

## Article

# Exploring the Antiviral Potential of Esters of Cinnamic Acids with Quercetin

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**Abstract:** Severe acute respiratory syndrome-related Coronavirus 2 (SARS-CoV-2) has infected more than 762 million people to date and has caused approximately 7 million deaths all around the world, involving more than 187 countries. Although currently available vaccines show high efficacy in preventing severe respiratory complications in infected patients, the high number of mutations in the S proteins of the current variants is responsible for the high level of immune evasion and transmissibility of the virus and the reduced effectiveness of acquired immunity. In this scenario, the development of safe and effective drugs of synthetic or natural origin to suppress viral replication and treat acute forms of COVID-19 remains a valid therapeutic challenge. Given the successful history of flavonoids-based drug discovery, we developed esters of substituted cinnamic acids with quercetin to evaluate their in vitro activity against a broad spectrum of Coronaviruses. Interestingly, two derivatives, the 3,4-methylenedioxy 6 and the ester of acid 7, have proved to be effective in reducing OC43-induced cytopathogenicity, showing interesting EC<sub>50</sub>s profiles. The ester of synaptic acid 7 in particular, which is not endowed with relevant cytotoxicity under any of the tested conditions, turned out to be active against OC43 and SARS-CoV-2, showing a promising EC<sub>50</sub>. Therefore, said compound was selected as the lead object of further analysis. When tested in a yield reduction, assay 7 produced a significant dose-dependent reduction in viral titer. However, the compound was not virucidal, as exposure to high concentrations of it did not affect viral infectivity, nor did it affect hCoV-OC43 penetration into pre-treated host cells. Additional studies on the action mechanism have suggested that our derivative may inhibit viral endocytosis by reducing viral attachment to host cells.

**Keywords:** quercetin; cinnamic acid esters; flavonoids; Coronaviruses; antivirals



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## 1. Introduction

Coronaviruses (CoVs) are a family of enveloped, positive-sense single-stranded RNA viruses. The family Coronaviridae is found within the order Nidovirales, suborder Coronavirineae, and is distinguished in the subfamily Orthocoronavirinae, which includes four

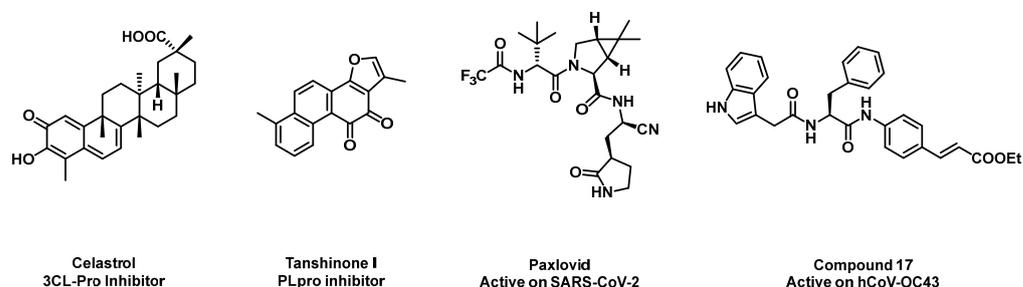
genera: alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus. The first two genera infect only mammalian species, while the others have a wider host range which also includes avian species [1].

The human coronaviruses HCoV-229E and HCoV-OC43, together with the more recently discovered HCoV-NL63 and HCoV-HKU1, cause mild respiratory infections associated with “common cold” symptoms, while the severe acute respiratory syndrome coronaviruses (SARS-CoV and SARS-CoV-2) and Middle East respiratory syndrome (MERS-CoV) are highly pathogenic in humans, infecting both bronchial epithelial cells and upper airway cells and [1] pneumocytes.

SARS-CoV-2 belongs to the Severe Acute Respiratory Syndrome-related coronavirus species in the subgenus Sarbecovirus. Since 2019 it has spread rapidly in the human population after likely spillover from bats and intermediate hosts, causing the COVID-19 pandemic known as the “coronavirus disease 2019”. Since the first case occurred on 17 November 2019, in Wuhan, the World Health Organization has confirmed more than 700 million cases among more than 200 countries, and more than 7 million deaths [2].

This pathogen affects the upper and lower respiratory tracts, has an efficient human-to-human transmission and causes typical clinical manifestations such as fever, dry cough, fatigue and in some cases even serious problems such as respiratory failure and death, as well as systemic involvement due to the massive inflammatory syndrome caused by the virus, especially in the elderly and in patients with comorbidities [3].

To date, several compounds have been shown to be active at the different stages of the viral cycle (Figure 1) such as entry and replication, and monoclonal antibody therapy may also be used. Nevertheless, the most effective method for a long-term prevention and control strategy is vaccination: for this reason, especially in recent years, different types of vaccines have been developed, through the employment of various platforms including recombinant vectors, DNA, mRNA in lipid nanoparticles, inactivated or live attenuated [4] viruses and protein subunits.



**Figure 1.** Natural and synthetic compounds active on SARS-CoV-2 and hCoV-OC43 [5–8].

Since the beginning of the COVID-19 pandemic, a considerable part of pharmaceutical research has been directed towards the discovery of new and effective antiviral drugs useful to treat and/or prevent the disease and to be available in case of future health emergencies. The search for potential antiviral agents has also led to the exploration of natural substances [9] and synthetic compounds that showcase great potential, biocompatibility and safety and represent, together with the re-proposal of proven antiviral molecules, a better and more practical approach to defeat the ongoing pandemic. With this purpose, several synthetic small molecules have been studied and were added to the first oral antiviral drug Paxlovid.

Quercetin, a flavonol abundant in fruit and vegetables and widely used as a food supplement to strengthen the immune system, is also studied for its biological properties, such as antioxidant, anti-inflammatory and immunomodulatory activities. The combination of said actions make it one of the most clinically studied supplements, although most studies in the literature report controversial results on its therapeutic role regarding COVID-19.

Several studies have highlighted the potential use of these flavonoids as antivirals [10–13], thanks to their ability to inhibit the initial stages of virus infection, interact

with viral proteases and reduce inflammation caused by infection. Cinnamic acid and its derivatives have been reported for their biological activity as anti-inflammatory and anti-proliferative compounds thanks to their cellular protection properties and reduction of factors that trigger metabolic syndrome [14–16]. Ferulic acid possesses a variety of biological properties, such as antioxidant [17,18], anti-inflammatory [19,20], antibacterial [21], anticancer [22] and antiviral [23–26] activities and cardiovascular protective effects [27].

Sinapic acid, another cinnamic family derivative, also possesses various disease-modifying properties implicated with oxidative stress [28,29].

3,4-Dihydroxycinnamic acid, also known as caffeic acid, can affect cancer, diabetes, atherosclerosis and Alzheimer's disease, as well as infections [30]. Moreover, substituted cinnamic acids are also studied for their antiviral activity, especially on the Zika virus and Hepatitis C virus [31,32].

In this study, we have described the first esters of cinnamic acids with quercetin, reported their activity on SARS-CoV-2 and hCoV-OC43 and shown their potential as adjuvants of approved antiviral agents.

## 2. Materials and Methods

### 2.1. Cells and Viruses

Cell lines were purchased from the American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of Coronaviruses were the following: Monkey kidney (Vero-76) [ATCC CRL 1587 Cercopithecus Aethiops], Monkey kidney (Vero C1008, clone E6) [ATCC CRL 1586 Cercopithecus Aethiops].

Human coronaviruses were: (i) Coronaviridae: Betacoronavirus strain OC43 (ATCC VR-1558), Alphacoronavirus strain 229E (ATCC VR-740), Betacoronavirus SARS-CoV-2 (strain VR PV10734) clinical isolate, kindly provided by Lazzaro Spallanzani Hospital, Rome, Italy. All experimental steps involving the SARS-CoV-2 virus were performed in a biosafety level 3 (BSL3) containment laboratory.

### 2.2. Cytotoxicity Assays

Vero-76 cells or Caco-2 (as reported in Supplementary Materials [33]) were seeded in 96-well plates at an initial density of  $3 \times 10^5$ /mL, in Minimum Essential Medium with Earle's salts (MEM-E), L-glutamine, 1 mM sodium pyruvate and 25 mg/L kanamycin, supplemented with 10% fetal bovine serum (FBS). Cell cultures were then incubated at 37 °C in a humidified, 5% CO<sub>2</sub>-enriched atmosphere, in the absence or presence of serial dilutions of test compounds. The test medium used for the cytotoxic assay as well as for the antiviral assay contained 1% of the appropriate serum. Cell viability was determined after 24, 72 (Quercetin and 7) or 120 h at 37 °C by the MTT method [34].

### 2.3. Antiviral Assays

The compound's activity against 229E, OC43 and EVA71 was based on the inhibition of virus-induced cytopathogenicity in Vero-76 cells acutely infected with a m.o.i. of 0.01. After 3 (229E), 5 (EVA71) or 5/6 (OC43) days of incubation at 35 °C or 37 °C (EVA71), cell viability was determined by the MTT method, as described previously [35]. The compound's activity against SARS-CoV-2 was determined by plaque reduction assays in infected cell monolayers, as described previously [36].

### 2.4. Yield Reduction Assay

Vero-76 cells were inoculated with OC43 at a m.o.i. of 0.1 in a maintenance medium and tested compounds (7) at non-cytotoxic concentrations. Following a 2 h adsorption period at 35 °C and 5% CO<sub>2</sub>, the inoculum was removed and replaced with fresh medium containing the same concentration of tested compounds. After 120 h at 35 °C and 5% CO<sub>2</sub>, each sample was harvested and diluted with serial passages, starting from 10<sup>-1</sup> up to 10<sup>-8</sup>. The titer of the serial dilutions of the virus-containing supernatant was determined

by the Reed and Muench method. Remdesivir and Hydroxychloroquine were used as reference compounds.

### 2.5. OC43 Virucidal Activity Assay

The title compound (20  $\mu\text{M}$ ) was incubated with  $1 \times 10^5$  TCID<sub>50</sub>/mL of OC43 at either 4 or 37 °C for 1 h. The mixture without a test sample was used as control. At the end of the incubation period, samples were serially diluted in media, and titers were determined on Vero-76 and Vero E6 cells at high dilutions, at which the compound was not active. Virus titers were determined by the Reed and Muench method in Vero-76 cells.

### 2.6. Cell Pretreatment Assay

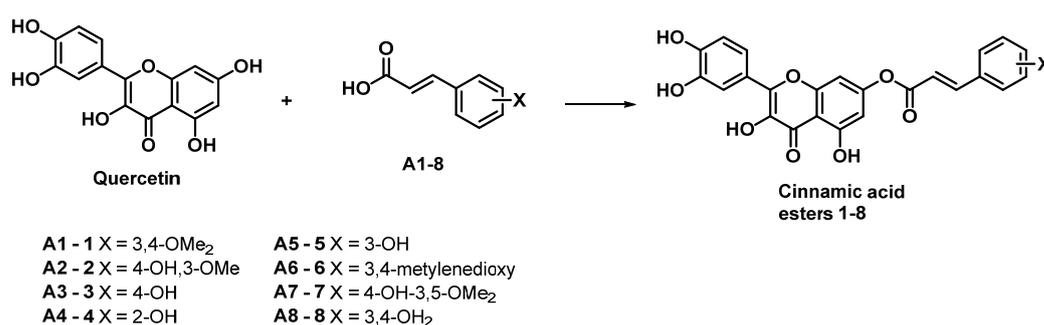
Vero-76 and Vero E6 cell monolayers in 24-well plates were incubated with 20  $\mu\text{M}$  concentration of compound 7 or references (100, 20, 4, 0.8  $\mu\text{M}$ ) for 2 h at 4 °C. After the removal of the compounds and two gentle washes, cells were infected with OC43 and SARS-CoV-2. After virus adsorption to cells, the inoculum was removed, and the cells were overlaid with medium and incubated for 4 days at 37 °C; then, finally, virus titers were determined by the Reed and Muench method and plaque assay, respectively.

### 2.7. Adsorption Assays

Vero-76 cells and E6 grown in 96- and 24-well plates were infected with OC43 and SARS-CoV-2, respectively, with an m.o.i. of 0.1, in the presence or absence of compound 7. Multiwells were incubated for 60 min at 4 °C. Medium containing unabsorbed virus was then removed, and cells were washed twice with PBS and overlaid with the medium. Plaques were counted after 72 h of incubation at 37 °C for SARS-CoV-2, while titer reduction was determined after 144 h of incubation at 35 °C by the Reed and Muench method for OC43.

## 3. Results and Discussion

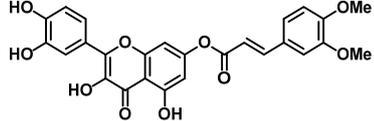
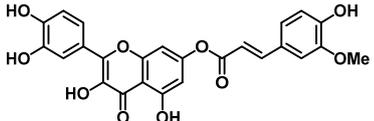
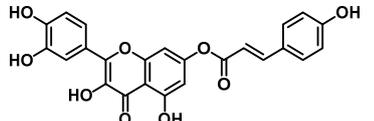
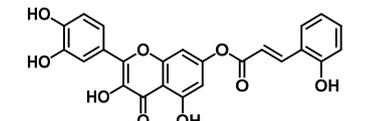
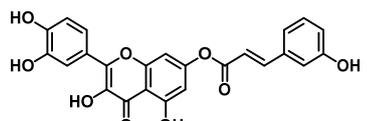
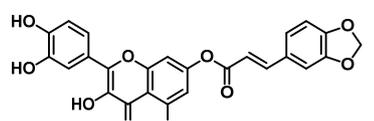
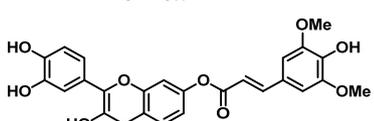
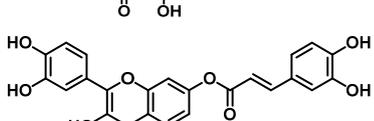
This study explored the antiviral properties of esters of hydroxycinnamic acids with quercetin in order to investigate their potential antiviral activity on a broad spectrum of coronaviruses. The esters were prepared through a reaction between substituted cinnamic acid and quercetin, using EDCI as coupling agents in the presence of HOBt in dry acetonitrile (Figure 2) solution as described in Supplementary Materials.



**Figure 2.** Synthesis of cinnamic acid esters.

Quercetin, esters, and the parent cinnamic acids were tested in cell-based assays for cytotoxicity (CC<sub>50</sub>), potency (EC<sub>50</sub>), and broad-spectrum antiviral activity against a panel of alpha and beta coronaviruses. To ascertain their selectivity against coronaviruses, the derivatives were also tested against enterovirus EVA71, another representative positive-single-stranded RNA virus (Table S2). As reported in Table 1, the esters showed an assorted profile of cytotoxicity.

**Table 1.** Cytotoxicity and antiviral activity of Quercetin and its esters with cinnamic acids against SARS-CoV-2, hCoV OC43, and hCoV-229E viruses in Normal Monkey kidney (Vero-76, supporting SARS-CoV-2 and OC43 replication) cells and Human Lung cancer (SK-MES-1, supporting 229E replication) cell line.

Compound	Structure	Vero-76	SARS-CoV-2	OC43	SK-MES-1	229E
		<sup>a</sup> CC <sub>50</sub>	<sup>c</sup> EC <sub>50</sub>		<sup>b</sup> CC <sub>50</sub>	<sup>d</sup> EC <sub>50</sub>
1		54.7	>54.7	>54.7	-	-
2		11.4	>11.4	>11.4	-	-
3		60	47.7 ± 0.32	>60	-	-
4		78	61	>78	-	-
5		75	37	>75	-	-
6		30	>30	2.4 ± 0.56	33	>33
7		100	46.6	6.5 ± 0.7	>100	>100
8		70	26	15	>100	>100
Quercetin	-	20	>20	>20	>100	51
Remdesivir	-	>100	1.6 ± 0.4	-	>100	5 ± 1
Hydroxychloroquine	-	60	-	1.9 ± 0.8	-	-

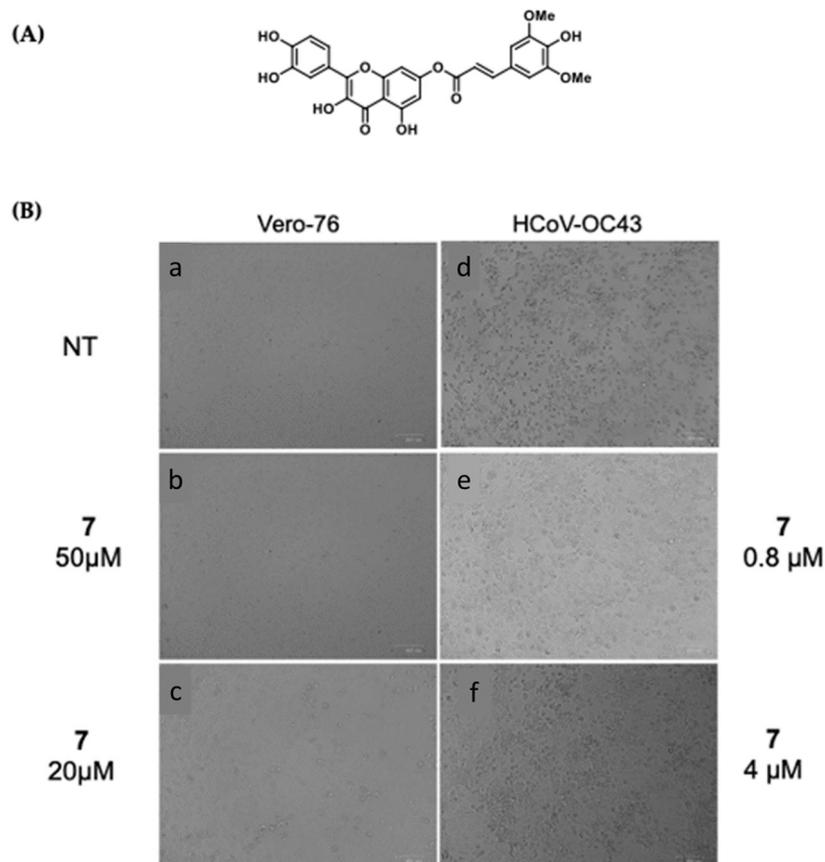
<sup>a</sup> Compd concn (μM) required to reduce by 50% the viability of mock-infected Vero-76 cells (<sup>a</sup> 120 h post-treatment), and SK-MES-1 (<sup>b</sup> 48 h post-treatment) cells, as determined by the MTT method. <sup>c</sup> Compd concn (μM) required to reduce by 50% the plaque number of SARS-CoV-2 or required to achieve 50% protection of Vero-76 cells from OC43 (<sup>d</sup>), induced cytopathogenicity, as determined by the MTT method. <sup>d</sup> Compd concn (μM) required to achieve 50% protection of SK-MES-1 cells from 229E, induced cytopathogenicity, as determined by the MTT method.

Cinnamic acids showed no cytotoxicity (Table S1), except for sinapic acid (CC<sub>50</sub> 44 μM). In general, the esters showed a lower level of cytotoxicity against the Vero-76 cell line than quercetin, with the sole exception of compound 2. Compound 1 showed CC<sub>50</sub> = 54.7 μM against Vero-76 and no activity on SARS-CoV-2 and OC43, while mono-substituted compound 3 showed a comparable cytotoxicity profile (CC<sub>50</sub> = 60 μM) and an EC<sub>50</sub> = 47.7 ± 0.32 μM against SARS-CoV-2. There was no evidence of activity against the

beta coronavirus OC43. Moving the -OH group from 4- to 2-position (compound 4) slightly reduced both cytotoxicity and antiviral activity, while analog ester 5 bearing the -OH in 3 position showed  $CC_{50} = 75 \mu\text{M}$  and an  $EC_{50} = 37 \mu\text{M}$  against SARS-CoV-2.

The 3,4-methylenedioxy derivative (compound 6) showed weak cytotoxicity ( $CC_{50} = 30 \mu\text{M}$ ) but effectively reduced the OC43-induced cytopathogenicity, showing an  $EC_{50} = 2.4 \pm 0.56 \mu\text{M}$ .

Interestingly, the ester of sinapic acid 7, which is not endowed with relevant cytotoxicity under any of the conditions tested ( $CC_{50} = 100 \mu\text{M}$ , Tables 1 and S2), proved active against OC43 (Figure 3B) and SARS-CoV-2, showing a promising  $EC_{50}$  profile ( $EC_{50} = 6.5 \pm 0.7 \mu\text{M}$  and  $EC_{50} = 46.6 \mu\text{M}$ , respectively).



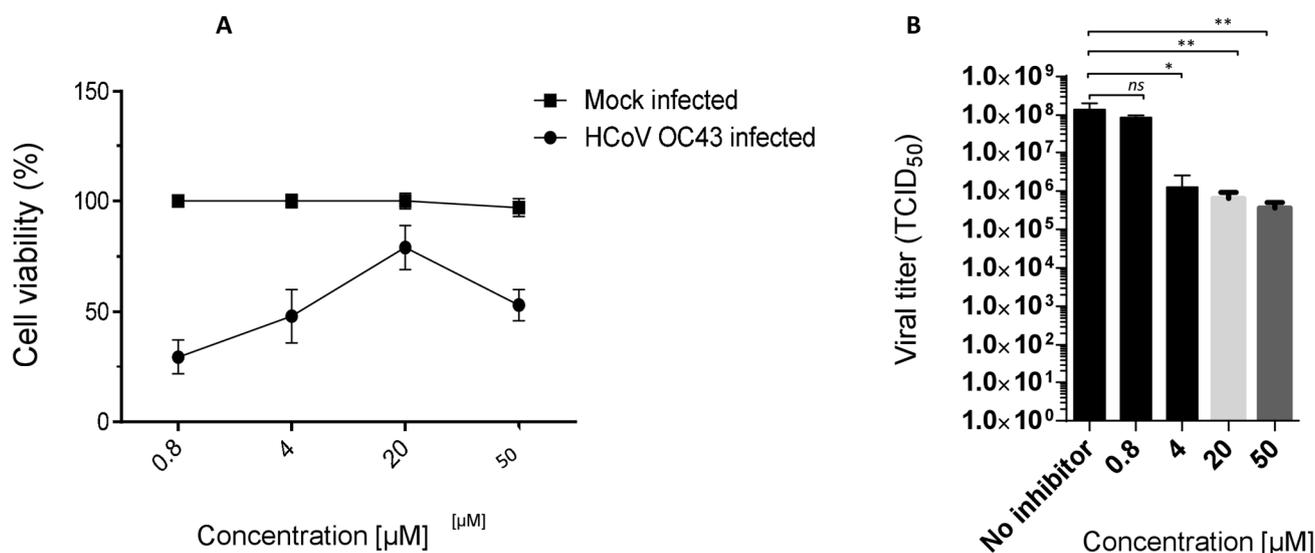
**Figure 3.** (A) The chemical structure of ester 7 and (B) validation of the effect of 7 (50, 20, 4, and 0.8  $\mu\text{M}$ ) on the Vero-76 HCoV-OC43-infected monolayers. Untreated control cells (a), treated and infected cells with 50  $\mu\text{M}$  (b), treated and infected cells with 20  $\mu\text{M}$  (c), treated and infected cells with 4  $\mu\text{M}$  (f), treated and infected cells with 0.8  $\mu\text{M}$  (e), infected cells (d). Pictures of cell morphology were taken at 120 h post-infection using a ZOE Fluorescent cell imager (Bio-Rad, Hercules, CA, USA) (bar size = 100  $\mu\text{m}$ , magnification, 20 $\times$ ).

Ester 7 proved the most interesting compound of the series (Figure 4A) against the OC43 virus, with the best selectivity index (Supplementary Materials = 15).

The ester of caffeic acid (8) also exhibited activity against SARS-CoV-2 ( $EC_{50} = 26 \mu\text{M}$ ) and OC43 ( $EC_{50} = 15 \mu\text{M}$ ) followed by weak cytotoxicity on Vero-76 ( $CC_{50} = 70 \mu\text{M}$ ).

These results highlighted that the presence of ether groups is generally related to the best profile of antiviral activity with low toxicity. The compounds 3–5, 8 bearing only hydroxy groups are endowed with low antiviral activity. However, the ether position and nature are crucial for its activity. Thus, the replacement of the 3,4-dimethoxyphenyl group of compound 1 with a 3,4-methylenedioxy group led to an improvement in activity (compound

6). The displacement of the ether groups in 3- and 5-positions and the introduction of a 4-hydroxy group afforded the best ester of series 7.



**Figure 4.** Cytotoxicity and anti OC43 activity of 7 and Yield reduction assay. (A) The viability of OC43-infected Vero-76 cells was estimated by MTT assay, six days after infection. The number of live cells was expressed as a percentage of mock-infected, untreated control cells. Data are expressed as means  $\pm$  SD of at least three independent measurements. (B) The yield of infectious HCoV-OC43 viruses produced in infected Vero-76 cells treated with 7. Vero-76 cells were infected with OC43 (m.o.i. 0.1). The infected monolayers were treated with 7, at indicated doses. Viral yields in the culture supernatant were determined by reed and Munch titration at 96 h post-infection. Statistical differences were analyzed via Student's *t*-test, a value of  $p \leq 0.05$  was considered significant, with \* =  $p \leq 0.05$ , \*\* =  $p < 0.01$ , ns: not significant.

The cytotoxicity of Quercetin and 7 were evaluated, in the short (24, 72 h) and long term (144 h), also against Caco-2 cells, human colorectal adenocarcinoma cells commonly employed as a model of the intestinal epithelial barrier. No cytotoxicity was detected for Quercetin against Vero-76 cells after 24 h; however, its toxicity increased with time (after 144 h  $CC_{50} = 20 \mu\text{M}$ ) followed by no activity on SARS-CoV-2 and OC43 (Table 1). Cytotoxicity to Caco-2 showed a similar trend,  $CC_{50}$  being 88.7  $\mu\text{M}$  and 61  $\mu\text{M}$  after 24 and 72 h, respectively (Table S2). No relevant cytotoxicity was confirmed for compound 7 against Caco-2 cells at 24 h, while a slight increase was observed at 72 h (Table S2).

To assess the selectivity of the lead compounds against beta-coronaviruses, they were also tested for cytotoxicity and antiviral activity against an alternative, representative positive-sense single-stranded RNA virus, EVA-71.

Notably, none of the esters was able to reduce the cytopathogenicity induced by EV-A71 (Table S2), confirming the active selectivity against the betacoronaviruses tested in this study.

### 3.1. Ester 7 Effect on Viral Yield

The antiviral activity of compound 7, selected as lead, against the OC43 strain was confirmed in a virus yield reduction assay (YRA) against Vero-76, as reported in Figure 4B. The concentrations of 50, 20, 4 and 0.8  $\mu\text{M}$  (not cytotoxic) were employed, and a significant dose-dependent reduction in the viral titer was observed. An interesting reduction in the OC43 titer (2 logs) was also detected at a low concentration of 4  $\mu\text{M}$  (Figure 4B).

### 3.1.1. Ester 7 Virucidal Activity Assay

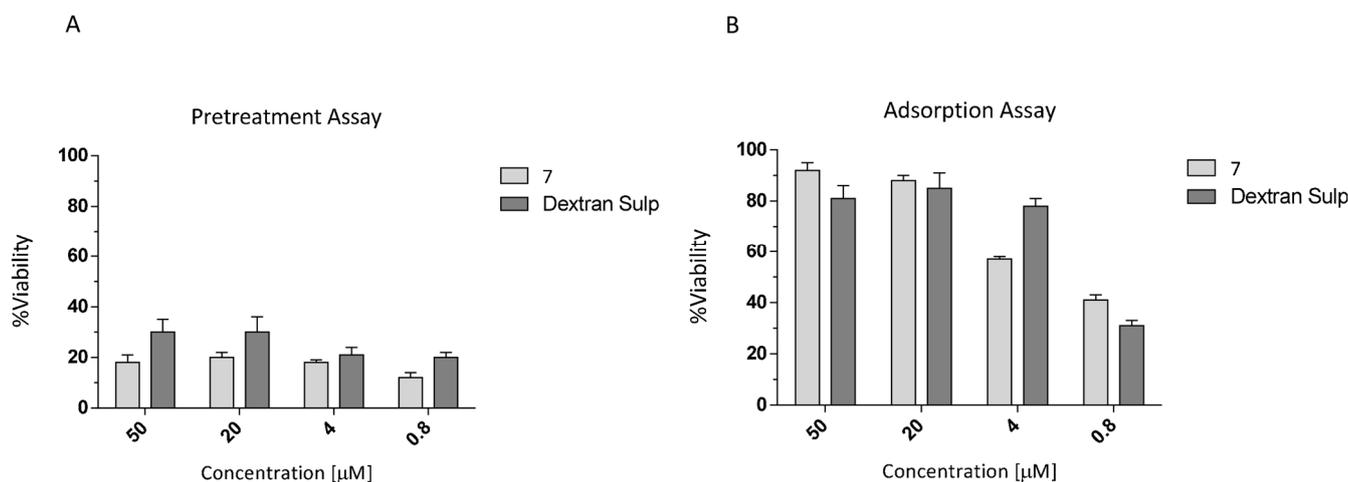
To investigate the potential mechanisms of antiviral action, ester 7 was first subjected to a direct virus inactivation assay. The effect of 7 on hCoV-OC43 inactivation infectivity, before cell infection, was analyzed at 0 °C and 37 °C in an OC43 virucidal activity assay. The virus titers of samples treated with 7 did not significantly differ from those determined in untreated samples. Then, ester 7 did not exert its inhibitory effect by a direct inactivation of the hCoV-OC43 virion.

### 3.1.2. Effect of Compound 7 on hCoV-OC43 Penetration into Pre-Treated Host Cells

To determine whether compound 7 was able to protect cells from hCoV-OC43 infection, a pre-treatment assay was then performed by incubating Vero-76 cell monolayers (2 h) with different concentrations of the conjugate (50, 20, 4, 0.8 µM). Dextran sulphate (DS), a broad-spectrum RNA/DNA-enveloped virus attachment inhibitor, was employed as a negative control.

The unbound compounds were washed off, and the Vero-76 cells were then infected with OC43. The percentage of cell viability was determined after six days.

The results reported in Figure 5A indicate that, under our experimental conditions, 7 failed to inhibit OC43 infection at the analyzed time point, as well as DS. These data show that pre-treatment with 7 does not protect monolayers from OC43 infection.



**Figure 5.** Cell Pretreatment Assay (A) and the dose-dependent inhibitory effect (B) of ester 7 on hCoV-OC43 adsorption. Data are expressed as means  $\pm$  SD of at least three independent measurements.

### 3.2. Adsorption Assay

We characterized the adsorption kinetic of hCoV-OC43 viral particles in the presence of ester 7. We investigated whether 7 was able to interfere with the binding of the virus to target cells by incubating OC43 and cell monolayers at 4 °C in the presence of the compound. As reported in Figure 5B, 7 and Dextran sulphate (as positive control) were efficiently able to block OC43 viral binding under these conditions.

## 4. Discussion

Medicinal plants and their constituents are the basic source of many drugs and should be thoroughly studied to prove their safety and efficacy in human therapy, according to the World Health Organization (WHO) [37]. Flavonoids are phenolic compounds that are widespread in the plant world: more specifically, they are secondary metabolites produced by plants and are classified into different types, according to their chemical structure.

Among the different flavonoids found in nature, the main subgroups are Anthoxanthins (flavanone and flavanol), flavans, flavanonols, flavanones, anthocyanidins, chalcones and isoflavonoids [10].

As these natural products have shown positive effects on human health, there has been an increasing effort to characterize their biological potential. In this context, we have carried out extensive research into flavonoids [36–40] and evaluated their efficacy as a complementary therapy to more specific antiviral compounds. More specifically, we have developed esters of cinnamic acids with quercetin to evaluate their cytotoxicity and potential antiviral activity in vitro.

Quercetin and cinnamic acids were evaluated alone and in parallel with their esters, against the coronavirus HCoV-229E, HCoV-OC43, and SARS-CoV-2.

Recently, several articles have been published on quercetin and its ability to protect against coronaviruses [41].

Yue Zhu and co-authors have described the inhibitory effects of quercetin on the replication of HCoV-229E in Huh-7 cells with an estimated  $EC_{50}$  value of 4.88  $\mu$ M [41] and on the Mpro activity of SARS-CoV-2 ( $IC_{50}$  = 6.79  $\mu$ M).

Interestingly, quercetin and sinapic acid showed no anti-beta coronavirus activity in our assay. No inhibitory effect was observed when ferulic acid was evaluated in vitro, in a cell-based assay against HCoV-229E, HCoV-OC43 or SARS-CoV-2, and quercetin showed only weak activity against the alfa coronavirus 229E ( $EC_{50}$  = 51  $\mu$ M).

Esters were evaluated for broad-spectrum anti-coronavirus activity, and 4, 7 and 8 were found to be active against OC43, while 7 and 8 also showed activity against SARSCoV-2. None of them showed activity against the 229E strain or EVA71, employed as a representative positive RNA virus, suggesting a selective activity against beta coronaviruses.

Ester 7 showed an interesting antiviral activity (Table 1 and Figure 3), and it did not exert any toxic effects on various human cells, thus proving to be safe at the concentrations tested. Considering that it had the best safety profile, 7 was further investigated. When evaluated in a Yield reduction assay, it significantly reduced the viral titer in a dose-dependent manner ( $p < 0.05$ ;  $p < 0.01$ ).

Cells pre-treated with 7 showed no inhibition of OC43 replication, suggesting that the compound did not act directly on cell receptors; moreover, the treatment of virions did not determine the direct inactivation of the virion. Furthermore, we showed that 7 reduced the adsorption and penetration of OC43 into cell monolayers by the adsorption assay when compared to untreated samples. Data showed that it was able to block the attachment of OC43 to the host cell. We can hypothesize that 7 most likely prevents the viral envelope from binding to the cell membrane, thereby blocking subsequent stages of infection.

The interpretation of these results must take into account the strengths and limitations of our study. The major strengths of our study include the characterization of new bioconjugates with broad beta coronavirus activity and the identification of ester 7, which is endowed with anti-OC43 and SARS-CoV-2 activity.

However, compared to other studies, under our experimental conditions, quercetin did not show relevant antiviral activity in vitro, confirming its low or no activity in vivo and highlighting the need for further evidence on the biological properties of many products of natural origin, which are often controversially proposed as solutions for serious diseases. In the meantime, it is necessary to clarify the effective mechanism of antiviral action of these promising bioconjugates.

## 5. Conclusions

Historically, natural products, either in the form of pure compounds or as standardised plant extracts, have been an incentive in the creation of new drugs. Given the recent pandemic emergency, and the resurgence of the emergency of new and neglected pathogens, the results highlight the importance of natural products as promising resources as medication for humans in the pharmaceutical industry. In this study, we have analyzed the antiviral properties of esters of hydroxycinnamic acids with quercetin. The ester of synaptic acid 7 in particular turned out to be active against OC43 and SARS-CoV-2, showcasing an ability to induce a significant dose-dependent reduction in viral titre.

Moreover, preliminary studies on the mode of action suggest a possible involvement of said esters in cellular viral penetration processes.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/v16050665/s1>, Table S1: Cytotoxicity and antiviral activity of the cinnamic acids; Table S2: Cytotoxicity of Quercetin and ester 7 against human cell lines.

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