ORIGINAL PAPER

PHYSICOCHEMICAL PARAMETERS USED FOR THE STUDY OF ANTIBACTERIAL ACTIVITY OF BENZIMIDAZOLE DERIVATIVES

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Abstract. *The antibacterial activity for a series 14 of benzimidazole derivatives was correlated with the structural descriptors, like the molecular shape descriptor, the energy descriptor ($\Delta E = E_{LUMO} - E_{HOMO}$), the information descriptors E and T and the fingerprint descriptors for molecular quantum states OMO/UMO. These descriptors have a greater or smaller contribution to the formation of the biological response corresponding to the studied compounds. With the help of the OMO/UMO fingerprint descriptors, information can be obtained on how molecules in the class interact with the active site of Gram-negative bacteria Pseudomonas aeruginosa. Of the atomic species existing in the studied benzimidazole derivatives, most contribute to the formation of biological activity exclusively in the electron-occupied molecular states (H, N and X).*

Keywords: *benzimidazole, descriptor, antibacterial activity.*

1. INTRODUCTION

The increase in the number of parasitic infections and their diversity has resulted in continuous search for substances with anti-infective activity. Benzimidazoles are compounds exhibiting different biological activities, such as antibacterial activities [1], antiviral [2], antifungal [3], anthelmintics, anti-HIV [4], antihistamines [5], antihypertensive activities [6] and neuroleptic actions [7]. In recent years, benzimidazole derivatives have also attracted attention due to their anticancer activity [8].

All of these make benzimidazoles a group of substances of great interest due to the diversity of their biological activity and their various applications in the medico-pharmaceutical field.

The biological activity of these compounds is expressed on the basis of the interaction of the drug molecule (called ligand) with specific molecules called receptors. The structure of a chemical is represented by different descriptors, which can be correlated with the analyzed pharmacological activity [9-14].

This paper aims to evaluate structural factors that influence the antibacterial activity of benzimidazole derivatives against *Pseudomonas aeruginosa* Gram-negative bacteria. Also,

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the study in this paper reaches the stage of identification of the molecule atoms that have a greater contribution to the formation of the antibacterial activity.

Such studies are necessary in the design of new benzimidazole compounds with optimized biological activity.

2. MATERIALS AND METHODS

This study was conducted on a set of 14 benzimidazole derivatives using molecular, informational and fingerprint descriptors for the quantum-molecular states of the studied chemical compounds. The preliminary molecular geometries for benzimidazole derivatives (Table 1), for which biological activity expressed by log 1/C is reported in literature [15], were obtained using the Molecular Mechanics (MM+) and then optimized using the PM3 parametrization [16]. The molecular geometries were used as input files to produce the mentioned molecular descriptors with the computer program MOPAC [17].

The obtained descriptors have been correlated with antibacterial activity AA using multiple regression analysis $AA = a_0 + \Sigma a_i X_i$, where X_i are the descriptors and a_0, a_1, a_2, … are the regression coefficients. The OMO-UMO fingerprint descriptors are obtained by reading the MOPAC output files, and their correlation with biological activity is a linear regression correlation.

3. RESULTS AND DISCUSSION

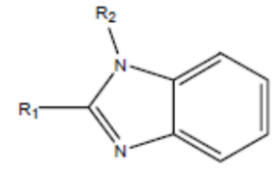
As may be seen in Table 1, the antibacterial activity given as AA = log(1/C) depends on the groups attached to the benzimidazole basic formula. Thus, the presence on the imidazole nucleus of an amino group ($-NH_2$) (compounds 1-7) causes a slight increase in antibacterial activity relative to the CH_3 group present at the same R_1 position of the nucleus (compounds 8-14). This may be explained by the fact that the hydrophilic $-NH_2$ group functions as a nucleophilic center and is capable of interacting with the receptor represented by the Gram-negative bacterium Pseudomonas aeruginosa.

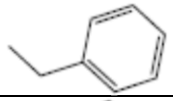
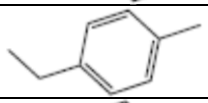
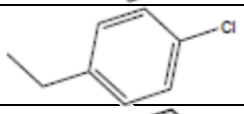
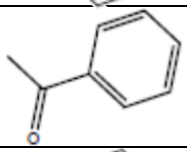
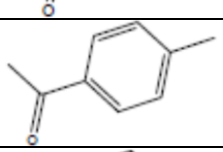
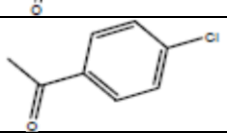
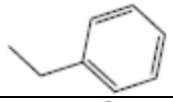
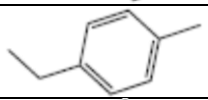
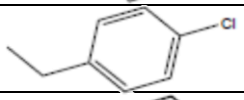
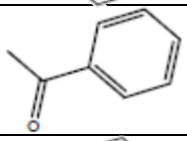
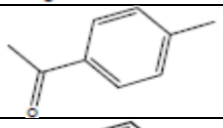
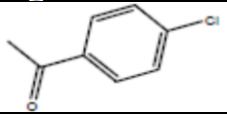
Interaction of the ligand - biological receptor, interaction based primarily on the molecular recognition of the two participants, depends on the molecular form of the ligand and the biological receptor (see Table 2) [18-21]. As shown in Table 2, the most active antibacterial compound (compound 7) has the highest values of the descriptors R_m, CSAA, E and T and also has the lowest value of the ΔE parameter.

The first two parameters (R_m and CSAA) are molecular form descriptors [22] and molecular form is very important in ligand-receptor interaction. The last descriptor, ΔE, represents the energy difference between HOMO and LUMO levels ($\Delta E = E_{LUMO} - E_{HOMO}$). This molecular descriptor explains the molecular stability [18], a small value (8.0883) indicates that the molecule is highly reactive (compound 7 with AA = 4.638).

Molecular orbitals HOMO - LUMO (Highest Occupied Molecular Orbital and Lowest Unoccupied Molecular Orbital) are orbital frontiers, that is the orbitals with which a molecule can interact with another molecule or other atom. For the most reactive compound (AA = 4.638), molecular orbital HOMO is represented in Fig. 1.

Table 1. Structure and antibacterial activity of studied compounds



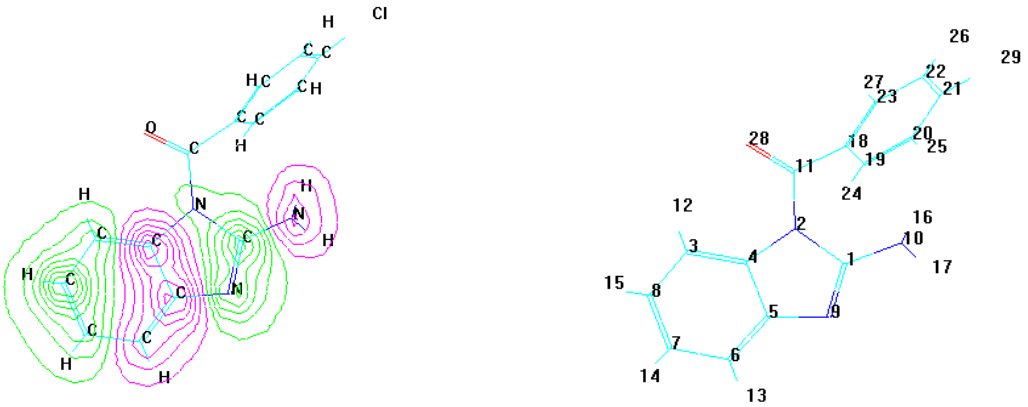
Compound	R_1	R_2	*AA=log1/C*
1	NH_2	H	3.425
2	NH_2		3.951
3	NH_2		4.278
4	NH_2		4.615
5	NH_2		3.676
6	NH_2		4.303
7	NH_2		4.638
8	CH_3	H	3.121
9	CH_3		3.648
10	CH_3		3.975
11	CH_3		4.312
12	CH_3		3.373
13	CH_3		4.000
14	CH_3		4.335

As seen in Fig. 1, at the formation of molecular orbital HOMO also contributes the hydrophilic group -NH_2 through which the molecule can interact with the biological receptor (the Gram-negative bacteria Pseudomonas aeruginosa) by electron transfer from the molecule to the receptor (Fig. 3).

Table 2. Molecular descriptors for benzimidazole derivatives

Compound	*AA*	*ΔH [kcal/mol]*	*R_m [cm^3/mol]*	*CSAA [$Å^2$]*	*ΔE [eV]*	*E_t [kcal/mol]*	*E*	*T*
1	3.425	219.46	42.6600	285.171	8.7070	-1611.94	34.4640	0.75348
2	3.951	347.39	70.6863	419.470	8.5699	-2590.13	58.0874	0.72041
3	4.278	315.28	75.7275	449.053	8.5540	-2663.21	62.0763	0.71616
4	4.615	320.18	75.4911	441.690	8.3534	-2950.21	65.7939	0.80614
5	3.767	234.81	70.6200	428.042	8.3042	-2882.54	60.9914	0.75160
6	4.303	202.70	75.6612	460.220	8.2837	-3038.38	65.0214	0.74633
7	4.638	207.60	77.4248	466.845	8.0883	-3158.38	68.9197	0.84098
8	3.121	165.03	44.1284	306.690	9.0156	-1731.94	34.5089	0.72627
9	3.648	292.96	72.1525	431.054	8.8660	-2434.66	58.2812	0.70982
10	3.975	260.85	77.1937	460.663	8.8270	-2914.85	62.2601	0.70610
11	4.312	265.75	76.9573	454.066	8.6350	-2789.52	66.0022	0.79455
12	3.373	180.38	72.0862	436.035	8.5535	-2868.42	61.1698	0.74007
13	4.000	148.27	77.1274	465.944	8.5890	-3050.31	65.1591	0.73453
14	4.335	153.17	76.8910	459.810	8.3933	-3087.94	68.7664	0.82697

ΔH - heat of reaction; R_m- molar refractivity; CSAA – Connolly accessible area; ΔE - energy difference between the levels HOMO and LUMO; E_t – total energy; E – information energy; T – information temperature



HOMO
(E = -8.85789)

Figure 1. Highest Occupied Molecular Orbital. The green color indicates a positive polarity and the color purple - the negative polarity.

The information descriptors E and T, having the highest values for compound **7** (68.9197 and 0.84098), express the information content of a molecular system and its ability to interact [23]. Thus, the highest value of information energy E belongs to the molecule that presents the highest order (total order) and which is the most stable (has the lowest total energy E_t, -3158.38 cal/mol). An explanation is given by the fact that information energy is applied on a probability field generated by the distribution of the electronic population on the quantum states of each atom. Because each atom has a certain electronic distribution, it means that E can be considered as a fingerprint of every atom in the molecule. In the studied benzimidazoles class (Table 1), the addition of some atoms in molecules determines the increase of the energy information E.

Correlation of antibacterial activity with these descriptors leads to satisfactory results (Table 3). With these correlations we can determine the structural factors that influence the antibacterial activity of the benzimidazole derivatives against Gram-negative bacteria *Pseudomonas aeruginosa*.

Table 3. Regression correlation
$AA = a_0 + a_iX_i$, X_i – descriptors

Descriptor	R^2 *(%)*
ΔH	7.5
R_m	51.8
CSAA	48.5
ΔE	49.6
Et	0.0
E	61.6
T	37.5
E, T	74.6
R_m, CSAA, ΔE	95.8
ΔE, E	75.5
ΔE, T	53.2
CSAA, E	71.0
ΔE, CSAA	65.6

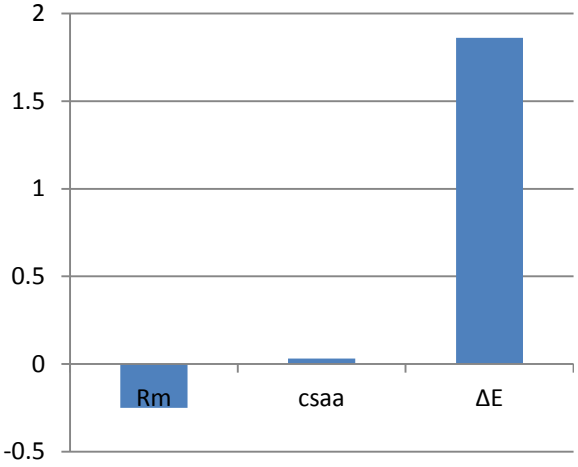


Figure 2. Contribution plot of descriptors X_i for correlation $AA = f(X_i)$.

The best correlation occurs between biological activity studied AA and the following physico-chemical parameters: R_m, CSAA and ΔE (R^2= 95.8) (Fig. 2). As we can see in this figure, the largest contribution is represented by the ΔE descriptor, it has a positive contribution of 86.91%. The other descriptors have a lower contribution to the formation of the biological response, namely, the Rm descriptor has a negative contribution of 11.63% and the CSAA descriptor has a very small positive contribution (1.45%). Therefore, in the design of new drugs with enhanced antibacterial activity (optimized), descriptors that have positive positive contributions should be used and descriptors with negative contributions should be avoided [24].

Presence of the energy descriptor ΔE in the AA = f (X_i) correlation equation undoubtedly suggests that the studied chemical structures interact with the active site of the Gram-negative bacteria Pseudomonas aeruginosa by transferring electrons from the ligand to the receptor [25]. These possible ligand-receptor interactions will be studied using the OMO / UMO fingerprint descriptors for quantum - molecular states.

If for the atom, the valence layer is responsible for the formation of chemical bonds, for the molecule it can be represented by the molecular orbital OMO / UMO. In other words, these orbitals are responsible for the interaction of molecules (Fig. 3).

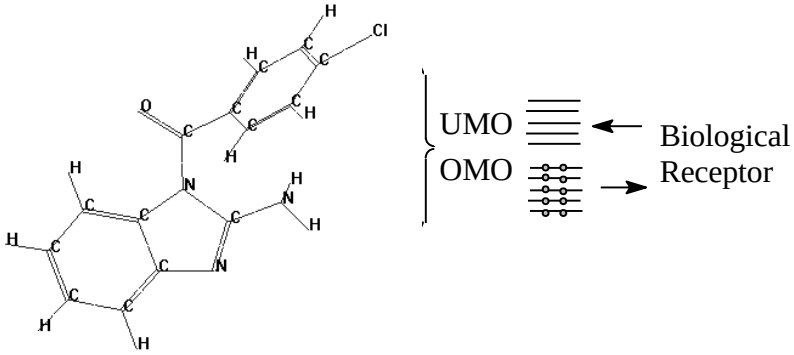


Figure 3. Chemical compound - biological receptor interaction [19].

The electron donor-acceptor capacity of a substance is described by its electronegativity. Tables 4 and 5 contain the estimated electronegativity fingerprint descriptors in the MOPAC output files for the studied compound class.

Table 4. Electronegativity fingerprint descriptors for quantumolecular states HOMO

AA	*HEL*	*HELAT*	*HELH*	*HEC*	*HEO*	*HEN*	*HEX*
3.425	6.421	6.421	0.000	2.922	0.000	3.499	0.000
3.951	6.322	6.224	0.099	1.419	0.000	2.804	0.000
4.278	6.291	6.196	0.096	1.482	0.000	2.714	0.000
4.615	6.301	6.205	0.096	1.487	0.000	2.717	0.000
3.767	6.364	6.271	0.093	1.526	0.001	2.744	0.000
4.303	6.346	6.249	0.097	1.559	0.002	2.688	0.000
4.638	6.334	6.237	0.097	1.603	0.001	2.633	0.000
3.121	6.734	6.715	0.019	1.446	0.000	3.269	0.000
3.648	6.729	6.680	0.049	2.277	0.000	3.403	0.000
3.975	6.732	6.683	0.049	1.271	0.000	3.411	0.000
4.312	6.722	6.675	0.047	1.296	0.000	3.373	0.006
3.373	6.344	6.331	0.013	1.797	0.042	2.493	0.000
4.000	6.473	6.440	0.033	1.760	0.142	2.538	0.000
4.335	6.353	6.335	0.018	1.798	0.042	2.494	0.001

Table 5. Electronegativity fingerprint descriptors for quantumolecular states LUMO

AA	*LEL*	*LELAT*	*LELH*	*LEC*	*LEO*	*LEN*	*LEX*
3.425	6.029	6.029	0.000	5.111	0.000	0.919	0.000
3.951	5.894	5.829	0.065	5.452	0.000	0.378	0.000
4.278	5.818	5.775	0.043	5.419	0.000	0.356	0.000
4.615	5.871	5.831	0.040	5.429	0.000	0.272	0.130
3.767	5.659	5.625	0.034	5.298	0.050	0.277	0.000
4.303	6.033	5.896	0.137	5.442	0.218	0.236	0.000
4.638	5.733	5.729	0.004	5.437	0.004	0.156	0.132
3.121	6.083	5.957	0.126	4.950	0.000	1.007	0.000
3.648	5.938	5.874	0.064	5.410	0.000	0.464	0.000
3.975	5.984	5.799	0.186	5.381	0.000	0.418	0.000
4.312	5.884	5.841	0.043	5.427	0.000	0.288	0.126
3.373	5.641	5.624	0.016	5.293	0.048	0.283	0.000
4.000	6.149	6.013	0.136	5.301	0.441	0.271	0.000
4.335	5.698	5.685	0.013	5.390	0.014	0.161	0.119

EL - total electronegativity of the molecular state; ELAT - electronegativity of heavy atoms (other than hydrogen atoms); ELH - electronegativity of hydrogen atoms; EC - electronegativity of the carbon atom; EN – the electronegativity of the nitrogen atom; EO – electronegativity of the oxygen atom; EX - the electronegativity of the halogen atom.

The prefix H refers to the HOMO state, the prefix L to the LUMO state. The descriptors in Tables 4 and 5 were correlated with linear statistical equations of the type AA = $a_0 + a_1X_1$, where X_1 = descriptor. Correlation coefficient R^2 gives us information on the contribution of atomic species H, C, O to the formation of AA activity. For the electronegativity of the atoms in the molecule, the values of the correlation coefficients estimated for these atomic species are given in Fig. 4 for the HOMO-LUMO molecular states.

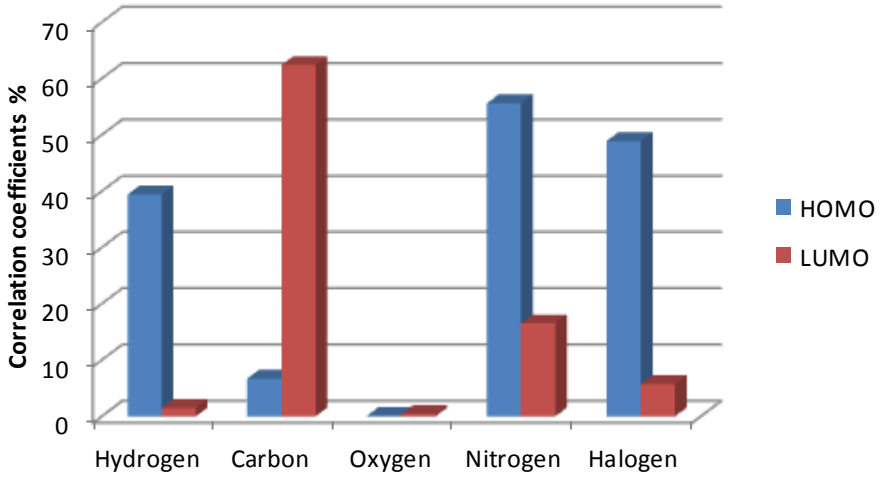


Figure 4. Correlation coefficient values for AA = $a_0 + a_1X_1$.

As can be seen in Fig. 4, among the atomic species existing in the benzimidazole derivatives studied, most of them contribute to the formation of biological activity exclusively in the electron-occupied molecular states (H, N and X). This means that between the mentioned atoms and the active sites of the Gram-negative bacteria Pseudomonas aeruginosa are formed chemical bonds by electron transfer of the ligand ➔ receptor type. The carbon atom contributes to the formation of biological activity in the quantum molecular state LUMO. This means that there is electron transfer between the carbon atoms and the bacteri of the receptor ➔ ligand type (Fig. 3). Indeed, analyzing compound 7 from the studied class, it can be seen that for the two electron-occupied molecular states (OMO) the nitrogen atom presents the following electronegativities (the results were appreciated from the MOPAC output file for the most active compound) (Table 6).

Table 6. Electronegativities of the atoms of compound 7 for MO 46 – 49*.

	OEL	**OEL-AT**	**OEL-H**	**OEL-C**	**OEL-O**	**OEL-N**	**OEL-X**
MO: 46	6.345	6.333	0.011	1.208	0.049	2.376	0.001
MO: 47	6.334	6.237	0.097	1.033	0.001	2.633	0.000
	UEL	**UEL-AT**	**UEL-H**	**UEL-C**	**UEL-O**	**UEL-N**	**UEL-X**
MO: 48	5.733	5.729	0.004	5.437	0.004	0.156	0.132
MO: 49	5.888	5.884	0.004	5.838	0.006	0.040	0.000

** Occupied Molecular Orbitals: 47; Unoccupied Molecular Orbitals: 49*

Table 7. Sequence of energy and molecular orbital levels for the molecule 7

Molecular orbitals	**46**	**47**	**48**
Energy [eV]	-9.17827	-8.85789	-7.69611
S N 2	-.00190	-.01172	.10364
PX N 2	-.00783	.01859	.02171
PY N 2	-.00330	.00176	-.03303
PZ N 2	.48695	.06917	.0027
S N 9	-.00814	.01046	.00492
PX N 9	.00822	.00258	.01664
PY N 9	.00305	-.01472	-.01019
PZ N 9	-.17588	-.22303	-.06970
S N 10	.05575	.15537	.00669
PX N 10	-.02654	.06664	.0067
PY N 10	.03797	.10323	.01994
PZ N 10	-.13921	-.40464	-.04479

It is observed that for the molecular state described by the molecular orbital MO = 47, the electronegativity of the nitrogen atom has the highest value (OEL-N = 2.633). As can be seen in Table 7, the molecular orbital MO = 47 which characterizes the highest molecular level occupied by electrons (i.e. the HOMO level energy -8.85789 eV) is practically located on the nitrogen atom numbered 10.

4. CONCLUSION

The interaction between the ligand atoms (the benzimidazole derivative) and the receptor (Pseudomonas aeruginosa Gram-negative bacteria) is achieved by transferring electrons from the ligand to the receptor. With the help of the OMO - UMO fingerprint descriptors, we can get information on how molecules interact with the active site of the biological receptor. Furthermore, fingerprints allow us to locate those atoms and identify those molecular fragments or chemical groups involved in the formation of the biological response. From the analysis of the quantum-molecular states corresponding to the 14 benzimidazole derivatives studied, it follows that most of the atomic species present in these compounds contribute to the formation of biological activity exclusively in the electron-occupied molecular states (H, N and X).

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