.7. The Arginine–Tetrazole Therapeutic Paradigm

The central hypothesis of this review is that arginine and tetrazole constitute complementary molecular-recognition elements. Arginine contributes a positively charged guanidinium group. Tetrazole, in its physiologically relevant anionic form, contributes a negatively charged tetrazolate group. This electrostatic complementarity creates a rational basis for designing molecules that recognize arginine-containing viral proteins (Figure 2).

Our paradigm is supported at the following levels. (i) Molecular, structural and epidemiological studies indicate that positively charged residues, including arginine, occur in important viral recognition and cleavage regions [24–28,50,53]. (ii) Pharmacological and

computational studies demonstrate that tetrazole-containing sartans can interact with protein targets relevant to SARS-CoV-2 [32–35,92]. (iii) DFT studies provide molecular-level indications for favorable interactions between selected ARBs/bisartans and ACE2, including interactions involving Arg273 [92]. (iv) The in silico bisartan studies have extended the concept to multiple viral proteins and virus families, including SARS-CoV-2, influenza A and respiratory syncytial virus (RSV) [34,35]. This emerging information suggests that tetrazolate can function as a molecular counterpoint to arginine. Importantly, the therapeutic paradigm does not imply that every arginine residue represents a druggable site.



Figure 2. Conceptual model of arginine-directed molecular interference by tetrazolate-containing compounds. An arginine-containing region of a viral protein generates a localized positively charged electrostatic microenvironment that may favor recognition of the negatively charged tetrazolate group. Subsequent tetrazolate–arginine-mediated molecular recognition is proposed to interfere with native molecular interactions or functional processes of the viral protein, potentially resulting in impaired viral protein function. Created in BioRender. Gadanec, L. (2026) https://BioRender.com/gf3tetb (accessed on 26 September 2026).

The most promising targets should satisfy several criteria: (i) the arginine should be solvent accessible; (ii) it should contribute significantly to protein function or molecular recognition; (iii) the surrounding region should present a definable molecular surface for suitable ligand binding; (iv) ligand binding should interfere with a biologically relevant process; (v) the interaction should be experimentally measurable; (vi) mutation of the target residue should alter binding or function; (vii) and the resulting molecular interaction should translate into a measurable antiviral phenotype.

This framework provides a rational basis for distinguishing functionally relevant arginine residues from incidental arginine residues. The development of bisartans is particularly significant because two tetrazole-containing pharmacophores may permit simultaneous or cooperative interactions with multiple cationic residues. Such multivalent recognition could potentially increase apparent affinity, improve residence time or allow recognition of extended basic surfaces.

The concept can also be extended beyond viral S protein. Arginine residues occur in viral proteases, polymerases, fusion proteins and receptor-binding domains [36,41]. If an arginine residue is essential for a conserved molecular interaction, it may represent a potential target for a suitably designed anionic ligand. The hypothesis remains to be fully validated experimentally, but its strength lies in its simplicity and testability.

Critical experiments should include comparison of tetrazole-containing and non-tetrazole analogues; comparison of mono-tetrazole sartans with bisartans; mutation of target arginine residues; direct binding measurements by SPR; furin-cleavage assays; S protein-ACE2 binding studies; pseudovirus assays; and, ultimately, authentic-virus studies. These could determine whether the arginine–tetrazole interaction is merely a computational observation or represents a genuine antiviral mechanism.

2.8. Bisartans as Next-Generation Arginine Blockers

Bisartans represent an important development of the sartan concept (Figure 3). The original bisartan series introduced a new class of sartan-like ARBs containing dual symmetric anionic biphenyl-tetrazole moieties [32,93,94]. The presence of two tetrazole-containing pharmacophores creates the possibility of multivalent recognition of positively charged residues and/or interaction with metal-containing catalytic sites. Computational investigations demonstrated interactions of bisartans with ACE2 and other SARS-CoV-2-related targets [32–35]. The 2024 study by Ridgway et al. extended this approach to influenza A and RSV, through inhibition of the RSV fusion protein and the influenza A H1N1 neuraminidase. The authors investigated the interaction of bisartans with conserved molecular features, including arginine-containing regions and metal-containing catalytic environments, using molecular docking and molecular dynamics. Particularly interesting interactions were reported for ACC519TT with SARS-CoV-2 non-structural protein 3/conserved macrodomain 1, influenza neuraminidase and the RSV fusion protein, as well as with the arginine-rich SARS-CoV-2 S1/S2 and S2′ regions. The studies indicate that anionic tetrazole groups can engage in strong interactions with cationic arginine residues and metal cations, such as Zn^{2+}, within viral and host-associated target sites. Moreover, the in silico binding profiles compared positively with those predicted for established Food and Drug Administration-approved antiviral agents, including Paxlovid and oseltamivir [35], making them novel candidates for further in vitro and in vivo testing.

These findings suggest a possible route toward broad-spectrum activity. Instead of targeting a single highly variable viral sequence, bisartans may recognize conserved physicochemical characteristics, such as cationic residues, anionic pockets or metal-dependent catalytic environments that are found in different viral proteins. However, this concept remains a hypothesis requiring experimental confirmation. A pan-antiviral drug cannot be established by docking across several viral proteins alone. The most compelling future evidence would require demonstration that the same compound binds multiple targets experimentally and inhibits corresponding viral functions in independent assays.

2.9. SPR and DFT as Validation Tools

Computational docking and molecular dynamics are valuable for identifying possible interactions, but they cannot by themselves establish molecular binding [95–97]. SPR provides an important complementary methodology. SPR permits real-time, label-free analysis of biomolecular interactions and can provide kinetic parameters, including association and dissociation rates, as well as apparent affinity under appropriate experimental conditions [98–100].

In regard to the arginine–tetrazole paradigm, SPR can be particularly informative. Potential experiments include immobilization of ACE2, S protein RBD, furin, selected viral proteins and synthetic arginine-rich peptides. Binding can then be compared among individual commercial sartans; novel benzimidazole ARBs (Figure 4); bisartans; tetrazole-deficient analogues; neutralized or chemically modified tetrazoles; arginine-to-neutral-residue mutants; and unrelated control compounds. This experimental strategy could directly test if tetrazolate groups contribute to binding and whether specific arginine residues are necessary for recognition.

A particularly informative experimental design would compare wild-type and arginine-to-alanine or arginine-to-lysine mutants. An arginine-to-alanine substitution removes the guanidinium functionality, whereas arginine-to-lysine preserves a positive charge but changes geometry and hydrogen-bonding characteristics. Differential effects could therefore help distinguish nonspecific electrostatic attraction from more specific

guanidinium–tetrazolate recognition. SPR should also be combined with orthogonal approaches. These include isothermal titration calorimetry, microscale thermophoresis, enzyme kinetics, competition assays and structural methods [101].

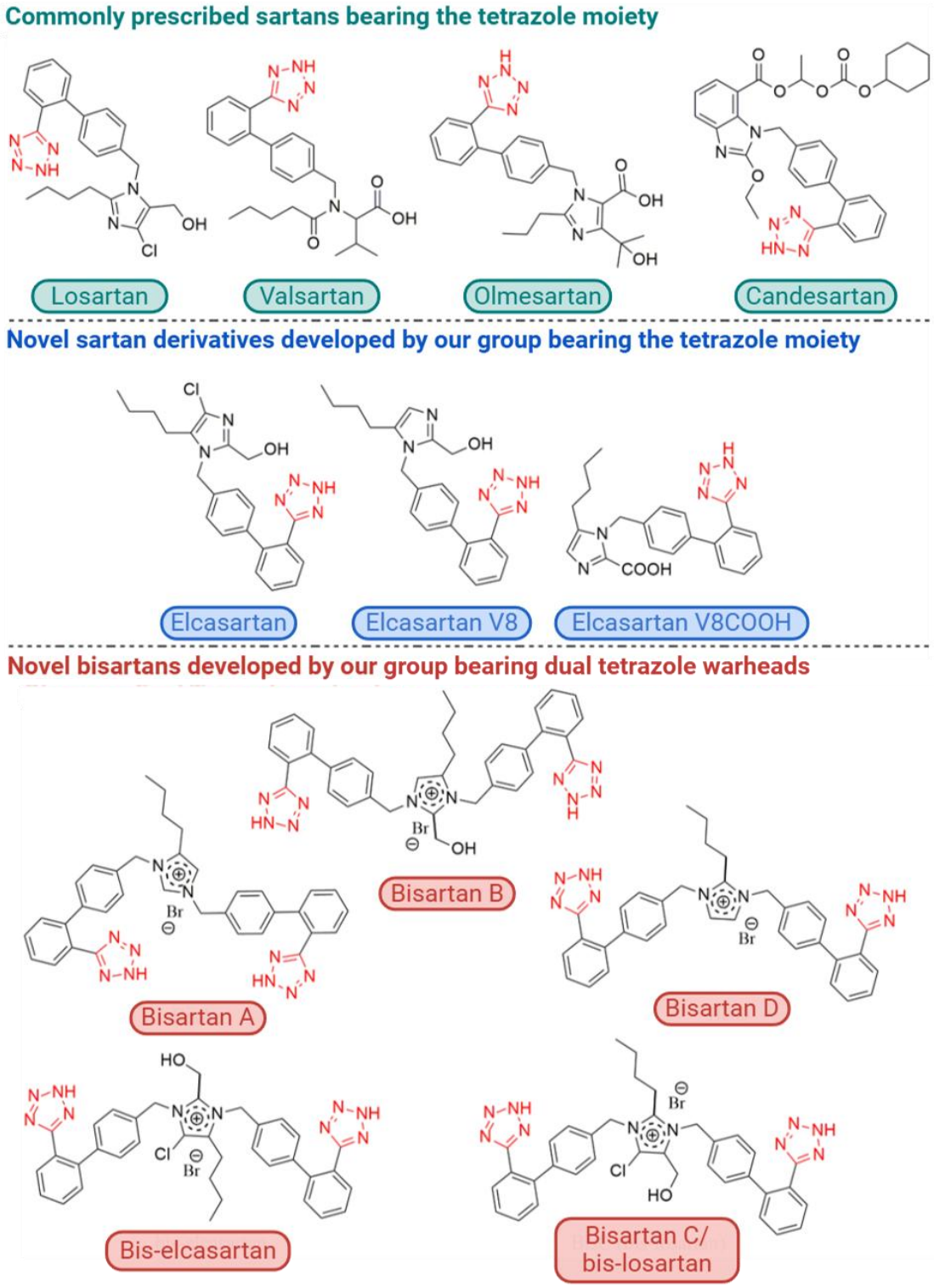


Figure 3. Chemical structures of widely prescribed sartans, and novel sartans and bisartans developed and synthesized in our laboratory.

The recent review by Węgrzyn and Matsoukas provides a detailed framework for the application of SPR to antiviral drug-discovery research and emphasizes both its strengths and its experimental limitations [98]. Data retrieved from SPR studies can be affected by

immobilization orientation, mass transport, nonspecific adsorption, avidity and ligand solubility. These factors are especially important for highly charged compounds, such as bisartans. Therefore, robust SPR experiments should include reference surfaces, concentration-dependent measurements, appropriate controls and independent confirmation of binding.

Furthermore, in silico and DFT studies have identified pronounced interactions involving tetrazole-containing compounds and ACE2's active site, including interactions involving Arg273. Specifically, eight drug candidates, including commercially available ARBs (i.e., candesartan, losartan, losartan carboxylic acid and telmisartan), coronavirus 2019 antiviral (i.e., nirmatrelvir) and the newly synthesized bisartans (i.e., benzimidazole-N-biphenyltetrazole (ACC519T), benzimidazole bis-N,N′-biphenyltetrazole (ACC519TT) and 4-Butyl-N,N′-bis{[[2′-(2H-tetrazol-5-yl)]biphenyl-4-yl]methyl}imidazolium bromide (Bisartan A)), were evaluated via DFT and enzymatic studies against the active site of ACE2. Notably, telmisartan and ACC519T had significant binding affinities with the active core of ACE2, interacting with the Arg273 guanidinium group [92].

SPR provides an important experimental approach for testing predicted molecular interactions. Currently, the available evidence has yet to establish clinical antiviral efficacy of ARBs and bisartans. Rather, it supports a coherent and experimentally testable hypothesis: arginine-containing viral molecular environments may be pharmacologically targeted by appropriately designed anionic tetrazole-containing ligands. This arginine–tetrazole paradigm provides a bridge between viral molecular biology, medicinal chemistry, computational drug discovery and biophysical validation and may contribute to the development of new antiviral and potentially broad-spectrum antiviral agents [34,91,92,98].

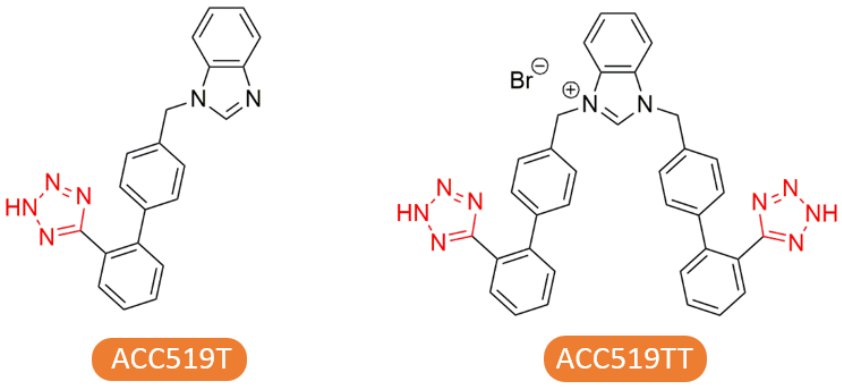


Figure 4. Chemical structures of sartan derivatives ACC519T and ACC519TT with benzimidazole as scaffold, developed and synthesized in our laboratories.

Table 1 summarizes the principal target/system categories of evidence described thus far, distinguishing computational predictions from biochemical/biophysical measurements and direct cellular or virological functional validation.

Table 1. Summary of the principal target/system categories described in the manuscript. "Computational" denotes predicted interactions; "biochemical/biophysical" denotes measurements made in purified systems; "cellular" and "virological" evidence require direct functional tests in cells or virus assays.

Compound(s)	Target/System	Method Reported	Supporting Evidence	Evidence Level/Limitation
Selected tetrazole-containing ARBs	ACE2/S protein–ACE2-associated system	Computational analysis; SPR and biochemical measurements reported in cited work [34]	Evidence relevant to interaction under assay conditions; does not itself prove viral-entry inhibition	Computational + biophysical/biochemical; no efficacy conclusion from binding alone
Selected ARBs and ACC519TT	ACE2 Arg273 region	DFT-based interaction model and ACE2 activity context [92]	Arg273–tetrazole contact proposed computationally	Specific residue contact not independently established by mutagenesis or structural determination in the submitted evidence
ACC519TT and related bisartans	Selected SARS-CoV-2, influenza A and RSV proteins	Docking and molecular dynamics simulations [35]	Predicted poses and interaction stability across selected targets	Computational only for cross-virus target engagement
Sartans/bisartans	Furin, TMPRSS2, cathepsins, S protein cleavage and S proteins-mediated entry	Direct functional evidence not established yet	These mechanisms remain to be tested directly	Hypothesis; protease, cleavage and entry assays required

2.10. From Molecular Recognition to Antiviral Activity

The transition from molecular interaction to antiviral activity requires several experimental levels. A compound may bind to a protein without affecting its biological function. Conversely, a compound may alter a biological pathway through mechanisms unrelated to the predicted binding site. Therefore, binding studies must be followed by functional assays. For SARS-CoV-2, relevant experiments include S protein–ACE2 binding assays, furin-cleavage assays, S protein-mediated cell-entry assays, pseudovirus assays and authentic-virus replication studies [53,65,89]. Regarding influenza, neuraminidase inhibition and viral replication assays are relevant [102]. For RSV, fusion-protein assays and cellular infection models are appropriate. This hierarchical validation is essential for establishing the biological significance of the arginine–tetrazole interaction.

An additional consideration is pharmacological exposure. A compound may demonstrate molecular binding at concentrations that are not achievable in vivo. Therefore, antiviral potency must ultimately be evaluated in relation to pharmacokinetics, cellular penetration, protein binding, toxicity and therapeutic index.

For repurposed sartans, their extensive clinical use provides substantial pharmacological and safety information; however, this does not establish antiviral efficacy. For newly developed bisartans, both antiviral pharmacology and conventional drug-development requirements remain to be addressed.

2.11. From Mechanism to Broad-Spectrum Activity

A particularly important question is whether this molecular principle can extend across phylogenetically and structurally unrelated viruses. As discussed previously, in silico studies of bisartans provide an initial basis involving SARS-CoV-2, influenza and RSV [35]. These viruses differ markedly in genome organization, virion architecture, and host–cell interactions, making a mechanism based on conventional sequence-specific

recognition unlikely to account for activity across all three viral systems. Therefore, a broad-spectrum pharmacological mechanism would be expected to target a conserved physicochemical feature that is shared across otherwise unrelated viral proteins or virion environments. Arginine residues and associated cationic molecular environments represent one potential common determinant. This concept is particularly intriguing because it shifts the framework for antiviral recognition from pathogen-specific sequence homology toward the recognition of conserved physicochemical properties. Such a mechanism could, in principle, provide a molecular basis for antiviral activity across diverse viral families. However, this remains a hypothesis: whether the proposed physicochemical interactions translate into genuine broad-spectrum antiviral activity, and whether they can do so at therapeutically relevant concentrations, will ultimately require rigorous experimental validation. Cytotoxicity, selectivity indices, assay-interference controls and independent replication are essential to further prove this hypothesis. Resistance-selection experiments, where appropriate, could assess whether viral escape occurs through changes at the proposed target or through alternative entry pathways.

2.12. From AngII to Sartans and Bisartans: Mechanism of AngII and Bisartans Action as Broad-Spectrum Antiviral Candidates—The Role of Tetrazole in Blocking Arginine

Pioneering research on AngII and the RAAS by Ridgway and coworkers, employing computational, enzymatic, SPR, and DFT approaches, has provided insights into the molecular driving forces underlying mutations in SARS-CoV-2 and the role of arginine in viral diseases. These studies demonstrated that bisartans exhibit stronger binding than conventional sartans at catalytic sites, highlighting their potential as therapeutic agents against a range of viral infections [1,32,34,35,50,91,92,98].

Structure–activity relationship analyses, nuclear magnetic resonance spectroscopy, and crystallographic studies of AngII have revealed a charge relay system (CRS) mechanism involving three aromatic residues (i.e., tyrosine, histidine and phenylalanine), and the C-terminal carboxylate group [103]. These structural features give rise to a tyrosine-derived anionic interaction that, together with phenylalanine, contributes to AT_1R activation, ultimately promoting increased blood pressure, vascular dysfunction, and congestive heart failure [104–106]. The guanidinium side chain of Arg2 plays a critical role in organizing this molecular cluster, stabilizing the tyrosine hydroxyl group and the CRS formed by the aromatic triad through electrostatic and π-π interactions. In addition, the guanidinium group forms a salt bridge with Asp263 of the receptor, thereby contributing to receptor-binding affinity [107]. The overall conformation of AngII is strongly influenced by proline, a sterically constrained amino acid that induces a gamma-turn in the peptide backbone, a structural feature essential for biological activity [108,109].

Previous studies investigating the design and development of losartan analogues led to the identification of a novel class of ARBs in which the substituents at positions 2 and 4 of the imidazole ring are interchanged relative to losartan, with the butyl and hydroxymethyl groups occupying the 4- and 2-positions, respectively [1,32,34,35,50,91–93,98]. Bisalkylated derivatives were subsequently developed based on these analogues, with the symmetrical incorporation of two biphenyl tetrazole moieties onto the imidazole ring. These compounds, termed bisartans, exhibit notable pharmacological properties with potential applications in the treatment of hypertension and coronavirus infections [1,32,34,35,50,91–93,98].

Bisartans are structurally distinct from conventional sartans, due to the substitution of both imidazole ring nitrogens with bulky biphenyl tetrazole or biphenylnitrile groups, resulting in a negative charge on the imidazole ring. This unusual substitution pattern alters the electronic character of the imidazole scaffold and, in the case of biphenyltetrazole derivatives, introduces additional anionic character, giving rise to larger, highly

functionalized zwitterionic molecules with physicochemical and pharmacological properties that differ substantially from those of conventional ARBs. The presence of two biphenyl units also introduces considerable conformational freedom through rotation about the inter-ring bonds, thereby increasing the structural repertoire available for molecular recognition and potentially enabling accommodation within diverse binding sites.

This conformational adaptability may be particularly relevant to interactions with proteins containing cationic or aromatic binding environments. In computational and structural models, bisartans have been proposed to engage the guanidinium groups of arginine residues through complementary electrostatic interactions between anionic tetrazole groups and positively charged arginine side chains. Additional stabilization may arise through π-mediated interactions involving aromatic residues, including phenylalanine and arginine [110]. Collectively, these interactions provide a plausible molecular basis for the ability of bisartans to recognize structurally diverse protein targets and may help explain their activity beyond the canonical AT_1R.

Importantly, the remarkable pharmacological profile of bisartans appears to extend beyond their established cardiovascular activity. Several studies have reported antiviral effects, with proposed mechanisms including inhibition of neuraminidase/sialidase enzymes involved in viral attachment and entry [35]. The AT1R has been extensively and in detail studied by several groups [86,107,111–119]. The found association with other receptors, renders ARBs and bisartans as promising repurposing candidates for a variety of diseases, through their pharmacological promiscuity. The found association with other receptors, including G protein coupled receptor (GPCRs), their concurrent ability to inhibit AT1R, together with their anti-inflammatory and immunomodulatory effects, may further enhance their therapeutic potential and can be regarded as an additional pharmacological benefit [120–132].

2.13. Limitations

This review synthesizes a hypothesis and a set of computational, biochemical and biophysical observations; it does not report new experimental validation. The available evidence is heterogeneous, and the different methods answer different questions. Computational predictions can prioritize interactions but cannot prove binding, selectivity or biological activity. Biophysical evidence of binding does not establish that a target is functionally inhibited. Enzyme activity measurements do not demonstrate viral-entry blockade, nor do they establish a reduction in viral replication. The specific ACE2 Arg273–tetrazolate contact requires direct residue-level testing. Direct inhibition of furin, TMPRSS2, cathepsins, S protein cleavage or viral entry by the sartans/bisartans discussed here is not established in the evidence summarized, but rather is a computational model. Finally, the proposed cross-virus mechanism remains speculative until each virus is tested using appropriate functional assays and host–cell safety controls.

Other literature on RAAS inhibitors reports immunomodulatory or neuroprotective effects, including in central nervous system inflammation; studies of myelin basic protein epitopes and citrullination concern multiple-sclerosis immunology, while topical losartan studies concern corneal scarring. This showcases the benefits administration of sartans has on many different diseases and thus potentially on viral infections as well [120–132].

3. Conclusions

Arginine residues can play important roles in viral infection through their participation in receptor recognition, proteolytic processing, protein–protein interactions and molecular assembly. Arginine-rich regions of SARS-CoV-2 S protein provide a particularly important example of this principle. The polybasic S1/S2 cleavage region and other basic

molecular environments contribute to viral protein activation and host–cell entry. Tetrazole-containing ARBs and bisartans provide a complementary chemical strategy because their anionic tetrazolate groups can interact with positively charged guanidinium groups and other cationic molecular environments. The resulting arginine–tetrazole therapeutic paradigm provides a rational framework for the development of new antiviral agents.

Several computational findings converge on this hypothesis. Structural and molecular studies establish the importance of arginine-containing viral regions; medicinal-chemistry studies demonstrate that tetrazole-containing ARBs can engage relevant protein targets; DFT and enzymatic studies provide mechanistic evidence for interactions between selected ARBs/bisartans and ACE2; computational bisartan studies extend the hypothesis to multiple viral proteins; and SPR provides a direct experimental methodology for testing molecular binding. Bisartans are particularly attractive because their two tetrazole-containing pharmacophores may enable multivalent recognition and potentially broader target engagement.

The central scientific challenge now is experimental validation. The most decisive experiments will combine SPR with site-directed mutagenesis, enzymatic assays, structure–activity studies and virological models. Demonstrating that removal or mutation of a target arginine reduces binding of a tetrazole-containing ligand would provide particularly strong evidence for the proposed mechanism. The concept should therefore be regarded as a testable therapeutic paradigm rather than an established antiviral mechanism. If validated, the approach could open a new direction in antiviral medicinal chemistry: targeting conserved physicochemical features such as functionally important cationic residues rather than relying exclusively on sequence-specific viral inhibitors.

The combination of arginine biology, tetrazole chemistry, ARB pharmacology, bisartan design, computational chemistry and SPR-based molecular validation represents a distinctive and potentially powerful strategy for antiviral drug discovery.

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Abbreviations

The following abbreviations are used in this manuscript:

ACC519T	Benzimidazole-N-biphenyltetrazole
ACC519TT	Benzimidazole bis-N,N′-biphenyltetrazole
ACE2	Angiotensin-converting enzyme 2
AngII	Angiotensin II
ARB	Angiotensin receptor blocker
AT_1R	Angiotensin II type 1 receptor
Bisartan A	4-Butyl-N,N′-bis{[[2′-(2H-tetrazol-5-yl)]biphenyl-4-yl]methyl}imidazolium bromide
CRS	Charge relay system
DFT	Density functional theory
DNA	Deoxyribonucleic acid
HSV	Herpes simplex virus
MBP	Myelin basic protein
MS	Multiple sclerosis
RAAS	Renin–angiotensin–aldosterone system
RBD	Receptor binding domain
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
S protein	Spike protein
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SPF	Surface plasmon resonance
TMPRSS2	Transmembrane protease, serine 2
VZN	Varicella-zoster virus

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