SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF COBALT(III) COMPLEX WITH PHENANTHROLINE AND MALTOSE

JOSÉ PARADA[1], ANA MARÍA ATRIA[1], GUILLERMO WIESE[2], ELIZABETH RIVAS[2], GINO CORSINI[2]*

[1]*Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.*
[2]*Centro de Investigación Biomédica, Universidad Autónoma de Chile. Santiago. Chile*

ABSTRACT

The mononuclear cobalt(III) complex derived from 1,10-phenanthroline and maltose ($[Co(phen)_2maltose]Cl_2 \cdot 3H_2O$) (**1**) has been synthesized and characterized in aqueous solution. Its characterization was based on its optical and spectroscopic properties.

The antimicrobial activity of this complex was screened *in vitro* against the microorganisms *Escherichia coli* DH5α, *Salmonella enterica* sv *Enteritidis* ISP/953, *Klebsiella pneumoniae* RYC492, *Pseudomonas aeruginosa* PAO1, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* GCA234, *Micrococcus* sp. *Staphylococcus aureus* ATCC25923.

Complex (**1**) showed antibacterial activity with a bacteriostatic effect over Gram positive and negative bacteria. The cytotoxicity of complex **(1)** was tested *in vitro* on human embryonic kidney cells.

Keywords: Carbohydrate-cobalt(III) complexes; circular dichroism; antibacterial activity.

1. INTRODUCTION

Transition metal complexes are of interest since they display a wide variety of application in fields ranging from materials science and catalysis to biological activity [1].

From the biological point of view, several studies have shown that the complexes may have antibacterial, antifungal and antitumor activity [2].

The biological activity of metal complexes is highly dependent on the nature of the metal ions and the donor sequence of the ligands because different ligands exhibit different biological properties [3].

An interesting group of ligands are the carbohydrates [4], whose complexes have shown significant antimicrobial activity against Gram-positive and Gram-negative bacterial strains as well as a few fungal strains [5]. Poller and Parkin reported the synthesis of organometallic derivatives of sucrose (lead, tin and germanium). In that study organotin was found to exhibit higher biocidal activities than would be expected from the tin content [6].

A few sugar-cobalt complexes with antibacterial activity have been reported. An important example is grafted PVA polymer with a derivative of erythro-ascorbic acid (pentulosono-lactone-2,3-enedianisoate) reported by Salih et al. It was evaluated for antimicrobial properties against four pathogenic bacteria (*Escherichia coli*, *Klebsiella pneumonae, Staphylococcus aureus, Staphylococcus albus*) and fungi (*Aspergillus niger, yeast*). This cobalt complex showed good activity against the various microbial isolates [7].

1,10-Phenanthroline is also one of the biologically important ligands, and several studies show that this ligand and a number of its complexes are effective against various strains of microorganisms. Examples are the complexes $[Cu(phen)_2mal)]\cdot 2H_2O$ and $[Ag_2(phen)_2(mal)]\cdot 2H_2O$ (phen = 1,10-phenanthroline, $malH_2$ = malonic acid) reported by B. Coyle et al. which inhibit the growth of *Candida albicans* [8].

Here the synthesis of the mixed ligand cobalt complex $[Co(phen)_2maltose]Cl_2 \cdot 3H_2O$ **(1),** as well as its spectral features and optical activity are reported.

UV-visible absorption spectroscopy and circular dichroism spectroscopy allow us to assign a Δ configuration to complex **(1).**

The complex was also screened for antibacterial activity against Gram positive and Gram-negative bacteria. We determined the antibacterial effects and evaluated their cytotoxic effect on human cells.

2. EXPERIMENTAL

2.1. Materials and instruments

All reagents and solvents employed were commercially available and were used as received without further purification.

The cobalt content of the complexes in the solutions as well as in the solid compounds was determined by atomic absorption spectroscopy with a Perkin Elmer 1100B spectrophotometer. Absorption spectra were recorded on a Unicam UV3 instrument. The circular dichroism (CD) spectra were monitored with a Yobin-Yvon CD 6 spectrometer. The elemental analyses were performed by Analyses Elemental CE Instruments EA 1108. The infrared spectrum (4000 - 600 cm^{-1}) was recorded using KBr pellets on a Perkin Elmer FT-IR C 97945 spectrometer. ^{1}H-NMR measurements were performed in DMSO-d_6 solution on a Bruker DXR 300 spectrometer (300 MHz).

2.2 Synthesis of the complex

Complex (**1**) was prepared by a procedure similar to that for the preparation of compounds $[Co(phen)_2L]^{2+}$ (L=arabinose **(2)** and galactose **(3)**) reported by Parada et al. An aqueous solution of $[Co(phen)_2Cl_2]Cl$ (10^{-2} M) was mixed with a two-fold molar excess of maltose and the pH was adjusted to 9.0 by addition of NaOH [9].

The complex was separated by chromatography on a Sephadex C25 column by elution with 0.1 M NaCl.

The eluate was evaporated to dryness under reduced pressure, followed by extraction with EtOH and MeOH to remove the NaCl. Anal. Calcd for $CoC_{36}H_{43}N_4O_{14}Cl_2$: Co, 6.65; C, 48.82; H, 4.85; N, 6.33, Found: Co, 7.15; C, 48.32; H, 4.69; N, 5.87.

2.3. Antimicrobial activity measurements.

All antimicrobial activity assays were performed in triplicate and confirmed by three separate experiments. The antimicrobial activity was investigated against some Gram-positive (*Enterococcus feacalis* ATCC29212, *Bacillus cereus* GCA234, *Micrococcus* sp. and *Staphylococcus aureus* ATCC25923) and Gram-negative bacteria (*Escherichia coli* DH5α, *Salmonella enterica* sv Enteritidis ISP/953, *Klebsiella pneumoniae* RYC492 and *Pseudomonas aeruginosa* PAO1).)

Bacteria were grown in Mueller Hinton agar (Difco) as well as Mueller Hinton broth (Difco) for 16 to 24 h at 37 ºC in an incubator.

The *in vitro* antibacterial activity of the complexes was tested using the paper disk diffusion method [12] and the minimal inhibitory concentration (MIC) was determined as the lowest concentrations of drug in the medium at which no microbial growth was visible [13].

2.4 Antibacterial effect

The *in vitro* antibacterial effect of the complexes was tested using a modified chromogenic plate test assay [14] *E. coli* HB101, which contains a chromosomal IPTG-inducible β-galactosidase gene. The chromogenic agar plates were prepared as follows: First, an inoculum with this strain was grown overnight in 2 mL of Mueller Hinton medium, at 37 ºC with shaking. Then, a top agar-incubation mix containing 5 mL of 0.8% agar previously melted at 45 ºC containing 0.1 mL of the bacterial cell inoculum, 0.01 mL of 1 mM IPTG, and 0.1 mL of X-Gal 50 mg/mL was vortex-mixed and carefully overlaid on Mueller Hinton agar plates prepared the day before. The disks containing the appropriate concentration of complex or antibiotic were deposited on the chromogenic agar plates. The plates were then incubated at 37 ºC for 12-24

e-mail: jparada@ciq.uchile.cl

h, the inhibition zones were visually inspected for color formation along de edges of the disks, and the plates were photographed. Only compounds causing cellular lysis produce a blue-colored edge at the inhibition zone.

2.5 Cytotoxic effect

The cytotoxicity of the complexes was tested *in vitro* on human cells using microplate cultures of human kidney embryonic cells (HEK293 cells) grown in DMEM medium plus 10% bovine fetal serum. The number of dead cells was determined by an MTT cell proliferation assay after 24 h of incubation with the complexes. [15] This method is a colorimetric assay system which measures the reduction of a tetrazolium component (MTT) to an insoluble formazan product by the mitochondria of viable cells. The amount of color produced is directly proportional to the number of viable cells.

3. RESULTS AND DISCUSSION

3.1 Spectroscopic characterization of $[Co(phen)_2maltose]Cl_2 \cdot 3H_2O$ complex (1)

The IR spectrum of the complex (1) showed characteristic bands of 1,10-phenanthroline and maltose ligands.

The spectrum shows a broad absorption band at 3462 cm^{-1} that can be associated with ν(O-H) of the maltose, and weak bands at 3052 and 2930 cm^{-1} that can be assigned to C-H stretching vibrations of maltose and phenanthroline, respectively. In the 1600-1400 cm^{-1} region the spectrum shows bands corresponding to ν(C=C, C=N) of the phenanthroline ligand. Bands between 1342 – 1316 cm^{-1} involve O-H, CH, CH_2 bending modes of the maltose .The peak at 1146 cm^{-1} have been assigned to C-C in-plane bending and the peaks at 1105 and 1195 cm^{-1} have been identified as bands associated with C-O-C bending. The νH (in plane) and δH(out plane) vibration of the phenanthroline are located in the 1038-700cm^{-1} region.

The ^{1}H-NMR spectrum of the complex reveals the resonance signals of the aliphatic hydrogen of the maltose (δ 3.0-5.0 ppm) and aromatic hydrogen of the phenanthroline (δ 7.6-9.1 ppm) to be well separated. The relation between two different signals is 1:3 which is in agreement with the proposed structure of the complex.

An absorption band at 272 nm is seen in the UV spectrum. The band is associated with p transitions of the 1,10-phenanthroline ligands. In this region the CD spectrum presents two bands of opposite signs which appear at 266 (+) and 280 (-) nm. The signs sequence is related to the Δ configuration of the chelate rings (excitonic effect) [10].

The complex showed a second positive CD signal under a shoulder in the UV region (322 nm), related to the $\pi \rightarrow \pi^*$ transition along the short axis of the phenanthroline rings.

In the visible region, the complex exhibited an absorption band at 486 nm corresponding to the $^1A_{1g} \rightarrow {}^1T_{1g}$ transition. In the CD spectra two signals (+) and (-) can be detected, which are correlated with the Δ configuration [11]. The CD spectra also showed a third signal (-) at 358 nm than can be assigned to the $^1T_{2g}$ state in octahedral symmetry.

The UV-VIS absorption and CD spectra of complex **(1)** are consistent with those reported earlier for $[Co(phen)_2L]Cl_2$, where L=arabinose for **(2)** and galactose for **(3)** as seen in Figures 1 and 2.

3.2 Biological studies

The *in vitro* antibacterial activity of complex (**1**) was evaluated against representative Gram positive and Gram negative bacteria. Its activity was compared with that presented by the homologous complexes with arabinose (**2**) and galactose (**3**) as ligands. The results of antibacterial activity and minimum inhibitory concentration (MIC) are reported in Tables 1 and 2, respectively.

Table 1 shows the antibacterial effect of the compounds using the paper disk diffusion method. The galactose complex **(3)** did not show any antibacterial effect on the tested bacteria, while the maltose complex **(1)** and the arabinose complex **(2)** display activity against *Escherichia coli, Salmonella Enteritidis, Klebsiella pneumonia, Bacillus cereus* and *Micrococcus sp.* bacteria. They did not show activity against *Pseudomonas aeruginosa, Enterococcus faecalis* and *Staphylococcus aureus*.

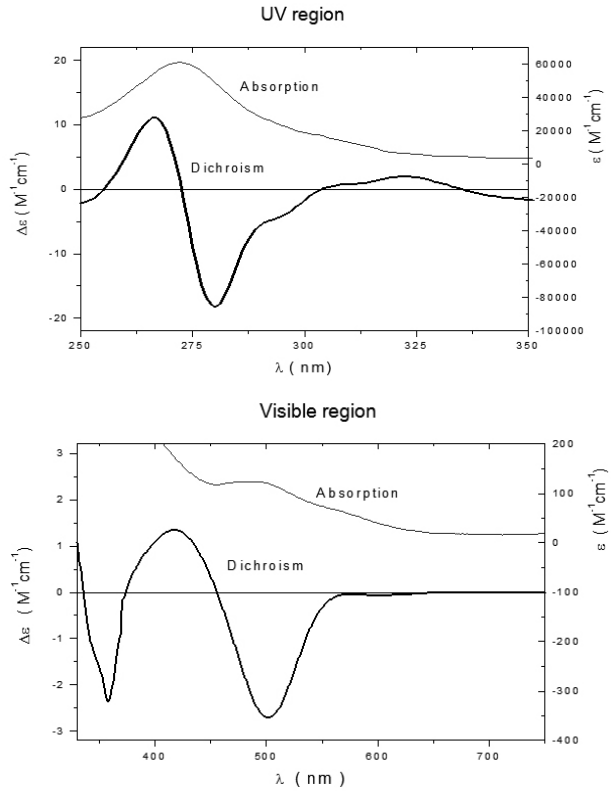

Figure 1. Absorption and circular dichroism (CD) spectra of $[Co(phen)_2maltose]^{2+}$ complex **(1).**

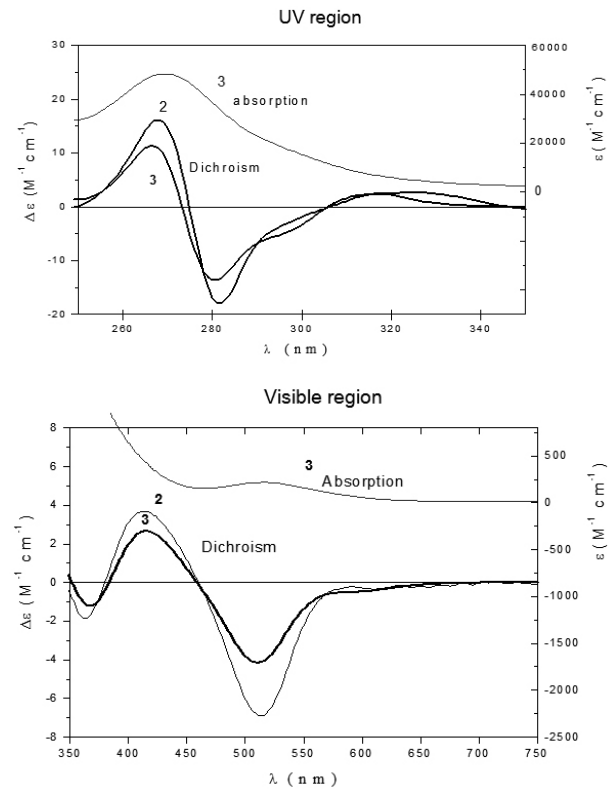

Figure 2. Absorption and circular dichroism (CD) spectra of $[Co(phen)_2arabinose]^{2+}$ (**2**) and $[Co(phen)_2arabinose]^{2+}$ complexes (**3**).

Table 1. Qualitative antibacterial activity of $[Co(phen)_2 maltose]^{2+}$ **(1)**, $[Co(phen)_2 arabinose]^{2+}$ **(2),** and $[Co(phen)_2 galactose]^{2+}$ **(3)** complexes.

Bacteria	Growth inhibition zone (mm)*				
	Co-MAL (1)	Co-ARA (2)	Co-GAL (3)	KAN	ZOX
Escherichia coli DH5α	14.1±1.1	17.0±0.8	R	21.4±0.4	ND
Salmonella Enteritidis	13.5±0.9	15.6±1.0	R	23.4±0.6	ND
Klebsiella pneumoniae	19.5±1.0	20.0±0.5	R	R	ND
Pseudomonas aeruginosa	R	R	R	R	ND
Enterococcus feacalis	R	R	R	R	ND
Bacillus cereus	14.4±0.3	15.4±0.7	R	22.1±0.6	ND
Micrococcus sp.	15.2±0.8	14.3±1.1	R	18.8±0.8	ND
Staphylococcus aureus	R	R	R	ND	34.7±0.7

* Disc with 400 μg of compound. Number of assays = 3. Co-MAL: $[Co(phen)_2 maltose]^{2+}$,
Co-ARA: $[Co(phen)_2 arabinose]^{2+}$, Co-GAL: $[Co(phen)_2 galactose]^{2+}$, KAN: Kanamycin (disk with 30 μg),
ZOX: Ceftizoxime (disk with 30 μg). R = resistant, ND = not determined.

Table 2. Antibacterial activity by minimal inhibition concentration (MIC) of $[Co(phen)_2 maltose]^{2+}$ and $[Co(phen)_2 arabinose]^{2+}$ complexes.

Bacteria	MIC (μg/mL)*	
	$[Co(phen)_2 maltose]^{2+}$ (1)	$[Co(phen)_2 arabinose]^{2+}$ (2)
Escherichia coli	40	40
Salmonella Enteritidis	40	60
Klebsiella pneumoniae	60	60
Bacillus cereus	60	80
Micrococcus sp.	60	80

* Number of assays = 3.

Both complexes showed moderate antibacterial activity when compared with the standard drug kanamycin. On the other hand, these complexes exhibited antibacterial activity against *Klebsiella pneumonia* compared to the reference drug.

Diffusion disks assay showed no difference in the antibacterial activity of complexes (**1**) and (**2**), which showed bacterial growth inhibition with MIC values ranging between 40-60 μg/mL and 40-80 μg/mL, respectively (Table 2).

We used a plate assay to distinguish bactericidal from bacteriostatic action modes of the antimicrobial complexes [16, 17]. This method was based on the use of β-galactosidase as an appropriate marker of cellular lysis. If lysis occurs, the enzyme activity is released outside the bacterium and detected on the plate. When the enzyme reaches the agar medium, it hydrolyzes 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal), a chromogenic compound included in the agar. After overnight incubation, X-Gal forms a blue circle staining the edge of the inhibition zone produced by the antibiotic application. Only compounds causing cellular lysis or membrane damage produce a blue-colored edge at the inhibition zone.

The assay showed that complexes (**1**) and (**2**) present bacteriostatic activity because they do not produce a blue circle staining the edge of the inhibition zone (Fig. 3). Ceftizoxime and chloramphenicol were used as bactericidal and bacteriostatic control agents, respectively.

In order to evaluate the ability of the compounds to damage eukaryotic cells, the *in vitro* cytotoxic effect of these complexes was tested on human embryonic kidney cells (Fig. 4).

Analysis of cytotoxicity curves reveals that the complexes studied are not toxic to human cell at MIC concentration. At 200 μg/mL concentrations there was about 80% survival of human cells, which is four times above the average of the MIC value for each compound. The results also show IC_{50} values for complexes (**1**) and (**2**) of 870 μg/mL and >1000 μg/mL, respectively. All this indicates that both complexes does not have an *in vitro* cytotoxic effect at concentrations of biological interest (4 times the MIC).

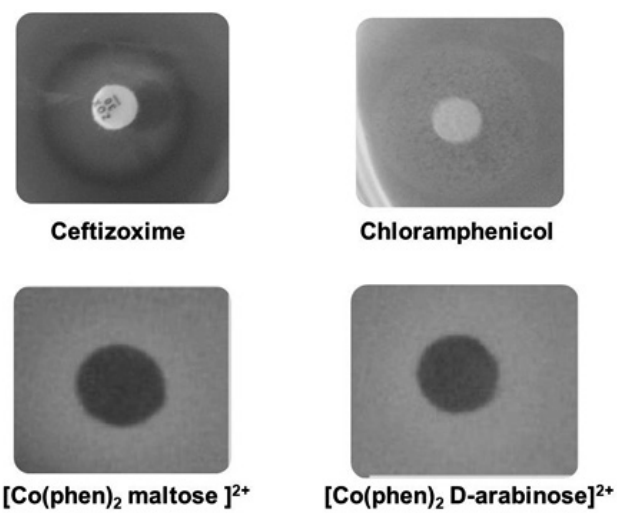

Figure 3. Plate assay showing bacteriostatic or bacteriolytic effect of complexes **(1)** and **(2)** over Gram-negative bacteria *Escherichia coli* BL21 (DE3). Used in assay disk with 400 μg of analyzed complex. Disc of ceftizoxime (30 μg/disk) and chloramphenicol (30 μg/disk) were used as bacteriostatic and bacteriolytic control respectively.

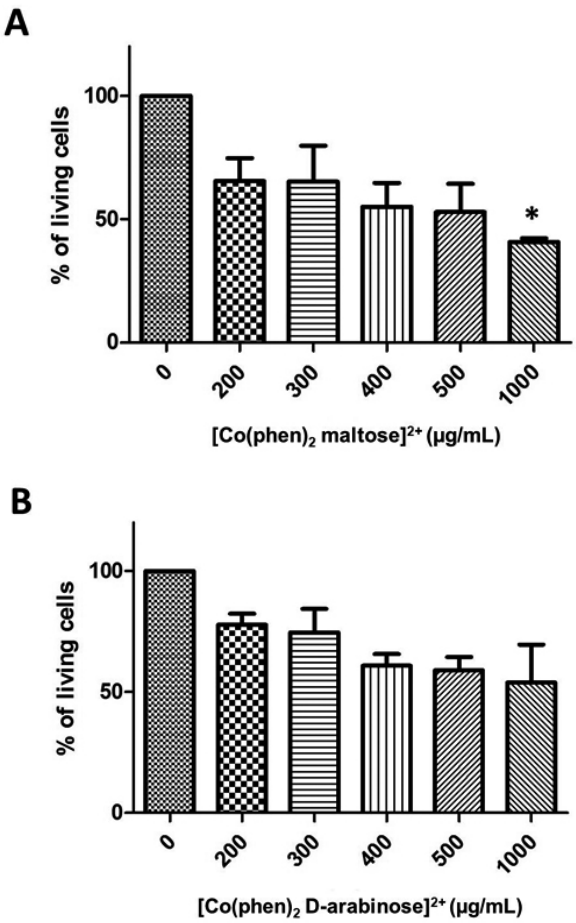

Figure 4. In vitro cytotoxicity effect of complexes on human cell (HEK293 cell).

CONCLUSIONS

Circular dichroism studies and UV-visible spectroscopy allowed to assign a delta configuration to complex **(1).** This complex showed antibacterial activity with a bacteriostatic effect over Gram-positive and Gram-negative bacteria, and the cytotoxicity test showed that the cobalt compound had low toxicity.

ACKNOWLEDGMENTS

Support under FONDECYT Project 1120125 is gratefully acknowledged.

REFERENCES

[1] K. D. Mjos, C. Orvig, *Chem. Rev*. **114**, 4540 (2014).
[2] B. S. Sekhon, *J. Pharm. Educ. Res*. **2**, 1, (2011).
[3] C. X. Zhang, S. J. Lippard, *Curr. Opin. Chem. Biol.* **7**, 481, (2003).
[4]) D. M. Whitfield, S. Stojkovski and B. Sarkar, *Coord. Chem. Rev*. **122**, 171, (1993); b) S. Yano, *Coord. Chem. Rev*. 92, 113, (1988); c) B. Gyurcsik and L. Nagy, *Coord. Chem. Rev*. **203**, 81, (2000).
[5] H. R. Appelt, J. S. Oliveira, R. C. V. Santos, O.E.D. Rodrigues, M. Z. Santos, E. F. Heck, and L. C. Rosa, *Int. J.Carbohydr. Chem.* **2013**, (2013). http://dx.doi.org/10.1155/2013/320892 Article ID 320892,
[6] (a) A. Parkin, R. C. Poller, Int. Pest. Control, 19, 5, (1977), b) R. C. Poller, A. Parkin, *ACS Symp. Ser*. **41**, 145, (1977).
[7] T. M. Salih, R. M. Rumez, M. A. Younus, B. I. Khalil and B. W. Khammas, *Jolst.* **1**, 51, (2013). doi: 10.12720/jolst.
[8] B. Coyle, K. Kavanagh, M. McCann, M. Devereux, M. Geraghty, *Bio Metals* **16**, 321, (2003).
[9] a) J. Parada, S. Bunel, C. Ibarra, G. Larrazábal, E. Moraga, *Carbohydr. Res*. **329**, 195, (2000); b) J. Parada, S. Bunel, C. Ibarra, G. Larrazábal, E. Moraga, N.D. Gillitt, C.A. Bunton, *Carbohydr. Res*. **333**, 185, (2001); c) J. Parada, S. Bunel, C. Ibarra, G. arrazábal, E. Moraga, N. Gillitt, C.A. Bunton, *Polyhedron* **21**, 2215, (2002); d) J. Parada, G. Larrazábal, *Polyhedron* **23**, 1341, (2004).
[10] a) C. J. Hawkins, Absolute Configuration of Metal Complexes, Wiley, New York, 1971. b) K. Nakanishi, N. Berova and R. W. Woody (Eds.), Circular Dichroism, Principles and Applications, VCH, Cambridge, 1994.
[11] a) I. Tinico, *Adv. Chem. Phys*. 4, 113, (1962); b) F.S. Richardson, *J. Chem. Phys*. **54**, 2453, (1971).
[12] K. L. Kwaniewska, *Bull. Environ. Contam. Toxicol*, **27**, (1981).
[13] National Committee for Clinical Laboratory Standards; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Fifth Edition: Approved Standard M7-A5. NCCLS, Wayne, PA, USA, 2000.
[14] G. Mardones, A. Venegas, *J. Microbiol. Meth*., **40**, 199, (2000).
[15] S. Maher, S. McClean, *Biochem. Pharmacol*. **71**, 1289, (2006).
[16] P. Cortés-Cortés, A. M. Atria, M. Contreras, O. Peña, K. Fernández, G. Corsin, *J. Chil. Chem. Soc*. **53**, 1527, (2008).
[17] A. M. Atria, P. Cortés-Cortés, M.T. Garland, R. Baggio, K. Morales, M. Soto, G. Corsini, *J. Chil. Chem. Soc*. **56**, 786, (2011).