Original Article

In vitro cytotoxicity and antiviral activity of aminocaproic acid against SARS-CoV-2

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ABSTRACT

Since the outbreak of the COVID-19 pandemic caused by the SARS-CoV-2 virus, there has been a pressing need for effective and accessible antiviral agents. Aminocaproic acid (Epsilon-aminocaproic acid, EACA), known primarily for its antifibrinolytic properties, has recently been in the spotlight for its potential cytotoxic and antiviral capabilities. This study aims to evaluate the in vitro cytotoxicity and antiviral activity of EACA against SARS-CoV-2.An in-depth in vitro analysis was conducted in isolated laboratories to determine the cytotoxic effects of EACA on cell cultures and its potential to inhibit SARS-CoV-2 replication. Assays to assess the direct in-vitro antiviral activity of EACA against the virus were performed (Vero E6 cell culture), considering viral entry inhibition and protein interaction disruption. The cytotoxic concentration (TTC50) for EACA on Vero E6 cell culture was set at 35,128 μg/ml ($p \leq 0.05$). EACA at concentrations of 1093 μg/ml, 2187 μg/ml, 4375 μg/ml, 8750 μg/ml, 17500 μg/ml, 25000 μg/ml, and 35000 μg/ml does not have therapeutic or virus inhibitory activity against the SARS-CoV-2 virus at a dose of 100 TCD50/0.2 ml. The use of EACA for the treatment or prevention of SARS-CoV-2 may not be effective. This highlights the need to continue searching for other effective candidates from the protease inhibitors group.

Keywords: Aminocaproic acid, SARS-CoV-2, COVID-19, Cytotoxicity, Antiviral agents, Protease inhibitors

Introduction

Since the initial official announcement of the Coronavirus Disease 2019 (COVID-19) in December 2019, the disease has posed significant challenges to public health, economic stability, and societal development globally [1].

The virus, SARS-CoV-2, responsible for COVID-19, has spread worldwide, causing a pandemic that has resulted in multitudinous loss of lives and a paralyzing effect on the global economy [2]. This backdrop underscores the critical need for developing new, effective, and accessible antiviral drugs [3].

SARS-CoV-2 structure shares similarities with other coronaviruses but also has some unique characteristics. It consists of a few vital components: viral envelope, spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, envelope (E) protein, and viral RNA [4-6]. For the SARS-CoV-2 virus replication cycle, the protease plays an essential role [7, 8]. Once the virus enters a host cell, it releases its RNA to hijack the cellular machinery and produce viral polyproteins [9, 10]. These polyproteins aren't functional in their initial form. Instead, they need to be cut into functional pieces to facilitate the formation of new viral particles [11, 12]. This cutting or cleaving process is where the protease comes into play. The protease enzyme of SARS-CoV-2 splits the polyproteins into individual proteins that are essential for the replication of the virus [13]. Without the action of the protease, the virus cannot reproduce and establish an infection effectively.

Given the pivotal role of protease in the virus's lifecycle, it becomes a promising target for antiviral drugs [14-16]. If the

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action of the protease can be inhibited, it might thwart the virus's ability to replicate, thereby curbing the infection [17]. This strategy isn't new and has been employed previously for other viruses [18]. For instance, protease inhibitors are a class of antiretroviral drugs used to treat HIV and Hepatitis C infections. With the advent of the COVID-19 pandemic, researchers worldwide have been investigating protease inhibitors that could be effective against the SARS-CoV-2 protease. Some existing drugs are being repurposed, while new ones are in development, all to disrupt the viral replication process.

Epsilon-aminocaproic acid (EACA) is an agent recognized primarily for its antifibrinolytic properties and has been widely used in medicine to prevent and treat hemorrhages [19]. EACA is an analog of the amino acid lysine [20]. In addition, EACA belongs to the protease inhibitors family. Recent studies have begun to uncover additional biological functions of EACA, including its potential cytotoxicity and antiviral activity [21, 22]. These properties have earmarked EACA as a prospective candidate for antiviral interventions, especially in the context of combating COVID-19 [23].

Cytotoxicity refers to the ability of a substance to cause damage or death to cells [24]. Within the scope of antiviral therapy, this attribute can be beneficial as viruses, including SARS-CoV-2, replicate inside host cells. Some research suggests that EACA might exert cytotoxic effects, thereby limiting the virus's replication [25].

Simultaneously, preliminary investigations also hint at EACA's direct antiviral activity. It might inhibit the virus's entry into the cell or interact with viral proteins, disrupting their functions [26, 27]. It's essential to highlight that current knowledge regarding EACA's cytotoxicity and antiviral activity against SARS-CoV-2 remains limited.

The heightened interest in EACA also stems from its widespread availability and relatively low cost, making it a potentially appealing option for countries with constrained resources. However, a cautious assessment of the possible risks and side effects associated with EACA's use as an antiviral is pivotal to ensure utmost safety and efficacy.

This research aimed to investigate the cytotoxicity and antiviral activity of EACA against SARS-CoV-2. Through a comprehensive analysis, we aspired to determine EACA's potential application in the battle against SARS-CoV-2, potentially paving new avenues for the treatment and prevention of this formidable disease.

Materials and Methods

Study design

Cell culture preparation

In this initial phase, the Vero E6 cell line was prepared for subsequent testing. This involved the cultivation, collection, and enumeration of viable cells. The living cells were then seeded onto 96-well plates to facilitate the subsequent stages of the research [28].

Cytotoxicity studies

This phase aimed to assess the potential cytotoxic effects of EACA on the Vero E6 cell line. Once the cell culture was prepared and plated, varying concentrations of EACA were introduced to the wells. The cells' viability post-exposure was then assessed. The gathered data underwent statistical analysis to decipher any significant cytotoxic trends associated with the acid [29].

Antiviral activity assessment

This critical phase was subdivided into three experiments, each exploring a different facet of the antiviral activity of EACA against SARS-CoV-2. This experiment aimed to understand the therapeutic potential of EACA. Specifically, its effectiveness post-infection was gauged. This would give insights into whether the compound can halt or reduce the viral load after the cells have been infected. In this setup, the Vero E6 cells were pre-treated with EACA before the introduction of the virus. The objective was to determine if pre-treatment could prevent or reduce the extent of infection, offering a potential preventive measure. This final experiment sought to evaluate the direct inhibitory effects of EACA on SARS-CoV-2. This would give insights into whether the compound can directly interfere with the virus's ability to function or replicate.

Study setting

The study was conducted at the Central Reference Laboratory (CRL) of the National Scientific Centre for Particularly Dangerous Infections named after M. Aikimbaeva (NNTSOOI).

Test substance

Aminocaproic acid, commonly referred to as 6-aminohexanoic acid or εaminocaproic acid (EACA), with the IUPAC designation 6-aminohexanoic acid, was the test compound of focus. Concentrations: 50000 µg/ml, 5000 µg/ml, 500 µg/ml, 50 µg/ml, 5 µg/ml, 0.5 µg/ml, 0.05 µg/ml, 0.005 µg/ml.

Equipment

During the study, a variety of specialized equipment was employed to ensure accurate and reproducible results. The CO2-incubators, models INCO 153 and CB 150 by MEMMERT and BINDER respectively, were utilized to maintain cells in precise environmental conditions with varying CO2 concentrations (ranging from 0-20%) and temperatures (between 20-45°C for INCO 153 and 5-50°C for CB 150). These incubators were vital for the proper growth and maintenance of cell cultures, ensuring that they were kept in optimal conditions simulating the physiological environment. The exact units were referenced with registration numbers CRL-CI-03 for the MEMMERT and both CRL-CI-03 and CRL-

CI-01 for the BINDER models, with the latter being specifically located in the BSL-3 section of the laboratory.

For accurate cell counting and viability assessments, the Countess™ II FL AMQAF1000 automated cell counter by INVITROGEN was employed. This device, registered under the code CRL-ACC-003, provided consistent and reliable measurements critical to maintaining the integrity of our experimental design and ensuring the right concentrations of cells for our assays.

Lastly, the ELX808 photometer system by BIOTEK, specifically designed for microplates and automated at 220V, was used for our immunofluorescence assays (IFA). Positioned in the III, BSL-3 section of the laboratory and recorded under registration numbers CRL-EL-001 and CRL-EL-003, this system was essential for detecting and quantifying any potential antiviral effects of EACA against SARS-CoV-2 by measuring the fluorescence emitted from treated samples compared to controls.

The MTT test was carried out according to the MTT kit protocol for cell viability and proliferation **(Table 1)**.

Table 1. Structure of a 96-well plate for the test substance.

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
A	background	background	background	background	background	background	background	background	background	background	background	background
B	background	PC	T1	T2	T3	T4	T5	T6	T7	T8	NC	background
C	background	PC	T1	T2	T3	T4	T5	T6	T7	T8	NC	background
D	background	PC	T1	T2	T3	T4	T5	T6	T7	T8	NC	background
E	background	PC	T1	T2	T3	T4	T5	T6	T7	T8	NC	background
F	background	PC	T1	T2	T3	T4	T5	T6	T7	T8	NC	background
G	background	PC	T1	T2	T3	T4	T5	T6	T7	T8	NC	background
H	background	background	background	background	background	background	background	background	background	background	background	background

T1-T8 – concentration of the test substance; NC – negative control; PC – positive control (DMSO or other cytotoxic substance). Background – background control wells: 50 µl MTT reagent + 50 µl cell culture medium (without cells).

To study the antiviral effectiveness of the SARS-CoV-2 virus model, the virus was used at a dose of 100 TCD50/0.2 ml.

Cell culture

The VERO cell line, designated as C1008 [Vero 76, clone E6, Vero E6], was employed for the propagation of the SARS-CoV-2 virus. Originating from the kidney of Cercopithecus aethiops, this epithelial cell line exhibits some contact inhibition, making it especially suitable for propagating viruses that replicate slowly. Recognized as a clone of Vero 76, Vero E6 cells are particularly adapted for the cultivation of the SARS-CoV-2 pathogen responsible for COVID-19. Renowned for their robustness in virological assays due to their broad viral permissiveness, these cells were obtained from ATCC (Catalogue: CRL-1586) and were stringently verified to be devoid of contaminants, ensuring the integrity of our experiments.

Virus strain

The SARS-CoV-2 strain hCoV19/Kazakhstan/KazNAU-NSCEDI-481/2020, further referenced as strain number 481, belongs to the Coronaviridae family, specifically the Betacoronavirus genus. This particular strain was isolated at the M. Aikimbaeva National Scientific Centre for Especially Dangerous Infections within a BSL-3 (Biosafety Level 3) laboratory in June 2020 from nasopharyngeal swabs collected from suspected COVID-19 cases in Almaty. Cultivated in the Vero E6 cell line, the presence of the virus was confirmed through PCR detection of the SARS-CoV-2 N-gene. A notable genetic feature of this strain is the D614G mutation in the spike protein, with the complete protein sequence available in the GISAID database under the accession number EPI_ISL_514093. Exhibiting an infectious activity of 6.00 lg TCID50/cm^3 in Vero E6 cells, this strain serves as a foundational element in the development and testing of immune-biological preparations, encompassing diagnostic test kits, immunoglobulins, and vaccines. Additionally, it is pivotal for assessing the antiviral efficacy of various substances both in vitro and in vivo. The strain's selection for this study was premised on its relevance to our research goals and was sourced from the aforementioned center, cataloged under the unique reference KKZI KA294. Rigorous checks ensured the strain was uncontaminated, thus upholding the precision and safety of our investigative methodologies.

The TCA50 value obtained in phase 2 of the experiment to determine the cytotoxicity of EACA on Vero E6 cell culture was used to conduct studies to assess the antiviral activity of EACA. DMEM (2% FBS) was used as a solvent for IV and to prepare a working dilution of the SARS-CoV2 virus. During the exposure, the antiviral activity of EACA against the SARS-CoV-2 virus was carried out according to a therapeutic, prophylactic, and virus inhibitory scheme, and to determine it, the test substance was prepared at six 2-fold concentrations of EACA starting from the TCA50 **(Table 2)**.

Table 2. Determination of preventive, therapeutic, virus-inhibiting activity of aminocaproic acid (EACA).

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	mg/ml, thus.	B	B	B	B	B	B	B	B	B
B	B	PC	T1/35.0	T1/35.0	T1/35.0	T1/35.0	T1/35.0	T1/35.0	T1/35.0	T1/35.0	NC	B
C	B	PC	T2/17.5	T2/17.5	T2/17.5	T2/17.5	T2/17.5	T2/17.5	T2/17.5	T2/17.5	NC	B
D	B	PC	T3/8.75	T3/8.75	T3/8.75	T3/8.75	T3/8.75	T3/8.75	T3/8.75	T3/8.75	NC	B
E	B	PC	T4/4.37	T4/4.37	T4/4.37	T4/4.37	T4/4.37	T4/4.37	T4/4.37	T4/4.37	NC	B
F	B	PC	T5/2.19	T5/2.19	T5/2.19	T5/2.19	T5/2.19	T5/2.19	T5/2.19	T5/2.19	NC	B
G	B	PC	T6/1.09	T6/1.09	T6/1.09	T6/1.09	T6/1.09	T6/1.09	T6/1.09	T6/1.09	NC	B
H	B	B	B	B	B	B	B	B	B	B	B	B

T1-T8 – concentration of the test substance; NC – negative control; PC – positive control (DMSO or other cytotoxic substance). B – background control wells: 50 µl MTT reagent + 50 µl cell culture medium (without cells).

Statistical analysis

Statistical analysis was conducted using SPSS software (version 25.0, IBM SPSS Inc., Chicago, Illinois, USA). To determine the dose-response, Tukey's multiple comparison tests were used.

Results and Discussion

This study assessed the cytotoxicity of EACA in Vero E6 cell culture. For the evaluation, a 96-well technique was used, where different concentrations of EACA were studied. The key indicator of the success of our study is the TCA50 (cytotoxic concentration at which 50% of cells die). As can be seen from **Table 3**, Vero E6 cells showed different sensitivity to EACA depending on the concentration. This is manifested by changes in the percentage of cell viability at different acid concentrations. The TCA50 cycle for EACA on Vero E6 cell culture was set at 35128 µg/ml ($p \leq 0.05$).

Table 3. Determination of cytotoxicity of aminocaproic acid (EACA).

N=3		PC DMSO	50000 T1	5000 T1	500 T3	50 T4	5 T5	0.5 T6	0.05 T7	0.005 T8	NC DMEM	
EACA	1	2	3	4	5	6	7	8	9	10	11	12
A	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM
B	DMEM	0.221	0.256	1.111	1.253	1.315	1.252	1.013	0.990	1.050	0.974	DMEM
C	DMEM	0.234	0.244	1.086	1.389	1.300	1.295	1.303	1.322	1.317	1.455	DMEM
D	DMEM	0.221	0.250	1.038	1.292	1.324	1.314	1.052	1.155	1.278	0.874	DMEM
E	DMEM	0.240	0.258	1.242	1.218	1.361	1.222	1.156	1.282	1.348	1.023	DMEM
F	DMEM	0.227	0.233	1.180	1.118	1.125	1.169	0.950	1.267	1.226	1.282	DMEM
G	DMEM	0.241	0.234	1.237	1.237	1.421	1.186	1.303	1.409	1.375	1.053	DMEM
H	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM	DMEM
ArithMean_group		**0.231**	**0.246**	**1.133**	**1.251**	**1.308**	**1.240**	**1.129**	**1.238**	**1.266**	**1.110**	
% Viability_B		22.7	26.3	114.1	128.6	135.0	128.6	104.0	101.7	107.8	100.0	
% Viability_C		16.1	16.8	74.6	95.4	89.3	89.0	89.5	90.9	90.5	100.0	
% Viability_D		25.2	28.6	118.8	147.8	151.5	150.3	120.4	132.2	146.2	100.0	
% Viability_E		23.5	25.2	121.4	119.1	133.1	119.4	113.0	125.3	131.8	100.0	
% Viability_F		17.7	18.2	92.0	87.2	87.8	91.2	74.1	98.8	95.6	100.0	
% Viability_G		22.9	22.3	108.4	117.5	135.0	112.7	123.7	133.9	130.6	100.0	
% Viability_Group		**21.3**	**22.9**	**104.9**	**115.9**	**121.9**	**115.2**	**104.1**	**113.8**	**117.1**	**100.0**	
SID_Group		3.6	4.7	18.1	22.1	26.7	23.2	19.2	18.8	22.4	0.0	
CC_{50}			**35128**									

Study of therapeutic activity

Within the experimental framework, the impact of epsilon-aminocaproic acid (EACA) was analyzed on Vero E6 cell cultures infected with the SARS-CoV-2 virus. As per **Table 4**, the therapeutic activity of EACA in vitro concerning the SARS-CoV-2 virus manifests across varied substance concentrations. A "+" denotes the presence of cytopathic effect (CPE), indicating the virus's adverse impact on the cells. Conversely, a "-" highlights the absence of CPE, potentially implying that EACA obstructs virus replication or blocks its effect on the cells.

For concentrations of 35.0, 17.5, 8.75, 4.37, 2.19, and 1.09 µg/ml, the presence of CPE is observed, suggesting the

insufficient efficacy of EACA at these dosages to prevent the virus's cytopathic effect on cell cultures.

Table 4. Virus-inhibitory activity of aminocaproic acid (EACA).

Prophylaxis (n=3)	1	2	3	4	5	6	7	8	9	10	11	12
A	D	D	Aminicaproic acid (EACA), mkg/ml, thous.								D	D
B	D	PC(+)	35.0(+)	35.0(+)	35.0(+)	35.0(+)	35.0(+)	35.0(+)	35.0(+)	35.0(+)	NC(-)	D
C	D	PC(+)	17.5(+)	17.5(+)	17.5(+)	17.5(+)	17.5(+)	17.5(+)	17.5(+)	17.5(+)	NC(-)	D
D	D	PC(+)	8.75(+)	8.75(+)	8.75(+)	8.75(+)	8.75(+)	8.75(+)	8.75(+)	8.75(+)	NC(-)	D
E	D	PC(+)	4.37(+)	4.37(+)	4.37(+)	4.37(+)	4.37(+)	4.37(+)	4.37(+)	4.37(+)	NC(-)	D
F	D	PC(+)	2.19(+)	2.19(+)	2.19(+)	2.19(+)	2.19(+)	2.19(+)	2.19(+)	2.19(+)	NC(-)	D
G	D	PC(+)	1.09(+)	1.09(+)	1.09(+)	1.09(+)	1.09(+)	1.09(+)	1.09(+)	1.09(+)	NC(-)	D
H	D	D	D	D	D	D	D	D	D	D	D	D

PC – positive control (DMSO or other cytotoxic substance)

The negative control (NC) illustrates a lack of CPE, confirming the virus's inactivity in this sample.

Study of prophylactic activity

An evaluation was conducted of the prophylactic activity of EACA concerning Vero E6 cell cultures infected with the SARS-CoV-2 virus.

All tested concentrations of EACA (35.0, 17.5, 8.75, 4.37, 2.19, and 1.09 µg/ml) display a cytopathic effect (CPE), marked by the "+" symbol. This indicates that the virus continues to adversely affect the cells despite the application of EACA.

The negative control (NC) demonstrates the absence of CPE, confirming the virus's inactivity in this sample.

Study of virus-inhibitory activity

The experiment aimed to evaluate the virus-inhibitory activity of EACA on cell model (Vero E6 cell cultures) infected with the SARS-CoV-2 virus.

All examined concentrations of EACA (25.0, 17.5, 8.75, 4.37, 2.19, and 1.09 µg/ml) show the presence of a cytopathic effect (CPE), denoted by the "+" symbol. This highlights that the virus continues to actively affect the cells even when EACA is applied.

The negative control (NC) illustrates a lack of CPE, underscoring the virus's inactivity in this sample **(Table 4)**. As a result of the studies, it was established that EACA in concentrations of 1093 µg/ml, 2187 µg/ml, 4375 µg/ml, 8750 µg/ml, 17500 µg/ml, 25000 µg/ml, and 35000 µg/ml in culture Vero E6 cells do not have therapeutic, prophylactic or virus inhibitory activity against the SARS-CoV-2 virus (strain hCoV-19/Kazakhstan/KazNAUNSCEDI-481/2020) at a dose of 100 TCD50/0.2 ml **(Table 5)**.

Table 5. Indicators of therapeutic, prophylactic, and virus-inhibitory activity of aminocaproic acid (EACA) against the SARS-CoV-2 virus.

Studied substance	Substance concentration, mg/ml	Viral load, $TCID_{50}$	Complete inhibition of cytopathic effect 100% (CIA100)
	Therapeutic activity		
Aminocaproic acid (EACA)	35 000	100	No
	17 500	100	No
	8 750	100	No
	4 375	100	No
	2 187	100	No
	1093	100	No
	Prophylactic activity		
Aminocaproic acid (EACA)	35 000	100	No
	17 500	100	No
	8 750	100	No
	4 375	100	No
	2 187	100	No
	1093	100	No
	Virus-inhibitory activity		
Aminocaproic acid (EACA)	35 000	100	No
	17 500	100	No
	8 750	100	No
	4 375	100	No
	2 187	100	No
	1093	100	No

Given the important role of proteolytic processing for viral propagation, one of the possible targets for chemotherapy of viral infection is to block the proteolytic cleavage of virus proteins [25]. The major protease (Mpro) is known to be vital for the replication of SARS-CoV-2, so SARS-CoV-2 Mpro is considered an important drug target for COVID-19 therapy [30]. To date, thousands of SARS-CoV-2 Mpro inhibitor candidate compounds have been screened to identify potent drug candidates [31-33]. However, many drugs have been shown to have little or no clinical potential as it is not common to achieve significant inhibition where high concentration is required [34].

The antiviral effect of EACA against some viruses has been demonstrated previously [34-37]. Moreover, EACA has been shown to reduce hemagglutinin cleavage and proteolytic activation of the virus in cultured cells, chick embryos, and lungs of infected mice [27]. Cleavage of hemagglutinin ensures the fusion of the viral and host cell membranes before the nucleocapsid is released into the cytoplasm. Some preclinical studies demonstrate that proteolytic enzyme inhibitors, including EACA, have therapeutic efficacy against influenza virus infection [21, 38]. EACA is known to be an active protease inhibitor that inhibits plasminogen activation and, at higher concentrations, noncompetitively inhibits plasmin [39]. Therefore, it may inhibit activation of the SARS-CoV-2 spike protein by plasmin [39]. However, its mechanism of action may not affect SARS-CoV-2 replication or its ability to cause cytopathic effects in Vero E6 cells.

The results of our cytotoxicity tests (the MTT test) showed that Vero E6 cells possess different sensitivity to EACA depending on the concentration, which is also consistent with similar previous studies to determine the cytotoxicity of substances [40, 41]. This is reflected in the change in the percentage of cell viability at different doses of EACA. The specific concentration of EACA at which 50% of cells die (TCC50) is 35128 μg/ml. This parameter serves as a key indicator of the toxicity of the compound to cells [42]. Previously, we studied the minimal cytotoxicity of EACA on another cell model (human peripheral blood mononuclear cells (PBMCs) [43].

Given the high TCA50 value, it can be assumed that EACA is relatively safe for Vero E6 cells at lower concentrations. In many studies assessing the antiviral activity of EACA, the protease inhibitor EACA showed this effectiveness only when used in combination with certain substances [25, 44].

In another study, the in vitro antiviral effect of EACA was investigated in Caco-2 cell culture by staining the SARS-CoV-2 antigen (adhesion protein), and visually assessing the cytopathogenic effect (CPE) [45]. The antiviral activity of EACA was manifested when non-toxic concentrations of the drug were used and did not depend on the time of application of ACC (before the introduction of the virus, simultaneously with the pathogen, after 1 hour of incubation) [45].

Lutsyuk *et al*. studied cytotoxicity and antiviral activity of 4-aminomethyl benzoic and 6-aminocaproic acids, based on the macrocyclic diaza-18-crown-6 platform against human influenza strains A/Hong Kong/1/68 (H3N2) and A/Puerto Rico/8/34 (H1N1) [35]. The authors used a model based on the Colpoda stein infusoria culture and the chorioallantoic membrane cells of chick embryos. The compounds showed significantly higher levels of antiviral activity compared to that of 4-aminomethylbenzoic and 6-aminocaproic acids on both strains of the influenza virus with no cytotoxicity demonstrated in the studied concentrations. The compounds with 6-aminocaproic acid fragments were more active toward both virus strains [35].

However, despite the promise that EACA can inhibit the vital proteases of SARS-CoV-2 (Mpro), in our study, EACA was not effective enough to block the cytopathic effects of SARS-CoV-2 on Vero E6 cell cultures at all concentrations tested.

Conclusion

It has been shown that some protease inhibitors hold potential as effective preventive measures against the onset and advancement of COVID-19. They act by obstructing the entry of the SARS-CoV-2 virus through mechanisms involving the angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) [46]. Furthermore, they can mitigate ensuing inflammation, coagulation disorders, and multi-organ failure. Based on this hypothesis, we tested the anti-viral ability of EACA (that belongs to the protease inhibitors family) against SARS-CoV-2. The results of in vitro studies demonstrated that EACA was not able to suppress replication of the SARS-CoV-2 virus (in vitro conditions). This indicates that the use of EACA for the treatment or prevention of SARS-CoV-2 in vitro may not be effective. This highlights the need to continue searching for and testing other potential compounds to combat the SARS-CoV-2 virus.

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