ORIGINAL PAPER

IDENTIFICATION OF MOLECULAR FRAGMENTS RESPONSIBLE FOR THE ANTIMICROBIAL ACTIVITY OF ACETAMIDE DERIVATIVES

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Abstract. *Amide compounds have an important role in various human activity domains. The aim of the paper is to determine the structural descriptors that influence the antimicrobial activity of the studied amide derivatives. With the help of the structure-activity correlation method and molecular docking, we have identified the atoms in the molecule that have a greater contribution to the formation of the antimicrobial activity. In the case of the studied molecules, the nitrogen atoms in the azole nucleus are highlighted, the results being supported also by the 2D diagrams of the applied docking method.*

Keywords: *amide derivatives, antimicrobial activity, molecular docking.*

1. INTRODUCTION

Amide derivatives are bioactive compounds that have multiple activities: antimicrobial [1], anti-HIV [2], antitumor [3], antipsychotics [4], analgesics [5], antidepressants, cardiotonic [6]. They are also used as first-line drugs in the treatment of tuberculosis [7]. Outside the medical field, amides are widely used as insecticides, herbicides and fungicides [8].

The wide variety of applications in medical and agrochemical applications has led to the appearance of a fairly large number of studies that enable the understanding of action mechanisms for these compounds. The biological activity of a drug compound (called ligand) appears as a result of the interaction of its molecule with a specific biological receptor [9-12]. The aim of this paper is to evaluate the structural parameters, called descriptors, which influence the biological activity of acetamide derivatives on Gram-negative bacteria *Escherichia coli* and the *Candida albicans* fungus.

2. MATERIALS AND METHODS

The molecules of the 9 acetamide compounds (Table 1, [13]) were modeled using Hyperchem 8 software [14]. The molecular geometries were used as input files to produce the

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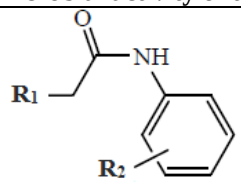
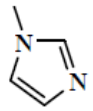
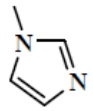
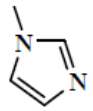
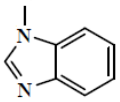
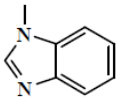
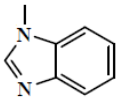
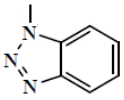
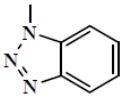
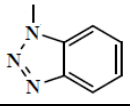
mentioned molecular descriptors with the computer software MOPAC (Molecular Orbital PACkage) [15]. Fingerprint descriptors of electronegativity type of the molecule atoms for quantum-molecular states O-MO/U-MO were evaluated from the MOPAC output files and regressively correlated with antimicrobial activity.

Finally, antimicrobial activity has been studied through molecular docking with FlexX docking software [16].

Table 1. Structure and antimicrobial activity of the studied derivatives [10].



Cmpd	R_1	R_2	*Antimicrobial activity*	
			E. coli	*C. albicans*
1a		H	8.44	7.11
1b		$4CH_3$	26.44	9.65
1c		4Cl	20.92	23.90
2a		H	9.16	14.72
2b		$4CH_3$	9.49	8.64
2c		4Cl	17.38	11.11
3a		H	12.19	9.30
3b		$4CH_3$	10.78	7.34
3c		4Cl	11.71	8.11

3. RESULTS AND DISCUSSION

As may be seen in Table 1, the most active compound on *Escherichia coli* Gram-negative bacteria is the compound 1b, the decreasing order of antimicrobial activity is indicated by the following series: 1b > 1c > 2c > 3a > 3c > 3b > 2b > 2a > 1a. The most active compound on the *Candida Albicans* fungus is the compound 1c (1c > 2a > 2c > 1b > 3a > 2b > 3c > 3b > 1a).

The biological response of a receptor to the action of a ligand (a drug substance) depends on the molecular shape of the two participants and their interaction [17]. Therefore, in our study, descriptors from the two categories were used, molecular descriptors and interaction descriptors. Their values are presented in Tables 2 and 3.

Table 2. Descriptors of molecular shape for the studied compounds

Cmpd	*Antimicrobial activity*		R_M [cm^3/mol]	*CSA* [Å^2]	*CSEV* [Å^3]	E_t [kcal/mol]	E_{HOMO} [eV]	E_{LUMO} [eV]	ΔE [eV]
	E. coli	*C. albicans*							
1a	8.44	7.11	57.91	385.184	168.299	38.204	-9.325	-0.134	9.1912
1b	26.44	9.65	62.95	415.295	185.036	40.257	-9.299	-0.151	9.1486
1c	20.92	23.90	62.71	408.909	182.362	40.811	-9.386	-0.471	8.6642
2a	9.16	14.72	75.28	446.742	207.222	35.088	-9.031	-0.108	8.9229
2b	9.49	8.64	80.32	475.867	223.851	40.407	-9.028	-0.123	8.9049
2c	17.38	11.11	80.08	469.943	220.970	40.856	-9.101	-0.437	8.9157
3a	12.19	9.30	77.58	448.359	202.004	34.855	-9.351	-0.405	8.9453
3b	10.78	7.34	82.34	478.824	218.623	33.456	-9.266	-0.397	8.8689
3c	11.71	8.11	82.18	472.243	215.916	34.045	-9.397	-0.478	8.9187

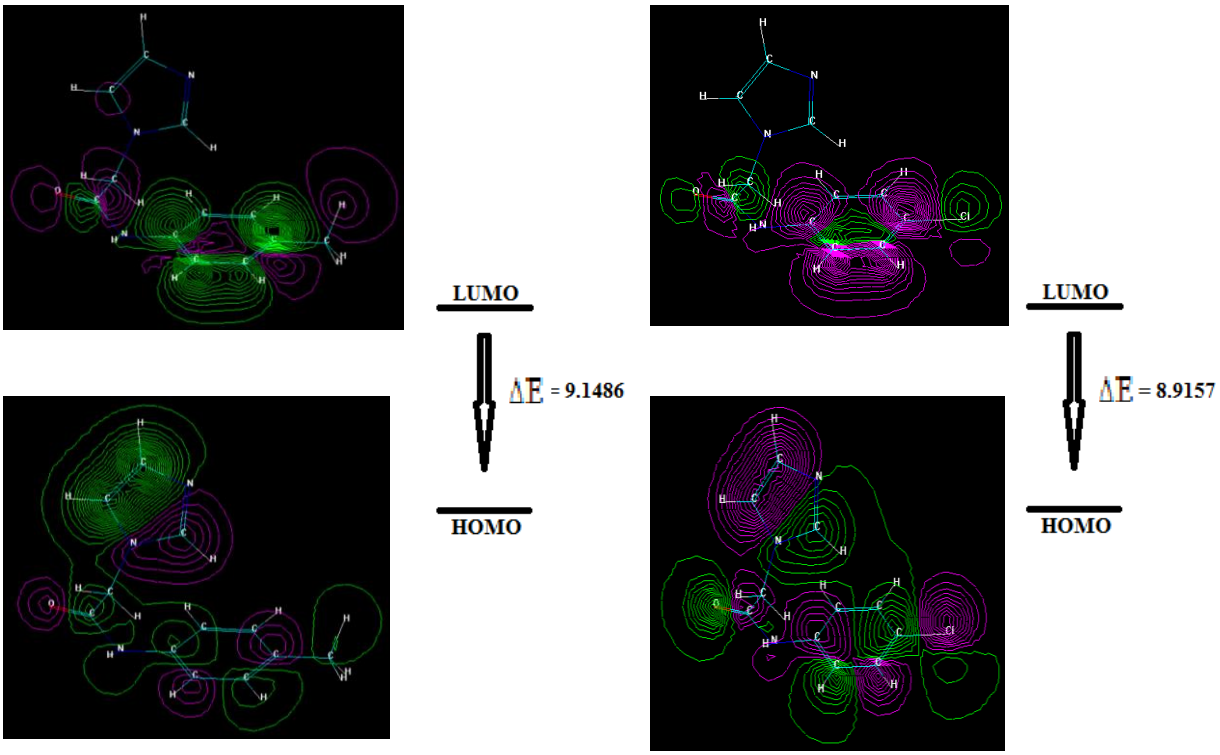
R_M - Molar Refractivity, CSA – Connolly Accessible Area, CSEV – Connolly Solvent Excluded Volume, ΔE - Energy difference between the levels HOMO and LUMO, E_t – Total Energy

The least active compound (1a), having the lowest antimicrobial activity on *E. coli* and on *C. albicans*, also has the lowest values of molecular shape descriptors CSA and CSEV (385.184 Å^2 and, respectively, 168.299 Å^3). These descriptors are very important in QSAR studies because the biological response of a chemical compound depends on its steric accommodation in the active receptor site of the biological receptor.

This compound also has the greatest energy difference ΔE, which explains its low activity compared to the other studied compounds [18]. The lower the value of this descriptor, the more reactive the chemical compound is. So, ΔE increases in the order 3a < 2a < 1a, and inhibitory activity on the *E. coli* bacteria manifested by these compounds increases in the order 1a < 2a < 3a.

A similar relationship between the variance of the energy descriptor ΔE and the inhibitory activity on the *C. albicans* fungus can also be made for compounds containing the heteroatom Cl: ΔE increases in the order 1c < 2c < 3c, and biological activity increases in the order 3c < 2c < 1c.

Molecular frontier orbitals HOMO – LUMO (Highest Occupied Molecular Orbital and Lowest Unoccupied Molecular Orbital) are the orbitals by which the molecule of a reactant interacts with the molecule of another reactant. In Fig. 1 are represented the frontier orbitals corresponding to the two most active compounds, 1 b and 1 c.



Compound 1b *Compound 1c*

Figure 1. Frontier molecular orbitals.
The green color indicates a positive polarity and the color purple - the negative polarity.

Electrostatic interactions between the ligand (acetamide derivative) and the biological receptor (*E. coli* or *C. albicans*) are simulated by interactions between a solute and a solvent. These interactions are calculated using the Gamess program, which incorporates the PCM model (Polarizable Continuum Model). Interactions between the two occur as a result of the electrostatic field of the solute molecule created by the presence of the continuous solvent medium (having certain physico-chemical properties: dielectric constant, polarizability etc.) [19].

Table 3. Electrostatic interaction energies (kcal/mol) for the studied compounds

Cmpd	*Antimicrobial activity*		*IES*	*EI*	*PCE*	*RFE*	*TI*	*TFES*
	E. coli	*C. albicans*						
1a	8.44	7.11	-413683	-6.95	17.79	3.04	13.89	-413670
1b	26.44	9.65	-438130	-6.97	19.57	3.33	15.93	-438114
1c	20.92	23.90	-700708	-7.84	18.72	2.77	13.65	-700694
2a	9.16	14.72	-509248	-6.52	21.35	3.61	18.44	-509229
2b	9.49	8.64	-533695	-6.29	23.14	3.95	20.80	-533674
2c	17.38	11.11	-796272	-7.39	22.28	3.49	18.38	-796254
3a	12.19	9.30	-519214	-6.27	20.91	3.55	18.19	-519196
3b	10.78	7.34	-519214	-6.12	18.91	3.43	16.19	-545196
3c	11.71	8.11	-806239	-7.19	21.82	3.37	18.00	-806221

IES = Internal Energy of Solute, EI = Electrostatic Energy, PCE = Pierroti Cavity Energy,
RFE = Repulsion Free Energy, TI = Total Interaction, TFES = Total Free Energy in Solvent

Upon introduction into the solvent, the solvate molecule forms a molecular cavity as in Fig. 2.

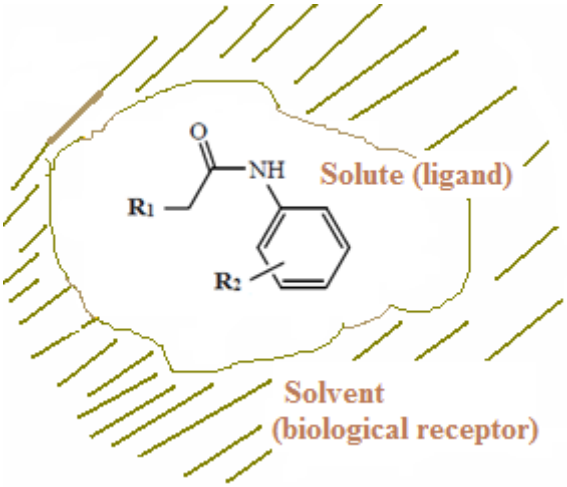


Figure 2. The ligand - receptor interaction in the PCM model vision [20].

Correlation of antimicrobial activities with these descriptors allows the finding of those structural parameters that influence the biological activity of the studied compounds. The results of these regression correlations are presented in Table 4.

As can be seen in Table 4, higher values of R^2 appear in both cases (*E. Coli* bacteria and *C. Albicans* fungus) if the activity is correlated with an energy descriptor and a molecular shape descriptor. Thus, a better correlation occurs between the studied antibacterial activity and descriptors E_t and CSA (R^2= 33.5), compared to the correlations of the same activity with the parameters considered separately (R^2 = 32.2 and, respectively, R^2 = 9.8).

Table 4. Linear correlation of biological activity = f(descriptor)

Descriptor	***E. coli***	***C. albicans***
	R^2 [%]	***R^2 [%]***
R_m	16.3	11.3
CSA	9.8	10.3
E_t	32.2	13.8
E_t, CSA	33.5	17.7
ΔE	0.6	3.7
ΔE, CSA	15.2	52.5
IES	2.1	9.4
EI	32.2	36.9
EI, CSA	32.8	37.4
PCE	3.7	2.7
RFE	16.1	26.6
TI	12.3	12.5
TFES	1.8	8.6

A similar situation also appears in the case of the antifungal activity of the studied compounds: in correlating the activity with EI and CSA, R^2= 37.4, and correlations with descriptors taken separately have much lower correlation coefficients, 36.9 and 9.8. Among the various forms of energy studied, it is observed that the electrostatic interaction EI has the best contribution to the formation of the antimicrobial properties, the R^2 correlation coefficients being the highest, 32.2 for *E. coli* and 36.9 for *C. albicans*.

The presence of the energy descriptor ΔE in the antimicrobial activity correlation equations with chemical structures expressed through molecular descriptors undoubtedly suggest that the studied molecules interact with the active sites of biological receptors by electron transfer [21]. This would be explained by the low values of the correlation coefficients, 0.6 for *E. coli* and 3.7 for *C. albicans*.

Table 5. The values of the electronegativity descriptors for the atoms in the molecule

Cmpd	***Antimicrobial activity***		***EL***	***ELAT***	***ELH***	***ELC***	***ELO***	***ELN***	***ELX***
	E. coli	***C. albicans***							
1a	8.44	7.11	180.463	92.492	87.971	63.629	5.573	23.290	0.000
1b	26.44	9.65	201.151	98.537	102.614	69.666	5.569	23.301	0.000
1c	20.92	23.90	180.483	100.206	80.277	63.578	5.592	23.266	7.770
2a	9.16	14.72	185.167	113.798	71.369	85.398	5.550	22.850	0.000
2b	9.49	8.64	240.520	122.718	117.803	94.331	5.547	22.840	0.000
2c	17.38	11.11	219.849	124.379	95.470	88.251	5.569	22.795	7.764
3a	12.19	9.30	213.225	118.296	94.929	83.012	5.488	29.795	0.000
3b	10.78	7.34	176.564	121.452	55.113	86.155	5.480	29.816	0.000
3c	11.71	8.11	177.876	122.550	55.326	81.503	5.500	29.764	5.784

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 – electronegativitatea atomului de oxigen, ELX - the electronegativity of the halogen atom.

Other parameters involved in the ligand-receptor interaction, which can describe the interaction between the atoms of the molecules of the two participants, are the electronegativity descriptors, defined as functions of the nature of the atoms and their electrical charge obtained from the electronic population partition after the formation of chemical bonds. These descriptors represent the "fingerprint" of the atoms and are called OMO/UMO fingerprint descriptors for quantomolecular states [22, 23]. Their values obtained for acetamide derivatives are given in Table 5.

Correlation of electronegativity descriptors with biological activity has led to the following results (Table 6). As can be seen in this table, higher correlation coefficients (13.3% against 0.2% for *E. coli* and 8.4% against 4.1% for *C. albicans*) recommends the electronegativity of heavy atoms descriptors (ELAT) as describing very well, in this case, the molecular structures and the interaction of these molecules with the active sites of biological receptors. These include carbon and oxygen atoms with approximately equal contributions to the formation of the antibacterial response against *E. coli* and, respectively, oxygen and halogen atoms to the formation the antifungal response of the studied compounds.

Table 6. Correlation results for electronegativity descriptors

	Descriptors	**R^2 [%]**		**Descriptors**	**R^2 [%]**
E. coli	EL	0.2	*C. albicans*	EL	4.1
	ELAT	13.3		ELAT	8.4
	ELH	6.6		ELH	0.2
	ELC	19.2		ELC	14.1
	ELO	21.0		ELO	29.4
	ELN	7.6		ELN	15.4
	ELX	13.5		ELX	28.7

This observation is interesting and suggests the possibility to use these descriptors in QSAR procedures by selecting only parts of the molecules or only certain atoms that are considered to play an essential role in the formation of the biological response or certain physico-chemical property.

The electronegativity fingerprint descriptors for the HOMO and LUMO quantomolecular states are presented in Tables 7a and 7b. Physical-chemical parameters (descriptors) presented in Table 7 were statistically correlated with antimicrobial activities. The correlation coefficients obtained provide information regarding the contribution of different atomic species to the formation of antimicrobial activity. The values of these coefficients are indicated in Table 8.

Table 7a. Fingerprint descriptors for quantomolecular state HOMO

HEL	***HELAT***	***HELH***	***HEC***	***HEO***	***HEN***	***HEX***
5.659	5.634	0.025	4.683	0.040	0.911	0.000
5.713	5.672	0.040	4.628	0.062	0.982	0.000
5.821	5.792	0.029	4.444	0.079	1.001	0.268
6.372	6.309	0.063	3.021	0.033	3.256	0.000
6.734	6.672	0.062	3.391	0.036	3.245	0.000
6.722	6.662	0.061	3.417	0.033	3.206	0.006
6.713	6.631	0.082	3.446	0.069	3.116	0.000
6.359	6.277	0.082	3.205	0.111	2.961	0.000
5.945	5.876	0.069	3.347	0.140	2.389	0.000

The prefix H refers to the HOMO state

Table 7b. Fingerprint descriptors for quantomolecular state LUMO

LEL	***LELAT***	***LELH***	***LEC***	***LEO***	***LEN***	***LEX***
5.844	5.819	0.025	5.728	0.056	0.035	0.000
5.910	5.731	0.178	5.652	0.047	0.033	0.000
5.831	5.808	0.023	5.598	0.038	0.032	0.141
5.846	5.821	0.024	5.725	0.057	0.040	0.000
5.914	5.739	0.176	5.648	0.052	0.039	0.000
5.835	5.814	0.022	5.602	0.039	0.033	0.139
6.415	6.393	0.022	3.480	0.003	2.910	0.000
6.051	6.029	0.022	3.113	0.003	2.912	0.000
6.082	6.060	0.022	3.130	0.005	2.925	0.000

The prefix L refers to the LUMO state

The statistical correlation of these descriptors with the antimicrobial activities of acetamide results in the following:

Table 8. Correlation coefficients R^2 [%]

Atom	***E. coli***				***C. albicans***			
	HOMO	***LUMO***	***O-MO***	***U-MO***	***HOMO***	***LUMO***	***O-MO***	***U-MO***
C	29.9	8.0	14.0	1.2	4.5	15.3	18.3	7.1
N	26.7	9.2	8.0	4.9	7.3	16.1	1.9	14.1
O	1.0	0.1	4.7	30.1	1.1	6.0	1.2	12.6
X	17.7	21.8	22.3	21.8	81.5	47.1	50.3	47.1

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

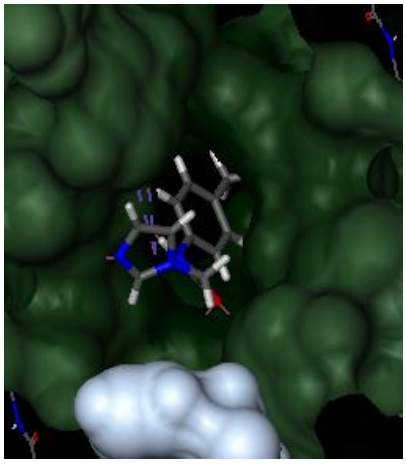
Among the species of atoms involved, it is noted that the nitrogen and carbon atoms of the HOMO molecular state contribute approximately with 26.7% (HEN) respectively 29.9% (HEC) to the formation of antibacterial activity and halogen atoms in the HOMO / LUMO molecular states which contribute approximately 17.7% (HEX) respectively 21.8% (LEX). For the formation of antifungal activity, it is noted that halogen atoms have higher correlation coefficients for both HOMO / LUMO molecular states (81.5% and 47.1%), as well as for O-MO/U-MO molecular states (50.3% and 47.1%).

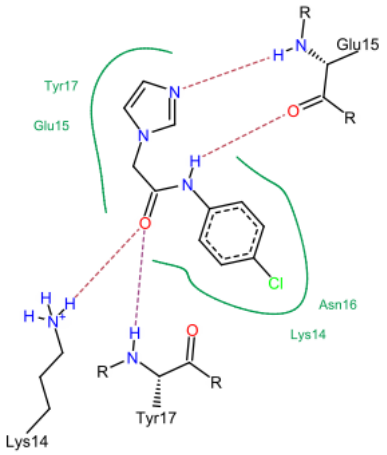
Correlation coefficient values R^2(%) for C and N atoms decrease for O-MO / U-MO molecular states sums, but increase for oxygen atoms. This shows that both the HOMO / LUMO and O-MO / U-MO states are reactive for the studied chemical structures.

The results obtained with fingerprint descriptors on how molecules in the studied class interact with active sites of biological receptors are also supported by molecular docking studies. In Fig. 3 two of the studied compounds 1b and 1c are represented, these being the most active compounds on gram-negative *Escherichia coli* bacteria and on *Candida albicans* fungus.

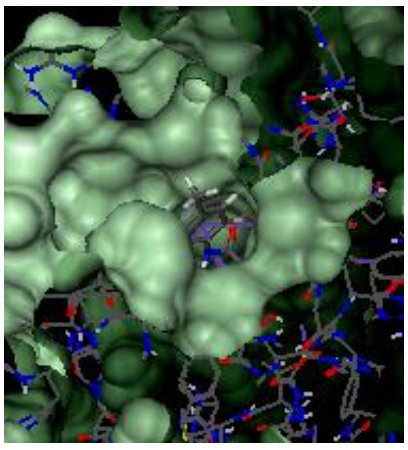
The docking technique allows visualization of interaction between the ligand and the biological receptor and predicts the optimized conformation of the stable complex formed by interaction. Hydrogen bonds made between the two participants to the interaction involve the participation of nitrogen atoms and carbonyl oxygen atoms from ligand molecules (acetamide derivatives). These results are consistent with those mentioned above, namely nitrogen atoms participate as electron donors (through the HOMO molecular states).

***C. albicans* – 1c complex**





***E. Coli* – 1b complex**



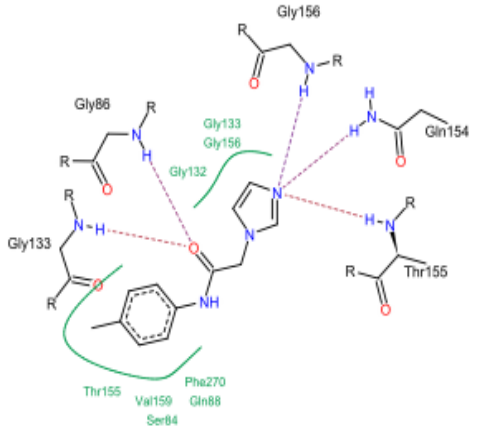


Figure 3. 3D and 2D interaction diagrams between chemical compounds and biological receptors [13]. The hydrogen bonds are represented by dotted lines.

In Table 9 are indicated the constitutive amino acids of the receptors used in our study with which the acetamide derivatives interact as inhibitors. It also shows the number of hydrogen bonds that occur at the interaction.

Table 9. Intermolecular interactions between studied compounds and receptors

	E. coli		*C. albicans*	
Cmpd	***Amino acids***	***Number of bonds***	***Amino acids***	***Number of bonds***
1a	Gly 86, Gly 133, Gly 156, Thr 155	5	Lys 14, Tyr 17, Glu 15	4
1b	Gly 86, Gly 133, Gly 156, Gin 154, Thr 155	5	Lys 14, Tyr 17, Glu 15	4
1c	Gly 86, Gly 133, Gly 156, Gin 154, Thr 155	5	Lys 14, Tyr 17, Glu 15	4
2a	Gly 86, Gly 133	2	Lys 14, Tyr 17, Glu 15	3
2b	Gly 86, Gly 156, Gin 154, Thr 155	5	Lys 14, Glu 15	2
2c	Gly 86, Gly 133	2	Lys 14, Tyr 17, Glu 15	4
3a	Gly 133, Gly 156, Gin 154, Thr 155	6	Lys 14, Tyr 17, Glu 15	4
3b	Gly 133, Gly 156, Gin 154, Thr 155	7	Lys 14, Asn 16	3
3c	Gly 133, Gly 156, Gin 154, Thr 155	7	Lys 14, Tyr 17, Glu 15	4

As can be seen in this table, for all the compounds studied, hydrogen bonds involve approximately the same amino acids of the *E. coli* bacterium or the *C. albicans* fungus, i.e., the same atomic groups. This suggests that the analyzed acetamide derivatives act all in the same active sites of biological receptors.

4. CONCLUSIONS

Identifying active parts in chemical structures makes it possible to design new compounds with optimized drug activity, opening up a new way to identify those molecular fragments or chemical groups that are most involved in drug activity formation. In the case of the studied acetamide derivatives, the groups of atoms contributing to the formation of antibacterial activities contain nitrogen and carbon atoms of the HOMO molecular state and halogen atoms in the HOMO / LUMO molecular states, and for the formation of antifungal activity, the halogen atoms of the molecular states O-MO / U-MO. Hydrogen bonds formed between the constitutive amino acids of the biological receptors and atomic groups of the acetamide derivatives are used to predict binding affinities and to develop binding models between derivatives and receptors.

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