SYNTHESIS AND CHARACTERIZATION OF SMALL PEPTIDE ANALOGUES WITH POTENTIAL INHIBITOR ACTIVITIES

Atanas Chapkanov[1], Tania Ignatova[1], Tatyana Dzimbova[1,2]

[1] South-West University "Neofit Rilski"
66 Ivan Mihailov str., 2700 Blagoevgrad, Bulgaria
[2] Institute of Molecular Biology "Rumen Tsanev"
21 Acad. Georgi Bonchev str., 1311 Sofia, Bulgaria
E-mail: tdzimbova@swu.bg

Received 11 January 2019
Accepted 30 July 2019

ABSTRACT

The bioactive peptides are specific protein fragments which have a positive effect on the body functions and conditions. These peptides have a hormone- or a drug like activity and play an important role.The antihypertensive peptides are a significant group of these peptides, which are used as angiotensin-converting enzymes inhibitors, because they are associated with the high blood pressure decrease and play an important role in cardiovascular diseases. Angiotensin-I Converting Enzyme (ACE) is one of the major components of the so-called Renin-Angiotensin System (RAS). Renin is responsible for the release of Angiotensin I (AI) in blood because of its catalytic action on angiotensinogen. The role of ACE connected with the inhibition of ACE enzymatic activity against AI is considered one of the major challenges in case of a hypertensive disease and a congestive heart failure.

The present investigation is focused on the synthesis of His-Leu and Val-Trp peptide analogues using well know procedures. The intermediates and the dipeptide analogues are characterized by TLC and spectral methods. The computer modeling and the docking studies are performed to determine the structure-activity relationship and to synthesize new potential ACE inhibitors.

Keywords: ACE, dipeptides, docking study, spectral analysis.

INTRODUCTION

The bioactive peptides of a hormone- or a drug like activity are specific protein fragments that have a positive impact on the body functions and conditions and play an important role in human health [1 - 3]. According to their functional properties, the bioactive peptides may be classified as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding and antioxidative [4]. The antihypertensive peptides are an important group of the bioactive peptides. They are used as angiotensin-converting enzymes inhibitors because they are associated with a decrease of the high blood pressure and play an important role in cardiovascular diseases. Angiotensin-I Converting Enzyme (ACE) is one of the major components of the so-called Renin-Angiotensin System (RAS). Renin is responsible for the release of Angiotensin I (AI) in blood because of its catalytic action on angiotensinogen (Fig. 1). The role of ACE in blood pressure maintaining connected with the inhibition of ACE enzymatic activity against AI is considered one of the major challenges in

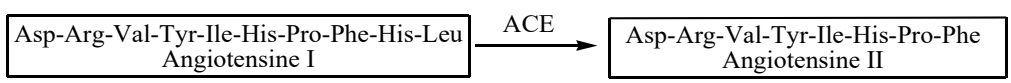

Fig. 1. Conversion of Angiotensin I to Angiotensin II.

case of a hypertensive disease and a congestive heart failure [5].

The present study is focused on the synthesis of His-Leu and Val-Trp peptide analogues using well know procedures. The intermediates and the dipeptide analogues are characterized by TLC and spectral methods.

The computational methods are playing an increasingly greater and more important role in drug discovery and development. They are expected to limit and focus on the chemical synthesis and the biological testing thereby greatly decreasing the traditional resource requirements [6 - 7].

The computer modeling and docking studies are performed aiming to determine the structure-activity relationship and to synthesize new potential ACE inhibitors.

The goals of the presented study refer to docking studies and the design of new potential ACE inhibitors, the synthesis of structural analogues of His-Leu (Fig. 2) after a modification and the investigation of the compounds structure-activity relationships.

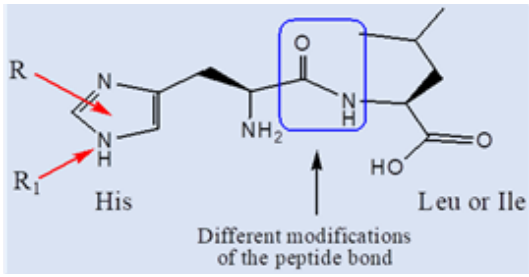

Fig. 2. Modifications in the structure of His-Leu.

EXPERIMENTAL

Materials and methods

Dipeptide analogues of His-Leu and Val-Trp were used in order to check their ability to bind ACE and act as its inhibitors. The peptide preparation was done with Avogadro (an open-source molecular builder and visualization tool - Version 1.0.3). The crystal structure of the ACE was obtained using RCSB (PDB id: 4aph). The docking studies were performed with the application of Genetic Optimization for Ligand Docking run on Scientific LINUX 5.5 operating system at the Center for Advanced Bioinformatics Research (CABR) at SWU "N. Rilski".

The IR-spectra of the analogues were recorded using Thermo Scientific Nicolet iS10 FT-IR spectrometer (4000 cm^{-1}-400 cm^{-1}). A spectral resolution of ± 4 cm^{-1} was used and 64 scans were accumulated. The FT-IR spectra in solid state were recorded using ATR accessory technique.

Synthesis

HCl.Leu-OEt and HCl.Val-OEt: 3.4 mM $SOCl_2$ were added dropwise to 15 ml of anhydrous ethanol at -10°C with stirring. After 15 min 1,5mM amino acid was added to the mixture. The reaction was completed within 2 h and the solvent was removed. The progress of the reaction was monitored by TLC in $CHCl_3$: MeOH : H_2O = 80:30:5 system. The precipitate obtained was filtered and dried over KOH. White crystals were obtained (a yield of 87 %).

Boc-His(Bzl)-Leu-OEt and Boc-Trp(Bzl)-Val-OEt: 0.9 mM HCl.Leu-OEt dissolved in 50 ml EtOAc were added with stirring to 0.9 mM of Et_3N. 0.9 mM of Boc-His(Bzl)-OH, 0.9 mM of DMAP and 0.9 mM of EDAC were sequentially added to the resulting mixture. The reaction proceeded at pH 8 - 9 within 24 h. The solvent was removed and 30 ml of CH_2Cl_2 were added. The solutions were sequentially washed with 10% $NaHCO_3$, 10% $NaHSO_4$ and neutralized with NaCl to pH = 7. The Boc-His (Bzl) –Leu-OEt and Boc-Trp (Bzl)-Val-OEt solutions were dried over anhydrous $MgSO_4$ and the solvent was removed. The products were stored over KOH (a yield of 32 %).

HCl.His (Bzl)-Leu-OEt and HCl-Trp (Bzl)-Val-OEt: 0.13 g of Boc-His (Bzl)-Leu-OEt were dissolved in 10 ml of CH_2Cl_2 and 1 ml of TFA with stirring. After 3 h the solvent was removed and the product dried under KOH (a yield of 86.4 %).

Boc-His (Bzl)-Leu-OH: 0.15 g of Boc-His (Bzl)-Leu-OEt were dissolved in 10 ml of dioxane and 10 ml of H_2O with stirring. Drops of thymolphthalein were added and 1N NaOH was introduced while the reaction mixture kept a constant blue color. The dioxane was evaporated and the aqueous solution was slightly acidified by dry $NaHSO_4$ and extracted with ethyl acetate. The organic layers were washed with water until pH 7 and the solvent was removed (a yield of 57 %).

RESULTS AND DISCUSSION

Docking studies

The docking is performed with a series of His-Leu analogues and angiotensin-converting enzyme (ACE). His-Leu interacts with ACE (Fig. 3) with a higher energy

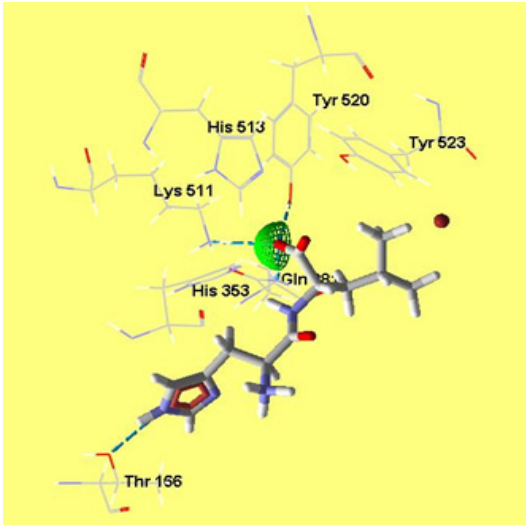

Fig. 3. Binding of His-Leu with ACE.

than that observed with the other analogues, - 89.462 kJ. His-Leu interacts electrostatically with Lys511, His513 and His353.

According to the docking studies the changes in the structure of His-Leu lead to analogues of a higher affinity to ACE. Their synthesis and evaluation will be the subject of a further research.

Synthesis of dipeptides

The synthesis and the spectral characterization of dipeptide His-Leu and Val-Trp are presented here. His-Leu and Val-Trp are prepared using DCC-method with a water-soluble reagent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl (EDAC), as shown in Fig. 4.

The final dipeptides are purified by column chro-

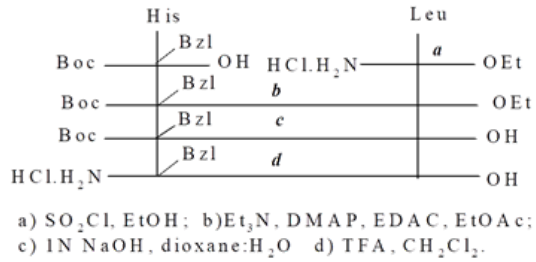

Fig. 4. Synthetic scheme of the dipeptide.

matography on silica gel with $CH_3CN:H_2O$ = 4:1. The starting and intermediate compounds are characterized by their physicochemical constants (Table 1) and the FT-IR spectral method.

FT-IR Spectral characterization

A spectroscopic analysis of the starting α-amino-acids

A comparative FT-IR spectral analysis of the reagents and some of the intermediate compounds obtained during the synthesis of each dipeptide is presented in this part.

It is known that the α-amino acids exist in a solid state in a zwitterion form and the molecules can be combined in their bipolar or whole neutral structures as shown in Scheme 1, [8].

The FT-IR analysis is carried out between 400 cm^{-1} and 4000 cm^{-1} using Thermo Scientific Nicolet iS10 FT-IR spectrometer. The IR- spectra of some investigated *α*-amino acids (His and Leu) in a solid state are shown in Fig. 5. There is a broad well-expressed absorption between 3500 cm^{-1} and 2000 cm^{-1} which is known in

a) b)

Scheme 1. Neutral and bipolar (zwitterion) molecular structures of: a) L-Histidine b) L-Leucine.

Table 1. Physicochemical constants.

Formula	Melting point, (m.p), °C	Optical activity, $[\alpha]^{20}D$	Color
L – Leu	>300º	+7,4º in H_2O	White
L – His	282º	_ 38,3º in H_2O	White
L-Val	297º	+27.5° in HCl	White
L-Trp	280-284º	-64.8 in g. CH_3COOH	White
Val- OEt. HCl.	102- 104º	-	White
Leu- OEt. HCl.	121 - 127º	+18-19º in EtOH	White
Boc –His(Bzl) -OH	181 - 184 º	+22º in MeOH	White
Boc-Thp-OH	135–137 º	–20-21º in DMF	-

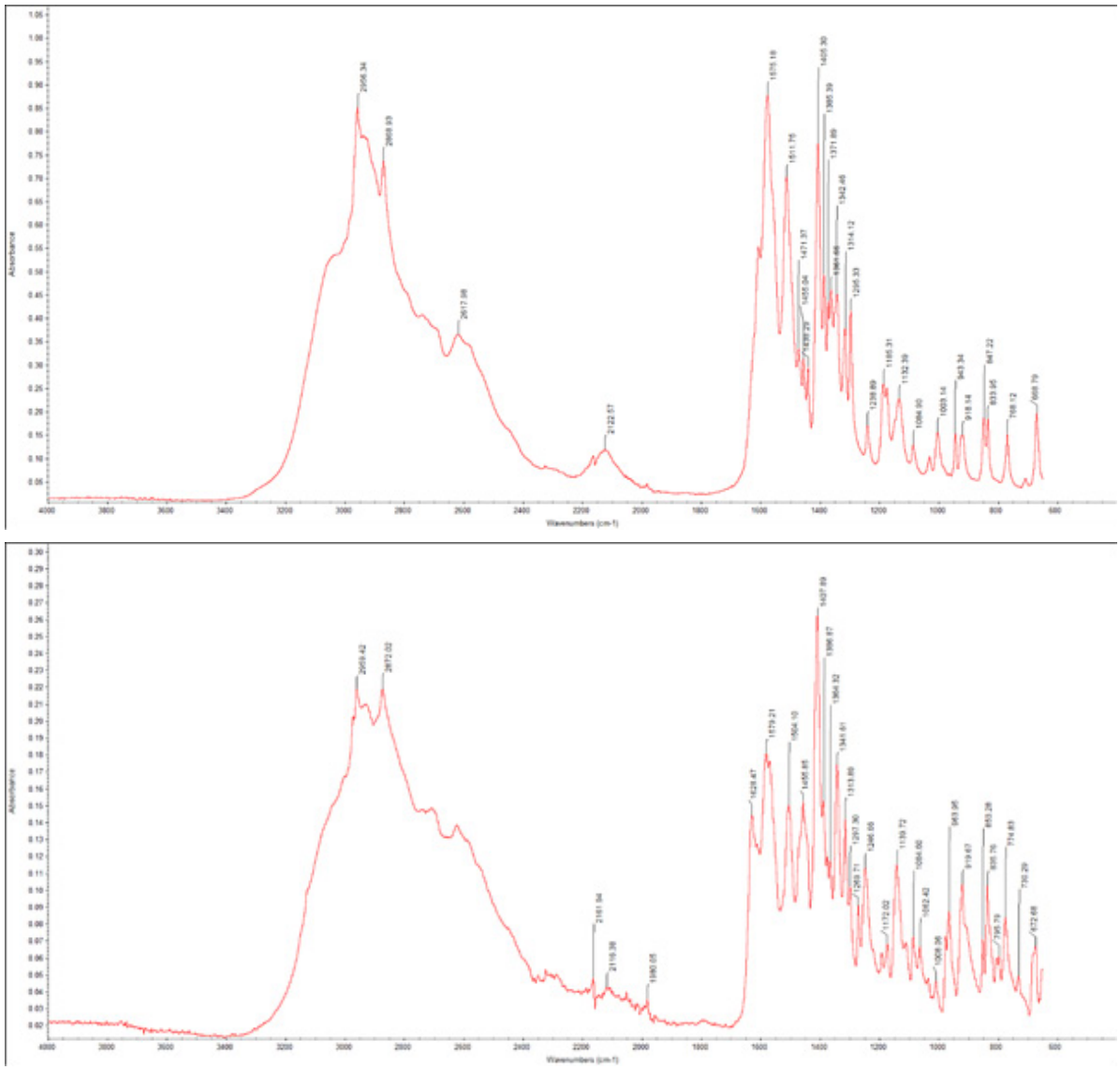

Fig. 5. FT-IR spectra of L-Leu (a) and L-His (b) in solid state.

IR spectroscopy as an "*ammonium band*". The absorption in this region is characterized by hydrogen bonded NH_3^+ stretching bands and a multiple fine structure on the lower wave number side of the band. Multiple combination and overtone bands also contribute to the extension of the absorption to about 2000 cm^{-1}. It is also a combination of two fundamental modes (a combination of δ_{CH3} bend and the rocking vibration of γ_{NH2} group (1084+1032) [9].

The maxima at 2956 cm^{-1}, 2868 cm^{-1}, 2959 $см^{-1}$ and 2872 $см^{-1}$ correspond to the stretching vibrations (ν^{as} and ν^{s}) of $-CH_2$ and $-CH_3$ groups of the side chain groups of Leu and His, while the band of Trp is recorded at 2851 cm^{-1}. The bands at 1340 cm^{-1} -1345 cm^{-1} (Leu and His) and 1356 cm^{-1} (Trp) correspond to deformation (δ_{CH3} and δ_{CH2}) vibrations. The peaks at 1575 cm^{-1} and 1405 cm^{-1} are assigned to the asymmetric and symmetric ($\nu^{as}_{COO^-}$ and $\nu^{s}_{COO^-}$) stretching modes of the carboxylate anion COO^- of Leu and His [10], while in case of Val and Trp they are outlined at 1427 cm^{-1} , 1590 cm^{-1} and 1413 cm^{-1}, correspondingly. The asymmetric NH bend of NH_3^+ (1511 cm^{-1}, 1506 cm^{-1} and 1504 cm^{-1}) and its symmetric bends (1471 cm^{-1}, 1463 cm^{-1} and 1455 cm^{-1}), respectively, are also clearly seen. This data confirms the bipolar structure of the pure amino acids. The presence of an isopropyl group in Leu and Val structures is confirmed by the doublet at 1385 cm^{-1} - 1371 cm^{-1} and the second pair of bands at 1185 cm^{-1} - 1170 cm^{-1} characterizing a

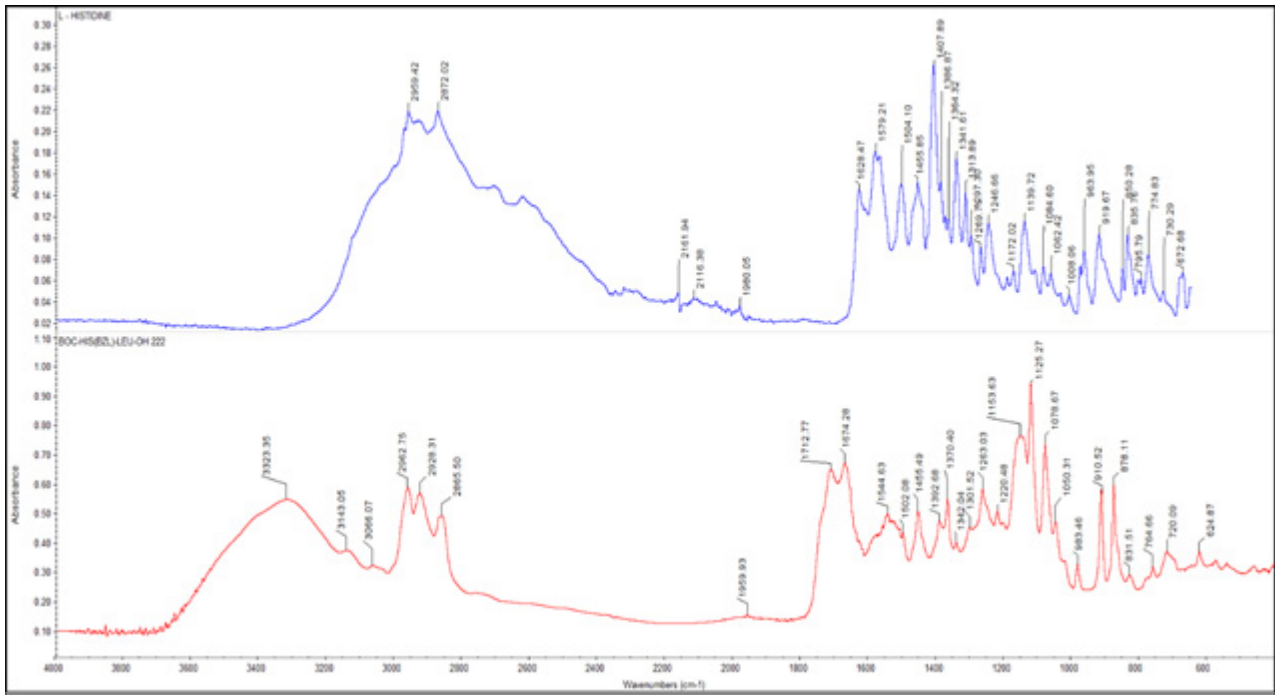

Fig. 6. FT-IR-spectra of L-His and Boc-His (Bzl) -Leu-OH in solid state.

scissor bending vibration. The band at 1628 cm^{-1} refers to the stretching $\nu_{C=N}$ modes of His molecule (imidazole heterocyclic) and is a result of skeletal interactions in the ring system.

Peaks appear within the frequency region of 1400 cm^{-1}-1200 cm^{-1}. They are found at 1361 cm^{-1}, 1342 cm^{-1} and 1316 cm^{-1} in case of Leu, at 1361 cm^{-1}, 1342 cm^{-1} and 1320 cm^{-1} for Val and at 1364 cm^{-1}, 1341 cm^{-1} and 1313 cm^{-1} in case of His. These bands are associated with the bending mode of CH_3 (Leu, Val), the stretching of C-C and the rocking of CH. The IR bands in the range of 1300 cm^{-1}-1200 cm^{-1} are associated with bending δ_{CH} modes. The corresponding bands are at 1295 cm^{-1} and 1238 cm^{-1} in case of Leu molecule, they are at 1297 cm^{-1}, 1269 cm^{-1} and 1246 cm^{-1} in case of His, while they are at 1271 cm^{-1} and 1230 cm^{-1} in presence of Val and Trp, correspondingly. There are single bands at 1028 cm^{-1} (Val), 1026 cm^{-1} (Trp), 1008 cm^{-1} (His) and 1003 cm^{-1} (Leu) due to the stretch of the CN group, while the bands appearing at 917 cm^{-1}, 919 cm^{-1}, 920 cm^{-1} and 926 cm^{-1} are associated with the C-C bond stretch [11].

A comparative FT-IR-spectral analysis of Boc-His(Bzl)-Leu-OH and L-Leu

There are several intensive and broad bands with a plurality of sub-maximum throughout the area in the 3500 cm^{-1} - 2250 cm^{-1} range (Fig. 6). The band at 3323 cm^{-1} is associated with the stretching ν_{NH} modes of the secondary amides, while those at 3143 cm^{-1} and 3066 cm^{-1} correspond to the stretching aryl (ν_{Ar-H}) vibrations of the benzyl moiety and the imidazole ring in the structure dipeptide. They may also belong to the stretching (ν_{NH}) modes, when the N-H group is incorporated in a strong hydrogen bonding [12 - 14]. In addition, these sub-maxima can be explained by Fermi-resonance interaction of overtones or combinations of fundamental and stretching N-H vibrations [10 - 11].

The band at 1712 cm^{-1} in the range of 1750 cm^{-1} - 1400 cm^{-1} corresponds to the stretching $\nu_{C=O}$ modes of -COOH group of Leu because of an unprotected ester group. The intense peak at 1674 cm^{-1} refers to the stretching symmetrical $\nu^{s}_{C=O}$ mode of the amide (-CO-NH-) group (*amide I band*), while the maximum at 1502 cm^{-1} (*amide II*) occurs *in trans-secondary* amides and responds to the complex contribution of the deformation δ_{NH} vibration and δ_{NH}+ ν_{CN} of the C-N bond. The presence of these bands confirms the hydrolysis of the leucine ester group.

CONCLUSIONS

Two dipeptides, His-Leu and Val-Trp are synthesized. They are characterized by their physicochemical constants as well as by chromatographic and spectral

analyses. The docking study is used to investigate the binding and the affinity of His-Leu and Val-Trp analogues to ACE.

REFERENCES

1. F. Shahidi, Y. Zhong, Bioactive peptides. Journal of AOAC International, 91, 2008, 914- 931.
2. S. Sharma, R. Singh, S. Rana, Bioactive peptides: a review, International Journal Bioautomotion, 15, 2011, 223-250.
3. B. Walther, R. Sieber, Bioactive proteins and peptides in foods. International Journal for Vitamin and Nutrition Research, 81, 2011, 181-191.
4. P. K. Arora and A. Chauhan "ACE inhibitors: A comprehensive review, International Journal of Pharmaceutical Sciences and Research 4, 2, 2013, 532-548.
5. C. Jao, S. Huang, K.C. Hsuc, Angiotensin I-converting enzyme inhibitory peptides: Inhibition mode, bioavailability and antihypertensive effects, Review article, BioMedicine, 2, 2012, 130-136.
6. I. Kapetanovic, Computer-Aided Drug Discovery and Development (CADDD): in silico-chemico-biological approach. Chemico-Biological Interactions, 171, 2, 2008, 165-176. doi: 10.1016/j.cbi.2006.12.006
7. A. Shukla, P. Sharma, O. Prakash, M. Singh, K. Kalani, F. Khan, D. Bawankule, S. Luqman, S. Srivastava, QSAR and docking studies on capsazepine derivatives for immunomodulatory and anti-inflammatory activity, PLoS One, 9, 7, 2014, e100797. doi: 10.1371/journal.pone.0100797
8. J. Emsley, Very Strong Hydrogen Bonding, Chemical Society Reviews, 9, 1981, 91-124.
9. R. Ittyachan, P. Sagayaraj, Growth and characterization of a new promising NLO L-histidine bromide crystal, Journal of Crystal Growth 249, 2003, 557-560.
10. P. Facanha Filho, P. Freir, K. Lima, J. Mendes Fieho, F. Melo, High temperature Raman spectra of L-Leucine crystals, Brazilian Journal of Physics 38, 1, 2008, 131-137.
11. S. Kumar, S. Rai , Spectroscopic studies of L-arginine molecule, Indian Journal of Pure and Applied Physics, 48,4, 2010, 251-255.
12. P. Anitha, N. Muruganantham, Growth and characterization of L-Alanine, International Journal of Science and Research, 3, 3, 2014, 51-56.
13. G. Barrett, J. Davies, B. Penke, G.Toth, G. Varasi, Analogue and conformational studies on peptides, hormones and other biologically active peptides, Amino Acids, Peptides and Proteins, 34, 1996, 55-148.
14. M. Majoube, Ph. Millie, G. Vergoten, Vibrational spectra of 4-methylimidazole: assignment of modes and calculation of Raman and resonance Raman intensities at the ab initio 6-31G level, Journal of Molecular Structure, 344, 1-2, 1995, 2136.