STATEMENT

by Prof. Rayko Dimitrov Peshev, Ph.D., DS, head of Dept "Epizootology and Infectious Diseases of Animals" in NDNIVMI, Sofia, according to the announced competition for "Docent" in professional direction 4.3. Biological Sciences, Scientific Speciality Virology (Molecular Virology), announced in SG No. 86 of 13.10.2023 for the needs of the Faculty of Biology at the "St. Cl. Ohridski" University.

In connection with the competition announced by the Faculty of Biology of the SU "St. Cl. Ohridski" Professional direction 4.3 Biological sciences, Scientific speciality Virology (Molecular virology), announced in SG, no. 86 of 13.10.2023 for the occupation of the academic position "Docent", the documents were submitted by a single candidate, Chief assistant Ph.D. d-r Anton Veselinov Hinkov. To participate in the competition, the following documents are submitted: curriculum vitae, diploma of completed higher education, diploma for scientific degree "Doctor", confirmation of the occupied academic positions of assistant and chief assistant, list of scientific publications, list of scientific publications for participation in the competition for associate professor, list of scientific contributions, minimum required points by groups of indicators, noticed citations, summary of the articles submitted for participation in the competition for "Docent".

Brief autobiographical data

Chief Assistant d-r Anton Veselinov Hinkov was born on 14.12.1981 in the city of Sofia. He completed his secondary education in 2000 at "Peter Bogdan" high school with teaching foreign language town Montana. From 2000 to 2004, he completed his Bachelor's degree at the University of Applied Sciences "St. Kliment Ohridski, majoring in "Molecular Biology". From 2004 to 2006, he completed a master's degree at the Sofia University "St. Kl. Ohridski", specialty "Molecular Biology". In 2012, he received a Doctor of the National Academy of Sciences, as the topic of his dissertation was: "Investigation of newly synthesized styrylquinolines for anti-HIV-1 activity in cell culture". From 2011 to 2012 he was a specialist virologist at the Faculty of Medicine, from 2012 to 2014 he was an assistant, and from 2014 to the present moment he was a chief assistant at the Faculty of Biology of Sofia University.

Scientific publication activity

A list of scientific articles is presented by the candidate. Of the scientific publications that are referenced and indexed in WEB of Science, Scopus with an impact factor or SJR, 23 peer-reviewed articles are indicated, and 9 publications are printed in journals without an impact factor. The results in the presented articles have a scientific-fundamental and applied nature. They describe studies related to the antiviral effect of various biological and physical factors.

In the competition for the acquisition of the academic position of Docent, the candidate presents 17 scientific publications. Contributions can be divided thematically into the following areas: I. Investigation of the antiviral activity of newly synthesized substances, II. Investigation of the antiviral activity of natural products, III. Study of the antiherpetic effect of physical factors.

Scientific Communication No. 1 reports the synthesis and biological activity data of HIV 1 PR inhibitors based on 4 new isosteres of Phe Pro and Pro Pro dipeptides. The isosteres contain 4 steroid centers and are synthesized by cyclization of epoxy amines derived from alpha amino acids. Their inhibitory activity was found to be in subnanomolar to micromolar amounts. It was established that Phe Pro inhibitors suppress HIV 1 replication in MT 2 cells with a weak cytotoxic effect on them and having a high therapeutic index. The authors believe that the isosteres have strong anti-HIV 1 PR activity. With regard to the study of the antiviral activity of newly synthesized substances, in Scientific Communication No. 2 the data from the synthesis of three ABC esters containing the amino acid glycine (Gly) and dipeptide esters (glycyl-glycine) were reflected and their activity against HIV replication was evaluated -1 III B in MT-4 cells. One of the newly synthesized esters – Gly-ABC has shown in the experiments low cytotoxicity and high anti-HIV-1 activity in MT-4 cells, as well as low mitochondrial toxicity and a high genetic barrier for resistance from HIV-1 protease inhibition. Peptidomimetics inhibiting HIV-1 protease are a potential source of molecules used in the fight against AIDS. Four new dihydroxyethylene isosteres of the dipeptides Phe-Pro and Pro-Pro were identified by establishing their activity in recombinant protease assays. The two Phe-Pro isostere-based inhibitors were tested in vitro and demonstrated their ability to suppress HIV-1 replication in infected MT-2 cells. The established low cytotoxicity against the same cells, which contributes to the high therapeutic index. The Phe-Pro dihydroxyethylene isostere can be used for the synthesis of HIV-1 protease inhibitors. In Scientific Communication No. 3, the results of the activity of methanol, ethanol and water extracts are reflected. of fresh and air-dried leaves of Haberlea Rhodopensis Friv. against human herpes virus type 1 and 2 and it was found that the total methanol extract of dry leaves had the highest activity – 61% and 60% for human herpes virus type 1 and 2, respectively. Scientific communication No. 4 is a review of the Bulgarian medicinal plants from different families Amaryllidaceae, Fabaceae, Geraniaceae, Lamiaceae, Onagraceae, Ranunculaceae, Rosaceae, Scrophulariaceae and Rhodophyta) for the last three decades and their effect in antiviral research. Elder (Sambucus ebulus L.), a popular medicinal plant used for centuries in the folk medicine of the Balkan Peninsula, was investigated in Scientific Communication No. 5. Preparations of S. ebulus have shown anti-inflammatory, antineoplastic and antimicrobial properties, in addition to abundant wound healing, antioxidant

and anti-ulcerogenic actions. A scheme was developed to isolate individual compounds using various chromatographic techniques, while structure elucidation was performed using 1D and 2D NMR. Five flavonoid glycosides, e.g. quercetin-3-O-laminaribioside [1], isorhamnetin-3-O-laminaribioside [2], quercetin-3-O-rutinoside [3], isorhamnetin-3-O-rutinoside [4], isorhamnetin-3-O- glucoside [5], have been identified. Compounds 1 and 2 are reported for the first time in the genus Sambucus. Several triterpeni-ursolic, oleanolic and olive acids were identified by GC-MS. The anti-herpes simplex virus type 1 properties and antioxidant effects were evaluated, and it was suggested that elderberry could serve as a potent source of valuable molecules for various purposes. In Scientific Communication No. 6, the results of the research on Catnip (Nepeta nuda ssp. nuda L.) are given and it is proved that the water extract of the herb has the highest antiherpetic activity. It was found to specifically affect viral adsorption to the cell surface and not affect the infectivity of extracellular virions. Scientific report No. 7 reflects the data from the research on Teucrium chamaedrys L. (Wall German), a widespread species of Teucrium (Lamiaceae), in the flora of Bulgaria, used as tea and in medical procedures. In an in vitro study, chloroform and methanol extracts were obtained by Soxhlet extraction, as well as a methanol extract obtained by thermostatic extraction and tested for antiviral activity. Two of the tested extracts inhibited herpes simplex virus type 2 (HSV-2) replication in MDBK cells significantly without apparent cytotoxicity. The 50% effective concentration (EC50) of the chloroform extract was 350 μg/ml. Virus replication was inhibited over 82% by the applied extract at the maximum permissible concentration (MTC). The methanolic extract of thermostat showed a weak antiviral effect (EC50 = 680 μg/ml). Extracts administered in MTC inactivated extracellular virus, viral adsorption and entry of HSV-2. The antiherpetic activity of Teucrium chamaedrys crude extracts was observed for the first time. In Scientific Communication No. 8, the data on the effect of an aqueous extract of mountain wormwood (Artemisia chamaemelifolia Vill.) on human herpes virus type 2 resistant to acyclovir and it has been proven that it preserves up to 80% of the survival of infected cells, thus making it possible to overcome of drug resistance. Scientific Communication No. 9 investigated the action of an aqueous extract of Nepeta nuda ssp. nuda L on the replication of human alpha herpesvirus (HHV) type 1 strain F (ACV-susceptible) and type 2 strain DD (ACV-resistant) in vitro. The virucidal assay showed that the aqueous extract did not reduce the infectivity of either of the two strains used at a concentration equal to the maximal non-toxic concentration. Antiviral activity was found not to be due to direct inactivation of extracellular virions. Rather, it is due to interference with adsorption, but not penetration (according to the results of the conducted experiment). The authors suggest that the aqueous extract exerts its

antiherpetic activity by affecting both early (adsorption) and late events of HHV replication. Metabolomic studies of the extract showed that the main phenolic acids present in the extract included rosmarinic, chlorogenic, gallic, vanillic, caffeic, protocatechinic, ferulic and cinnamic acids; while the presence of flavonoids is noted by circimaritin, chrysoeriol, vanillin, rutin and quercetin. In article No. 10, the activity of esters of the antiherpetic drugs ganciclovir and penciclovir used in medical practice with bile acids (cholic, chenodeoxycholic and deoxycholic) and amino acid esters of acyclovir was determined against human herpes virus type 1 and type 2. The modified analogues are more little active compared to the generic substances, which showed that this type of modification is not suitable for increasing the bioavailability of ganciclovir, acyclovir and penciclovir in the cell. In Scientific Communication No. 11, the authors set out to evaluate some specific growth conditions of Pediococcus pentosaceus ST65ACC and its bacteriocin expression via ABC transporters; to purify the bacteriocin and determine its sequence; and to evaluate the cytotoxic potential of the purified bacteriocin(s). The bacteriocin produced by P. pentosaceus ST65ACC is similar to coagulin, with low cytotoxicity, strong antimicrobial activity and possible additional metabolic pathways in the producer cell. In addition to MRS broth, bacteriocin was also produced in medium containing XOS as the sole carbon source. In scientific communication No. 12, the results of the studies of the impact of physical factors on the replication and extracellular virions of HHV 1 are reflected. For this purpose, nutrient medium and water were treated with a surface wave non-equilibrium gas discharge plasma to establish an antiviral and virucidal effect on human herpesvirus and was demonstrated a 1.67 log10 decrease in virus titer in the virus sample compared to the control. Paper No. 13 reports the results of studies on hemolymph from Rapana venosa (hRv), Helix lucorum (Hl) and Eriphia verrucosa (hEv), mucus from Helix aspersa (Ha) and the α-HaH structural subunit of hemocyanin from H. aspersa (sHa) versus replication of herpesviruses sensitive to the antiviral drug acyclovir. Hemolymph fractions from R. venosa (MW 30-100 kDa) and from E. verrucosa (MW 3-100 kDa) showed the highest inactivating activity (over 99% inactivation of extracellular virion infectivity). In Scientific Communication No. 14, he reports data from phylogenetic studies of the plant Nepeta nuda ssp. nuda L and the metabolic response in variable growth conditions. In article No. 15, the data on the impact of soil on plant metabolism and active molecules acting on antiviral activity are reflected. The plant Teuricum chamaedris L was studied against human alphaherpes virus. Plants were obtained from different regions of the country with a view to establishing the impact of the soil on the accumulation of active molecules against the herpes virus, and it was found that plants collected from the area of the village of Dobromirtsi, are the most active. They determined that

the chloroform Soxhlet extracts, inhibited up to 99.7% of extracellular virions, and the methanol thermostatic extracts, had 99.99% activity. The impact on the viral replication cycle was weak, with the most active extract having a selectivity index of 1.87. An opinion has been expressed that the places where these plants grow should be controlled in order to obtain more active molecules. The antiherpetic activity of cell-free supernatants of ten newly isolated strains of lactic acid bacteria was reported in communication No. 16. For 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. Scientific Communication No. 17 reported data from the application of a standardized extract of the aerial parts of the plant Astragalus glycyphyllos L and was shown to inhibit up to 70% the replication of both acyclovir-susceptible and acyclovir-resistant strains of human herpesvirus. The tabletability of the standardized plant extract and potential for adjuvant antiviral application in further trials has been demonstrated.

Fulfillment of minimum national requirements

From the reference for the minimum national requirements under indicator - 1 A for a defended dissertation the candidate has 50 points, under point B indicator 2 – no points, under point B indicators 3 and 4 there is a total of 100 points with 2 articles in Q 1 – 50 points, 1 article in Q 2 – 20 points and 2 articles in Q 3 – 30 points. According to indicator D - a sum of indicators from 5 to 10, according to the requirements of the Law, there should be 200 points, and he exceeds this indicator and collects 206 points as follows: in Q 1 there are 2 pcs. articles 50 pts, from 3 articles in Q 2 there are 60 pts in Q 3 from 4 articles there are 60 points and in Q 4 from 3 articles there are 36 pts. In indicator group D, the sum of the points for indicator 11 should be 50, and it presents 28 citations, which carry 56 points, exceeding the requirements for this indicator. From the presented scientific reports and citations, it can be seen that the candidate fulfills the minimum national requirements for the Docent.

Additional evidence of the candidate's research activity

From the applicant's participation certificate it is clear that he participated in 20 scientific research projects as a lead contractor. He was the academic supervisor of 9 graduates, for the acquisition of the OKS Master's degree, who successfully defended their diploma theses, and of 4 for the acquisition of the OKS Bachelor's degree. Chief assistant Dr. Hinkov has participated in 13 scientific research projects financed by the Ministry of Education and Culture and Science and Technology of St Kl Ohridski. As a result of the research conducted since 2011, 23 articles have been published in scientific journals with impact factor or JSR, 9 articles

in journals without impact factor or SJR. From the presented citations, it can be seen that Chief Assistant d-r. Hinkov so far has citations to his scientific articles, which speaks of their being recognizable to the scientific community. The candidate has presented 21 communications published in full text in proceedings of international scientific forums and 8 publications of national scientific forums. The candidate, in co-authorship with K. Shishkova, has written and published a guide for practical classes in virology, according to which students and graduates of the Biological Faculty of the SU are trained. From the reference made from the individual study plans, it is also visible the large study employment of the candidate, as for the last 6 years there are 2228.7 hours of study employment, of which 1503.5 hours are classroom employment.

Conclusion

The scientific research and applied achievements presented to me by the Chief assistant d-r. Anton Veselinov Hinkov, and the obtained results in the field of virology give me full right to conclude that he meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Application in the Biological Faculty of the SU. His participation in the education of Biology students is palpable, which can be seen from both classroom and study hours. The scientific indicators have been fulfilled and meet the requirements for acquiring the academic position "Docent". The candidate has worked in the field of virology for 13 years, and during this period significant research and applied results have been achieved, which have been presented to the scientific community. They are valued and this is evident from the citations of the scientific reports. I will vote positively and allow myself to recommend to the members of the Scientific Jury and to the members of the Scientific Council of the Faculty of Biology at Sofia University to vote positively for the awarding of the academic position "Docent" to the Chief assistant Dr. Anton Veselinov Hinkov in professional field 4.3 Biological Sciences.

12/1/2024 Wrote the statement:

Sofia (Prof. Rayko Peshev, Ph.D. D.S.)