ORIGINAL PAPER

STUDY OF THE INTERACTION BETWEEN OXICAM DERIVATIVES AND COX1 USING FINGERPRINT DESCRIPTORS AND MOLECULAR DOCKING

MANUEL AMZOIU[1], FLORENTINA CRISTOVICI[2], DENISA AMZOIU[1*], FLORICA POPESCU[2], ALEXANDRA NITULESCU[2], EMIN CADAR[3]

Manuscript received: 10.03.2021; Accepted paper: 03.06.2021; Published online: 30.06.2021.

Abstract. *Oxicam derivatives play an important role in the treatment of pain and inflammation. The mechanism of action of these compounds consists in the inhibition of cyclooxygenase (COX) and the blockade of isoform 1 of this enzyme (COX-1) is considered to generate adverse effects. The aim of this paper is to establish which of the atoms in the oxicams molecules are responsible for their inhibitory activities, using electronegativity as fingerprint descriptor. Using this descriptor and molecular docking programs, the atoms in the molecule that have a greater contribution to COX-1 inhibition have been identified. In the case of the studied molecules, the oxygen atoms and the nitrogen atoms are highlighted. The oxygen atoms participate in the interaction as electron acceptors through U-MO molecular levels (74.1%) and the nitrogen atoms participate in the interaction both as a nucleophilic center through the molecular state of HOMO (13.7%) and as an electrophilic center through the molecular state of LUMO (13.2%). In the case of three out of four of the studied compounds, the 4-hydroxyl group of the thiazine ring participates in the interaction with COX-1. The results are also supported by the 2D and 3Ddiagrams of the applied docking method.*

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

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used classes of drugs worldwide, especially for the treatment of inflammation and pain [1-3]. Their analgesic and anti-inflammatory actions are mainly due to blocking the synthesis of prostaglandins from arachidonic acid by the inhibition of cyclooxygenase. Prostaglandins have protective effects especially in the gastrointestinal tract or kidneys, but they are also involved in the development of inflammation [1, 4, 5].

The discovery of cyclooxygenase isoforms (COX-1 and COX-2) led to the concept that COX-1, a constitutive enzyme, tends to have a homeostatic function, whereas COX-2, an inducible enzyme during inflammation, tends to facilitate the inflammatory response. Thus,

[1] University of Medicine and Pharmacy, Faculty of Pharmacy, 200349 Craiova, Romania. E-mail: m_amzoiu@yahoo.com; damzoiu@yahoo.com
[2] University of Medicine and Pharmacy, Faculty of Medicine, 200349 Craiova, Romania. E-mails: fcristovici@gmail.com; prof_floricapopescu@hotmail.com; luci.alexa94@yahoo.com
[3] Ovidius University of Constanta, Faculty of Pharmacy, 900527 Constanta, Romania. E-mail: emin.cadar@gmail.com.

the two forms of COX enzyme play an extremely important role in the body. Blocking one of them can cause devastating effects, which is why the cyclooxygenases COX-1 and COX-2 must be maintained in a harmonious balance. Most NSAIDs inhibit both COX isoforms, but based on the discovery of the existence of COX isoforms, selective COX-2 inhibitors have been introduced into therapy, with the idea that they have fewer side effects (especially gastrointestinal) [6-8]. Currently, attempts are being made to obtain new anti-inflammatory agents that keep the 2 cyclooxygenases COX-1 and COX-2 in balance. This is possible if it is known how the existing anti-inflammatory drugs interact with the biological target, *i.e*. which areas of the anti-inflammatory compound molecule are responsible for their inhibitory activities.

Therefore, the aim of this study is to establish which of the atoms present in the molecules of oxicams are part of these active zones. The fingerprint descriptors used in this regard are the electronegativities of the molecular states of the studied compounds and the electrical charges placed on the atoms in the molecule, charges arising from the formation of chemical bonds. The use of these descriptors allows obtaining additional information about the mechanism by which the atoms responsible for the manifestation of the studied biological activity are involved in the drug-cyclooxygenase interaction [9, 10].

The identification of these atoms is done according to a statistical analysis [11] based on an equation which contains a single descriptor:

$$A = a_0 + a_i \cdot X_i,$$

where A is the biological activity, the contributions a_i are the regression coefficients and X_i represents the fingerprint descriptors corresponding to the chemical compounds.

Electronegativity descriptors show the ability of atoms in the molecule to gain or lose electron densities, that is they characterize the involvement of external atomic orbitals in the formation of chemical bonds. According to Mulliken [12], absolute electronegativity describes the easiness of an atom to loose/gain electrons and is defined by the relationship:

$$\chi \approx \frac{I + A}{2} = \frac{E_{HOMO} + E_{LUMO}}{2},$$

where I represent the ionization potential, A is the electron affinity, E_{HOMO} is the energy of the highest occupied molecular orbital and E_{LUMO} is the energy of the lowest unoccupied molecular orbital.

Unlike atoms, in which the release-acceptance of electrons involves the valence layers, a molecule interacts with another molecule (or with a biological receptor) through its molecular states, namely through the O-MO (Occupied Molecular Orbitals) layers in electron release processes and with the U-MO (Unoccupied Molecular Orbitals) layers in the electron acceptors processes. In the case of a molecule, in other words, the interaction of two molecules, that of the ligand (drug) and the biological one (the receptor), can be achieved by electron transfer between the molecular states O-MO and U-MO [13-16].

The interactions between compound and biological receptor takes place by electron transfer from ligand (compound - tenoxicam) to receptor (COX) and vice versa (Fig. 1).

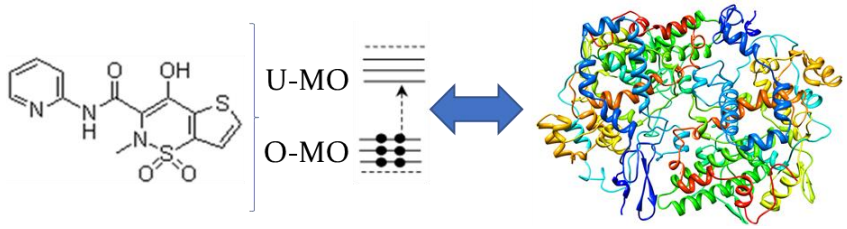


Figure 1. The interaction compound – COX.

The electronegativity of the O-MO / U-MO molecular states can be estimated with the following formula [16]:

$$X_i = \sum_j \overline{C}_{ij}^2 \chi_j(Q_j),$$

where χj(Qj) represents the electronegativity of the atoms in molecule and $\overline{C}_{ij}^2$ the contribution of "j" atom equal to the sum of the Mulliken partition coefficients of all its valence atomic orbitals.

The total electronegativity of the O-MO/U-MO quantum states is defined by the relation:

$$EL = ELH + EC + EO + EN,$$

in which the contributions of each atomic species to the total value are as follows: ELH is the electronegativity brought about by the hydrogen atoms, EC - carbon, EN - nitrogen, EO - oxygen etc. For all descriptors in this paper, the prefix H refers to the molecular state HOMO and the prefix L to the state LUMO:

$$HEL = HELH + HEC + HEO + HEN$$

and

$$LEL = LELH + LEC + LEO + LEN.$$

Electronegativity descriptors are estimated taking into account the electrical charges of the atoms in the molecule [16,17]. These descriptors are obtained by forming chemical bonds between atoms, as partitions of the electronic population on the atoms in the molecule.

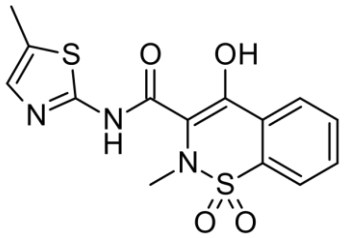
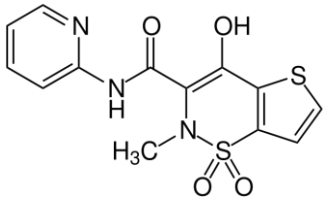
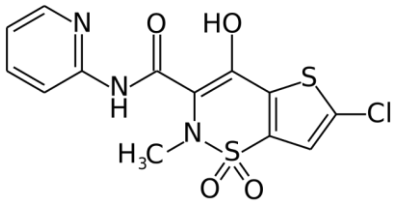
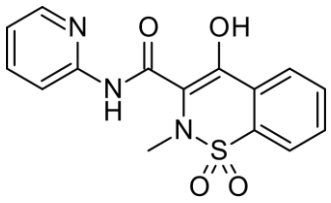
In the second part of the study, the interaction between a ligand (drug) and the biological receptor is studied by the molecular docking method, which gives the possibility of theoretical interpretation of the binding of two molecules, by calculating the intermolecular binding energies [19, 20].

2. MATERIALS AND METHODS

The partition coefficient values of the oxicam compounds were taken from the literature [21], its values being presented in Table 1. The partition coefficient is a measure of the hydrophobicity phenomenon (lipophilicity), the concept of hydrophobic interaction being applied today in the design of new drugs. Chemical modeling of the molecules of the 4 oxicams was performed using the ArgusLab program [22]. Molecular structures were used as

input data for MOPAC 6 software (Molecular Orbital PACkage), offered as a shareware program for academic research [23]. FlexX software was used to study how to bind oxicam compounds to the active site of the receptor (the COX-1 isoform) [24]. The receptor molecule (*i.e.*, COX-1) was taken from the Protein Data Bank [25].

Table 1. Structure and partition coefficient of the studied compounds [21]

Compound	Structure	logP (octanol/water)
Meloxicam		0,10
Tenoxicam		1,90
Lornoxicam		2,62
Piroxicam		3,06

3. RESULTS AND DISCUSSION

The participation of the ligand (drug) to the ligand-biological receptor interaction is described in this study with electronegativity parameters (descriptors), which represent the "fingerprint" of atoms and are called O-MO/U-MO fingerprint descriptors for quantum molecular states [26, 27]. Their values are presented in Table 2.

Table 2. The values of the electronegativity descriptors for the atoms in the molecule

Compound	EL	ELAT	ELH	ELC	ELO	ELN
Meloxicam	248.128	146.374	101.754	82.287	18.737	19.070
Tenoxicam	189.898	134.346	55.551	69.961	18.621	19.001
Lornoxicam	188.365	141.014	47.350	69.760	18.647	17.379
Piroxicam	248.581	145.589	102.991	89.509	18.595	18.913

EL - total electronegativity, ELAT - electronegativity of heavy atoms (other than hydrogen atoms), ELH - electronegativity of hydrogen atoms, ELC - electronegativity of the carbon atom, ELN – the electronegativity of the nitrogen atom, ELO – the electronegativity of the oxygen atom.

The correlation of the electronegativity descriptor with the hydrophobicity of molecules led to the results presented in Table 3.

Table 3. Correlation results for electronegativity descriptors

Descriptor	R2 [%]
EL	9.0
ELAT	4.2
ELH	9.5
ELC	0.9
ELO	84.8
ELN	18.4

As can be seen in this table, the high correlation coefficients recommend the use of the electronegativity descriptor of heavy atoms (ELO and ELN) which are representative for the molecular structures and the interaction of these molecules with the active sites of biological receptors. The largest contribution to the formation of hydrophobicity is the electronegativity of oxygen atoms (84.8%), followed by that of nitrogen atoms (18.4%).

This observation is interesting and suggests the possibility of using these descriptors in chemical modulation, in order to reduce the rather pronounced side effects presented by the studied anti-inflammatory compounds.

Electronegativity fingerprint descriptors characteristic of HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) quantum molecular states are presented in Tables 4a and 4b, and the results of the regression correlation of these descriptors with log P are shown in Table 5. The correlation coefficients obtained provide information about the contribution of different atomic species to the hydrophobicity formation.

Table 4a. Fingerprint descriptors for quantomolecular state HOMO

HEL	HELAT	HELH	HEC	HEO	HEN
5.624	5.456	0.168	3.261	0.659	1.421
5.575	5.323	0.252	3.275	0.301	1.320
5.680	5.467	0.213	3.268	0.259	1.129
5.627	5.458	0.170	3.255	0.654	1.435

The prefix H refers to the HOMO state

Table 4b. Fingerprint descriptors for quantomolecular state LUMO

LEL	LELAT	LELH	LEC	LEO	LEN
6.170	6.155	0.015	4.950	0.179	0.187
5.716	5.684	0.032	4.277	0.070	0.253
5.723	5.693	0.029	4.243	0.062	0.247
6.182	6.169	0.013	4.953	0.179	0.183

The prefix L refers to the LUMO state

Table 5. Correlation coefficients R2 [%]

Atom	HOMO	LUMO	O-MO	U-MO
C	0.4	9.4	77.0	23.4
N	13.7	13.2	41.4	37.8
O	10.7	9.7	2.8	74.1

HOMO = Highest Occupied Molecular Orbital,
LUMO = Lowest Unoccupied Molecular Orbital,
O-MO/U-MO = the last and, respectively, the first three occupied / unoccupied molecular states

It is clear from Table 5 that oxygen atoms will participate in the interaction as electron acceptors through U-MO molecular levels (74.1%). Carbon atoms, on the other hand, have a contribution to hydrophobicity through O-MO molecular states (77.0%), which means that they participate as nucleophilic centers. The equal participation of nitrogen atoms in the interaction should be noted, both as a nucleophilic center through the molecular state of HOMO (13.7%) and as an electrophilic center through the molecular state of LUMO (13.2%). Also, the same atoms have better involvement in the interaction but through the deep molecular levels, O-MO/U-MO (41.4% and 37.8% respectively).

As with electronegativity fingerprint descriptors, fingerprint descriptors based on the electrical charges of the atoms in the molecule confirm the participation of the atoms in the molecule in the inhibitory activity of the oxicam derivatives studied. The values of these descriptors are presented in Tables 6a and 6b.

Table 6a. Fingerprint descriptors for quantomolecular state HOMO

HQ	HQ-AT	HQ-H	HQ-C	HQ-O	HQ-N
-0.186	-0.187	0.001	-0.044	-0.042	-0.115
-0.185	-0.188	0.003	-0.078	-0.024	-0.108
-0.178	-0.181	0.003	-0.094	-0.021	-0.092
-0.185	-0.187	0.001	-0.043	-0.042	-0.115

The prefix H refers to the HOMO state

Table 6b. Fingerprint descriptors for quantomolecular state LUMO

LQ	LQ-AT	LQ-H	LQ-C	LQ-O	LQ-N
-0.056	-0.056	0	-0.121	-0.024	-0.013
-0.136	-0.136	0	-0.176	-0.009	-0.018
-0.136	-0.136	0	-0.176	-0.008	-0.018
-0.054	-0.054	0	-0.120	-0.025	-0.013

The prefix L refers to the LUMO state

The results of the statistical correlation of these descriptors with the inhibitory activity of the studied oxicam derivatives ($\log P = a_0 + a_1X_1$, X_1 - electric charge descriptor) are presented in Table 7.

Table 7. Correlation coefficients R2 [%]

Atom	*HOMO*	*LUMO*	*O-MO*	*U-MO*
C	11.2	8.4	96.8	79.1
N	14.1	9.1	53.8	93.8
O	10.5	7.3	86.4	87.8

HOMO = Highest Occupied Molecular Orbital,
LUMO = Lowest Unoccupied Molecular Orbital,
O-MO/U-MO = the last and, respectively, the first three occupied / unoccupied molecular states

For the electric charge descriptors of atoms in electron-occupied (O-MO) and non-electron-occupied (U-MO) molecular states, as shown in the regression analysis (Table 7), the values of the correlation coefficients R^2 (%) indicate that between all heavy atoms other than hydrogen atoms, carbon atoms participate most in the log P activity with molecular orbitals O-MO: QC 96.8, and nitrogen atoms participate in the inhibitory activity with molecular orbitals U-MO: QN 93.8. Oxygen atoms have approximately the same involvement, with 86.4% contributing to O-MO levels and 87.8% to U-MO levels.

In other words, carbon, nitrogen and oxygen atoms are involved in the formation of the biological response through electrostatic interactions between their electrical charges and the electrical charges of the atoms at the biological receptor site.

Going into detail and considering only the highest electron-occupied molecular state (HOMO) and the lowest unoccupied molecular state (LUMO), the results obtained and presented in Table 7 indicate a participation in the electrostatic interactions with the biological receptor of all heavy atoms (others other than hydrogen atoms):

HOMO R^2(%): HQ-C 11.2, HQ-O 10.5, HQ-N 14.1 and

LUMO R^2(%): LQ-C 8.4, LQ-O 7.3, LQ-N 9.1.

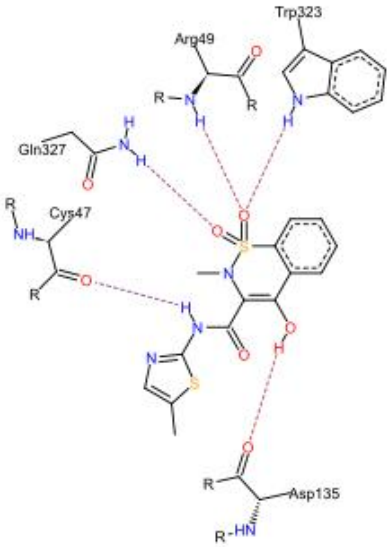
These observations show that the molecular states HOMO/LUMO and O-MO/U-MO are reactive for the chemical structures studied.

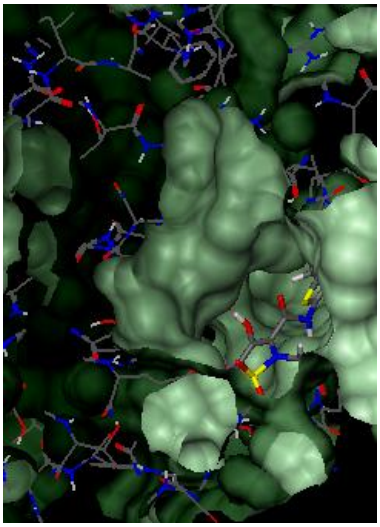
An advantage of fingerprint descriptors is the possibility of locating those atoms, molecular fragments or chemical groups from which new chemical structures with optimized activity can be designed in principle.

The results obtained with these fingerprint descriptors are supported by the study performed using the molecular docking method presented below. This method allows the visualization of the interaction between the ligand (oxicam derivatives) and the biological receptor represented by cyclooxygenase and predicts the optimized conformation of the stable complex formed by the interaction.

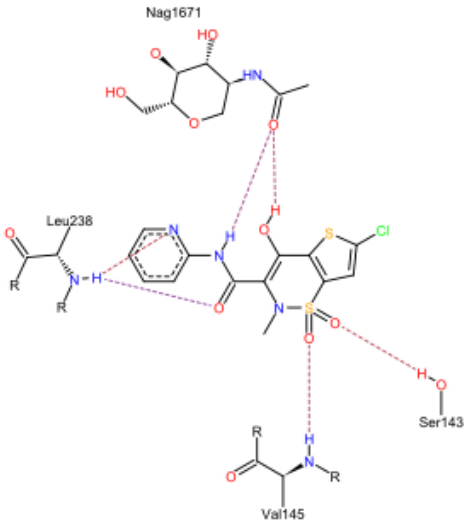
Fig. 2 shows the oxicam–COX-1 complexes.

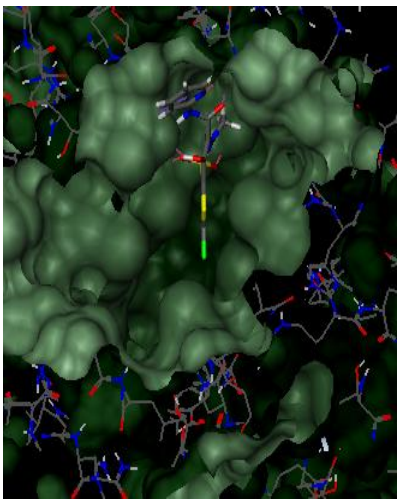
Meloxicam



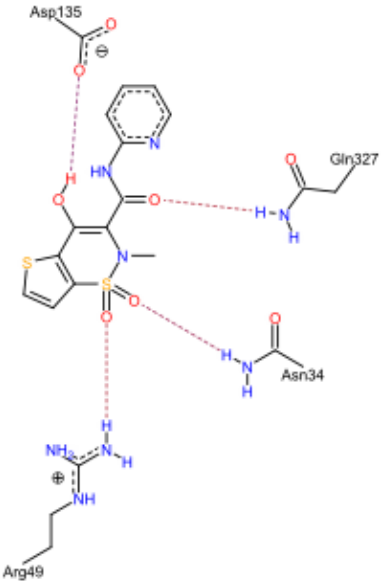


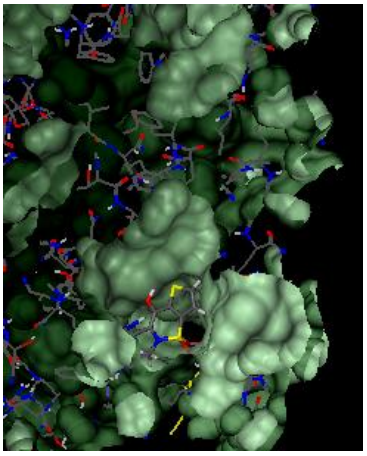
Lornoxicam



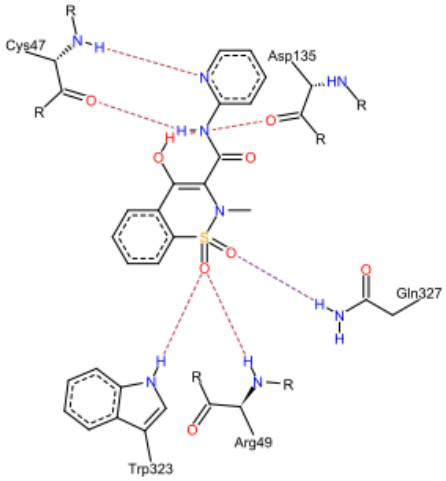


Tenoxicam





Piroxicam



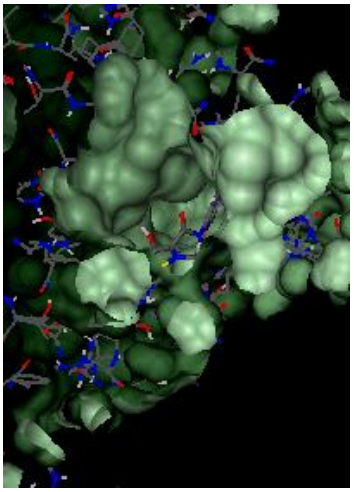


Figure 2. 3D and 2D interaction diagrams between chemical compounds and biological receptors. The hydrogen bonds are represented by dotted lines.

The hydrogen bonds made between the two participants in the interaction involve the participation of oxygen atoms and nitrogen atoms in the ligand molecules. Thus, the involvement of the sulfonyl dioxide group with Arg49 and Gln327 (for meloxicam), with Val145 and Ser143 (for Lornoxicam), with Asn34 and Arg49 (for tenoxicam), and with Gln327, Trp323 and Arg49 (for piroxicam) is observed in all derivatives. Also, three of them form hydrogen bonds involving the H atom of the carboxamide side group, with Nag1671 (for lornoxicam) and the amino acid residue Cys47 (for meloxicam and piroxicam). The 4-hydroxyl group of the thiazine ring participates in the interaction with cyclooxygenase (via Asp135 or Nag1671) only in the case of meloxicam, lornoxicam and tenoxicam.

By rotating the ligand in the binding site, stable complexes are obtained, whose binding energy (affinities) (kJ/mol) calculated using the HEX 8.0 program [27] is presented in Table 8.

Table 8. Values of binding energies of oxicam–COX-1 complexes

Compound	Meloxicam	Tenoxicam	Lornoxicam	Piroxicam
$E_{binding}$ [kJ/mol]	- 273.47	- 266.37	- 353.96	- 262.89

From the data presented in Table 8, it can be concluded that the molecular edifice Lornoxicam–COX-1 (Fig. 2), having the lowest energy value, is the strongest agent for inhibiting the cyclooxygenase COX-1.

4. CONCLUSIONS

The identification of atoms in the chemical structures of oxicam derivatives that make hydrogen bonds with amino acids in the structure of cyclooxygenase 1 (COX-1) opens the way to the design of new compounds with optimized drug activity.

In other words, the localization of atoms with the help of fingerprint descriptors studied in this paper opens a new way of identifying molecular groups or fragments that contribute to the formation of the biological response.

In the case of the studied oxicam derivatives, the groups of atoms containing oxygen atoms will participate in the interaction as electron acceptors through the U-MO molecular levels, those with nitrogen atoms participate in the interaction, both as the nucleophilic center through the molecular state HOMO, and as an electrophilic center by the molecular state LUMO. Also, nitrogen atoms have a good involvement in the interaction through the deep molecular levels, O-MO/U-MO. The hydrogen bonds formed between the atom groups of oxicam derivatives and the amino acids constituting COX-1 show that the molecular states HOMO/LUMO and O-MO/U-MO are reactive for the chemical structures studied.

The results obtained by molecular docking indicate the participation of the same atoms, *i.e.* nitrogen and oxygen atoms, in the formation of hydrogen bonds within the oxicam-cyclooxygenase complex.

The study brings new information on the interaction of oxicam derivatives with cyclooxygenase, representing the basis for the development of new compounds with anti-inflammatory action, more effective and with fewer side effects.

REFERENCES

[1] Szczęśniak-Sięga, B. M. et al., *Bioorganic & Medicinal Chemistry*, **27**(8), 1619, 2019.
[2] Jones, R., Rubin, G., Berenbaum, F., Scheiman, J., *Am. J. Med*., **121**, 464, 2008.
[3] Patrono, C., Rocca, B., *Pharmacol. Res*., **59**, 285, 2009.
[4] Seo, M. J., Oh, D. K., *Prog. Lipid. Res*., **66**, 50, 2017.
[5] Amzoiu, D., Amzoiu, E., Popescu, F., *Curr. Health Sci. J*., **36**(2), 101, 2010.
[6] Rai, N., Sarkar, M., Raha, S., *Pharmacol. Rep*., **67**, 1215, 2015.
[7] Zarghi, A., Arfaei, S., *Iran J. Pharm. Res*., **10**(4), 655, 2011.
[8] Cristea, A. N., eds. *Tratat de farmacologie*, Ed. Medicală, Bucureşti, 2005.
[9] Anoaica, P. G., Amzoiu, E., Bozzini, F., Averis, L. M. E., Bubulica, M. V., *Rev. Chimie (Bucharest)*, **66**(3), 390, 2015.
[10] Anoaica, P. G., Amzoiu, E., Lepădatu, C. I., *Revue Roum. Chim*., **52**(8-9), 789, 2007.
[11] Kubinyi, H., *QSAR: Hansch analysis and related approaches*, VCH Weinheim, New York, Basel, Cambridge, Tokyo, 1993.
[12] Mulliken, R. S. *Chem. Phys*., **2**, 782, 1934.
[13] Amzoiu, E., Anoaica, P. G., Lepădatu C. I., *Rev. Roum. Chim*., **56**(7), 711, 2011.
[14] Amzoiu, E., Amzoiu, M., Sirbu, R., Stanciu, G., Erand, M., Sandulovici, R., *J. Sci. Arts*, **1**(42), 191, 2018.

[15] Amzoiu, M.O., Cristea, O.M., *Curr. Health Sci. J.*, **42**(2), 125, 2016.
[16] Radulescu, C., Tarabasanu-Mihaila, C., Hossu, A.M., Ionita, I., *Rev. Chim. (Bucharest)*, **55**(12), 1006, 2004.
[17] Radulescu, C., Tarabasanu-Mihaila, C., *Rev. Chim. (Bucharest)*, **55**(2), 102, 2004
[18] Lepadatu, C.I., Culita, D.C., Patron, L., *Optoelectr. Advan. Mat. – rapid commun.*, **4**(2), 160, 2010.
[19] Amzoiu, M. et al., *J. Sci. Arts*, **2**(47), 469, 2019.
[20] Amzoiu, D., Stoian, A.M., Amzoiu, E., Rău, G., *Rev. Chimie*, **66**(12), 2013, 2015.
[21] https://pubchem.ncbi.nlm.nih.gov/compound
[22] http://www.arguslab.com/arguslab.com/ArgusLab.html
[23] Stewart, J.J.P., *J. Comp. Chem.*, **10**, 209, 1989.
[24] Rarey, M., Kramer, B., Lengauer, T., Klebe, G., *J. Mol. Biol.*, **261**(3), 470, 1996.
[25] Sidhu, R.S., Lee, J.Y., Yuan, C., Smith, W.L. *Biochemistry*, **49**, 7069, 2010.
[26] Amzoiu, E. et al., *J. Sci. Arts*, **1**(46), 177, 2019.
[27] Agrawal, P., Singh, H., Srivastava, H.K. et al., *BMC Bioinformatics*, **19**, 426, 2019.