Attention of the members of the scientific jury,

determined by Order No. РД-38-609/14.11.2023,

issued by the Rector of SU"St. Kliment Ohridski" - Sofia

R E V I E W

of the scientific production of chief assistant Dr. Anton Vesselinov Hinkov, PhD, "Virology" Laboratory, Faculty of Biology (BF), Sofia University (SU) "St. Kliment Ohridski", Sofia

in connection with the procedure for occupying the academic position of "ASSOCIATE PROFESSOR" in direction 4.3 Biological sciences (Virology - molecular virology), announced in State Gazette (SG) No. 86/ 13.10.2023

written by Prof. Dr. Radka Mladenova Argirova, medical doctor-virologist, speciality "virology", Acibadem City Clinic UMBAL "Tokuda", Sofia

DEAR RESPECTED MEMBERS OF THE SCIENTIFIC JURY,

Here I present to your attention a review in connection with the announcement in SG, no. 86 of 13.10.2023 of a competition for the academic position of "docent" at the "Virology" Laboratory, BF, at the University of St. Kliment Ohridski" - Sofia and according to Order No. RD-38-609/14.11.2023, issued by the Rector of SU "St. Kliment Ohridski" - Sofia. The only candidate in the competition is the chief assistant at the Laboratory "Virology", Faculty of Biology, SU "St. Kliment Ohridski", Sofia. Anton Vesselinov Hinkov, doctor of biology.

Chief Assistant Dr. Anton Vesselinov Hinkov was born in 1981 in Sofia. In 2006 he graduated at Sofia University "St. Kliment Ohridski", Faculty of Biology, specialization "Molecular Biology". In 2012, he defended his doctoral thesis on the topic: "RESEARCH OF NEWLY SYNTHESIZED STYRYLQUINOLINES FOR ANTI-HIV-1 ACTIVITY IN CELL CULTURE". Since 2012, he has been an assistant, and since 2014, a chief assistant at the Laboratory "Virology", Faculty of Biology, SU "St. Kliment Ohridski", Sofia. He speaks English well.

Chief Assistant Dr. Anton Vesselinov Khinkov is mainly engaged in teaching, research and expert activities. In the current competition, Dr. Anton Hinkov presents himself with 1 dissertation (50 points according to the quartile system), there are a total of 32 scientific articles, of which he participated in the competition with 17 - 206 points according to the quartile system), 101 citations (50 points according to the quartile system system), participation in 13

scientific projects, scientific supervision of 9 graduates to obtain a master's degree and 4 to obtain a "bachelor's" degree. Hinkov presents 21 participations in international fora and 8 – in national scientific fora, all closely related to the scientific specialty. In addition, he has a rich teaching activity, which I will review separately. From 2020 until now, he is a member of the Quality Commission of the Faculty of Biology, SU "St. Cl. Ohridski".

According to the candidate himself, the thematic grouping of his contributions leads to three categories: I. Investigation of the antiviral activity of natural products, II. Investigation of the antiviral activity of newly synthesized substances, III. Study of the antiherpetic effect of physical factors.

In fact, the first experimental direction is already a traditional activity of the Laboratory of Virology in BF at SU "St. Cl. Ohridski". It is imposed as a constant task and requirement in modern medicine due to the increasingly frequently observed resistance and allergic reactions when using standard anti-herpes remedies. A large number of extracts, as well as their fractions and active substances isolated from lower and higher plants, invertebrates and microorganisms, have been studied for inhibitory and inactivating activity. The goal of the experiments is practically a screening of natural products; then the use of those having shown anti-herpes activity is particularly relevant and recommended. This allows finding of new antiviruses, incl. such exhibiting activity against viruses resistant to the chemotherapeutics used in medical practice. Several strains of human herpesvirus type 1, human herpesvirus type 2, and an acyclovir-resistant strain of human herpesvirus type 2 were used as experimental models. More important contributions related to these studies are as follows:

I.1. The total methanolic extract of dry leaves of *Haberlea Rhodopensis* /endemic species for the Balkans/ showed the highest activity - 61% and 60% respectively for human herpes virus type 1 and 2 /1/. Several consecutive publications described/2,3,4/ particularly well-expressed antiherpetic properties exclusively in cell culture for 4 plants often used in folk medicine. Also, deepening of the study in the mechanism of action of the studied plants in the life cycle of herpes viruses /publications 5,6,7/ is impressive. I highly appreciate the search for new anti-herpes remedies among the Bulgarian flora and the use of folk medicine sources.

II.2. The well-known "catnip" /*Nepeta nuda ssp L.*/ deserves attention and continued research. Haemolymph from Rapana venosa (hRv), Helix lucorum (Hl) and Eriphia verrucosa (hEv), mucus from Helix aspersa (Ha) and α-HaH structural subunit of hemocyanin from H. aspersa (sHa) versus replication of susceptible to the antiviral drug acyclovir (ACV) strains F and BA of human herpesvirus type 1 and type 2. The hemolymph fractions from R. venosa (MW 30-100 kDa) and from E. verrucosa (MW 3-100 kDa) showed the highest antiviral activity (over 99% inactivation of the infectivity of the extracellular virus of HSV-1 and HSV-2 types of viruses, respectively), sufficient to consider this herb for actual pharmacological application /publication 10/.

I.3. The effect of cell-free supernatants of ten newly isolated strains of lactic acid bacteria from traditional fermented foods was also investigated. [11]. The antiherpetic activity of secondary

metabolites isolated from Lactic Acid Bacteria isolated from fermented products /14/ has been investigated and proven, some of which are promising for future studies due to their high selective index.

The probiotic strains *Lactobacillus delbrueckii subsp. Bulgaricus KZM 2-11-3* and *Lactiplantibacillus plantarum KC 5-12* were found to have strong activity against human herpes virus type 2 with a selectivity index above 45, which is a good premise for further research. It is worth noting that the interest in these studies is very high, which is confirmed by the fact that 6 articles were cited 62 times by foreign and Bulgarian authors.

II. The second section of the applicant's contributions is somewhat double worded. On the one hand, targeted studies of new synthetic compounds have been conducted in search of an antiviral, not only an antiherpes, effect. On the other hand, substances already known for their antiviral effect were modified in order to intensify this effect or minimize side effects, and this is also considered a promising direction. Regarding this section, it is important to emphasize that Hinkov is part of a team study, which fact does not diminish either his participation or his contributions. The more important contributions in this area are found in publications 12,13 and 14 and are as follows:

II.1. The HIV-1 protease has been one of the most exploited viral targets for the development of therapeutic inhibitors. The work discussed and cited by Hinkov is a joint work with an Italian team, in which the activity of four newly synthesized dihydroxyethylene isosteres of the dipeptides Phe-Pro and Pro-Pro against the aspartate protease of HIV-1 was demonstrated in cell culture. The two inhibitors based on the Phe-Pro isostere were also further tested in vitro, proving their ability to suppress HIV-1 replication in infected MT-2 cells. Low cytotoxicity was found for the same cells. These results indicate that the Phe-Pro dihydroxyethylene isostere can be used for the synthesis of HIV-1 protease inhibitors [12]. This work has been cited 9 times, but more significantly, Hinkov was practically the most active in the development and publication of a rapid screening method for the evaluation of HIV-1 protease inhibitors.

II.2. Three ABC esters containing the amino acid glycine (Gly) and dipeptide esters (glycyl-glycine) were synthesized at the Southwestern University in Blagoevgrad. Their anti-HIV-1 III B-activity was proven in cell culture, Hinkov's participation was precisely and especially active at this stage of the research. One of the newly synthesized esters – Gly-ABC showed low cytotoxicity, high anti-HIV-1 activity in MT-4 cells, low mitochondrial toxicity and high genetic barrier to resistance. This publication has been cited 3 times.

II.3. Modification of nucleoside analogues could increase their bioavailability in the cell and reduce the therapeutic dose in the therapy of herpes virus infections. With this aim and in this sense, the activity of esters of the antiherpes drugs ganciclovir and penciclovir with bile acids (cholic, chenodeoxycholic and deoxycholic) and amino acid esters of acyclovir against human herpes virus type 1 and type 2 was studied. Unfortunately, it has been shown that the modified analogues were less active than the generic substances, indicating that this type of modification

is not suitable for increasing bioavailability. The latest experiments are an important experience, albeit with a negative result.

III. Study of the impact of physical factors on the replication and extracellular virions of HSV-1. For the first time , the impact of nutrient medium and water treated with surface wave non-equilibrium gas discharge plasma (constructed by a Bulgarian team of scientists) for antiviral and virucidal action was investigated. Human herpes virus was used. When studying the virucidal effect of a plasma-treated virus suspension diluted 1:2 with dH2O, a reduction of the virus titer in the virus sample was found compared to the control by 1.67 log10 [15]. Personally, I cannot well judge the significance and application of this physical effect, but I trust that future experiments will make it clear.

All studies are performed with reliable and modern virological and molecular-biological research methods, they are up-to-date, with the perspective of being continued in the future, they were carried out in teams of different specialists, proven by the candidate's participation in 13 projects with departmental and external funding. This fully corresponds to the interdisciplinary nature of modern science, the conclusions are convincing. Of course, the most convincing are the citations of the published results. On the other hand, the collective mind and interdisciplinary approach today make it quite difficult to determine the role of the individual researcher in a given development. In this sense, I would make a critical note for more clearly expressed personal participation and personal initiative, not only as an activity in the laboratory, but also as shaping the publications, generating scientific projects, etc., since I still do not see his own handwriting and initiative in the common research activity. However, the candidate's current achievements are a good basis for the candidate's future prospects and development.

Anton Hinkov's teaching and pedagogic activity consists of academic employment after 2018 in 408-480 hours per year, of which more than half is classroom employment. Hinkov has lectures on Microbiology with Virology - for "Master", specialty Pharmacy, practical exercises and training practices on "Molecular Virology", "Virology" and "Microbiology with Virology". He is the supervisor of 9 graduates – for the degree "Master" and 4 - for "Bachelor". Together with Assoc. Prof. Kalina Shishkova, he co-authored the "Guide for Practical Exercises in Virology", which is omitted from the list of his publications, but is important for the process of training and work in the virology laboratory.

I fully agree with the distribution into 3 groups of the main topics of the candidate's scientific activities, as well as with the conclusions and contributions of his research. Since in his development Anton Hinkov has inherited and continues to develop the directions of antiviral therapy with natural and synthetic substances, it is quite understandable that he - together with the other colleagues from the Lab. Virology - expands this field of research with an active search for analogues of known antivirals in order to improve their antiviral activity and reduce resistance and adverse events in the course of therapy. Therefore, there is continuity in topics, expansion of the scope of the target natural sources - the object of the research and enrichment of the previously known conclusions with new contributions from these researches.

The academic position "associate professor" also requires serious teaching work, which is the first duty in the university, in this case - BF. Anton Hinkov's educational workload includes both teaching students, with practical trainings and lectures, as well as guiding masters in developing their diploma theses.

I personally know Dr. Anton Hinkov as an accurate researcher, with enduring interests in virology and pharmacology, as evidenced by his scientific output, which exceeds the requirements for an "associate professor" at BF.

In conclusion, the complex assessment of the candidate's scientific research and teaching activities, his contributions and participation in successful and cited research activities, exceeding the requirements for the scientific position "docent", his experience in training in modern virology, as well as his personal qualities - hard work and persistence, gives me the confidence to vote positively for the appointment of chief assistant Dr. Anton Veselinov Hinkov, Ph.D., to the academic position of "Associate Professor" in the BF of SU "St. Kliment Ohridski", Sofia. I especially emphasize Hinkov's perspectives mainly in terms of his scientific achievements, which even today exceed the minimum required for an "associate professor", that is why I strongly suggest to the members of the respected scientific jury to vote positivelly.

Sofia, 06/02/2024 REVIEWER:

molecules or as structural and functional subunits) isolated from the Black Sea rapana (*Rapana hemocyanin*) and representatives of the genus *Mollusca, Eriphia verrucosa (hEv), Helix aspersa (Ha)* and structural subunit α was demonstrated for the first time -HaH from H. *aspersa* hemocyanin (sHa) to HSV-1 replication /12,13/. The antiherpetic activity of secondary metabolites isolated from **Lactic Acid Bacteria** isolated from fermented products /14/ has been investigated and proven, some of which are promising for future studies due to their high selective index.

The rational synthesis of new substances with anti-herpes, anti-influenza and anti-coronavirus action is based on the knowledge of the structure and functions of the applied antivirals. For example, the molecular structures of the newly synthesized analog compounds of the ion channel inhibitors amantadine and rimantadine were thoroughly investigated using single crystal X-ray analysis. Molecular docking studies showed that two of the investigated compounds, namely 2A and 4A, showed promising binding affinity to HCoV-229E and 2 SARS-CoV-2 sites - RNA-dependent RNA polymerase site and SARS-CoV-2 Nsp3 (207-379, MES site) respectively /21/. This article was published in 2023, it's still early for citations, but I'm sure it will resonate widely in the scientific community.

The most important contribution in the field of "Medical virology" is the demonstration for the first time in the country of Torque Teno viruses by adapting different primer systems. The

presence of the viruses was proven in samples from blood donors, patients with registered viral hepatitis, patients with a primary brain tumors, patients with respiratory diseases, kidney transplant patients and patients of unknown disease etiology. I have already said that this is still one of the few studies in our country.

In this direction, the establishment of human papillomavirus (HPV) in a risk group of patients positive for the presence of periodontitis-causing bacteria is also important. The most frequently detected genotype of the papillomavirus associated with periodontitis is HPV58.

All studies are performed with reliable and modern virological and molecular biological research methods, they are up-to-date, with the prospect of being continued in the future, they were carried out in a team of different specialists, as evidenced by the participation in 20 projects with departmental and external funding, which fully corresponds to the interdisciplinary nature of modern science, the conclusions are convincing.

The teaching and pedagogical activities of Kalina Shishkova include lectures since 2002 on Virology - OKS "Bachelor"; "Antiviral Immunity", "Phages and Lysogeny" - OCS "Master", practical exercises and educational practices in "Molecular Virology", "Virology" and "Microbiology and Virology". Kalina Shishkova is also a co-author of the "Guide for practical exercises in virology", which is omitted from the list of publications , but important for the process of training and work in the laboratory of virology.

I fully agree with the distribution into 4 groups of the main topics of the candidate's scientific activities, as well as with the conclusions and contributions of her research. Since in her development Kalina Shishkova has inherited and continues to develop the directions of antiviral therapy with natural substances, it is quite understandable , that she has expanded this field of research with an active search for analogues of known antivirals in order to improve their antiviral activity and reduce resistance and adverse events in the therapy

process. **Therefore, there is continuity in thematic respect, combined with updating the research methods, expanding the scope of the target natural sources - the object of the research and enriching the previously known conclusions with new contributions from these researches**.

The academic position "associate professor" requires serious teaching work, which is the first duty in a university, in this case - BF. Kalina Shishkova's teaching and workload includes both teaching students, with exercises and lectures, as well as guiding masters in developing their diploma theses. Kalina Shishkova is a supervisor of 9 graduates.

I personally know Dr. Kalina Shishkova as a thorough, accurate researcher with lasting interests in virology, which is also proven by her scientific output.

In conclusion, the comprehensive evaluation of the candidate's research and teaching activities, contributions and participations and in promoting scientific achievements in her field, her long experience in teaching modern virology, as well as her personal qualities, give me the confidence to vote positively for the appointment of the main assistant Dr. Kalina August Shishkova, Ph.D., to the academic position of "associate professor" in the Faculty of Biology of the University of St. Kliment Ohridski", Sofia. Her achievements exceed the minimum required for an "associate professor" position, so I strongly propose that the members of the esteemed scientific jury also vote positively.

Sofia, Nov.6th, 2023 REVIEWER:

София, 05.11.2023 РЕЦЕНЗЕНТ: