BIOLOGICAL EVALUATION OF AROMATIC COMPOUNDS AS β-LACTAMASE INHIBITORS

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Summary

The appearance of bacterial resitance due to β-lactamase enzyme is frequent in Colombia and it has important consequences in term of morbidity and mortality. The inhibitory evaluation of the β-lactamase enzyme of 5 compounds gave as a result more powerful compounds than clavulanic acid. The more active chalcones establish hydrogen bonds with Ser, Tyr and Lys amino acids, the high number of hydrogen bonds between the compound and the amino acid increases the affinity and the inhibitory activity. In accordance with the results, the compounds might inhibit irreversibly the β-lactamase enzyme.

KEY WORDS: β-lactamase, clavulanic acid, enzyme-inhibitor, amoxicillin, chalcone

The threat of bacterial infection to human health has increased in recent years as bacterias have become resistant to presently available antibiotics (1, 2). The resistance rate because of producing microorganisms of β-lactamase has enhance in the last years (3), therefore, the research and formulation of alternative solution in order to reduce the microbial pharmacoresistance constitute a fundamental necessity and the development of inhibitors of β-lactamases to be coadministered with normal β-lactam antibiotics is a good strategy to control these illnesses, thus, understanding of the catalytic mechanism of β-lactamases is crucial for the design of new antibiotics (2).

Methods

Enzyme kinetics: Amoxicillin (36, 54, 72, 90, 108 μM) used as sustrate, chalcones (1)-(5) and clavulanic acid (5.4 μM) used as inhibitors, and β-lactamase enzyme (clase C de *Enterobacter cloacae*, 0.6μM) were runned in phosphate buffer (pH 7.3) at 37°C and monitored spectrophotometrically at 250 nm each 5 min for 10 hr. Hydrolysis of amoxicillin decreases the absorbance (4).

Syntheses: 5 compounds were synthezised following the Brian et al procedure (5): 1,3-diphenylpro-2-en-1-one (**1**), 1-(4-methylphenyl)-3-phenylprop-2-en-1-one (**2**), 1-(4-methylphenyl)-3-(4-nitrophenyl)-prop-2-en-1-one (**3**), 3-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**4**), 3-(2,3-dimethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**5**). The clavulanic acid and amoxicillin were reference patterns.

Computational Details: All the computational studies were performed using Gaussian 03 software. The geometric parameters and energy of the compounds were optimized using HF/6-31G∗ level. The compounds (**4**) and (**5**) intermediates were optimized using the Kamaljit and Pratt reference (6, 7).

Results

The results of inhibitory evaluation of β-lactamase enzyme are given in table 1.

Table 1. Inhibition Constants (*K*i), Vmax, Km and Energy of the inhibitor compounds (**1**)-(**5**) , clavulanic acid and amoxicillin.

Compound	Vmax (μM/min)	Km (μM)	Ki (μM)	E (Hartree)	Inhibition
(1)	**$1.63x10^{-4}$**	**154.2**	**4158**	**-649.86**	**Non competitive**
(2)	**$3.46x10^{-4}$**	**296.6**	**5.8**	**-688.90**	**Competitive**
(3)	**$3.45x10^{-4}$**	**366.4**	**3.9**	**-892.37**	**Competitive**
(4)	**$3.44x10^{-4}$**	**395.3**	**3.5**	**-916.63**	**Competitive**
(5)	**$3.41x10^{-4}$**	**407.8**	**3.3**	**-916.64**	**Competitive**
amoxicillin	**$3.40x10^{-4}$**	**154.0**	**-------**	**-1552.08**	**---------**
clavulanic acid	**$3.47x10^{-4}$**	**217.2**	**13.2**	**-736.86**	**Competitive**

Michaelis-Menten constant constitutes the starting points for determining the inhibition and wether the attack of the serine residue in the enzyme is on the carbonyl gorup in the chalcone. The substituents on phenyl group of the chalcones change the site binding with the β-lactamase enzyme, these compounds change from non-competitive to competitive inhibitors. By comparison of inhibition constants values it appeared that the compounds (**4**) and (**5**) are the molecules with the highest inhibition power, probably due to their ability to interact with the enzyme through of hydrogen bonds between the Serine, Tyrosine and Lysine amino acids of the active site of the enzyme and the carbonyl carbon and methoxyl groups of the chalcones, the figure 1 shows the rates of enzyme inactivation at each inhibitor concentration.

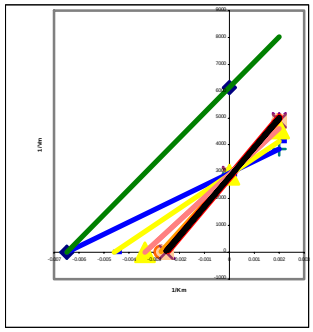
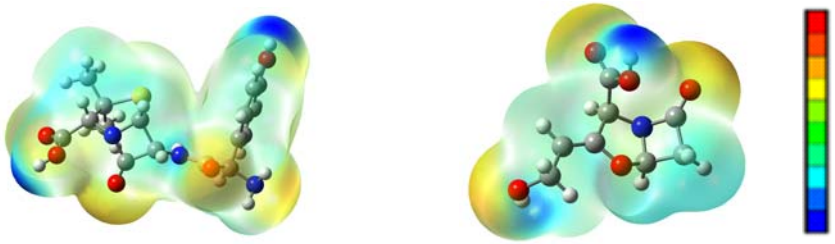


Figure 1. Lineweaver-Burk plot of the evaluated compounds: green: (**1**), pink: (**2**), dark yellow: (**3**), violet: (**4**), red: (**5**), yellow: clavulanic acid, blue: amoxicillin

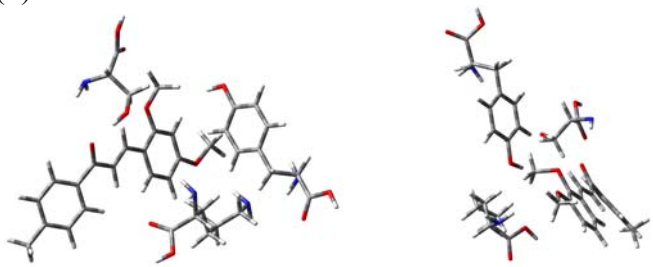
In accordance with the Mulliken charges, the carbonyl carbon of the clavulanic acid is more susceptible to nucelophilic attack of hydroxyl of the Serine residue in the enzyme, because the stronger positive charge located on the carbonyl carbon of the β-lactam ring than on the carbonyl group of the β-lactam ring in amoxicillin, the total electron density are shown in figure 2. In the chalcones case, the carbonyl carbons are equally charged doing all of them susceptible to nucelophilic attack of hydroxyl of the serine residue in the enzyme.
According to the energy of the compounds, the chalcones and clavulanic acid are more susceptible to nucleophilic attack than amoxicillin, therefore, the chalcones might be potential inhibitors of the enzyme activity.

(a)



amoxicillin clavulanic acid

(b)



chalcone (**4**) chalcone (**5**)

Figure 2. (a) charge density surface for amoxicillin and clavulanic acid (b) Stereoviews of optimized structures of the interaction between compounds (**4**) and (**5**) intermediate formed on interaction of the Serine, tyrosine and Lyisine amino acids. Red: (greatest negative) nucleophiles, blue: (highest positive) electrophiles with intermediate colors indicating regions of values between these extremes.

In order to understand the interaction of the compounds (**4**) and (**5**) with the enzyme they were modelled with Ser, Tyr and Lys amino acids according to reference (6, 7). The molecular modelling shows hydrogen bonds between the Ser amino acid and carbonyl carbon, Tyr amino acid and 2-metoxy group and Lys amino acid and 4-methoxyl groups in the chalcone (**4**). The Tyr amino acid is located between 2-metoxy group and 3-metoxy group in the chalcone (**5**), which might stablish 2 hydrogen bonds with this compound one with each methoxyl group alternately, and probably this fact might increase the number of hydrogen bonds of the chalcone (**5**) in the active site of the enzyme and therefore, it increases the affinity, the bond lenghts of each intermediate are shown in table 2.

Table 2-Hydrogen bond distance between funtional groups of the chalcones (**4**) and (**5**) and Serine, Tyrosine and Lysine amino acids.

intermediates	Hydrogen bond	Distance (Å)
chalcone(4)-amino acid	C=O---HO-Ser	1.811
	2-CH_3O---HO-Tyr	3.510
	4-CH_3O---H_2N-Lys	2.640
chalcone(5)-amino acid	C=O---HO---Ser	2.527
	2-CH_3O---HO-Tyr	3.520
	3-CH_3O---HO-Tyr	2.620
	3-CH_3O---H_2N-Lys	3.020

The formation of strong hydrogen bonds with both funtional groups might reveal the difference between the Km of the β-lactamase enzyme with the chalcone (**5**) and chalcone (**4**).

Discussion

A comparison of the results of the electronic profile of the clavulanic acid, amoxicillin and inhibitors can help to identify the molecular sites involved in the interactions with the β-lactamase enzyme and it would be a point of view to design new compounds with improved inhibitory properties.

The obtained results show that the addition of substituents on the aromatic rings of chalcones change the affinity of the compounds for the enzyme. The electron-donating groups like CH_3O in the chalcones might contribute to improve the inhibitory activity and the 2 and 3-phenyl position of the methoxyl group in the chalcones seem to enhance the affinity of the inhibitors for the enzyme because they would be able to stablish more hydrogen bonds in active site of the enzyme and therefore might encourage the interaction with amino acid in the active site of the enzyme.

The enzyme does not recover the enzyme activity after 10 hr, this might suggest all of the evaluated inhibitors might inhibit irreversible the β-lactamase enzyme.

Our results on the molecular modelling agree with this experimental evidence. It is therefore very important to establish theoretical patterns, allowing one to obtain accurate predictions of the inhibitory potential of new compounds.

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