OPINION

by Prof. Dr. Ivanka Stankova - South – West University ‘’Neofit Rilski’’

Scientific field - Natural sciences, mathematics and informatics

Professional field - 4.2 Chemical Sciences

Scientific specialty - „Organic Chemistry, chemistry of natural and physiologically active compounds“

Author - Boryana Krasimirova Yakimova

Topic - Design and synthesis of biologically active peptides as potential inhibitors for angiotensin converting enzyme (ACE I)

For awarding the **Doctor degree**

Supervisor : Prof. Ivanka Stoineva, DSc, Institute of organic chemistry with centre of phytochemistry, Bulgarian Academy of Sciences

Hypertension is a leading risk factor for human health worldwide, and in Bulgaria it is one of the leading places in terms of disease. In clinical practice for the treatment of hypertension are used: vasodilators, blockers of Ca-channels, blockers of AngII receptors and angiotensin converting enzyme-I (ACEI). More services that ensure the functioning of the renin-angiotensin system (RAS) have been proven to be the most effective drugs and achieve the most significant effect in normalizing blood pressure. It is only a matter of time before "new normality" interest in the study of new enzyme-converting enzyme inhibitors (ACEIs) as modulators of the renin-angiotensin system (RAS) increases. This is necessary not only for their importance as drugs for the treatment of hypertension, but because of their potential to study the development of coronavirus infection (COVID-19). All this determines the relevance of research. The research in the dissertation can be related to structured and synthesized design of short-chain peptides, with potential properties as angiotensin-converting enzyme inhibitors (ACE), synthesis of monoacid amino acid esters and design as potential angiotensin-converting enzyme inhibitors (ACE). and checking the kinetic studies of the newly synthesized peptide inhibitors using fluorogenic substrates.

For the preparation of short-chain peptides, conventional synthesis methods used in peptide chemistry were used - liquid-phase synthesis for proline di- and tripeptides, using Fmoc-Cl as a condensing agent, as well as solid-phase peptide synthesis using Fmoc strategy and 2- chlorotrityl chloride resin, which gave 7 tripeptides and one tetrapeptide. The tripeptides H-Ile-Ala-Lys-OH and H-Val-Ala-Trp-OH were obtained by solid phase peptide synthesis using Fmoc strategy and Wang resin. According to data in the literature, mono- and polyacylated sugars with amino acids show ACE

inhibitory activity. In the dissertation a transesterification reaction of amino acid esters was used to obtain monoacylated sucrose and glucose. Cyanmethyl esters of N-protected amino acids have been used as aminoacylating agents because they are easily transesterified due to the lack of spatial interference. The other reason why cyanmethyl esters are used is their easy synthesis and high yields of the final product. NMR spectroscopy (2DCOSY, DEPT135, HSQC) was applied to detect the synthesized esters and the structure of OH-substituted sucrose esters at the 2nd, 3rd and 6th positions in the glucopyranosyl ring and OH-substituted esters was proved on the 1st position in the fructofuranosyl ring with the amino acids - valine, proline and isoleucine. The compounds show inhibitory activity. The cis-trans isomerization of the dipeptide H-Val-Pro-OH was examined by NMR spectroscopy. It was found that it is strictly pH dependent, which is in accordance with the theoretical calculations. Infrared spectroscopy has shown that the strong intramolecular hydrogen bonds NH2 .... O = CN (Amide) and O = C-OH ... NH2 in the tripeptides H-Val-Pro-Pro-OH and H-Ile-Pro- Pro-OH inhibits the degradation of tripeptides by proteolytic enzymes. The biological activity of the newly synthesized compounds in vitro and ex vivo on the activity of ACEI was studied using lisinopril as a reference. The results show showing significant differences. In the first case, changes in the amount of substrate used are directly investigated, while in the second, activity is judged indirectly by the contractile response of the ileum to the metabolite formed in the reaction (AT-II).

The main contributions of the PhD student are: the developed procedure for solid-phase synthesis of new short-chain peptides, not described in the literature so far and proven as inhibitors of angiotensin-converting enzyme; application of high performance liquid chromatography and suitable conditions for the separation of regioisomeric esters of sugars. Appropriately selected proline peptides that show pronounced antihypertensive action.

The dissertation is written consistently and clearly and contains original research and results. Scientific results have a certain value and are achieved through appropriate methods and approaches. The impression of the precisely conducted experiment, of the correctly described procedures is very good. It can be seen that the educational tasks of the doctoral program have been fulfilled.

The scientific results have been published in 4 publications, 3 of them with impact factor and one with SJR. 11 citations were noted. The results of the dissertation are presented at 14 scientific forums.

I have no doubt in the significant contribution of the doctoral student in the implementation of the set goals and objectives.

The abstract of the dissertation reflects in summary form the content of the dissertation and is written in accordance with the approved rules.

Conclusion

The dissertation shows that the candidate, Boryana Krasimirova Yakimova possesses deep theoretical knowledge and professional skills, demonstrating qualities and skills for conducting research with obtaining original and significant scientific offerings. Because of the above, I am convinced of my positive assessment of the research, presented in the dissertation, abstract, achieved results and contributions, and propose to the Honorable Scientific Jury to award the scientific degree "Doctor" to Boryana Krasimirova Yakimova in the field of higher education "Natural sciences, mathematics and informatics", professional field 4.2 "Chemical sciences" (Organic Chemistry, chemistry of natural and physiologically active compounds).

18.05.2021 Prof. Dr. Ivanka Stankova