ORIGINAL PAPER

BISPHOSPHONATES-PDA: CORRELATION BETWEEN STRUCTURE AND PHYSICOCHEMICAL PROPERTIES

MARIA VIORICA CIOCILTEU[1], EMILIA AMZOIU[1], MANUEL OVIDIU AMZOIU[1,*], GABRIELA RAU[1], CATALINA GABRIELA PISOSCHI[1], ROBERTO ANGELO POSTELNICU[1], JOHNY NEAMTU[1], ALEXANDRA COSTACHI[1], DANIEL CORD[2], ANDREEA GABRIELA MOCANU[1]

Manuscript received: 11.01.2023; Accepted paper: 02.03.2023; Published online: 30.03.2023.

Abstract. *Bisphosphonates are used to treat a number of bone related diseases such as osteosarcoma, malignant hypercalcemia, osteomyelitis. Developing novel drug delivery systems may overcome the adverse reactions caused by traditional administration. This study uses a combination of molecular docking studies and correlation techniques between structure – physical and chemical properties to assess how different bisphosphonates (alendronate, risedronate, pamidronate, zoledronate) interact with polydopamine in order to later design new formulations. The structure of polydopamine is still under discussion therefore, its bisphosphonate binding properties have not been completely established. Polydopamine was modeled by repeated docking of tetrameric subunits combined in two ways which led to simple and mixed oligomers. Fingerprint descriptors, namely electronegativity of the OMO/UMO quantum molecular states, were used for the correlation studies. The correlation coefficients suggest that several atom species such as nitrogen and carbon have increased contributions to the formation of both HOMO and LUMO molecular states. The results showed that the most stable complex was obtained with risedronate for both simple and mixed dopamine oligomers (-186.00 kJ/mol and -184.92 kJ/mol).*

Keywords: *molecular docking; fingerprint descriptors; bisphosphonates.*

1. INTRODUCTION

Bone related diseases such as osteomyelitis, osteosarcoma, osteoporosis are difficult to treat due to several limiting factors such as decreased perfusion of the bone, low bioavailability, toxicity and side effects. Therefore, designing drug delivery systems based on newer carriers such as microparticles, liposomes or nanoparticles that target the bone is a highly researched field [1-8].

Bisphosphonates induce apoptosis in osteoclasts [1], inhibit resorption of the destructive bone and the release of tumor stimulating factors [2]. Depending on the structure of the two terminal phosphate groups bisphosphonates may be divided into non-nitrogen containing bisphosphonates such etidronate and clodronate and nitrogen containing bisphosphonates such as alendronate, pamidronate, zoledronate, risedronate.

[1] University of Medicine and Pharmacy of Craiova, 200638 Craiova, Romania.
E-mail: m_amzoiu@yahoo.com; emanro2002@yahoo.com.
[2] University Titu Maiorescu Bucharest, 031593 Bucharest, Romania.

The latter displays a better bone affinity [9,10]. Furthermore, bisphosphonates improve quality of life for patients with bone tumors by reducing both pain and bone damage [2].

Polydopamine (PDA), a polymer obtained through dopamine oxidation, has the ability to attach to different types of compounds creating a coating with a thickness that may vary between a few and 100 nm. Moreover, the PDA coating can be subsequently functionalized with either metallic nanoparticles or nucleophile molecules [11-13].

The exact structure of PDA is still under discussion. Therefore, there is not a complete characterization regarding its bisphosphonate binding properties. Knowing how bisphosphonates interacts as a ligand to PDA which acts as a receptor plays a key role in safely designing new formulations. Furthermore, it helps to fully understand their involvement in different fundamental biochemical processes [11-13].

Using both molecular docking studies and correlation techniques between structure – physical and chemical properties allows a complete approach of the binding problems that may arise. This may yield new probable binding conformations between the bioactive compounds and the biological active target on the receptor. Furthermore, understanding the structure generates new information that helps comprehend how the physiological response is obtained [14].

Fingerprint descriptors such as electronegativity of the Occupied Molecular Orbital with electrons/ Unoccupied Molecular Orbital with electrons OMO-UMO quantum molecular states were used for the correlation studies.

Furthermore, they proved to be reliable for the QSAR/QSPR studies. The OMO/UMO molecular states are formed with the contribution of atoms from the molecule. Moreover, their intervention in the ligand (drug)-receptor interaction identifies them as reactive states for the studied chemical structures [15-17].

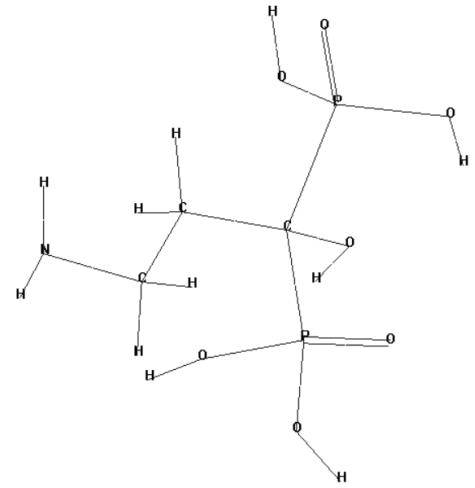
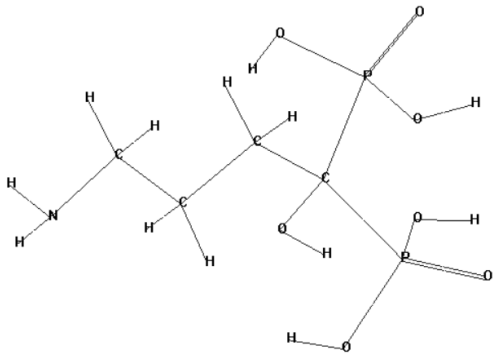
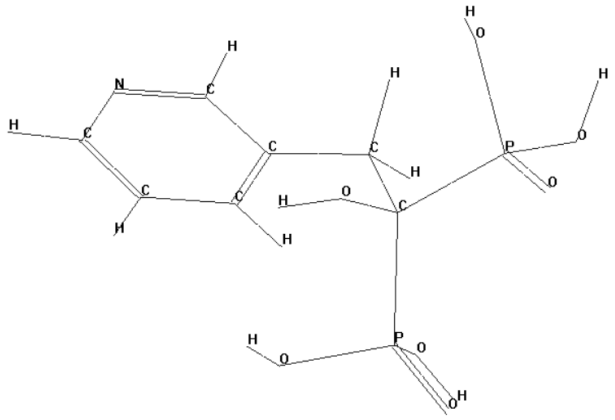
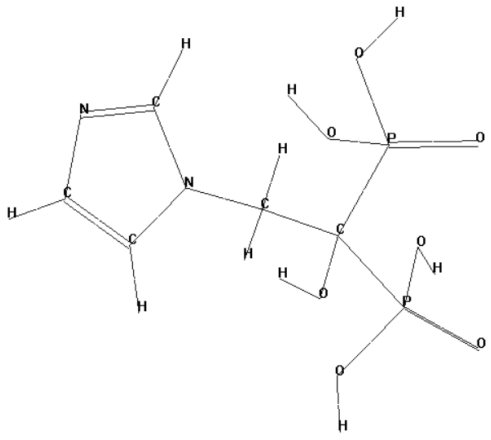
The aim of the study is to determine how each atom from the bisphosphonate structure is involved in a physical and chemical property or a biological activity by combining the molecular docking technique with the structure – binding energy correlation technique. This allows the design of new chemical structures using molecular fragments or identified chemical bonds. Moreover, these compounds have a predictable and optimizable response function.

2. MATERIALS AND METHODS

2.1. MATERIALS

The studied compounds are represented by 4 bisphosphonates for which the specialized literature provides different relative potencies against osteoporosis (Table 1) [18].

Table 1. Bisphosphonates relative potency in osteoporosis.

Compound	Relative potency
	100
	100-500
	1000
	5000

2.2. METHODS

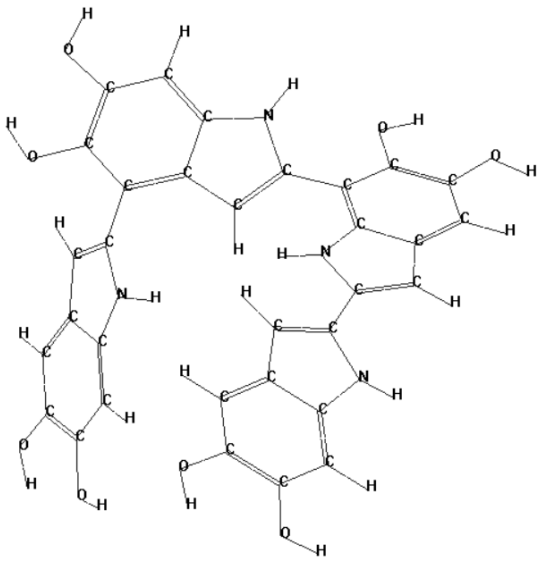
The bisphosphonates geometry was modeled with HyperChem Release 8.0 Professional (Hypercube Inc.) software using the semi-empirical parametric method 3 PM3.

Ligand (bisphosphonate) – receptor (PDA) interaction energy was determined by molecular docking with Hex 8.0 program. Due to its amorphous state, the structure of PDA does not currently exist in the PDB database [https://www.rcsb.org/]. Therefore, we made oligomeric structures by repeated docking of tetrameric subunits and obtained layered aggregates as predicted by studies carried out on PDA [19-21].

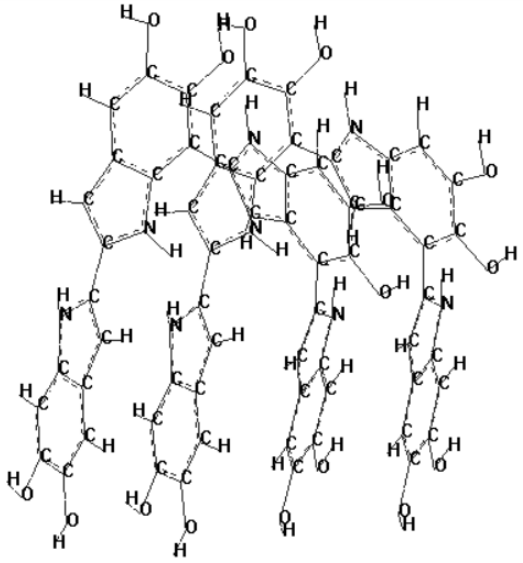
The fingerprint descriptors were determined with an in-house program known as Elwindow which performs LCAO-MO calculations for the studied molecules. Moreover, it uses the output files of the MOPAC package [MOPAC 7.0 for UNIX, Quantum Chemistry Program Exchange, Project 688.] to read the information (electrical charges, mixing coefficients, atomic contributions, etc.).

3. RESULTS AND DISCUSSION

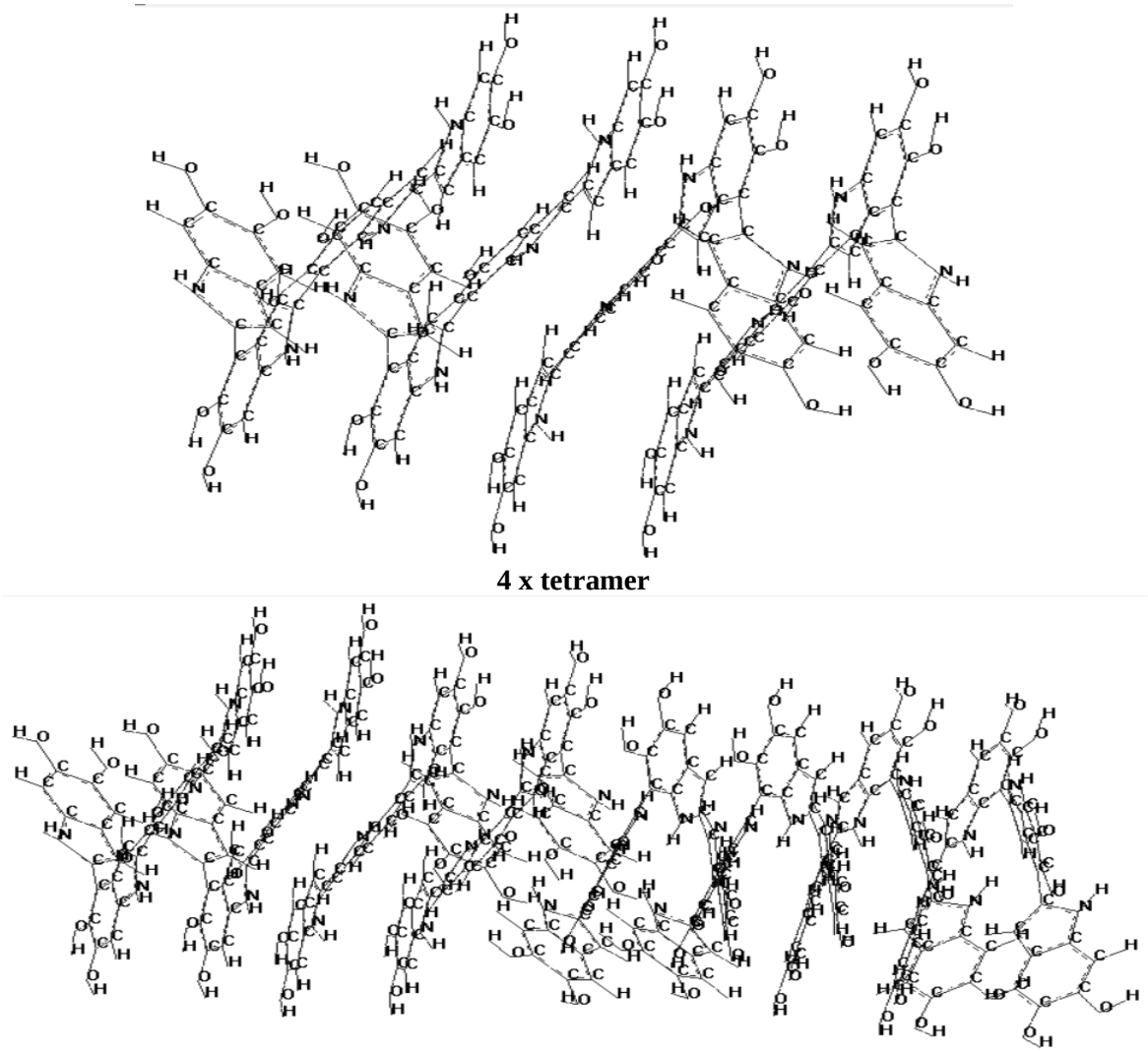
The dopamine target was obtained by repeated docking of tetrameric subunits, the latter being combined in two ways. The first form is obtained by the chemical binding of the same repeat unit – dihydroxy indole resulting in simple oligomers. The second form of docking uses tetramers obtained by mixing 4 different subunits in an equimolecular ratio (dopamine, dihydroxy indole, leukodopaminechrome and dopaminechrome) which result in mixed oligomers. The chemical bonding of the repeat units was implemented in the most reactive positions, namely 2, 4 and 7 (Fig. 1).



1 x tetramer



2 x tetramer



4 x tetramer

8 x tetramer

Figure 1. Tetramer- tetramer Hex docking experiments.

It should be noted that with our docking process the tetramers have almost planar stable conformations and stack up nicely. This is in accordance with literature data that mentions that it is important to use almost planar molecular models in order to simulate the self-assembly mechanism of PDA [22].

By docking bisphosphonates with all these oligomeric forms yielded interaction energies that vary almost in the same manner for both simple and mixed forms (Tables 2 and 3). Therefore, in almost all cases the bisphosphonate – dopamine oligomer interaction energy varies in the increasing order risedronate> zoledronate> alendronate> pamidronate.

Table 2. Bisphosphonate – dopamine oligomer (simple oligomer) interaction energies [kJ/mol]

Compound	1 x tetramer	2 x tetramer	4 x tetramer	8 x tetramer
Alendronate	-150.00	-158.68	-160.07	-153.73
Pamidronate	-138.72	-151.86	-158.04	-156.23
Risedronate	-177.61	-187.01	-193.12	-186.00
Zoledronate	-163.63	-174.11	-183.50	-185.25

Table 3. Bisphosphonate – dopamine oligomer (mixed oligomer) interaction energies [kJ/mol]

Compound	1 x tetramer	2 x tetramer	4 x tetramer	8 x tetramer
Alendronate	-152.24	-141.40	-177.29	-156.21
Pamidronate	-146.14	-140.96	-156.33	-153.46
Risedronate	-183.19	-164.89	-204.00	-184.92
Zoledronate	-154.98	-157.99	-179.75	-179.43

In addition, the interaction energies between bisphosphonates and mixed oligomers is slightly lower than in the case of bisphosphonates and simple oligomers as shown in the tables 2 and 3 (for risedronate the interaction energy with the mixed oligomer is -204.00 kJ/mol whereas the interaction energy with the simple oligomer is -193.12 kJ/mol). This shows that a localization of the bisphosphonate in the active sites with mixed content PDA is beneficial for an increased stability of the complex obtained by interaction. On the other hand, this is supported by the fact that the structure of PDA is amorphous, containing oligomers with variable sizes and mers in oxidized forms that have a major impact on the molecular structure of PDA and, implicitly, on the interaction.

Therefore, the most stable bisphosphonate-PDA complex is risedronate which has the lowest bonding energy (-186.00 kJ/mol and, respectively, -184.92 kJ/mol). This can also explain its delayed release from the administration form and thus, may present a better therapeutic action compared to the others (zoledronate, alendronate and pamidronate).

Computational studies carried out with the help of fingerprint descriptors such as electronegativity of the OMO/UMO quantum molecular states allow the understanding of the mechanism of action of bisphosphonates through the ability of the atoms in the molecule to gain or lose electron densities when interacting with PDA.

Elwindow program allows the estimation of descriptors such as the electronegativity for each OMO/UMO molecular state, EL = ELH + ELC + ELO + ELN as well as the contributions of each atomic species ELH (for hydrogen), ELC (for carbon), ELN (for nitrogen), ELO (for oxygen). The values for these descriptors are shown in Tables 4 and 5.

Table 4. Electronegativity of the HOMO molecular states for the studied compounds

$E_{binding}$ [kJ/mol]	HEL	HELH	HELC	HELO	HELN
-156.21	7.006	0.891	0.386	0.146	5.374
-153.46	6.789	0.550	0.904	1.157	2.637
-184.92	6.342	0.402	1.766	1.879	0.004
-179.43	5.609	0.022	4.679	0.023	0.872

HEL - total electronegativity, HELAT - electronegativity of heavy atoms (other than hydrogen atoms);
HELH - electronegativity of hydrogen atoms;
HELC - electronegativity of the carbon atom;
HELO – the electronegativity of the oxygen atom, H prefix refers to the HOMO state;
HELN – the electronegativity of the nitrogen atom.

Table 5. Electronegativity of the LUMO molecular states for the studied compounds

$E_{binding}$ [kcal/mol]	LEL	LELH	LELC	LELO	LELN
-156.21	18.296	0.337	0.584	1.319	0.000
-153.46	18.229	0.329	0.660	1.260	0.004
-184.92	6.658	0.070	5.122	0.085	0.308
-179.43	18.415	0.407	0.697	1.199	0.017

LEL - total electronegativity, LELAT - electronegativity of heavy atoms (other than hydrogen atoms);
LELH - electronegativity of hydrogen atoms;
LELC - electronegativity of the carbon atom;
LELO – electronegativity of the oxygen atom, L prefix refers to the LUMO state;
LELN – the electronegativity of the nitrogen atom.

The bisphosphonates-PDA interaction energies were statistically corelated ($E = a_0 + a_1X_1$; X_1 are the fingerprint descriptors) with the chemical structure of the studied compounds. Table 6 shows the results expressed in the form of correlation coefficients R^2 [%].

Table 6. The correlation coefficients R^2 [%] for the electronegativity of the molecular states

Atom	HOMO	LUMO	OMO	UMO
H	51.50	24.30	71.90	98.10
C	45.20	48.30	99.90	72.90
O	7.90	53.60	85.30	84.30
N	71.60	51.40	5.90	67.90

Among the species of atoms from the studied molecules that contribute more to the formation of quantomolecular states, oxygen atoms stand out as they contribute more to the formation of the LUMO state (LELO) with a percentage of 53.6% than to the formation of HOMO states (7.9%) as presented in table 6. Nitrogen atoms, on the other hand, have large contributions to the formation of both HOMO and LUMO molecular states, 71.6% (HELN) and 51.4% (LELN). Also, the carbon atoms have almost equal contributions for the HOMO/LUMO states in forming the biological response 45.2% (HELC) and 48.3% (LELC).

The table shows even bigger contributions from all the atoms in the molecule to the formation of the other 2 molecular states OMO and UMO. The values for the correlation coefficients suggest the possibility of an electron transfer between atoms of bioactive molecules (bisphosphonates) and atoms in the active site of the biological receptor (dopamine) (Fig. 2).

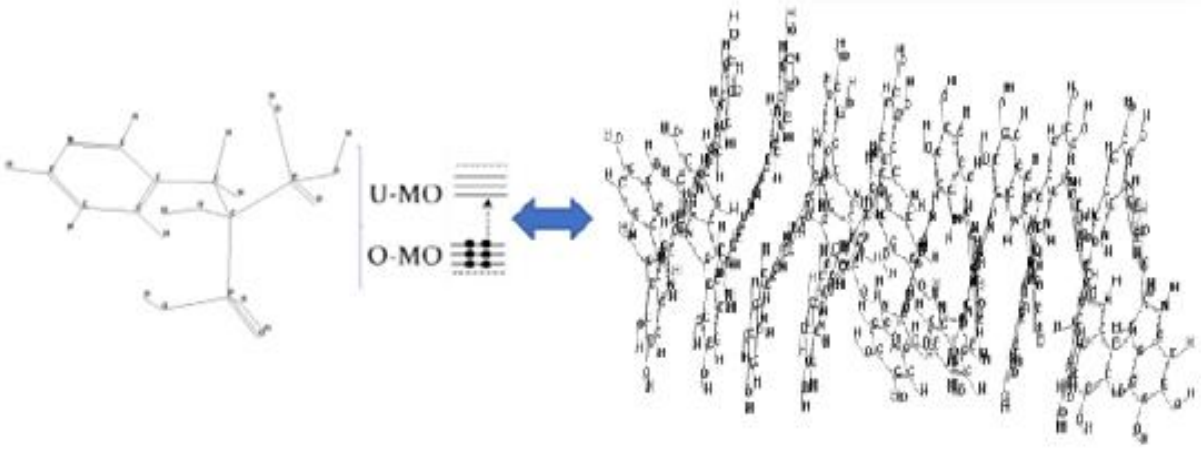


Figure 2. Bisphosphonate - dopamine oligomer interaction.

4. CONCLUSIONS

The bisphosphonate - dopamine oligomer interaction energy varies for both simple and mixed oligomers in the same order, namely risedronate > zoledronate > alendronate > pamidronate. This suggests that the bisphosphonate- risedronate complex is the most stable which may lead to a delayed release after administration. Both nitrogen atoms and carbon atoms have high contributions to the formation of quantomolecular states namely HOMO and LUMO molecular states. Furthermore, nitrogen, carbon and oxygen atoms display high contributions to the formation of both OMO and UMO molecular states. This suggests that an electron transfer between atoms from the bisphosphonate and atoms from the dopamine receptor might occur. Thus, the atoms will participate in the interaction as electron acceptor and donor centers.

Acknowledgement: *This work was supported by the grant POCU/993/6/13/153178, "Performanță în cercetare" – "Research performance" co-financed by the European Social Fund within the Sectorial Operational Program Human Capital 2014-2020.*

Authors' contributions: All authors have equal contributions to the manuscript.

REFERENCES

[1] Vaculikova, E., Placha, D., Pisarcik, M., Peikertova, P., Dedkova, K., Devinsky, F., Jampilek, J., *Molecules*, **19**(11), 17848, 2014.
[2] Zhong, Y., Li, S., *Drug Design, Development and Therapy*, **15**, 4939, 2021.
[3] Pan, Y., Wang, J., Jiang, Z., Guo, Q., Zhang, Z., Li, J., Hu, Y., Wang, L., *Scientific Reports*, **12**, 12290, 2022.
[4] Bucurica, I. A., Popescu, I.V., Radulescu, C., Cimpoca, Gh.V., Dulama, I.D., Teodorescu, S., Gurgu, I.V., Let, D.D., *Bulletin of Materials Science*, **41**, 71, 2018
[5] Poinescu, A.A., Radulescu, C., Vasile, B.S., Ionita, I., *Revista de Chimie*, **65**(10), 1245, 2014.
[6] Zarafu, I., Matei, L., Bleotu, C., Ionita, P., Tatibouët, A., Paun, A., Nicolau, I., Hanganu, A., Limban, C., Nuta, D.C., Nemes, R.M., Diaconu, C., Radulescu, C., *Molecules*, **25**(14), 3308, 2020
[7] Zarafu, I., Limban, C., Radulescu, C., Dulama, I.D., Nuta, D.C., Chirita, C., Chifiriuc, M.C., Badiceanu, C.D., Popa, M., Bleotu, C., Dragu, L.D., Stirbescu, R.M., Bucurica, I.A., Stanescu, S.G., Ionita, P., *Coatings*, **12**, 45, 2022
[8] David, M., Serban, A., Radulescu, C., Danet, A.F., Florescu, M., *Bioelectrochemistry*, **129**, 124, 2019
[9] Grey, A., Reid, I. R., *Therapeutics and Clinical Risk Management*, **2**(1), 77, 2006.
[10] Chen, Y., Wu, X., Li, J., Jiang, Y., Xu, K., Su, J., *Frontiers in Pharmacology*, **13**, 909408, 2022.
[11] Liebscher, J., Mrówczyński, R., Scheidt, H. A., Filip, C., Hădade, N. D., Turcu, R., Bende, A., Beck, S., *Langmuir*, **29**(33), 10539, 2013.
[12] Ball, V., *Frontiers in Bioengineering and Biotechnology*, **6**, 109, 2018.
[13] El Yakhlifi, S., Ball, V., *Colloids and Surfaces B: Biointerfaces*, **186**, 110719, 2020.
[14] Reiss, A., Mitran, R. A., Băbeanu, C., Amzoiu, E., Amzoiu, M. O., Văruţ, R. M., Gănescu, A., *Revista de Chimie*, **71**(10), 11, 2020.
[15] Amzoiu, E., Anoaica, P. G., Lepadatu, C. I., *Revue Roumaine de Chimie*, **56**(7), 711, 2011.
[16] Amzoiu, M., Cristovici, F., Amzoiu, D., Popescu, F., Nitulescu, A., Cadar, E., *Journal of Science and Arts*, **2**(55), 529, 2021.
[17] Lepadatu, C. I., Culita, D. C., Patron, L., *Optoelectronics And Advanced Materials-Rapid Communications*, **4**(2), 160, 2010.
[18] Tripathi, K. D., *Essentials of Medical Pharmacology*, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 2013.
[19] Yana, J., Chiangraeng, N., Nimmanpipug, P., Lee, V. S., *Journal of Molecular Graphics and Modelling*, **107**, 107946, 2021.
[20] Szewczyk, J., Pochylski, M., Szutkowski, K., Kempinski, M., Mrowczynski, R., Iatsunskyi, I., Gapinski, J., Coy, E., *Materials Today Chemistry*, **24**, 100935, 2022.
[21] Alfieri, M. L., Panzella, L., Oscurato, S. L., Salvatore, M., Avolio, R., Errico, M. E., Pasqualino, M., Napolitano, A., d'Ischia, M., *Biomimetics*, **3**, 26, 2018.
[22] Szewczyk, J., Pochylski, M., Szutkowski, K., Kempinski, M., Mrowczynski, R., Iatsunskyi, I., Gapinski, J., Coy, E., *Materials Today Chemistry*, **24**, 100935, 2022.