



Review

Novel CYP11A1-Derived Vitamin D and Lumisterol Biometabolites for the Management of COVID-19

Shariq Qayyum ^{1,2,3} , Radomir M. Slominski ⁴, Chander Raman ¹ and Andrzej T. Slominski ^{1,5,*} ¹ Department of Dermatology, University of Alabama at Birmingham, Birmingham, AL 35294, USA² Research Program in Men's Health: Aging and Metabolism, The Center for Clinical Investigation, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115, USA³ Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA⁴ Department of Genetics, Informatics Institute, University of Alabama at Birmingham, Birmingham, AL 35294, USA⁵ Pathology and Laboratory Medicine Service, VA Medical Center, Birmingham, AL 35294, USA

* Correspondence: aslominski@uabmc.edu

Abstract: Vitamin D deficiency is associated with a higher risk of SARS-CoV-2 infection and poor outcomes of the COVID-19 disease. However, a satisfactory mechanism explaining the vitamin D protective effects is missing. Based on the anti-inflammatory and anti-oxidative properties of classical and novel (CYP11A1-derived) vitamin D and lumisterol hydroxymetabolites, we have proposed that they would attenuate the self-amplifying damage in lungs and other organs through mechanisms initiated by interactions with corresponding nuclear receptors. These include the VDR mediated inhibition of NF κ B, inverse agonism on ROR γ and the inhibition of ROS through activation of NRF2-dependent pathways. In addition, the non-receptor mediated actions of vitamin D and related lumisterol hydroxymetabolites would include interactions with the active sites of SARS-CoV-2 transcription machinery enzymes (M^{Pro}; main protease and RdRp; RNA dependent RNA polymerase). Furthermore, these metabolites could interfere with the binding of SARS-CoV-2 RBD with ACE2 by interacting with ACE2 and TMPRSS2. These interactions can cause the conformational and dynamical motion changes in TMPRSS2, which would affect TMPRSS2 to prime SARS-CoV-2 spike proteins. Therefore, novel, CYP11A1-derived, active forms of vitamin D and lumisterol can restrain COVID-19 through both nuclear receptor-dependent and independent mechanisms, which identify them as excellent candidates for antiviral drug research and for the educated use of their precursors as nutrients or supplements in the prevention and attenuation of the COVID-19 disease.

Keywords: vitamin D; lumisterol; SARS-CoV-2; anti-inflammatory; ACE2; M^{Pro}; RdRp

Citation: Qayyum, S.; Slominski, R.M.; Raman, C.; Slominski, A.T. Novel CYP11A1-Derived Vitamin D and Lumisterol Biometabolites for the Management of COVID-19. *Nutrients* **2022**, *14*, 4779. <https://doi.org/10.3390/nu14224779>

Academic Editor: Federica I. Wolf

Received: 15 August 2022

Accepted: 8 November 2022

Published: 11 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

COVID-19 is still the top health issue in the world. Vaccines approved for COVID-19 provide protection against some strains of SARS-CoV-2; however, new mutant strains develop in continuity and some of them escape immunity provided by current vaccines [1]. The infection from SARS-CoV-2 has severe adverse outcomes with a significantly higher mortality rate than influenza. The major cause of death in COVID-19 is acute respiratory distress syndrome (ARDS) caused by cytokine storm [2,3]. This enhanced hyperactivated innate immune response against the virus causes severe damage to the patient's body/organs which might be fatal (Figure 1). In this mini-review we will discuss how vitamin D and its derivatives can be helpful against the infection caused by SARS-CoV-2.

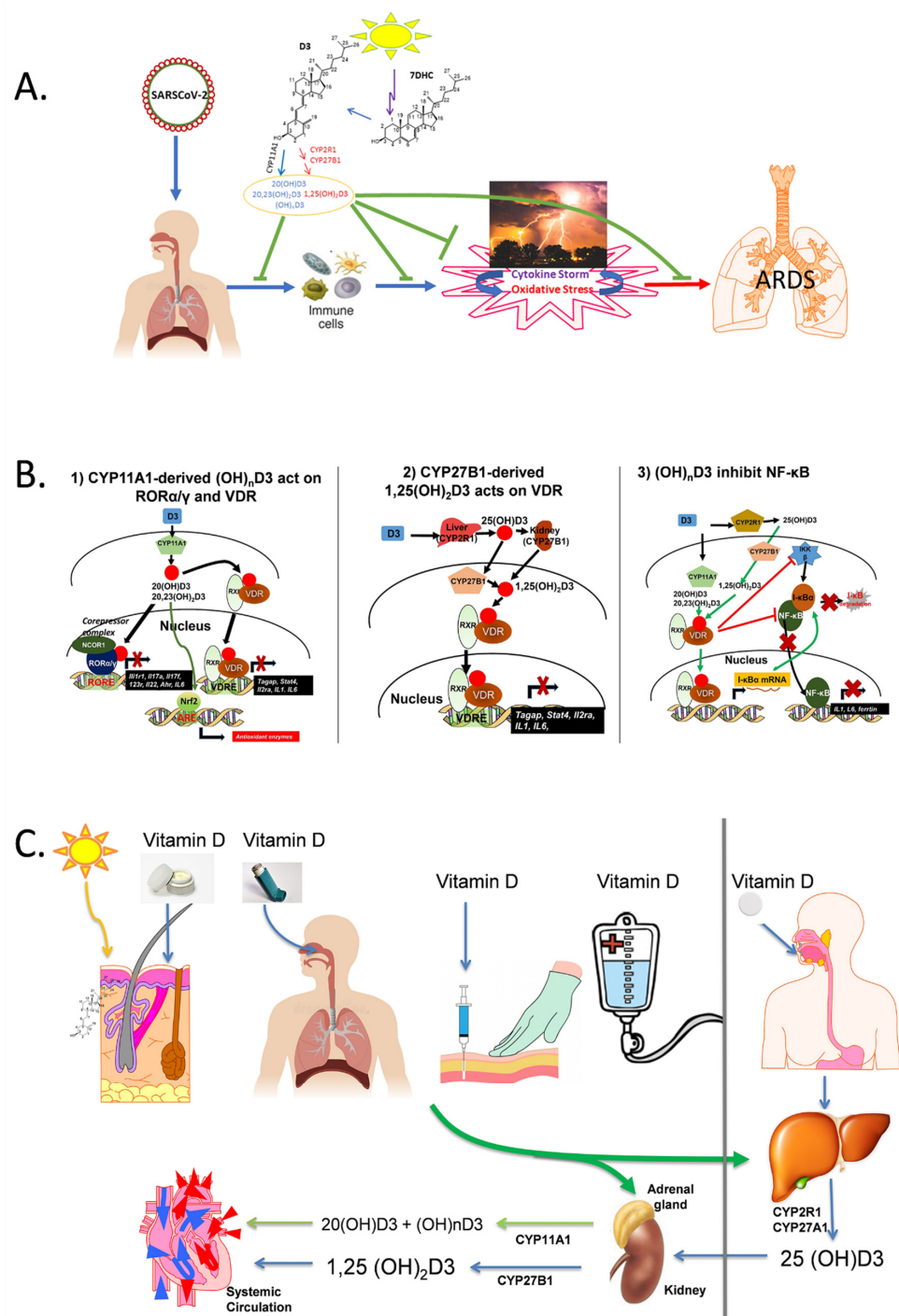


Figure 1. Possible mechanisms by which vitamin D can counteract the COVID-19 illness. In panel (A) it is proposed that the novel hydroxyderivatives of vitamin D₃, in similar manner as 1,25(OH)₂D₃, inhibit cytokine storm and oxidative stress, with net attenuating effect on ARDS and multiorgan failure induced by COVID-19. Panel (B) proposes a mechanism of action of canonical and non-canonical vitamin D-hydroxyderivatives. Vitamin D signaling in mononuclear cells involves the activation of the VDR or inverse agonism on ROR γ with downstream inhibition of inflammatory genes and the suppression of oxidative stress through the activation of NRF2. VDR, vitamin D receptor; RXR, retinoid X receptor; ROR, retinoic acid orphan receptor, RORE, ROR response element; ARE, antioxidant response element; VDRE, vitamin D response element; NRF2, transcription factor NF-E2-related factor 2. Panel (C) shows how different routes of vitamin D delivery impact vitamin D hydroxylation/activation patterns. Reprinted with permission from the publisher [86].

Vitamin D, a prohormone, is a fat soluble secosteroid, which is formed in the skin after the absorption of UVB energy by the B ring of 7-dehydrocholesterol (7DHC) [4–6]. The prolonged exposure of 7-DHC to UVB leads to the phototransformation of pre-vitamin D3 to tachysterol and lumisterol [4–6]. It can also be ingested from the diet and supplements. As a prohormone, it must be activated to exert the biological activity. In the classical activation pathway vitamin D3 is metabolized by several cytochromes P450 (CYPs) enzymes before being transformed to its known active form 1,25-dihydroxyvitamin D3 (1,25(OH)₂D3) [7–10]. 1,25(OH)₂D3 activates the nuclear vitamin D (VDR) receptor, which controls not only body calcium metabolism [9,11–13] but also many important physiological functions, including the regulation of the innate and adaptive immunity [6,9,14–17]. Vitamin D can be activated by two pathways known as a canonical, with sequential hydroxylation at C25 and C1α [18], and a non-canonical, activated by CYP11A1 [19,20]. The canonical pathway includes the metabolism of vitamin D3 to 25-hydroxyvitamin D3 (25(OH)D3) by CYP2R1 and CYP27A1 in the liver and final C1α hydroxylation in the kidney to the biologically active form 1,25(OH)₂D3 by CYP27B1 [7–9]. This pathway also operates in the peripheral tissues, including skin [8,18,21–24].

The phenotypic effect of 1,25(OH)₂D3 is predominantly mediated through an interaction with the VDR leading to the transcriptional activation of more than 3000 genes [4,6,8–10]. Non-genomic regulatory actions for 1,25(OH)₂D3 were also described [6,8,10]. 1,25(OH)₂D3, in addition of regulating body calcium metabolism, also regulates diverse functions on the systemic, tissue, and cellular levels [4,6,8–15,18–20]. Of general interest are the anti-oxidative and anti-inflammatory properties of 1,25(OH)₂D3, which have been appreciated for almost two decades [4,6,8–15,18–20]. The latter includes the downregulation of pro-inflammatory cytokines production through the inhibition of NFκB [6,12–14].

Recently discovered non-canonical pathways of vitamin D activation are initiated by an obligatory enzyme of steroidogenesis, CYP11A1, in a complex process involving several CYPs and producing more than a dozen of hydroxyderivatives [8,20,25–27]. CYP11A1 is not only expressed in adrenals, placenta, and gonads [28] but also in immune cells [29] and other peripheral organs including skin [19,30]. The CYP11A1-derived hydroxyderivatives are non-calcemic or low calcemic [31–34] and can therefore be used at high concentrations for therapeutic purposes [35–39]. They are also detectable in natural products including honey [40] and human serum [25,41,42]. Similarly, to 1,25(OH)₂D3, they can alter gene expression by binding on the genomic site of the VDR [33,43–46]. CYP11A1-derived vitamin D3 hydroxyderivatives also bind to other nuclear receptors, including aryl hydrocarbon receptor (AhR) [47–49], retinoic acid orphan receptors (ROR)α and γ [44,50], liver X receptors (LXR)α and β [51] and can change their expression and activities [27]. The CYP11A1-derived hydroxymetabolites of vitamin D3, including 20(OH)D3 and 20,23(OH)₂D3, have demonstrated anti-inflammatory and anti-oxidative effects [27,36,42,46,52–56], which are similar to the effects described for the classical active form of vitamin D3, 1,25(OH)₂D3 [6,12–14,18–20].

Novel pathways of 7DHC transformation by CYP11A1 [30,57,58], with the further phototransformation of the 5,7-dienal products to corresponding secosteroids [27,59–64] and the hydroxylations of lumisterol by CYP11A1 and CYP27A1, were also discovered [65–67], with their products being biologically active and detectable in human body and acting on RORα and γ as inverse agonists and as agonists on LXR α and β and on the non-genomic site of the VDR [51,66,68]. Most recently, an enzymatic activation of tachysterol was reported with the metabolic products acting on the VDR, AhR, LXRs, and RORs [63]. These metabolites exert similar biological effects as the classical active form of vitamin D [1,25(OH)₂D3] and they have their unique activity pattern towards various nuclear receptors, aside of the classical VDR/RXR complex [9].

Although SARS-CoV-2 infection in human cells involves multiple factors, in this review we are focusing on two main interactions listed below. The spike protein (S) of SARS-CoV-2 facilitates the entry of the virus into human cells by engaging angiotensin-converting enzyme 2 (ACE2) as their entry receptor [69] and further cellular serine protease TMPRSS2 is used for priming of S protein [70–72]. The association between ACE2 and Spike protein is critically im-

portant and current vaccines (mRNA) are developed to inhibit this interaction [73]. The actions of CYP11A-derived vitamin D₃-hydroxymetabolites, canonical 1,25(OH)₂D₃ and lumisterol hydroxymetabolites with SARS-CoV-2 replication machinery enzymes were previously explored [74], which included molecular modeling on classical vitamin D compounds [75]. The significance of these studies is further discussed in this review.

The SARS-CoV-2 virus replicates within host cells using its cellular and enzymatic components. M^{Pro}, also termed 3CL protease, is a 33.8-kDa cysteine protease which helps in the maturation of functional polypeptides involved in the assembly of replication-transcription machinery [76–78]. M^{Pro} digests the polyprotein at no less than 11 conserved sites, starting with the autolytic cleavage of this enzyme itself from pp1a and pp1ab, which are individual nonstructural proteins essential for viral genome replication [76]. Another enzyme important to the life cycle of SARS-CoV-2 is RdRp (RNA-dependent RNA polymerase), which catalyzes the replication [79] of RNA from an RNA template. SARS-CoV-2 use an RdRp complex for the replication of their genome and for the transcription of their genes [79]. M^{Pro} and RdRp are enzymes required for viral replication and are not homologous to any gene in the human genome. Hence, they are very attractive targets for the development of anti-viral drugs against COVID-19. Therefore, we will further discuss how the hydroxymetabolites of vitamin D and of lumisterol can counter SARS-CoV-2 infection at different stages of this process.

2. CYP11A1-Derived Vitamin D and Lumisterol Hydroxymetabolites Exert Anti-Inflammatory and Antioxidant Effects

The cytokine storm is a response to viral infection, which causes immune cells to release several pro-inflammatory cytokines/chemokines (interferons, interleukins 1, 6 and 17, chemokines, colony-stimulating factors, and tumor necrosis factors (TNF)), leading to hyper inflammation and organ damage [80–82]. This process in the lung leads to acute lung injury and ARDS. Along with = ARDS, another factor which plays a role in damage to the tissue and cells is oxidative stress. It is secondary to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [83–85]. CYP11A1-derived vitamin D₃ and lumisterol hydroxymetabolites exhibit potent anti-inflammatory activities through the inhibition of IL-1, IL-6, IL-17, TNF α and INF γ production and/or other pro-inflammatory pathways [25,36,37,43,46,50,52,54–56,68], which are similar to those mediated by classical 1,25(OH)₂D₃ [4,6,8–15,18–20]. The anti-inflammatory effects of active forms of vitamin D can be mediated through the downregulation of NF- κ B, involving action on VDR and inverse agonism on ROR γ leading to the attenuation of Th17 responses (Figure 1B). These compounds also induce anti-oxidative and reparative responses with mechanism of action involving the activation of NRF2 and p53 signaling pathways [27,42,52,53,68,86]. Interestingly, the anti-viral role of NRF2 is also recognized [87]. The use of 1,25(OH)₂D₃ has its limitations because of the toxicity that includes hypercalcemia [7,88]. However, CYP11A1-derived 20(OH)D₃, 20(OH)D₂, and 20,23(OH)₂D₃ are not calcemic even at very high doses [31–34]. Hence, vitamin D and its metabolites can be used as economic nutritional supplements to counter the effects of the SARS-CoV-2 infection like cytokine storm [86,89].

3. Inhibition of the Interaction between ACE2 and SARS-CoV-2 Spike RBD

The ACE2 interacts with the receptor-binding domain (RBD) region of the spike protein [90,91]. SARS-CoV-2 and SARS-CoV-2 RBD are typically in standing up state and resting state, respectively. SARS-COV-2 RBD has higher binding affinity to ACE2, but its lying-down state makes it less accessible to ACE2 and other inhibitory or neutralizing agents [90,91]. The use of host protease (furin, TMPRSS2, cathepsins etc.) for its activation helps as a strategy to overcome the lying down state and maintaining its high binding affinity to ACE2 [90–92]. This interaction is critical and important for drug development against COVID-19 infection. Molecular modeling and simulation [93] evaluated the binding of vitamin D₃ and its hydroxyderivatives to SARS-CoV-2 RBD, and their potential to inhibit

its interaction with ACE2. The study showed that vitamin D3 and its hydroxyderivatives can function as inhibitors of TMPRSS2 and inhibit the SARS-CoV-2 receptor binding domain (RBD) binding to the ACE2 [93]. Molecular dynamics (MD) simulations for the interactions of 1,25(OH)₂D3 have shown the favorable binding free energy of ACE2, SARS-CoV-2 RBD, and TMPRSS2 with 1,25(OH)₂D3 [93]. The binding free energy of ACE2, SARS-CoV-2, and TMPRSS2 with 1,25(OH)₂D3 were -18.55 ± 4.16 , -16.97 ± 1.69 , and -21.04 ± 1.53 kcal/mol separately, further indicating that ACE2, SARS-CoV-2 RBD, and TMPRSS2 show favorable binding with 1,25(OH)₂D3 [93]. The predicted interaction of 1,25(OH)₂D3 with SARS-CoV-2 RBD and ACE2 could result in the conformation and dynamical motion changes of the binding surfaces between SARS-CoV-2 RBD and ACE2, leading to the interruption of the binding of SARS-CoV-2 RBD with ACE2 [93]. The interaction of 1,25(OH)₂D3 with TMPRSS2 also caused the conformational and dynamical motion changes of TMPRSS2, which could affect TMPRSS2 to prime SARS-CoV-2 spike proteins [93]. These studies [93] have indicated that vitamin D3 and its biologically active hydroxymetabolites have the theoretical potential to prevent the cellular entry of SARS-CoV-2 by serving as the inhibitor of TMPRSS2 and blocking the binding of SARS-CoV-2 RBD with ACE2.

Molecular modeling was also used for the virtual screening of antiviral compounds to SARS-CoV-2 non-structural proteins [94]. The described interactions between spike protein and ACE2 can be also disrupted by other compounds, including vitamin D as described by other research groups [95–97]. These included interactions with vitamins, retinoids, steroids, vitamin D derivatives, and dihydrotachysterol as examples. The detailed mechanisms of action were discussed in [95–97]. In addition, vitamin D was identified as a potential inhibitor of COVID-19 Nsp15 endoribonuclease binding sites [98].

To confirm our predictions on novel vitamin D3 and lumisterol hydroxymetabolites, we have used an inhibitor screening kit (SARS-CoV-2 inhibitor screening kit, Acro Biosystems) (Table 1). The kit uses a colorimetric ELISA platform, which measures the binding of immobilized SARS-CoV-2 S protein and biotinylated human ACE2. Top compounds with best binding energy were theoretically predicted previously [93], selected for this assay, showing the inhibition of the interaction between ACE2 and RBD (Table 1). 20(OH)L3 showed the highest level of inhibition of the RBD-ACE2 interaction followed by 25S27(OH)L3 and 20(OH)D3 at concentrations of 2×10^{-7} M. The compounds were observed to be effective in μ M concentrations, which is promising for future clinical and preclinical testing.

Table 1. Inhibition of ACE2 and RBD interaction by the hydroxymetabolites. Inhibition by the selected metabolites was observed concentration of 2×10^{-7} M using a SARS-CoV-2 inhibitor screening kit from Acro Biosystems. The assay followed the manufacture’s protocol of the SARS-CoV-2 (B.1.617.2) Inhibitor Screening Kit (Spike RBD) (1 Innovation Way, Newark, DE 19711, USA). Data were analyzed by one way ANOVA using GraphPad Prism statistical software.

No.	Name of the Ligand	Inhibition in Enzyme Activity (%)	p-Value
1.	20(OH)D3	46.057	0.013
2.	1,20(OH) ₂ D3	29.222	NS *
3.	1,25(OH) ₂ D3	36.876	0.034
4.	20,23(OH) ₂ D3	36.152	0.018
5.	24(OH)L3	32.343	0.0265
6.	20(OH)L3	74.552	0.001
7.	25S27(OH)L	51.722	0.005
8.	20,22(OH) ₂ L3	13.074	NS *

NS * Not significant.

In addition, the treatment of HaCaT keratinocytes with these hydroxymetabolites changed the expression of ACE2 and TMPRSS2 in a metabolite-specific manner. 1,20(OH)₂D₃, 1,25(OH)₂D₃, and 24(OH)L₃ suppressed the expression of ACE2, and TMPRSS2 expression was inhibited by 20(OH)D₃, 1,20(OH)₂D₃, 1,25(OH)₂D₃, 20(OH)L₃, and 24(OH)L₃ (Supplementary Figure S1). This suggests that these compounds can inhibit the bonding of ACE2 and RBD not only by directly blocking the binding but also by altering the expression of these receptors' genes.

4. Inhibition of the Activity of the Replication Enzymes of SARS-CoV-2

SARS-CoV-2 replicates inside the host cell after its entry. The viral particles utilize host resources, but viral replication machinery plays crucial role in its replication. These viral specific factors do not share homologies with human proteins and are therefore targets for drug development against COVID-19 [74]. We selected two SARS-CoV-2 replication enzymes, RdRp or nsp12 and 3C-like protease (3CL^{pro} or M^{pro}), based on their recognized importance for drug development [76]. Although there are reports that vitamin D and its metabolites have potential to inhibit other viral protein, we selected these proteins as we have had experimental potential to confirm our results [74]. 3CL-Chymotrypsin, such as Protease or Main protease (M^{pro}), is one of the two proteolytic enzymes that helps in cleaving the replicase polyprotein 1ab in SARS-CoV-2 at 11 specific sites, the recognition sites being Leu-Gln (Ser, Ala, Gly) in order to release 12 nsps (nsp4, nsp6-16) that are essential for viral replication as well as viral assembly [3,99]. This enzyme shares no common cleavage site with any human protease and its functional importance in the life cycle of the virus makes it an attractive target for drug development. Similarly, RNA-dependent RNA Polymerase (RdRp) is an enzyme that is responsible for the replication of RNA from an RNA template [100]. RdRp is another conserved protein of retroviruses and is also a proven target for the development of antiviral drugs [79]. We performed molecular docking on the active sites of these two enzymes and found that the hydroxyderivatives of vitamin D₃ and lumisterol were binding efficiently on the active sites of these enzymes with similar affinities to known therapeutics danoprevir, lopinavir, and ritonavir serving as positive controls [74]. We further confirmed the inhibition of the enzyme activity in the presence of the compounds.

Danoprevir, lopinavir, and ritonavir were used as a standard for the comparison of predicted energies of the top 10 selected compounds [74,94]. A virtuous complementarity to the M^{pro} binding pocket was observed for top compounds, which indicates a possibility that these metabolites have ability to hinder the substrate accessibility, inhibiting the enzymatic activity in process. Significant interactions between the selected metabolites and the critically important residues of the M^{pro} substrate-binding pocket were observed (Figure 2) and predicting a block to the substrate-binding pocket of COVID-19 M^{pro} (Figure 2) [74]. The detailed analysis of the residues interaction with these metabolites was reported previously [74]. The inhibition of M^{pro} was confirmed using 3CL Protease, MBP-tagged (SARS-CoV-2) Assay (BPS Biosciences). The 25(OH)L₃, 24(OH)L₃, and 20S(OH)7DHC being most effective at inhibiting M^{pro} activity by 10–19% at a concentration of 2×10^{-7} M (Figure 3A). Similarly, selected metabolites showed interactions with critically essential residues of SARS-CoV-2 RdRp (Figure 4) [74]. These sterols and secosteroids presented a similar binding pattern to inhibitor remdesivir on RdRp active sites [74,101]. The binding prototype of the compounds showed a virtuous complementarity to the SARS-CoV-2 RdRp binding pocket predicting that they can inhibit the enzymatic activity (Figure 3B). These metabolites showed inhibitory activity ranging from 40–60% at a concentration of 10^{-7} M (Figure 3B) with 25(OH)L₃ with an IC₅₀ of 0.5 μM followed by 1,25(OH)₂D₃ and 20S(OH)L₃, which had an IC₅₀ of 1 μM. Thus, our published work [74] has demonstrated that novel 7DHC, lumisterol, and vitamin D₃ hydroxymetabolites have the potential to inhibit SARS-CoV-2 infection by restricting its replication cycle. Interestingly, unbiased retrospective analyses of microarray data obtained with epithelial cells indicated the antiviral effects of 20,23(OH)₂D₃ with similar effects for 1,25(OH)₂D₃ [86]. A potential role of

hydroxylumisterols appears to be strengthened by recent findings, showing their similarity in structure to 25(OH)L3, where 25-hydroxycholesterol can act as a potent SARS-CoV-2 inhibitor [102], and that cholesterol 25-hydroxylase inhibits SARS-CoV-2 [103] and oxysterols show anti-viral activity [104].

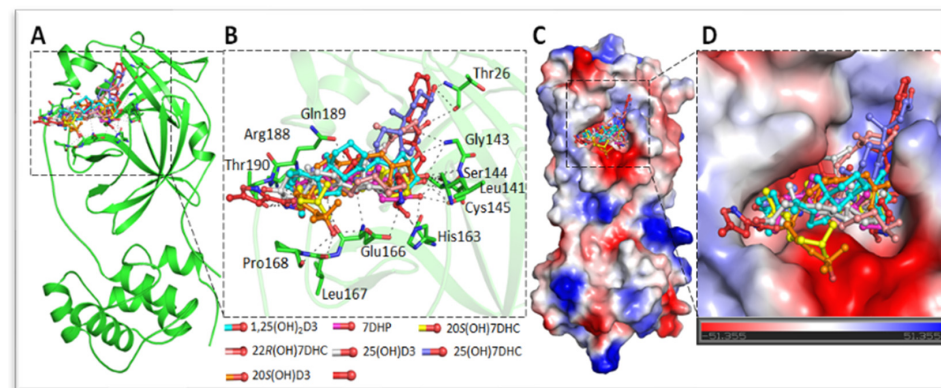


Figure 2. The binding pattern of identified compounds with SARS-CoV-2 M^{Pro}. (A) structural representation of the protein in complex with selected sterols and secosteroids. (B) selected compounds blocking the binding pocket and making significant interactions with the functionally important residues of SARS-CoV-2 M^{Pro}. (C) surface representation of conserved substrate-binding pocket of SARS-CoV-2 M^{Pro} in complex with selected compounds. (D) zoomed view of the substrate-binding pocket of SARS-CoV-2 M^{Pro} in complex with selected compounds. Reprinted with permission from the publisher [74].

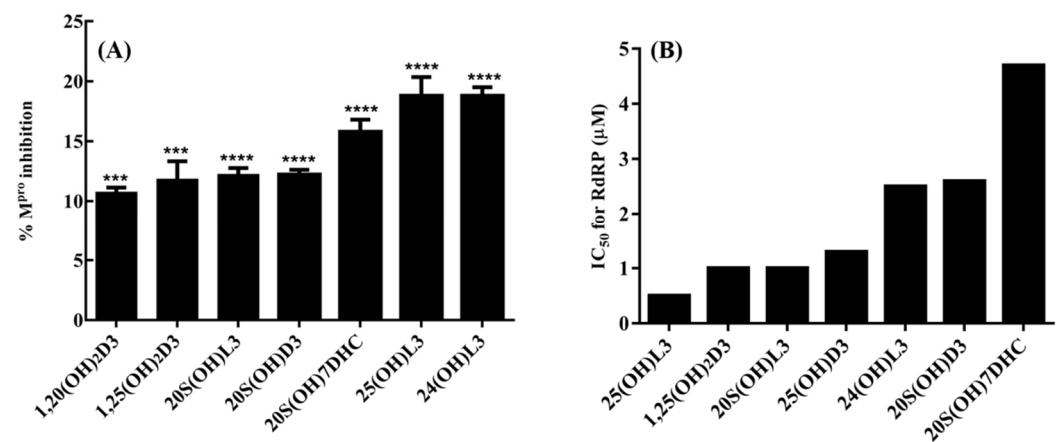


Figure 3. Enzyme inhibition by the selected sterols and secosteroids. (A) the M^{Pro} enzyme inhibition by the selected metabolites at concentration of 2×10^{-7} M. The inhibition percentages were calculated using the formula: % inhibition = $100 \times [1 - (X - \text{Minimum}) / (\text{Maximum} - \text{Minimum})]$. Minimum = negative control without any enzyme (0% enzyme activity); Maximum = positive control with enzyme and substrate (100% enzyme activity). The test sets included enzymes, substrates, and the test compounds, and excitation at a wavelength of 360 nm and the detection of emission at a wavelength of 460 nm was observed for change in enzyme activity. The statistical significance of differences was evaluated by one-way ANOVA; *** $p < 0.001$ and **** $p < 0.0001$ for all conditions relative to ethanol blank, $n = 3$. (B) the RdRp enzyme activity inhibition by selected sterols and secosteroids. The inhibition percentages were calculated using the formula: % inhibition = $100 \times [1 - (X - \text{Minimum}) / (\text{Maximum} - \text{Minimum})]$. Minimum = negative control without any enzyme (0% enzyme activity); Maximum = positive control with enzyme and substrate (100% enzyme activity). The statistical significance of differences was evaluated by one-way ANOVA; *** $p < 0.001$ and **** $p < 0.0001$ for all conditions relative to the ethanol blank, $n = 3$. RdRp, RNA-dependent RNA polymerase. Reprinted with permission from the publisher [74].

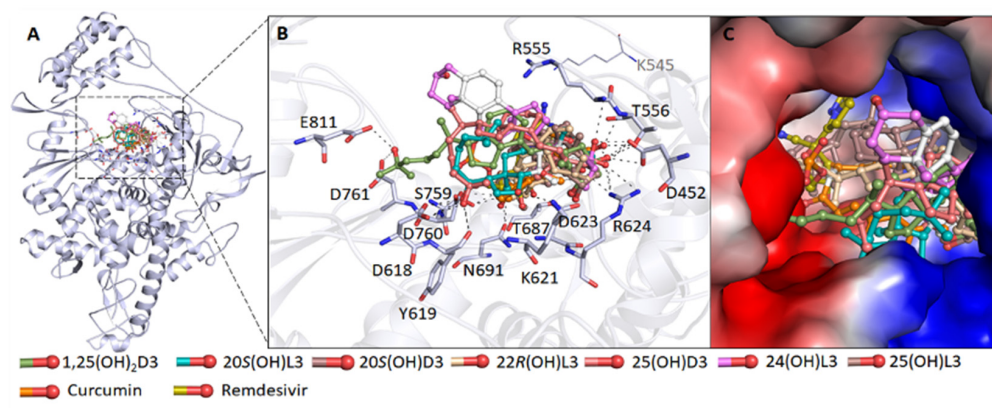


Figure 4. The binding pattern of identified sterols and secosteroids with SARS-CoV-2 RdRp. (A) structural representation of the protein in complex with selected compounds. (B) active site residues of the RdRp-binding pocket making significant interactions with each of the identified compounds. (C) surface view of the RdRp active site with the electrostatic potential from red (negative) to blue (positive) in complex with selected compounds. RdRp, RNAdependent RNA polymerase. Reprinted with permission from the publisher [74].

5. Hypothesis

Overall, the above considerations provide strong support for the ability of D₃, L₃, and 7DHC hydroxymetabolites to counter the different stages of SARS-CoV-2 infection and most of them are non-calcemic. These metabolites can attenuate cytokine storm and have an ability to inhibit the viral replication enzymes. A deficiency of these hydroxymetabolites may contribute to the transition of SARS-CoV-2 patients from asymptomatic to symptomatic. Vitamin D deficiency in the body will lead to reduced levels of the vitamin D hydroxymetabolites and consequently diminished capacity to attenuate cytokine storm. Anti-inflammatory effects by vitamin D and lumisterol hydroxymetabolites were observed at 0.1 μ M concentration. Similarly, 20(OH)L₃, 25S27(OH)L₃, and 20(OH)D₃ were able to inhibit RBD-ACE2 interaction, which is necessary for cellular entry of the virus. For M^{Pro}, a significant inhibition has been observed at 0.1 μ M of 25(OH)D₃, which is close to its plasma concentration [8]. Of note, low pre-infection 25(OH)D₃ levels are associated with a higher severity of the COVID-19 illness [105]. For RdRp, the IC₅₀ for 25(OH)D₃ was 1.3 μ M, approximately one order of magnitude above its plasma concentration. For the hydroxylumisterols tested, plasma concentrations are unknown except for 20(OH)L₃ where a value of 0.25 μ M has been reported [66]. Also based on the enzymology, 25(OH)L₃, which had the lowest IC₅₀ (0.5 μ M) for the inhibition of RdRp, is likely to have substantially higher concentrations. Interestingly, similar in structure 25(OH)cholesterol has been shown to have anti-SARS-CoV-2 activities [102,103], while cholesterol 25-hydroxylase generated anti-inflammatory environments [106] and oxysterols are recognized for anti-viral activities [104].

Therefore, defects in vitamin D or lumisterol delivery either orally as nutrients or supplements or their production in the skin after UVB exposure can lead to the deficiency of corresponding hydroxymetabolites; which have demonstrated anti-viral potential [74,86,93]. This family of compounds contains dozens of molecules [27], which can influence the different stages of SARS-CoV-2 infection. This will require further validation, as has been carried out for other molecules, including classical vitamin D₃ derivatives [48,75,94–98]. Vaccines against SARS-CoV-2 are clearly a major advance in controlling COVID-19; however, new viral variants emphasize the need for alternative therapeutic or nutritional approaches. Therefore, consideration for novel vitamin D and L₃ metabolites for anti-viral drugs that could attenuate COVID-19 is warranted.

6. Concluding Remarks

There are reports demonstrating a strong association for the pre-infection deficiency of vitamin D in hospitalized COVID-19 patients [100] and increased disease severity and

mortality [102,103]. The oral supplementation of vitamin D may affect SARS-CoV-2 infection outcomes. Several clinical trials are ongoing, assessing the ability of vitamin D to prevent COVID-19 infection and disease severity [104–110], showing its importance in managing COVID-19. However, the precise mechanism of vitamin D action against COVID-19 is still unresolved. Here, we have formulated a hypothesis for the mechanism of action of vitamin D and lumisterol hydroxymetabolites against COVID-19. It includes the enzymatic activation of vitamin D3 or sterol precursors with receptor-independent [74,93] or nuclear receptor-dependent activities downstream of VDR, LXR, and AhR activation or inverse agonism on RORs [27,51,86]. Similarly, tachysterol and its metabolites represent additional candidates for at least receptor-mediated activities [63]. We also acknowledge other mechanism-oriented work on classical vitamin D hydroxyderivatives in the prevention or therapy of COVID-19 that has been reported or reviewed recently [75,96,111–117]. Therefore, further clinical testing for their therapeutic use would represent an important step in understanding the beneficial actions of vitamin D3, lumisterol, and possibly tachysterol derivatives [63,97] in COVID-19 with significant implications for the nutritional approach. Vitamin D, lumisterol, or tachysterol ingested through the diet or as supplements would serve as prohormones for further activation in the body towards biologically active forms.

7. Patent

Patent application pending (WO2022006446A1), which includes the experimental portion of this work.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu14224779/s1>, Figure S1: The change in the expression of *ACE2* and *TMPRSS2* in keratinocyte HaCaT cell line after treatment with listed vitamin D and lumisterol metabolites at 2×10^{-7} M [39].

Author Contributions: S.Q., R.M.S., C.R. and A.T.S.—conceptualization, formal analysis, writing, figure preparation, review, and editing. All authors have read and agreed to the published version of the manuscript.

Funding: NIH (grants nos. 1R01AR073004, R01AR071189, and R21AI149267-01A1) and VA merit (grant no. 1I01BX004293-01A1).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable. The discussed papers were selected through a PubMed search and current reading of articles on COVID-19 and the role of vitamin D and sterol compounds in the therapy and prevention of the disease.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* **2020**, *5*, 536–544. [[CrossRef](#)] [[PubMed](#)]
2. Mehta, P.; McAuley, D.F.; Brown, M.; Sanchez, E.; Tattersall, R.S.; Manson, J.J.; on behalf of the Hlth Across Speciality Collaboration, U.K. COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet* **2020**, *395*, 1033–1034. [[CrossRef](#)]
3. Zhang, C.; Shi, L.; Wang, F.S. Liver injury in COVID-19: Management and challenges. *Lancet Gastroenterol. Hepatol.* **2020**, *5*, 428–430. [[CrossRef](#)]
4. Holick, M.F.; Frommer, J.E.; McNeill, S.C.; Richtand, N.M.; Henley, J.W.; Potts, J.T., Jr. Photometabolism of 7-dehydrocholesterol to previtamin D3 in skin. *Biochem. Biophys. Res. Commun.* **1977**, *76*, 107–114. [[CrossRef](#)]
5. Holick, M.F.; Clark, M.B. The photobiogenesis and metabolism of vitamin D. *Fed. Proc.* **1978**, *37*, 2567–2574. [[PubMed](#)]
6. Wacker, M.; Holick, M.F. Sunlight and Vitamin D: A global perspective for health. *Dermato-Endocrinol.* **2013**, *5*, 51–108. [[CrossRef](#)]
7. Holick, M.F. Vitamin D deficiency. *N. Engl. J. Med.* **2007**, *357*, 266–281. [[CrossRef](#)]
8. Tuckey, R.C.; Cheng, C.Y.S.; Slominski, A.T. The serum vitamin D metabolome: What we know and what is still to discover. *J. Steroid Biochem. Mol. Biol.* **2019**, *186*, 4–21. [[CrossRef](#)]

9. Bikle, D.D. Vitamin D: Newer Concepts of Its Metabolism and Function at the Basic and Clinical Level. *J. Endocr. Soc.* **2020**, *4*, bvz038. [[CrossRef](#)]
10. Jenkinson, C. The vitamin D metabolome: An update on analysis and function. *Cell Biochem. Funct.* **2019**, *37*, 408–423. [[CrossRef](#)]
11. Zmijewski, M.A.; Carlberg, C. Vitamin D receptor(s): In the nucleus but also at membranes? *Exp. Dermatol.* **2020**, *29*, 876–884. [[CrossRef](#)] [[PubMed](#)]
12. Carlberg, C. Vitamin D Genomics: From In Vitro to In Vivo. *Front. Endocrinol.* **2018**, *9*, 250. [[CrossRef](#)] [[PubMed](#)]
13. Haussler, M.R.; Jurutka, P.W.; Mizwicki, M.; Norman, A.W. Vitamin D receptor (VDR)-mediated actions of 1 α ,25(OH)₂vitamin D(3): Genomic and non-genomic mechanisms. *Best Pr. Res. Clin. Endocrinol. Metab.* **2011**, *25*, 543–559. [[CrossRef](#)] [[PubMed](#)]
14. Abhimanyu; Coussens, A.K. The role of UV radiation and vitamin D in the seasonality and outcomes of infectious disease. *Photochem. Photobiol. Sci.* **2017**, *16*, 314–338. [[CrossRef](#)] [[PubMed](#)]
15. Dankers, W.; Colin, E.M.; van Hamburg, J.P.; Lubberts, E. Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential. *Front. Immunol.* **2016**, *7*, 697. [[CrossRef](#)] [[PubMed](#)]
16. Chun, R.F.; Liu, P.T.; Modlin, R.L.; Adams, J.S.; Hewison, M. Impact of vitamin D on immune function: Lessons learned from genome-wide analysis. *Front. Physiol.* **2014**, *5*, 151. [[CrossRef](#)]
17. Bouillon, R.; Marcocci, C.; Carmeliet, G.; Bikle, D.; White, J.H.; Dawson-Hughes, B.; Lips, P.; Munns, C.F.; Lazaretti-Castro, M.; Giustina, A.; et al. Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. *Endocr. Rev.* **2019**, *40*, 1109–1151. [[CrossRef](#)]
18. Bikle, D.D. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem. Biol.* **2014**, *21*, 319–329. [[CrossRef](#)]
19. Slominski, R.M.; Raman, C.; Elmets, C.; Jetten, A.M.; Slominski, A.T.; Tuckey, R.C. The significance of CYP11A1 expression in skin physiology and pathology. *Mol. Cell Endocrinol.* **2021**, *530*, 111238. [[CrossRef](#)]
20. Slominski, A.T.; Li, W.; Kim, T.K.; Semak, I.; Wang, J.; Zjawiony, J.K.; Tuckey, R.C. Novel activities of CYP11A1 and their potential physiological significance. *J. Steroid Biochem. Mol. Biol.* **2015**, *151*, 25–37. [[CrossRef](#)]
21. Holick, M.F.; Smith, E.; Pincus, S. Skin as the site of vitamin D synthesis and target tissue for 1,25-dihydroxyvitamin D₃. Use of calcitriol (1,25-dihydroxyvitamin D₃) for treatment of psoriasis. *Arch. Dermatol.* **1987**, *123*, 1677–1683a. [[CrossRef](#)] [[PubMed](#)]
22. Reichrath, J.; Saternus, R.; Vogt, T. Endocrine actions of vitamin D in skin: Relevance for photocarcinogenesis of non-melanoma skin cancer, and beyond. *Mol. Cell Endocrinol.* **2017**, *453*, 96–102. [[CrossRef](#)] [[PubMed](#)]
23. Bikle, D.D. Vitamin D: An ancient hormone. *Exp. Dermatol.* **2011**, *20*, 7–13. [[CrossRef](#)]
24. Slominski, A.T.; Kim, T.K.; Li, W.; Tuckey, R.C. Classical and non-classical metabolic transformation of vitamin D in dermal fibroblasts. *Exp. Dermatol.* **2016**, *25*, 231–232. [[CrossRef](#)]
25. Slominski, A.T.; Kim, T.K.; Li, W.; Postlethwaite, A.; Tieu, E.W.; Tang, E.K.Y.; Tuckey, R.C. Detection of novel CYP11A1-derived secosteroids in the human epidermis and serum and pig adrenal gland. *Sci. Rep.* **2015**, *5*, 14875. [[CrossRef](#)]
26. Slominski, A.T.; Kim, T.K.; Shehabi, H.Z.; Semak, I.; Tang, E.K.; Nguyen, M.N.; Benson, H.A.; Korik, E.; Janjetovic, Z.; Chen, J.; et al. In vivo evidence for a novel pathway of vitamin D(3) metabolism initiated by P450scc and modified by CYP27B1. *FASEB J.* **2012**, *26*, 3901–3915. [[CrossRef](#)]
27. Slominski, A.T.; Chairprongsuk, A.; Janjetovic, Z.; Kim, T.K.; Stefan, J.; Slominski, R.M.; Hanumanthu, V.S.; Raman, C.; Qayyum, S.; Song, Y.; et al. Photoprotective properties of vitamin D and lumisterol hydroxyderivatives. *Cell Biochem. Biophys.* **2020**, *78*, 165–180. [[CrossRef](#)] [[PubMed](#)]
28. Miller, W.L.; Auchus, R.J. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr. Rev.* **2011**, *32*, 81–151. [[CrossRef](#)] [[PubMed](#)]
29. Slominski, R.M.; Tuckey, R.C.; Manna, P.R.; Jetten, A.M.; Postlethwaite, A.; Raman, C.; Slominski, A.T. Extra-adrenal glucocorticoid biosynthesis: Implications for autoimmune and inflammatory disorders. *Genes Immun.* **2020**, *21*, 150–168. [[CrossRef](#)]
30. Slominski, A.; Zjawiony, J.; Wortsman, J.; Semak, I.; Stewart, J.; Pisarchik, A.; Sweatman, T.; Marcos, J.; Dunbar, C.; Tuckey, R.C. A novel pathway for sequential transformation of 7-dehydrocholesterol and expression of the P450scc system in mammalian skin. *Europ. J. Biochem.* **2004**, *271*, 4178–4188. [[CrossRef](#)]
31. Slominski, A.T.; Janjetovic, Z.; Fuller, B.E.; Zmijewski, M.A.; Tuckey, R.C.; Nguyen, M.N.; Sweatman, T.; Li, W.; Zjawiony, J.; Miller, D.; et al. Products of vitamin D₃ or 7-dehydrocholesterol metabolism by cytochrome P450scc show anti-leukemia effects, having low or absent calcemic activity. *PLoS ONE* **2010**, *5*, e9907. [[CrossRef](#)] [[PubMed](#)]
32. Wang, J.; Slominski, A.; Tuckey, R.C.; Janjetovic, Z.; Kulkarni, A.; Chen, J.; Postlethwaite, A.E.; Miller, D.; Li, W. 20-hydroxyvitamin D inhibits proliferation of cancer cells with high efficacy while being non-toxic. *Anticancer Res.* **2012**, *32*, 739–746. [[PubMed](#)]
33. Slominski, A.T.; Kim, T.K.; Janjetovic, Z.; Tuckey, R.C.; Bieniek, R.; Yue, J.; Li, W.; Chen, J.; Nguyen, M.N.; Tang, E.K.; et al. 20-Hydroxyvitamin D₂ is a noncalcemic analog of vitamin D with potent antiproliferative and prodifferentiation activities in normal and malignant cells. *Am. J. Physiol. Cell Physiol.* **2011**, *300*, C526–C541. [[CrossRef](#)]
34. Chen, J.; Wang, J.; Kim, T.K.; Tieu, E.W.; Tang, E.K.; Lin, Z.; Kovacic, D.; Miller, D.D.; Postlethwaite, A.; Tuckey, R.C.; et al. Novel vitamin D analogs as potential therapeutics: Metabolism, toxicity profiling, and antiproliferative activity. *Anticancer Res.* **2014**, *34*, 2153–2163. [[PubMed](#)]
35. Slominski, A.; Janjetovic, Z.; Tuckey, R.C.; Nguyen, M.N.; Bhattacharya, K.G.; Wang, J.; Li, W.; Jiao, Y.; Gu, W.; Brown, M.; et al. 20S-hydroxyvitamin D₃, noncalcemic product of CYP11A1 action on vitamin D₃, exhibits potent antifibrogenic activity in vivo. *J. Clin. Endocrinol. Metab.* **2013**, *98*, E298–E303. [[CrossRef](#)] [[PubMed](#)]

36. Postlethwaite, A.E.; Tuckey, R.C.; Kim, T.K.; Li, W.; Bhattacharya, S.K.; Myers, L.K.; Brand, D.D.; Slominski, A.T. 20S-Hydroxyvitamin D₃, a secosteroid produced in humans, is anti-inflammatory and inhibits murine autoimmune arthritis. *Front. Immunol.* **2021**, *12*, 678487. [[CrossRef](#)]
37. Myers, L.K.; Winstead, M.; Kee, J.D.; Park, J.J.; Zhang, S.; Li, W.; Yi, A.K.; Stuart, J.M.; Rosloniec, E.F.; Brand, D.D.; et al. 1,25-Dihydroxyvitamin D₃ and 20-Hydroxyvitamin D₃ Upregulate LAIR-1 and Attenuate Collagen Induced Arthritis. *Int. J. Mol. Sci.* **2021**, *22*, 13342. [[CrossRef](#)]
38. Skobowiat, C.; Oak, A.S.; Kim, T.K.; Yang, C.H.; Pfeffer, L.M.; Tuckey, R.C.; Slominski, A.T. Noncalcemic 20-hydroxyvitamin D₃ inhibits human melanoma growth in in vitro and in vivo models. *Oncotarget* **2017**, *8*, 9823–9834. [[CrossRef](#)]
39. Janjetovic, Z.; Postlethwaite, A.; Kang, H.S.; Kim, T.K.; Tuckey, R.C.; Crossman, D.K.; Qayyum, S.; Jetten, A.M.; Slominski, A.T. Antifibrogenic Activities of CYP11A1-derived Vitamin D₃-hydroxyderivatives Are Dependent on RORgamma. *Endocrinology* **2021**, *162*, bqaa198. [[CrossRef](#)]
40. Kim, T.K.; Atigadda, V.; Brzeminski, P.; Fabisiak, A.; Tang, E.K.Y.; Tuckey, R.C.; Slominski, A.T. Detection of 7-dehydrocholesterol and vitamin D₃ derivatives in honey. *Molecules* **2020**, *25*, 2583. [[CrossRef](#)]
41. Jenkinson, C.; Desai, R.; Slominski, A.T.; Tuckey, R.C.; Hewison, M.; Handelsman, D.J. Simultaneous measurement of 13 circulating vitamin D₃ and D₂ mono and dihydroxy metabolites using liquid chromatography mass spectrometry. *Clin. Chem. Lab. Med.* **2021**, *59*, 1642–1652. [[CrossRef](#)] [[PubMed](#)]
42. Slominski, A.T.; Janjetovic, Z.; Kim, T.K.; Wasilewski, P.; Rosas, S.; Hanna, S.; Sayre, R.M.; Dowdy, J.C.; Li, W.; Tuckey, R.C. Novel non-calcemic secosteroids that are produced by human epidermal keratinocytes protect against solar radiation. *J. Steroid Biochem. Mol. Biol.* **2015**, *148*, 52–63. [[CrossRef](#)] [[PubMed](#)]
43. Kim, T.K.; Wang, J.; Janjetovic, Z.; Chen, J.; Tuckey, R.C.; Nguyen, M.N.; Tang, E.K.; Miller, D.; Li, W.; Slominski, A.T. Correlation between secosteroid-induced vitamin D receptor activity in melanoma cells and computer-modeled receptor binding strength. *Mol. Cell Endocrinol.* **2012**, *361*, 143–152. [[CrossRef](#)] [[PubMed](#)]
44. Slominski, A.T.; Kim, T.K.; Hobrath, J.V.; Oak, A.S.W.; Tang, E.K.Y.; Tieu, E.W.; Li, W.; Tuckey, R.C.; Jetten, A.M. Endogenously produced nonclassical vitamin D hydroxy-metabolites act as “biased” agonists on VDR and inverse agonists on RORalpha and RORgamma. *J. Steroid Biochem. Mol. Biol.* **2017**, *173*, 42–56. [[CrossRef](#)] [[PubMed](#)]
45. Lin, Z.; Chen, H.; Belorusova, A.Y.; Bollinger, J.C.; Tang, E.K.Y.; Janjetovic, Z.; Kim, T.K.; Wu, Z.; Miller, D.D.; Slominski, A.T.; et al. 1alpha,20S-dihydroxyvitamin D₃ interacts with vitamin D receptor: Crystal structure and route of chemical synthesis. *Sci. Rep.* **2017**, *7*, 10193. [[CrossRef](#)]
46. Lin, Z.; Marepally, S.R.; Goh, E.S.Y.; Cheng, C.Y.S.; Janjetovic, Z.; Kim, T.K.; Miller, D.D.; Postlethwaite, A.E.; Slominski, A.T.; Tuckey, R.C.; et al. Investigation of 20S-hydroxyvitamin D₃ analogs and their 1alpha-OH derivatives as potent vitamin D receptor agonists with anti-inflammatory activities. *Sci. Rep.* **2018**, *8*, 1478. [[CrossRef](#)]
47. Slominski, A.T.; Kim, T.K.; Janjetovic, Z.; Brozyna, A.A.; Zmijewski, M.A.; Xu, H.; Sutter, T.R.; Tuckey, R.C.; Jetten, A.M.; Crossman, D.K. Differential and overlapping effects of 20,23(OH)₂D₃ and 1,25(OH)₂D₃ on gene expression in human epidermal keratinocytes: Identification of AhR as an alternative receptor for 20,23(OH)₂D₃. *Int. J. Mol. Sci.* **2018**, *19*, 3072. [[CrossRef](#)]
48. Song, Y.; Slominski, R.M.; Qayyum, S.; Kim, T.K.; Janjetovic, Z.; Raman, C.; Tuckey, R.C.; Song, Y.; Slominski, A.T. Molecular and structural basis of interactions of vitamin D₃ hydroxyderivatives with aryl hydrocarbon receptor (AhR): An integrated experimental and computational study. *Int. J. Biol. Macromol.* **2022**, *209*, 1111–1123. [[CrossRef](#)]
49. Brzeminski, P.; Fabisiak, A.; Slominski, R.M.; Kim, T.K.; Janjetovic, Z.; Podgorska, E.; Song, Y.; Saleem, M.; Reddy, S.B.; Qayyum, S.; et al. Chemical synthesis, biological activities and action on nuclear receptors of 20S(OH)D₃, 20S,25(OH)₂D₃, 20S,23S(OH)₂D₃ and 20S,23R(OH)₂D₃. *Bioorg. Chem.* **2022**, *121*, 105660. [[CrossRef](#)]
50. Slominski, A.T.; Kim, T.K.; Takeda, Y.; Janjetovic, Z.; Brozyna, A.A.; Skobowiat, C.; Wang, J.; Postlethwaite, A.; Li, W.; Tuckey, R.C.; et al. RORalpha and ROR gamma are expressed in human skin and serve as receptors for endogenously produced noncalcemic 20-hydroxy- and 20,23-dihydroxyvitamin D. *FASEB J.* **2014**, *28*, 2775–2789. [[CrossRef](#)]
51. Slominski, A.T.; Kim, T.K.; Qayyum, S.; Song, Y.; Janjetovic, Z.; Oak, A.S.W.; Slominski, R.M.; Raman, C.; Stefan, J.; Mier-Aguilar, C.A.; et al. Vitamin D and lumisterol derivatives can act on liver X receptors (LXRs). *Sci. Rep.* **2021**, *11*, 8002. [[CrossRef](#)] [[PubMed](#)]
52. Chaiprasongsuk, A.; Janjetovic, Z.; Kim, T.K.; Tuckey, R.C.; Li, W.; Raman, C.; Panich, U.; Slominski, A.T. CYP11A1-derived vitamin D₃ products protect against UVB-induced inflammation and promote keratinocytes differentiation. *Free Radic. Biol. Med.* **2020**, *155*, 87–98. [[CrossRef](#)] [[PubMed](#)]
53. Chaiprasongsuk, A.; Janjetovic, Z.; Kim, T.K.; Jarrett, S.G.; D’Orazio, J.A.; Holick, M.F.; Tang, E.K.Y.; Tuckey, R.C.; Panich, U.; Li, W.; et al. Protective effects of novel derivatives of vitamin D₃ and lumisterol against UVB-induced damage in human keratinocytes involve activation of Nrf2 and p53 defense mechanisms. *Redox Biol.* **2019**, *24*, 101206. [[CrossRef](#)] [[PubMed](#)]
54. Slominski, A.T.; Kim, T.K.; Li, W.; Yi, A.K.; Postlethwaite, A.; Tuckey, R.C. The role of CYP11A1 in the production of vitamin D metabolites and their role in the regulation of epidermal functions. *J. Steroid Biochem. Mol. Biol.* **2014**, *144PA*, 28–39. [[CrossRef](#)] [[PubMed](#)]
55. Janjetovic, Z.; Tuckey, R.C.; Nguyen, M.N.; Thorpe, E.M., Jr.; Slominski, A.T. 20,23-dihydroxyvitamin D₃, novel P450scc product, stimulates differentiation and inhibits proliferation and NF-kappaB activity in human keratinocytes. *J. Cell Physiol.* **2010**, *223*, 36–48. [[PubMed](#)]

56. Janjetovic, Z.; Zmijewski, M.A.; Tuckey, R.C.; DeLeon, D.A.; Nguyen, M.N.; Pfeffer, L.M.; Slominski, A.T. 20-Hydroxycholecalciferol, product of vitamin D3 hydroxylation by P450scc, decreases NF-kappaB activity by increasing IkappaB alpha levels in human keratinocytes. *PLoS ONE* **2009**, *4*, e5988. [[CrossRef](#)]
57. Slominski, A.T.; Kim, T.K.; Chen, J.; Nguyen, M.N.; Li, W.; Yates, C.R.; Sweatman, T.; Janjetovic, Z.; Tuckey, R.C. Cytochrome P450scc-dependent metabolism of 7-dehydrocholesterol in placenta and epidermal keratinocytes. *Int. J. Biochem. Cell Biol.* **2012**, *44*, 2003–2018. [[CrossRef](#)]
58. Guryev, O.; Carvalho, R.A.; Usanov, S.; Gilep, A.; Estabrook, R.W. A pathway for the metabolism of vitamin D3: Unique hydroxylated metabolites formed during catalysis with cytochrome P450scc (CYP11A1). *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14754–14759. [[CrossRef](#)]
59. Zmijewski, M.A.; Li, W.; Zjawiony, J.K.; Sweatman, T.W.; Chen, J.; Miller, D.D.; Slominski, A.T. Photo-conversion of two epimers (20R and 20S) of pregna-5,7-diene-3beta, 17alpha, 20-triol and their bioactivity in melanoma cells. *Steroids* **2009**, *74*, 218–228. [[CrossRef](#)]
60. Zmijewski, M.A.; Li, W.; Chen, J.; Kim, T.K.; Zjawiony, J.K.; Sweatman, T.W.; Miller, D.D.; Slominski, A.T. Synthesis and photochemical transformation of 3beta,21-dihydroxypregna-5,7-dien-20-one to novel secosteroids that show anti-melanoma activity. *Steroids* **2011**, *76*, 193–203. [[CrossRef](#)]
61. Slominski, A.; Kim, T.K.; Zmijewski, M.A.; Janjetovic, Z.; Li, W.; Chen, J.; Kusniatsova, E.I.; Semak, I.; Postlethwaite, A.; Miller, D.D.; et al. Novel vitamin D photoproducts and their precursors in the skin. *Dermato-Endocrinology* **2013**, *5*, 7–19. [[CrossRef](#)] [[PubMed](#)]
62. Zmijewski, M.A.; Li, W.; Zjawiony, J.K.; Sweatman, T.W.; Chen, J.; Miller, D.D.; Slominski, A.T. Synthesis and photo-conversion of androsta- and pregna-5,7-dienes to vitamin D3-like derivatives. *Photochem. Photobiol. Sci.* **2008**, *7*, 1570–1576. [[CrossRef](#)] [[PubMed](#)]
63. Slominski, A.T.; Kim, T.K.; Slominski, R.M.; Song, Y.; Janjetovic, Z.; Podgorska, E.; Reddy, S.B.; Song, Y.; Raman, C.; Tang, E.K.Y.; et al. Metabolic activation of tachysterol3 to biologically active hydroxyderivatives that act on VDR, AhR, LXRs, and PPARgamma receptors. *FASEB J.* **2022**, *36*, e22451. [[CrossRef](#)] [[PubMed](#)]
64. Li, W.; Chen, J.; Janjetovic, Z.; Kim, T.K.; Sweatman, T.; Lu, Y.; Zjawiony, J.; Tuckey, R.C.; Miller, D.; Slominski, A. Chemical synthesis of 20S-hydroxyvitamin D3, which shows antiproliferative activity. *Steroids* **2010**, *75*, 926–935. [[CrossRef](#)]
65. Tuckey, R.C.; Slominski, A.T.; Cheng, C.Y.; Chen, J.; Kim, T.K.; Xiao, M.; Li, W. Lumisterol is metabolized by CYP11A1: Discovery of a new pathway. *Int. J. Biochem. Cell Biol.* **2014**, *55*, 24–34. [[CrossRef](#)]
66. Slominski, A.T.; Kim, T.K.; Hobrath, J.V.; Janjetovic, Z.; Oak, A.S.W.; Postlethwaite, A.; Lin, Z.; Li, W.; Takeda, Y.; Jetten, A.M.; et al. Characterization of a new pathway that activates lumisterol in vivo to biologically active hydroxylumisterols. *Sci. Rep.* **2017**, *7*, 11434. [[CrossRef](#)]
67. Tuckey, R.C.; Li, W.; Ma, D.; Cheng, C.Y.S.; Wang, K.M.; Kim, T.K.; Jeayeng, S.; Slominski, A.T. CYP27A1 acts on the pre-vitamin D3 photoproduct, lumisterol, producing biologically active hydroxy-metabolites. *J. Steroid Biochem. Mol. Biol.* **2018**, *181*, 1–10. [[CrossRef](#)]
68. Chaiprasongsuk, A.; Janjetovic, Z.; Kim, T.K.; Schwartz, C.J.; Tuckey, R.C.; Tang, E.K.Y.; Raman, C.; Panich, U.; Slominski, A.T. Hydroxylumisterols, photoproducts of pre-vitamin D3, protect human keratinocytes against UVB-induced damage. *Int. J. Mol. Sci.* **2020**, *21*, 9374. [[CrossRef](#)]
69. Li, W.; Moore, M.J.; Vasilieva, N.; Sui, J.; Wong, S.K.; Berne, M.A.; Somasundaran, M.; Sullivan, J.L.; Luzuriaga, K.; Greenough, T.C.; et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **2003**, *426*, 450–454. [[CrossRef](#)]
70. Matsuyama, S.; Nagata, N.; Shirato, K.; Kawase, M.; Takeda, M.; Taguchi, F. Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. *J. Virol.* **2010**, *84*, 12658–12664. [[CrossRef](#)]
71. Glowacka, I.; Bertram, S.; Müller, M.A.; Allen, P.; Soilleux, E.; Pfefferle, S.; Steffen, I.; Tsegaye, T.S.; He, Y.; Gnirss, K.; et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J. Virol.* **2011**, *85*, 4122–4134. [[CrossRef](#)]
72. Shulla, A.; Heald-Sargent, T.; Subramanya, G.; Zhao, J.; Perlman, S.; Gallagher, T. A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. *J. Virol.* **2011**, *85*, 873–882. [[CrossRef](#)] [[PubMed](#)]
73. Hasegawa, H. Development of Corona-virus-disease-19 Vaccines. *JMA J.* **2021**, *4*, 187–190. [[CrossRef](#)] [[PubMed](#)]
74. Qayyum, S.; Mohammad, T.; Slominski, R.M.; Hassan, M.I.; Tuckey, R.C.; Raman, C.; Slominski, A.T. Vitamin D and lumisterol novel metabolites can inhibit SARS-CoV-2 replication machinery enzymes. *Am. J. Physiol. Endocrinol. Metab.* **2021**, *321*, E246–E251. [[CrossRef](#)] [[PubMed](#)]
75. Oristrell, J.; Oliva, J.C.; Subirana, I.; Casado, E.; Domínguez, D.; Toloba, A.; Aguilera, P.; Esplugues, J.; Fafián, P.; Grau, M. Association of Calcitriol Supplementation with Reduced COVID-19 Mortality in Patients with Chronic Kidney Disease: A Population-Based Study. *Biomedicines* **2021**, *9*, 509. [[CrossRef](#)] [[PubMed](#)]
76. Jin, Z.; Du, X.; Xu, Y.; Deng, Y.; Liu, M.; Zhao, Y.; Zhang, B.; Li, X.; Zhang, L.; Peng, C.; et al. Structure of M(pro) from SARS-CoV-2 and discovery of its inhibitors. *Nature* **2020**, *582*, 289–293. [[CrossRef](#)]
77. Amin, S.A.; Banerjee, S.; Singh, S.; Qureshi, I.A.; Gayen, S.; Jha, T. First structure-activity relationship analysis of SARS-CoV-2 virus main protease (Mpro) inhibitors: An endeavor on COVID-19 drug discovery. *Mol. Divers.* **2021**, *25*, 1827–1838. [[CrossRef](#)]

78. Yang, J.; Lin, X.; Xing, N.; Zhang, Z.; Zhang, H.; Wu, H.; Xue, W. Structure-Based Discovery of Novel Nonpeptide Inhibitors Targeting SARS-CoV-2 M(pro). *J. Chem. Inf. Model.* **2021**, *61*, 3917–3926. [[CrossRef](#)]
79. Mishra, A.; Rathore, A.S. RNA dependent RNA polymerase (RdRp) as a drug target for SARS-CoV2. *J. Biomol. Struct. Dyn.* **2021**, *40*, 6039–6051. [[CrossRef](#)]
80. Pugin, J.; Ricou, B.; Steinberg, K.P.; Suter, P.M.; Martin, T.R. Proinflammatory activity in bronchoalveolar lavage fluids from patients with ARDS, a prominent role for interleukin-1. *Am. J. Respir. Crit. Care Med.* **1996**, *153*, 1850–1856. [[CrossRef](#)]
81. Tisoncik, J.R.; Korth, M.J.; Simmons, C.P.; Farrar, J.; Martin, T.R.; Katze, M.G. Into the eye of the cytokine storm. *Microbiol. Mol. Biol. Rev.* **2012**, *76*, 16–32. [[CrossRef](#)] [[PubMed](#)]
82. Ragab, D.; Salah Eldin, H.; Taeimah, M.; Khattab, R.; Salem, R. The COVID-19 Cytokine Storm; What We Know So Far. *Front. Immunol.* **2020**, *11*, 1446. [[CrossRef](#)] [[PubMed](#)]
83. Schieber, M.; Chandel, N.S. ROS function in redox signaling and oxidative stress. *Curr. Biol.* **2014**, *24*, R453–R462. [[CrossRef](#)]
84. Ye, S.; Lowther, S.; Stambas, J. Inhibition of reactive oxygen species production ameliorates inflammation induced by influenza A viruses via upregulation of SOCS1 and SOCS3. *J. Virol.* **2015**, *89*, 2672–2683. [[CrossRef](#)] [[PubMed](#)]
85. Vlahos, R.; Stambas, J.; Bozinovski, S.; Broughton, B.R.; Drummond, G.R.; Selemidis, S. Inhibition of Nox2 oxidase activity ameliorates influenza A virus-induced lung inflammation. *PLoS Pathog.* **2011**, *7*, e1001271. [[CrossRef](#)]
86. Slominski, R.M.; Stefan, J.; Athar, M.; Holick, M.F.; Jetten, A.M.; Raman, C.; Slominski, A.T. COVID-19 and Vitamin D: A lesson from the skin. *Exp. Dermatol.* **2020**, *29*, 885–890. [[CrossRef](#)] [[PubMed](#)]
87. Herengt, A.; Thyrsted, J.; Holm, C.K. NRF2 in Viral Infection. *Antioxidants* **2021**, *10*, 1491. [[CrossRef](#)]
88. Podgorska, E.; Sniegocka, M.; Mycinska, M.; Trybus, W.; Trybus, E.; Kopacz-Bednarska, A.; Wiechec, O.; Krzykawska-Serda, M.; Elas, M.; Krol, T.; et al. Acute hepatologic and nephrologic effects of calcitriol in Syrian golden hamster (*Mesocricetus auratus*). *Acta Biochim. Pol.* **2018**, *65*, 351–358. [[CrossRef](#)]
89. Slominski, A.T.; Slominski, R.M.; Goepfert, P.A.; Kim, T.K.; Holick, M.F.; Jetten, A.M.; Raman, C. Reply to Jakovac and to Rocha et al.: Can vitamin D prevent or manage COVID-19 illness? *Am. J. Physiol. Endocrinol. Metab.* **2020**, *319*, E455–E457. [[CrossRef](#)]
90. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Kruger, N.; Herrler, T.; Erichsen, S.; Schiergens, T.S.; Herrler, G.; Wu, N.H.; Nitsche, A.; et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**, *181*, 271–280 e278. [[CrossRef](#)]
91. Perrotta, F.; Matera, M.G.; Cazzola, M.; Bianco, A. Severe respiratory SARS-CoV2 infection: Does ACE2 receptor matter? *Respir. Med.* **2020**, *168*, 105996. [[CrossRef](#)] [[PubMed](#)]
92. Shang, J.; Wan, Y.; Luo, C.; Ye, G.; Geng, Q.; Auerbach, A.; Li, F. Cell entry mechanisms of SARS-CoV-2. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 11727–11734. [[CrossRef](#)] [[PubMed](#)]
93. Song, Y.; Qayyum, S.; Greer, R.A.; Slominski, R.M.; Raman, C.; Slominski, A.T.; Song, Y. Vitamin D3 and its hydroxyderivatives as promising drugs against COVID-19: A computational study. *J. Biomol. Struct. Dyn.* **2021**, *17*, 11727–11734. [[CrossRef](#)] [[PubMed](#)]
94. Singh, A.; Dhar, R. A large-scale computational screen identifies strong potential inhibitors for disrupting SARS-CoV-2 S-protein and human ACE2 interaction. *J. Biomol. Struct. Dyn.* **2021**, *17*, 1–14. [[CrossRef](#)] [[PubMed](#)]
95. Shoemark, D.K.; Colenso, C.K.; Toelzer, C.; Gupta, K.; Sessions, R.B.; Davidson, A.D.; Berger, I.; Schaffitzel, C.; Spencer, J.; Mulholland, A.J. Molecular simulations suggest vitamins, retinoids and steroids as ligands of the free fatty acid pocket of the SARS-CoV-2 spike protein. *Angew. Chem. Int. Ed.* **2021**, *60*, 7098–7110. [[CrossRef](#)]
96. Mansouri, A.; Kowsar, R.; Zakariazadeh, M.; Hakimi, H.; Miyamoto, A. The impact of calcitriol and estradiol on the SARS-CoV-2 biological activity: A molecular modeling approach. *Sci. Rep.* **2022**, *12*, 717. [[CrossRef](#)]
97. Fidan, O.; Mujwar, S.; Kciuk, M. Discovery of adapalene and dihydrotachysterol as antiviral agents for the Omicron variant of SARS-CoV-2 through computational drug repurposing. *Mol. Divers.* **2022**, 1–3. [[CrossRef](#)]
98. Shalayel, M.H.; Al-Mazaideh, G.M.; Aladaileh, S.H.; Al-Swailmi, F.K.; Al-Thiabat, M.G. Vitamin D is a potential inhibitor of COVID-19: In silico molecular docking to the binding site of SARS-CoV-2 endoribonuclease Nsp15. *Pak. J. Pharm. Sci.* **2020**, *33*, 2179–2186. [[CrossRef](#)]
99. Dai, W.; Zhang, B.; Jiang, X.M.; Su, H.; Li, J.; Zhao, Y.; Xie, X.; Jin, Z.; Peng, J.; Liu, F.; et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science* **2020**, *368*, 1331–1335. [[CrossRef](#)]
100. Lung, J.; Lin, Y.S.; Yang, Y.H.; Chou, Y.L.; Shu, L.H.; Cheng, Y.C.; Liu, H.T.; Wu, C.Y. The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase. *J. Med. Virol.* **2020**, *92*, 693–697. [[CrossRef](#)]
101. Yin, W.; Mao, C.; Luan, X.; Shen, D.D.; Shen, Q.; Su, H.; Wang, X.; Zhou, F.; Zhao, W.; Gao, M.; et al. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science* **2020**, *368*, 1499–1504. [[CrossRef](#)] [[PubMed](#)]
102. Zu, S.; Deng, Y.-Q.; Zhou, C.; Li, J.; Li, L.; Chen, Q.; Li, X.-F.; Zhao, H.; Gold, S.; He, J.; et al. 25-Hydroxycholesterol is a potent SARS-CoV-2 inhibitor. *Cell Res.* **2020**, *30*, 1043–1045. [[CrossRef](#)] [[PubMed](#)]
103. Wang, S.; Li, W.; Hui, H.; Tiwari, S.K.; Zhang, Q.; Croker, B.A.; Rawlings, S.; Smith, D.; Carlin, A.F.; Rana, T.M. Cholesterol 25-Hydroxylase inhibits SARS-CoV-2 and other coronaviruses by depleting membrane cholesterol. *EMBO J.* **2020**, *39*, e106057. [[CrossRef](#)] [[PubMed](#)]
104. Wnętrzak, A.; Chachaj-Brekiesz, A.; Kuś, K.; Lipiec, E.; Dynarowicz-Latka, P. Oxysterols can act antiviral through modification of lipid membrane properties—The Langmuir monolayer study. *J. Steroid Biochem. Mol. Biol.* **2022**, *220*, 106092. [[CrossRef](#)] [[PubMed](#)]

105. Dror, A.A.; Morozov, N.; Daoud, A.; Namir, Y.; Yakir, O.; Shachar, Y.; Lifshitz, M.; Segal, E.; Fisher, L.; Mizrachi, M.; et al. Pre-infection 25-hydroxyvitamin D3 levels and association with severity of COVID-19 illness. *PLoS ONE* **2022**, *17*, e0263069. [[CrossRef](#)]
106. Takahashi, H.; Nomura, H.; Iriki, H.; Kubo, A.; Isami, K.; Mikami, Y.; Mukai, M.; Sasaki, T.; Yamagami, J.; Kudoh, J.; et al. Cholesterol 25-hydroxylase is a metabolic switch to constrain T cell-mediated inflammation in the skin. *Sci. Immunol.* **2021**, *6*, eabb6444. [[CrossRef](#)]
107. Chiodini, I.; Gatti, D.; Soranna, D.; Merlotti, D.; Mingiano, C.; Fassio, A.; Adami, G.; Falchetti, A.; Eller-Vainicher, C.; Rossini, M.; et al. Vitamin D Status and SARS-CoV-2 Infection and COVID-19 Clinical Outcomes. *Front. Public Health* **2021**, *9*, 736665. [[CrossRef](#)]
108. Kaya, M.O.; Pamukçu, E.; Yakar, B. The role of vitamin D deficiency on COVID-19: A systematic review and meta-analysis of observational studies. *Epidemiol. Health* **2021**, *43*, e2021074. [[CrossRef](#)]
109. Leaf, D.E.; Ginde, A.A. Vitamin D3 to Treat COVID-19: Different Disease, Same Answer. *JAMA* **2021**, *325*, 1047–1048. [[CrossRef](#)]
110. Kazemi, A.; Mohammadi, V.; Aghababae, S.K.; Golzarand, M.; Clark, C.C.T.; Babajafari, S. Association of Vitamin D Status with SARS-CoV-2 Infection or COVID-19 Severity: A Systematic Review and Meta-analysis. *Adv. Nutr.* **2021**, *12*, 1636–1658. [[CrossRef](#)]
111. Bae, J.H.; Choe, H.J.; Holick, M.F.; Lim, S. Association of vitamin D status with COVID-19 and its severity: Vitamin D and COVID-19: A narrative review. *Rev. Endocr. Metab. Disord.* **2022**, *23*, 579–599. [[CrossRef](#)] [[PubMed](#)]
112. Rastogi, A.; Bhansali, A.; Khare, N.; Suri, V.; Yaddanapudi, N.; Sachdeva, N.; Puri, G.D.; Malhotra, P. Short term, high-dose vitamin D supplementation for COVID-19 disease: A randomised, placebo-controlled, study (SHADE study). *Postgrad. Med. J.* **2022**, *98*, 87–90. [[CrossRef](#)] [[PubMed](#)]
113. Martineau, A.R.; Forouhi, N.G. Vitamin D for COVID-19: A case to answer? *Lancet Diabetes Endocrinol.* **2020**, *8*, 735–736. [[CrossRef](#)]
114. Lau, F.H.; Majumder, R.; Torabi, R.; Saeg, F.; Hoffman, R.; Cirillo, J.D.; Greiffenstein, P. Vitamin D insufficiency is prevalent in severe COVID-19. *medRxiv* **2020**. [[CrossRef](#)]
115. Luo, X.; Liao, Q.; Shen, Y.; Li, H.; Cheng, L. Vitamin D Deficiency Is Associated with COVID-19 Incidence and Disease Severity in Chinese People. *J. Nutr.* **2021**, *151*, 98–103. [[CrossRef](#)]
116. Chauss, D.; Freiwald, T.; McGregor, R.; Yan, B.; Wang, L.; Nova-Lamperti, E.; Kumar, D.; Zhang, Z.; Teague, H.; West, E.E.; et al. Autocrine vitamin D signaling switches off pro-inflammatory programs of TH1 cells. *Nat. Immunol.* **2022**, *23*, 62–74. [[CrossRef](#)]
117. Quesada-Gomez, J.M.; Lopez-Miranda, J.; Entrenas-Castillo, M.; Casado-Díaz, A.; Nogues, Y.S.X.; Mansur, J.L.; Bouillon, R. Vitamin D Endocrine System and COVID-19: Treatment with Calcifediol. *Nutrients* **2022**, *14*, 2716. [[CrossRef](#)]