REVIEW

by the official reviewer of the dissertation by Khaydarova Saken Zharqynuly on the topic "Study of the antiviral activity of drugs against the SARS-CoV-2 virus in vitro, " submitted for the degree of Doctor of Philosophy (PhD) in the specialty "8D05110 - Virology"

№	Criteria	Meets the criteria (underline one answer)	Justification of the official reviewer's position (comments should be highlighted in italics)
1.	The topic of the dissertation (as of the date of its approval) corresponds to the directions of scientific development and/or state programs	1.1 Compliance with priority areas of scientific development or state programs:	The dissertation was completed within the framework of the grant funding project AP09260672 on the topic: "Study of the antiviral activity of tenofovir alafenamide and other pharmacological drugs against the SARS-CoV-2 virus in vitro and in vivo" (2021–2023), funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.
		1) the dissertation was completed within the framework of a project or target program financed from the state budget (indicate the name and number of the project or program);	
		2) the dissertation was completed within the framework of another state program (indicate the name of the program);	
		3) the dissertation corresponds to the priority direction of scientific development approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan (indicate the direction).	
2.	Scientific significance.	The work makes / does not make a significant contribution to science, and its importance is well demonstrated / not demonstrated.	The dissertation contributes to molecular virology and experimental pharmacology, as it demonstrates using a SARS-CoV-2 strain circulating in the Republic of Kazakhstan, the conservativeness of RdRP as an antiviral target and provides a comparative assessment of the efficacy of several RdRP inhibitors, including the determination of their selectivity and safe concentrations for the first time.
3.	Principle of independence.	Level of independence:	The aim of the study, the defined objectives, and the obtained experimental results generally indicate that the candidate conducted the research independently, including
		1) high;	
		2) average;	

		3) low;	performing experiments, collecting and performing initial data analysis, as well as interpreting the results. The work covers an extensive range of experimental models — from *in vitro* virological assays to immunological experiments with IL-33 in mice. The depth of description of the *in vitro* experiments significantly exceeds that of the molecular and immunological sections.
		4) no independence.	
4.	Principle of internal consistency	4.1 Justification of the dissertation's relevance:	The dissertation is relevant in the fields of molecular virology and experimental pharmacology: well-known antiviral pharmaceutical agents were evaluated in cell lines; however, the pathogenetic mechanisms of their action on RdRP can be considered indirect, and the assessment of their effects on mouse immune cells is of a preliminary or indirect nature.
		1) substantiated;	
		2) partially substantiated;	
		3) not substantiated.	
		4.2 The content of the dissertation reflects the dissertation topic:	The content of the dissertation topic generally corresponds to the stated research theme: it presents the molecular-genetic characteristics of SARS-CoV-2 isolated in Kazakhstan, as well as the evaluation of the antiviral activity of pharmaceutical agents *in cell* lines and *in vivo*.
		1) reflects;	
		2) partially reflects;	
		3) does not reflect.	
		4.3. The aim and objectives correspond to the dissertation topic:	The aim and objectives clearly reflect the topic of the dissertation, ensuring consistency between the experimental work, the obtained data, and their interpretation.
		1) correspond;	
		2) partially correspond;	
		3) do not correspond.	
		4.4 All sections and provisions of the dissertation are logically interconnected:	The main sections of the dissertation (Introduction, Materials and Methods, Results, Discussion) follow a logical sequence and reflect the experimental work with SARS-CoV-2, including the assessment of antiviral activity of the agents, cytotoxicity, and RdRP inhibition. At the same time, the connection between *in vitro* studies on the Vero E6 cell line and *in vivo* experiments with IL-33 and lymphoid cells is presented indirectly; that is, a direct explanation of how cellular data translate to the immune response in mice is not fully elucidated.
		1) completely interconnected;	
		2) partial connection;	
		3) no connection.	

		4.5 The author's proposed new solutions (principles, methods) are substantiated and compared with existing solutions:	The dissertation includes an assessment of cytotoxicity and RdRP inhibition efficacy using a SARS-CoV-2 model for the known antiviral agents Tenofovir, Ribavirin, and Favipiravir. It was established that Dexamethasone exhibits no antiviral activity.
		1) critical analysis is present;	
		2) partial analysis;	
		3) analysis does not represent the author's own opinions, but rather quotations from other authors;	
		4) no analysis.	
5.	The principle of scientific novelty	5.1 Are the scientific results and findings new?	1. The interaction of SARS-CoV-2 with host cells has been experimentally substantiated, and the mechanisms of pharmacological inhibition of viral replication have been elucidated. It is known that the biologically relevant Vero E6 cell system (non-human) exhibits high permissiveness to SARS-CoV-2, which confirms the experimental justification of the direct effects of pharmaceutical agents — Tenofovir (Tenvir) in two forms: the tablet form TDF (Tenofovir Disoproxil Fumarate) and the pure compound TAF (Tenofovir Alafenamide), tablet forms of Ribavirin, Favipiravir (Fabiflu), and the glucocorticoid steroid Dexamethasone — on viral replication in living cells. The registered pharmaceutical forms involved belong to different pharmacological groups with distinct pathogenetic mechanisms of action against the virus, but share a common effect on RdRP (NSP12). 2. A comparative assessment of cytotoxicity and antiviral activity of the pharmaceutical agents was performed. In particular, the use of CCK-8 and MTT assays allowed differentiation between cell death resulting from virus-induced cytopathic effects and drug-related toxicity, thereby demonstrating the absence of changes in cellular metabolic activity during viral replication inhibition. 3. The comparative characterization of the activity of one
		1) completely new;	
		2) partially new (25-75% new);	
		3) not new (less than 25% new).	

			of the key viral replication enzymes, RdRP (NSP12), in the Alpha variant circulating in Kazakhstan versus the Wuhan strain demonstrated that the replication complex activity of both strains is susceptible to inhibition by pharmaceutical agents. 4. Variation of the multiplicity of infection (MOI) and analysis of viral titer established RdRP inhibition under conditions of high viral load, indicating that the pharmaceutical agents shift the balance toward preservation of cellular function, reducing virus-induced cell damage in addition to their virus-inhibitory activity. In other words, the agents are capable of mitigating virus-induced cellular damage while maintaining cell viability. 5. Experimental studies with IL-33 in mice demonstrated that the innate immune response in lung tissue can be activated without direct cytopathic damage.
		5.2 Are the dissertation's conclusions new? 1) Completely new; 2) Partially new (25-75% new); 3) Not new (less than 25% new).	The most important achievement is the confirmation of the efficacy of the pharmaceutical agents: the maintenance of host cell functionality with the elucidation of pathogenetic mechanisms and the inhibition of viral replication. This can be regarded as the fundamental significance of the study for the development of universal antiviral approaches against coronavirus infections and RNA viruses, as well as for the evaluation of domestic pharmaceutical agents.
		5.3 Are the technical, technological, economic, or managerial decisions new and justified? 1) Completely new; 2) Partially new (25-75% new); 3) Not new (less than 25% new).	The novelty of the dissertation lies in the experimental biological evaluation of the antiviral activity of pharmaceutical agents and in the methodological approaches to interpreting the obtained results. The dissertation does not propose independent technical, technological, economic, or managerial solutions formalized as laboratory or industrial protocols, implementation algorithms, or economic assessments, and it is not aimed at the development of technical or managerial solutions.

6.	Justification of the main conclusions	All main conclusions are either based on scientifically sound evidence or, in the case of qualitative research and fields in arts and humanities, are sufficiently well substantiated.	The dissertation is significant for the screening evaluation of RdRP inhibitors. It is relevant for the phenotypic screening of antiviral compounds, including agents with known or potential RdRP-targeted activity. At the same time, the preclinical and clinical antiviral efficacy, as well as direct RdRP inhibition, were not investigated in the study.
7.	The main statements presented for defense	It is necessary to answer the following questions for each statement separately:	The statements presented in the dissertation for defense are generally based on experimental data. Experimentally, suppression of SARS-CoV-2 replication in Vero E6 cell cultures was demonstrated under the influence of the investigated pharmaceutical agents, confirming the presence of a direct antiviral effect *in vitro*. A comparative assessment of cytotoxicity and antiviral activity using CCK-8 and MTT assays was performed correctly and corresponds to widely accepted methodological approaches in cell biology. The application of these methods made it possible to distinguish virus-induced cytopathic effects from drug-related toxicity and to demonstrate the preservation of cellular metabolic activity during inhibition of viral replication. Within the selected experimental model, this conclusion is well justified and supported, despite the known limitations of metabolic assays, which do not allow evaluation of subcellular mechanisms of damage. The statement regarding the comparative activity of RdRP (NSP12) of the SARS-CoV-2 Alpha variant circulating in Kazakhstan and the Wuhan strain is based on indirect indicators of replication, viral titer, and cytopathic effect. Variation of the multiplicity of infection (MOI) and analysis of viral titer demonstrated a reduction in virus-induced cell damage and maintenance of cell viability even
		7.1 Is the proposition proven?	
		1) proven;	
		2) rather proven;	
		3) rather not proven;	
		4) not proven;	
		5) in its current formulation, it is impossible to verify the provenness of the proposition.	
		7.2 Is it trivial?	
		1) yes;	
		2) no;	
		3) in its current formulation, it is impossible to verify the triviality of the proposition.	
		7.3 Is it new?	
		1) yes;	
		2) no;	
		3) in its current formulation, it is impossible to verify the novelty of the proposition.	
		7.4 Level of application:	
		1) narrow;	
		2) medium;	
		3) broad;	

		4) in its current formulation, it is impossible to verify the level of application of the proposition.	under high viral load. This result indicates a pronounced antiviral and cytoprotective effect of the investigated agents. Experimental studies using recombinant IL-33 in a mouse model demonstrated an activation of the innate immune response in lung tissue without signs of significant cytopathic damage. Overall, it should be noted that the dissertation is based on a substantial body of experimental data and demonstrates the correct application of cell and virological models.
		7.5 Is it proven in the article?	
		1) yes;	
		2) no;	
		3) in its current formulation, it is impossible to verify the provenness of the proposition in the article.	
8.	Principle of reliability.	8.1 The choice of methodology is justified and described in sufficient detail:	The choice of methodology in the dissertation is generally well justified and corresponds to the research objectives, as the author employed a combination of in vitro and in vivo approaches, allowing assessment of both the direct effects of pharmaceutical agents on viral replication in cell systems and the characteristics of the innate immune response at the whole-organism level. The use of the Vero E6 cell model to evaluate antiviral activity and cytotoxicity of pharmaceutical agents can be considered methodologically appropriate for primary antiviral drug screening. The application of MTT and CCK-8 assays enables correct differentiation between virus-induced cytopathic effects and drug-related toxicity, confirming the adequacy of the chosen in vitro methods. The inclusion of in vivo experiments in mice using IL-33 expands the methodological scope of the study and demonstrates an attempt to extrapolate the obtained data to the level of the systemic innate immune response in lung tissue. At the same time, the in vivo experiments are auxiliary in nature and do not directly model the antiviral efficacy of the agents under SARS-CoV-2 infection conditions.
	Reliability of sources and the information provided.	1) yes;	
		2) no.	

		8.2 The results of the dissertation were obtained using modern scientific research methods and data processing and interpretation techniques, with the application of computer-based technologies:	Results of the dissertation were obtained majorly through modern methods of scientific research. In vitro experiments employed standard and widely accepted cell models (Vero E6), cytotoxicity assays (MTT, CCK-8), as well as quantitative determination of viral load using qRT-PCR, which is consistent with current approaches in virology and pharmacology. Data processing and interpretation methods included the construction of standard qPCR curves, Cq calculation, determination of IC□□, IC□□, and IC□□□ values, and the use of the selectivity index (SI), which are appropriate for evaluating the antiviral efficacy of pharmaceutical agents and their cytotoxicity. The application of computer-based technologies for statistical processing and analysis of in vitro and in vivo experimental data indicates the use of modern data analysis tools and overall confirms the methodological and technological validity of the applied approach.
		1) yes;	
		2) no.	
		8.3 The theoretical conclusions, models, identified relationships, and patterns are proven and supported by experimental research (for fields of study in the pedagogical sciences, the results are substantiated on the basis of a pedagogical experiment):	Theoretical conclusions, models, and identified relationships presented in the dissertation are generally well substantiated and supported by experimental research. In vitro experiments with SARS-CoV-2 and cell lines demonstrated a correlation between the concentrations of antiviral agents and a reduction in viral replication, as well as enabled an assessment of drug effects on cell viability. A comparative analysis of RdRP (NSP12) activity between the Alpha variant and the Wuhan strain revealed patterns of sensitivity of the replication complex to inhibition. In vivo experiments involving IL-33 in mice confirmed the possibility of activating the innate immune response of lung tissue without direct cytopathic damage, thereby demonstrating the biological validity of the theoretical assumptions.
		1) yes;	
		2) no.	

		8.4 The key statements are supported / partially supported / not supported by references to relevant and reliable scientific literature.	Key statements of the dissertation are supported by references to relevant and reliable scientific literature. Sources addressing the antiviral activity of Favipiravir, Ribavirin, and Tenofovir, the mechanisms of RdRP inhibition, and the immunological effects of IL-33 were used.
		8.5 The cited literature sources are sufficient / insufficient to support the literature review.	The cited literature sources generally correspond to the aims and objectives of the study and allow for an adequate understanding of the current state of research in the field of antiviral therapy against SARS-CoV-2.
9	Principle of practical significance	9.1 The dissertation has theoretical significance:	This dissertation has theoretical significance, as it elucidates the mechanisms of SARS-CoV-2 interaction with the host cell and the antiviral action of various drugs at the RdRP level. Theses findings advance our understanding of pharmacological suppression of viral replication and form the basis for further research in the field of antiviral therapy.
		1) yes;	
		2) no.	
		9.2 The dissertation has practical significance, and there is a high probability of applying the obtained results in practice:	This dissertation has practical significance, as the obtained results allow us to evaluate the efficacy of antiviral drugs against SARS-CoV-2 in vitro and in vivo models and can be used to optimize dosage and drug selection in clinical practice. These data are highly likely to be used in developing treatment strategies for COVID-19 in the early stages of the disease, as well as for screening new RdRP inhibitors and other antiviral agents.
		1) yes;	
		2) no.	
		9.3 The proposals for practical application are:	Statements presented in the dissertation are novel, as they are based on experimental data on the antiviral activity of Tenofovir (TDF/TAF), Ribavirin, and Favipiravir against SARS-CoV-2, including an analysis of RdRP inhibition and an assessment of cytotoxicity. The study proposes new methodological approaches to interpreting the antiviral effects of pharmaceutical agents and to the use of dosage forms under conditions of high viral load, which have not previously been systematized or formalized for practical application.
		1) completely novel;	
		2) partially novel (25–75% of the proposals are novel);	
		3) not novel (less than 25% of the proposals are novel).	

10.	Quality of writing and design	Quality of academic writing: 1) high; 2) average; 3) below average; 4) low.	The dissertation is written in clear and logical language, the structure is consistent, the chapters and subsections are interconnected and consistently develop the research topic. Scientific terminology is used correctly, and references are formatted according to requirements. The level of academic writing meets dissertation research standards.
11.	Comments on the dissertation	1. The topic of this dissertation is of interest to experimental virology and pharmacology. It should be noted that the main results were obtained using the Vero E6 cell line, which imposes certain limitations when extrapolating the data to clinical settings. Therefore, it seems appropriate to confirm the obtained results in models based on human cells. 2. The study used viral load parameters (MOI, TCID□□), but their physiological relevance in the clinical context was not the subject of a special analysis. It seems possible to further discuss the relationship of the selected parameters with the level of viral replication in vivo in more detail, which would allow for a broader interpretation of the obtained data. 3. The use of dexamethasone as a control drug requires additional methodological justification, since this drug is not a direct-acting antiviral agent. This aspect, however, does not diminish the overall scientific value of the obtained results. 4. In this dissertation, RdRp (NSP12) is considered one of the key molecular targets of the drugs being studied. However, direct experimental data confirming the inhibition of RdRp activity within the replication-transcription complex were not presented in this study. Conducting relevant molecular studies may be considered as a promising area for future work. Recommendations include expanding research using human cell models and applying molecular methods for analyzing RdRp activity to enhance the practical and clinical significance of the results. The data obtained in this study can be used as a basis for further preclinical and translational research.	
12.	The scientific level of the doctoral student's articles on the research topic (in the case of defending a dissertation in the form of a series of articles, official reviewers comment on the scientific level of each article of the doctoral student on the research topic)	The scientific level of the doctoral student's published articles corresponds to the topic of his dissertation research. Overall, the scientific level of the articles meets the publication requirements for the dissertation topic. Presented works are based on generally accepted in vitro models and standard cell-based assays, allowing the obtained results to be considered valid at the stage of primary experimental screening for antiviral activity against the SARS-CoV-2 virus.	

13.	Decision of the official reviewer (in accordance with paragraph 28 of these Model Regulations)	award a Doctor of Philosophy (PhD) degree

In reviews, official reviewers indicate one of the following solutions:

1) award a Doctor of Philosophy (PhD) degree or a doctorate in a particular field;

Copies of reviews from official reviewers are given to the doctoral student no later than 5 (five) working days before the dissertation defense.

Official reviewer:
JSC Scientific Center for Anti-Infectious Drugs
candidate of biological sciences
(place of work, academic title)

КАДР ОТДЕЛ КАДРОВ

(signature)

Ibragimova Nailya Akhtamovna
(Full name)