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*Current Topics in Medicinal Chemistry*, 2015, *15*, 1743-1749 

# Molecular Docking Studies of the Antitumoral Activity and Characterization of New Chalcone

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**Abstract:** Phytochemical investigation of *Azorella madreporica* led to the isolation of four known compounds and an unknown chalcone. The structure of the new compound was identified by spectroscopy, including two-dimensional NMR techniques and comparison with published spectral data. The antioxidant activity of chalcone (compound **1**) was measured using the 1,2-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay, and the bioactivity was evaluated against five bacteria (*Mycobacterium smegmatis* ATCC 14468, clinical isolates of *Staphylococcus aureus, Klebsiella granulomatis, Morganella morganii* and *Escherichia coli*) and four cancer cell lines. Docking studies with the tested cancer related proteins revealed nearby values of energy between doxorubicin and compound **1**. Besides, protein-ligand interactions correlate with these energy values.

**Keywords:** Antitumoral, *Azorella madreporica*, Chalcone, Diterpenes, Docking, Yareta.

## 1. INTRODUCTION

The word chalcone comes from the Greek chalcos, which means bronze. Chalcones and related compounds, also called aromatic ketones, form the core of a range of important biomolecules. Chalcones share the common structural framework of 1, 3-diphenyl-2-propen-1-one; similar to α,β unsaturated ketone encoded by an ethylene ketone group. As auxochromes, chalcones provide floral pigments, antibiotics, UV protectants and insect repellents to plants. Chalcones are among others found in heartwood, bark, leaves, fruit and roots [1,2], and they are biosynthetic precursors of flavonoid/isoflavonoids [3]. Those compounds are easily cycled by Michael addition to the α,β double bond, forming a flavanone. Interestingly, A and B rings have different energy barriers to rotation. Given their relatively simple structure, the variety and different form of cycling and widespread availability, chalcones are a useful template in the search for molecules with therapeutic potential. The simplest chalcone can be prepared by aldol condensation between a benzaldehyde and an acetophenone using a base as a catalyst [4].

Chalcones and their derivatives display a wide array of pharmacological activities: anti-inflammatory, antifungal, antimalarial, anti-tumor, anti-microbial, antiviral and anti-tuberculosis. Moreover, antioxidant, antimitotic, antileishmanial, antiplatelet and anti-cancer potentials have been observed [5-12]. The application of "*in vitro*" assays enables to characterize if molecules are promising bioactive products.

In this study, we describe the isolation and the characterization of the biological properties of a new chalcone and four known compounds (two diterpenes and two flavonoids), using *Azorella madreporica*, a species of the Tribu Mulinae plant, known as a producer of biologically active and unique diterpenes, as source [13-15].

## 2. EXPERIMENTAL

### 2.1. Chemistry

#### *2.1.1. General Procedure*

$^{1}$H-NMR spectra were registered by dissolving the samples in $CDCl_3$ at 400.13 MHz and for $^{13}$C-NMR at 125.03 MHz in a multidimensional Bruker spectrometer. Chemical displacements are expressed as values relative to TMS as an internal standard. Bidimensional spectra were obtained using standard Bruker software. Purification took place in Merck Hitachi equipment using high performance chromatographic column (HPCC) of silica gel (Si Ultrasphere 5 μm, 10 - 250 mm). Columns chromatography were performed over 0.040-0.063 μm Merck silica gel. Exclusion chromatography was

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1873-5294/15 $58.00+.00 

carried out using Sephadex LH-20. UV spectra were measured in a Shimadzu spectrophotometer, UV-160, using $CHCl_3$ as a solvent. IR spectra were measured using NaCl tablets in the 500-4000 $cm^{-1}$ region and were recorded on a FT-IR Thermo Nicolet Nexus 670 spectrophotometer, with 0.125 $cm^{-1}$ spectral resolution. Mass spectra were taken in a Micromass Q-TOF spectrometer.

#### *2.1.2. Plant Material*

The plant was gathered at Valle Nevado (Metropolitan Region, Chile) in December 2012. A sample of the species (No. 3360) was left at the Herbarium of the University of Chile's Faculty of Sciences.

#### *2.1.3. Extraction and Isolation*

The whole *A. madreporica* plant (1.1 Kg) was ground into a fine powder, which was extracted with *n*-hexane at room temperature. After filtering, the solvent was vacuum evaporated at a low temperature producing a rubber (80.0 g). The concentrated *n*-hexane was adsorbed on silica gel (130.0 g) and fractionated in a column containing silica gel (800.0 g), which was eluted using an *n*-hexane-EtOAc gradient with increasing amounts of EtOAc. The fraction that displayed an orange pigment (80.0 mg) was purified using HPCC, obtaining 15 mg of a new compound **1.** From the subsequent fractions, mulin-11,13-dien-20-oic acid, **2** (150 mg), alpinum isoflavone, **3** (30 mg), 7-acetoxymulin-9,12-diene **4** (30 mg) and 4-hydroxylonchochalcone **5** (5 mg) were purified. The identification of those compounds were made by comparison with authentic samples and with literature data [16-18].

**Compound 1.** [(E)-4-(5-hydroxy-6-(3-(4-hydroxyphenyl)acryloyl)-2-methyl-2H-chromen-2-yl)butan-2-one]. An amorphous yellow powder. UV ($CHCl_3$) $\lambda_{max}$ (log ε): 375 (1.32), 283 (1.57 ), 257 (1.07) nm. $\nu_{max}$ IR (NaCl): 3350, 3300, 3051, 3033, 1723 and 1650 $cm^{-1}$. EIMS m/z (rel.int) 378.4 (5), 364.0 (2), 308.0 (10) 147(100). NMR of $^{1}H$ and $^{13}C$, see Table **1**.

### 2.2. Biological Activity

#### *2.2.1. Antimicrobial Activity*

The antibacterial activity of the compounds was studied *in vitro* using the paper disk assay method. Test organisms included *Mycobacterium smegmatis* ATCC 14468, clinical isolates of *Staphylococcus aureus, Klebsiella granulomatis*, *Morganella morganii* and *Escherichia coli*. Bacterial strains were inoculated into LB broth and incubated at 37 °C. After 18 h of growth, the turbidity was adjusted to a 0.5 McFarland standard with sterile saline solution. Standardized bacterial cultures were individually swabbed on the surface of Luria-Bertani (LB) agar plates. Sterile filter paper disks (Whatman N° 1; 6 mm diameter) were impregnated with different concentrations of the compounds, placed on the agar surface and incubated at 37 °C for 18 h. Disks impregnated with DMSO and broad- spectrum antibiotic were included as negative and positive controls respectively. Plates were examined for zones of inhibition and the diameter (mm) of halos around disks was recorded. The MICs were determined by a broth macrodilution method according to CLSI guidelines [19]. The bacterial strains were cultured in Luria-Bertani (LB) broth supplemented with glycerol (0.4 %) and Tween 80 (0.05 %). A penicillin/streptomycin solution was used as positive control and DMSO as negative control. The MIC was defined as the lowest concentration of compound that inhibits bacterial growth. All the assays were carried out in triplicate.

#### *2.2.2. Free Radical Scavenging Assay (DPPH)*

Samples were dissolved in DMSO to adjust a concentration of 1 mg/mL and then diluted to prepare serial concentrations for the antioxidant assay. The final concentration of DMSO was less than 2%. Reference chemicals were used for comparison in all the assays. The scavenging activity was estimated using DPPH as the free radical model as per the method adapted from Brand-Williams and Molyneux [20,21]. Briefly, an aliquot of 1 mL of compound (10, 50 and 100 μg/mL) and control (2% DMSO final), respectively, were mixed with 2 mL of DPPH solution. The mixture was shaken vigorously and left to stand at room temperature for 5 minutes, in darkness. The mixture was measured spectrophotometrically at 515 nm. The free radical scavenging activity was calculated as a percentage of DPPH decoloration using the following equation:

$$\% \text{ scavenging DPPH free radical} = 100 \times (1\text{-}AE/AD)$$

where AE, is the absorbance of the solution after adding the compound and AD is the absorbance of the blank DPPH solution. Quercetin was used as the reference compound.

#### *2.2.3. Cell Culture*

Colon cell carcinoma RKO cells, breast cancer MCF-7 cells, prostatic carcinoma PC-3 cells and human astrocytoma D384 cells were cultured in RPMI-supplemented medium (100 units/mL penicillin G, 100μg/mL streptomycin, 0.25μg/mL amphotericin B), 2mM L-glutamine with 10% fetal bovine serum (FBS, v/v, Invitrogen) in a humidified incubator (37°C, 5% $CO_2$).

#### *2.2.4. Cell Proliferation Analysis*

Cell viability was analyzed using the MTS assay (Invitrogen) to assess the viability and/or the metabolic state of the cancer cells based on mitochondrial respiratory activity. A total of $5 \times 10^3$ cells were seeded into each well of 96-well plates and allowed to adhere for 24h. After 24h, the cells were treated with the molecule isolate 50μM. Each concentration/assay was performed 3 times in triplicate. Negative control cells were treated with DMSO to get the final concentration of 0.1% v/v, and doxorubicin 1μM was used as a positive control. The cells were then incubated with treatments for 48h. After 42h MTS (5 mg/mL) was added and cells were further incubated for 4h at 37°C. The absorbance was measured at 570 nm against the reference wavelength of 650 nm. The percentage of viability was calculated based on the formula: Viability (%) = (absorbance of treated cells/absorbance of control cells) x 100%.

### 2.3. Molecular Modeling

All molecular modeling studies were done on an Intel core i3 2.53 GHz running on Windows 7. The protein crystal structure of Poly (ADP-ribose) polymerase-1 (PDB code: 1UK1), vascular endothelial growth factor receptor kinase 2

**Table 1.** $^{1}$H-NMR, $^{13}$C- NMR, COSY and HMBC of compound 1.

| Number | $^{13}$C | $^{1}$H | COSY | HMBC |
|---|---|---|---|---|
| 1 | 128.0 | | | |
| 2 and 6 | 131.0 | 7.55 (2H, d, J=8.2) | 6.88 | β', 4 |
| 3 and 5 | 116.4 | 6.88 (2H, d, J=8.2) | 7.55 | 1, 4 |
| 4 | 158.6 | | | |
| 1' | 114.6 | | | |
| 2' | 161.3 | | | |
| 3' | 109.2 | | | |
| 4' | 160.1 | | | |
| 5' | 108.3 | 6.32 (1H, d, J=8.8) | 7.70 | 1', 4' |
| 6' | 131.2 | 7.70 (1H, d, J=8.8) | 6.30 | 4', β |
| 2'' | 80.2 | | | |
| 3'' | 126.5 | 5.46 (1H, d, J=10.2) | 6.83 | 2'', 3' |
| 4'' | 117.6 | 6.83 (1H, d, J=10.2) | 5.46 | 2'', 4' |
| 5'' | 27.9 | 1.44 (3H, s) | | 6'', |
| 6'' | 35.8 | 2.05 and 1.95 | 2.60 | 2'', 3'' |
| 7'' | 38.9 | 2.60 | 2.05 | 6'', |
| 8'' | 209.1 | | | |
| 9'' | 30.6 | 2.14 (3H, s) | | 8'' |
| α | 118.1 | 7.41 (15.4) | 7.83 | 1, β |
| β | 192.4 | | | |
| β' | 144.7 | 7.83 (15.4) | 7.41 | β, 2' |
| OH | - | 13.8 | | 3', 1', |

(PDB code: 1VR2) and DNA topoisomerase II isozyme (PDB code: 1ZXM) were downloaded from the Protein Data Bank (PDB) [22].

Hydrogen atoms were added to the protein using the "add hydrogen" option in Autodock Tools and the ligand structures were built in the same software [23]. The docking procedure took place using the Autodock vina program. The tridimensional figures of the protein-ligand relationship, residue interactions and distances were obtained using PyMol [24].

## 3. RESULTS AND DISCUSSION

### 3.1. Structure Determination

*Azorella madreporica'*s petroleum ether extract was studied using the usual chromatographic techniques (open columns and high effectiveness chromatography) with Sephadex LH-20 and silica gel as a base, carrying out elution using blends of petroleum ether and ethyl acetate of increasing polarity. This allowed compound **1** to be isolated as an amorphous powder. The ultraviolet spectrum showed absorptions at $\lambda_{max}$ 257, 283 and 375 nm. The infrared spectrum showed absorptions of the hydroxyl group (3350 cm$^{-1}$) and carbonyl functions (1650 and 1723.9 cm$^{-1}$). Its molecular formula was found using MS and combined analysis of the $^{1}$H and $^{13}$C-NMR spectra as well as $C_{23}H_{22}O_5$ (molar mass: 378.42 Da). The $^{13}$C-NMR spectrum confirms the presence of 23 carbons, of which 2 are methyl, 8 methines (two signals which correspond to 2 carbons each), 2 methylenes and 9 quaternary carbons. Furthermore the existence of two carbonyl groups was detected at δ 209.1 and 192.4 ppm, which were assigned respectively to an aliphatic ketone and α,β unsaturated ketone, the olefinic carbons of which appeared at δ 144.7 and 118.1 ppm. In the $^{1}$H-NMR spectrum, the double bond was confirmed by signals at δ 7.83 (1H, J = 15.4 Hz) and 7.41 (1H, J = 15.4 Hz) ppm. The ion fragment at m/z 147 ($[M]^{+}$ -$C_9H_7O_2$), formed by rupture of the C=O-C-1 bond in the EIMS, confirmed the presence of this fragment. The large coupling constant (J= 15.4 Hz) between the $H_\alpha$ and $H_\beta$' signals suggests the E geometry of the double bond $C_\alpha$–$C_\beta$'. The molecule has an aromatic system with *para* substitution, as shown by the double signals from the $^{13}$C-NMR spectrum at δ 131.0 and at 116.4 ppm and by a pair of doublets for two hydrogens in the $^{1}$H-NMR spectrum at

δ 6.88 (2H, J = 8.2 Hz) and 7.55 (2H, J = 8.2 Hz) ppm. Another olefinic system showed signals in both types of spectrum at $\delta_C$ 117.6 and 126.5 ppm, $\delta_H$ 5.46 (1H, d, J = 10.2Hz) and 6.83 (1H, d, J = 10.2 Hz) ppm. Another aromatic AX system appeared at $\delta_C$ 108.3 (CH) and 131.2 (CH) ppm and $\delta_H$ 6.32 (1H, d, J = 8.8 Hz) and 7.70 (1H, d, J = 8.8 Hz) ppm. The two-methylene groups are also coupled, appearing at $\delta_C$ 38.9 ($CH_2$) and 35.8 ($CH_2$) and $\delta_H$ 2.60 (2H, m) and 2.05(1H, m), 1.95 (1H, m) ppm. The presence of a trans-*p*-cumaroyl [(2E)-3-(4- hydroxyphenyl)-2-propenoyl] fragment was established by observing correlations between H-2/H-6 and H-3/H-5 and Hα and Hβ', in the COSY $^1H$-$^1H$ spectrum, and also by the corresponding long-range $^{13}C$-$^1H$ interaction observed in the HMBC spectrum (Table **1**). Finally, the existence of a methyl ketone was detected by the signals at $\delta_C$ 30.6 ($CH_3$) and Z $\delta_H$ 2.14 (s, 3H) ppm, and the other methyl group at $\delta_C$ 27.9 ppm and $\delta_H$ 1.44 (s, 3H) ppm is bonded to a quaternary carbon ($\delta_C$ 80.2 ppm). The correlation with $J^2$ and $J^3$ found by the HMBC pulse program, summarized in Table **1**, leads us to conclude that compound **1** has the structure of Fig. (**1**). To our knowledge, it is the first time this has been reported from natural sources.

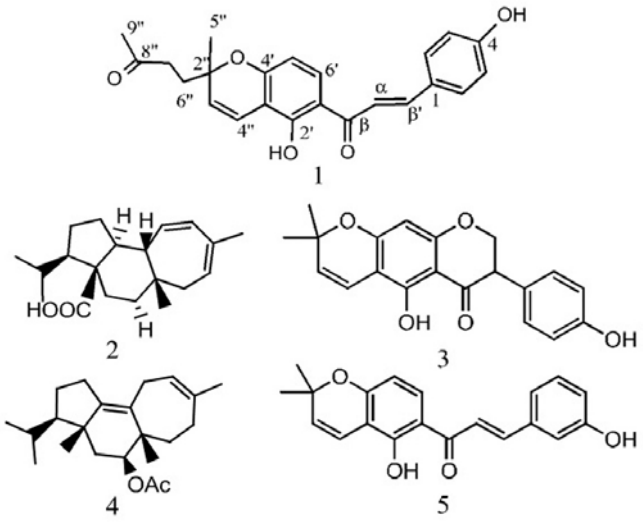


**Fig. (1).** Structure of compounds isolated from *A. madreporica*.

### 3.2. Biology

#### *3.2.1. Antibacterial Bioassay*

The MIC results are presented in Table **2** for all compounds against five different bacteria, with penicillin- streptomycin as positive control. The results showed that 7-acetoxymulin-9,12-diene **4** is active against *S. aureus* and *M. smegmatis* (ATCC 14468) and mulin-11,13-dien-20-oic acid **2** is active against *K. granulomatis, M. smegmatis* (ATCC 14468) and *S. aureus*, in decreasing order. Both mulinane compounds showed better activity against Gram- positive bacteria. The low susceptibility of Gram-negative bacteria to plant diterpenoids has been reported [25]. Gram- negative bacteria are generally more resistant to antimicrobial substances. This is related to the presence of an outer- leaflet lipopolysaccharide (LPS) which serves as a protective barrier against antimicrobial agents [26]. Both compounds exhibited similar activity against the fast-growing, non-pathogenic organism *M. smegmatis*. The antimycobacterial activity of mulinane compounds has been reported. Thus, compound **2** showed moderate activity against drug sensitive and drug-resistant strains of *M. tuberculosis* [14]. Interestingly, methylated derivatives of this compound showed higher anti-tuberculosis activity than the parent metabolite [27].

In the present work, the alpinum isoflavone did not show antibacterial activity. This is in contrast to other studies, which have reported that alpinum isoflavone **3** inhibits the growth of both Gram-negative and Gram-positive bacteria [28,29]. The compound **1** did not show antibacterial activity at any of the concentrations tested. This is in disagreement with previous reports, which show that natural chalcones and chalcone derivatives significantly inhibit the growth of Gram-positive [29-32] and Gram-negative organisms [33,34]. Perhaps an explanation to this disagreement is that in our case clinical isolates were used. Further structural modifications of compound **1** and investigations on structure-activity relationships are in progress in order to improve the *in vitro* antimicrobial activity.

#### *3.2.3. Free Radical Scavenging Assay (DPPH)*

The trapping activity of the DPPH radical of the compound **1** was determined at 100, 50 and 10 μg/mL, obtaining

**Table 2. Minimum inhibitory concentrations (μg/mL) of compounds isolated from *Azorella madreporica* against some selected bacteria.**

| | MIC | | | | |
|---|---|---|---|---|---|
| **Compound** | ***S. aureus*** | ***M. smegmatis*** | ***K. granulomatis*** | ***E. coli*** | ***M. morganii*** |
| 1 | - | - | - | - | - |
| 2 | 33.1 | 39.0 | 78.0 | - | - |
| 3 | - | - | - | - | - |
| 4 | 49.0 | 43.8 | - | - | - |
| 5 | - | - | - | - | - |
| Penicillin streptomycin | 15.6 | 0.49 | 15.6 | - | - |

**Table 3. Effect of compound 1 on the growth of human cancer cell lines.**

| | % of Inhibition ± SEM | | | |
|---|---|---|---|---|
| | Human Cancer Cell Lines | | | Cytotoxicity |
| Treatment | RKO | MCF-7 | PC-3 | D384 |
| 1 | 55.5 ± 0.3 | 42.3 ± 1.1 | 46.3 ± 1.7 | 44.5 ±3.7 |
| Doxorubicin | 56.4 ± 2.4 | 36.1 ± 3.1 | 47.8 ± 4,7 | 82.6 ± 1.2 |

*Each data is given as the mean and its standard error (SEM) of at least three independent experiments.

trapping percentages of 97.2 ± 1.8; 72.3 ± 2.5 and 44.4 ± 2.2 % respectively, with an estimated $IC_{50}$ value of 17.2 ± 1.5 μg/mL, comparable with quercetin, which has an estimated $IC_{50}$ value of 5.8 ± 0.4 μg/mL.

**Table 4. Affinity values (Kcal/mol) for the compounds using Autodock Vina.**

| | Enzyme | | |
|---|---|---|---|
| Compound | 1ZXM | 1UK1 | 1VR2 |
| 1 | -7.6 | -6.8 | -7.7 |
| Doxorubicin | -8.6 | -7.4 | -9.3 |

**Table 5. Calculated polar interactions for the compound 1-protein complexes expressed in Å.**

| Protein | Residue | Organic Function Involved | Distance |
|---|---|---|---|
| 1UK1 | Treonine 738 | Carbonyl 8´´ | 2.94 |
| | Glutamine 912 | Carbonyl 8´´ | 2.86 |
| | Phenylalanine 744 | Carbonyl β | 2.96 |
| 1ZXM | Arginine 323 | Carbonyl β | 2.90 |
| | Lysine 321 | Hydroxyl C-4 | 2.86 |

### *3.2.4. Antitumor Assay*

A viability assay was performed to measure cell proliferation of several human cancer lines at a standard concentration of compound **1** (50 μM). 3-(4,5-dimethylthiazol-2-yl)-5-(carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was conducted for this purpose. This is a simple, reliable method to assess cell viability or drug toxicity. The ability of live cells to cleave the tetrazolian ring and convert it to formazan crystals permits testing tumor cell sensitivity to anticancer agents [35].

Compound **1** was assessed for its ability to decrease the proliferation of colon, breast and prostate cancer cells. These biological models represents some of the most aggressive and prevalent manifestations of this disease, which currently account for about one in every seven deaths worldwide - more than HIV/AIDS, tuberculosis and malaria combined [36]. Under the conditions of our assays, the viability of RKO colon cells treated with compound **1** decreased, showing a comparable effect with the positive control, doxorubicin. A similar effect is observed in breast MCF-7 and prostate PC-3 cells which are valid models to assess new chemopreventive agents against cancer [37].

Astrocytoma D384 are representative cells involved in the brain defense system, serving as a potential first line of defense by regulating the volume and composition of extracellular space. In general, astrocytes, the most abundant glial cell types in the brain, are critically involved in maintaining and regulating the trafficking across the blood brain barrier [38]. The apoptosis of primary cultured astrocytes is a model for assessing the potential cytotoxic effects of several compounds [39] see Table **3**.

In the present study, the astrocyte cell line demonstrated a medium tolerance to compound **1**. Further studies oriented to reduce potential toxic effects are needed, since this compound is a good candidate to develop a chemopreventive agent to fight against different cancer types.

### 3.3. Molecular Docking Studies

The resultant antitumor activity of the tested compound led us to perform molecular docking studies in order to understand the ligand-protein interactions. The compound **1** and doxorubicin were studied using AutoDock vina. This program evaluates the affinity energy resulting for each conformation of compounds under study. The studied proteins form a complex ligand-protein through an electrostatic force, like a hydrogen bridge, between the compounds and a residue of protein. The minimal distance between the compounds and the residue of active site is 1 Å. Furthermore, the protein crystal structure of Poly (ADP-ribose) polymerase-1, vascular endothelial growth factor receptor kinase 2 and DNA topoisomerase II isozyme were used. In general, the new chalcone and doxorubicin has similar affinities in all systems used (Table **4**). Thus, with vascular endothelial growth factor receptor kinase 2, the compound **1** forms three links with residues of the active site: the OH of the *p*-phenol donates a proton to NH from the Cys 919 residue at a distance of 3.2 Å and two interactions with $NH_2$ of Lys 868, one is the α,β unsaturated ketone and the other is the OH in C-2'. The distances of these interactions were determined as 3.2 and 3.1 Å respectively. In the same system, doxorubicin has three interactions through the amino and hydroxyl groups with the Lys 868 residue, at 3.1 Å in each. Thus, both compounds showed interactions with the same residue of Lys 868. However, the difference between both compounds is the interaction of **1** with Cys 919 residue instead Lys 868

residue. These results are shown in Fig. (**2**). The interactions of both compounds and the active site of 1VR2 are found in Fig. (**3**). The results found with the other proteins are summarized in Table **5**. These facts may be correlated with the antitumoral activity based on the affinity values obtained from the different enzyme systems that were used.

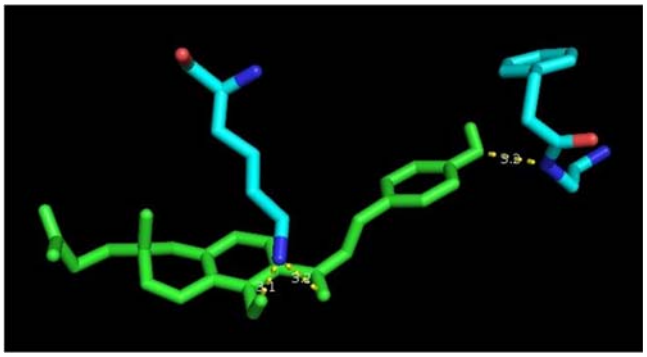

**Fig. (2).** Polar interactions of the compound **1** with Cys 919 and Lys 868 residues in vascular endothelial growth factor receptor kinase 2.

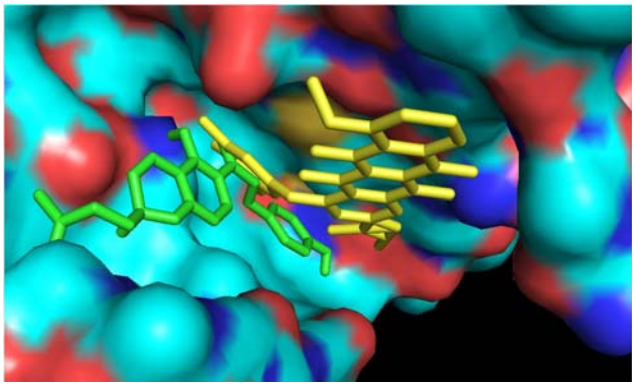

**Fig. (3).** Interactions of **1** and dexorubicin in binding site of 1VR2 vascular endothelial growth factor receptor kinase 2.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

## ACKNOWLEDGEMENTS

This research was supported by Fondecyt project Nº 1120199 and project PIEI-Quim-Bio, University of Talca. Universidad Técnica Particular de Loja and Universidad de Cuenca cooperate under the frame of the Bioactive Products Research Network, ascribed to the Ecuadorian Research and Posgrads Network, REDU.

## REFERENCES

[1] Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M.; Koizumi, M.; Ito, C.; Furukawa, H. Quantitative Study of Flavonoids in Leaves of *Citrus* Plants. *J. Agric. Food Chem.,* **2000**, *48*, 3865-3871.

[2] Crozier, A.; Burns, J.; Aziz, A.; Stewart, A.; Rabiasz, H. Antioxidant Flavonols from Fruits, Vegetables and Beverages: Measurements and Bioavailability. *Biol. Res.,* **2000**, *33*, 79-88.

[3] Falcone, M. L.; Rius, S. P.; Casati, P. Flavonoids: Biosynthesis, Biological Functions, and Biotechnological Applications. *Front. Plant Sci.,* **2012**, *3*, 222.

[4] Noyce, D. S.; Pryor, W. A. Carbonyl Reactions. I. Kinetics and Mechanism of the Acid-Catalyzed Aldol Condensation of Benzaldehyde and Acetophenone. *J. Am. Chem. Soc.,* **1955**, *77*, 1397-1401.

[5] Nowakowska, Z.; Kedzia, B.; Schroeder, G. Synthesis, Physicochemical Properties and Antimicrobial Evaluation of New (E)-Chalcones. *Eur. J. Med. Chem.,* **2008**, *43*, 707-713.

[6] Lahtchev, K. L.; Batovska, D. I.; Parushev, S. P.; Ubiyvovk, V. M.; Sibirny, A. A. Antifungal Activity of Chalcones: A Mechanistic Study Using Various Yeast Strains. *Eur. J. Med. Chem.,* **2008**, *43*, 2220-2228.

[7] Domínguez, J. N.; León, C.; Rodrigues, J.; Gamboa de Domínguez, N.; Gut, J.; Rosenthal, P. J. Synthesis and Evaluation of New Antimalarial Phenylurenyl Chalcone Derivatives. *J. Med. Chem.,* **2005**, *48*, 3654-3658.

[8] Nowakowska, Z. A Review of Anti-Infective and Anti-Inflammatory Chalcones. *Eur. J. Med. Chem.,* **2007**, *42*, 125-137.

[9] Lin, Y. Chalcones and Flavonoids as Anti-Tuberculosis Agents. *Bioorg. Med. Chem.,* **2002**, *10*, 2795-2802.

[10] Gacche, R. N.; Dhole, N. A.; Kamble, S. G.; Bandgar, B. P. *In-Vitro* Evaluation of Selected Chalcones for Antioxidant Activity. *J. Enzyme Inhib. Med. Chem.,* **2008**, *23*, 28-31.

[11] Ducki, S. The Development of Chalcones as Promising Anticancer Agents. *IDrugs,* **2007**, *10*, 42-46.

[12] Boeck, P.; Bandeira Falcão, C. A.; Leal, P. C.; Yunes, R. A.; Filho, V. C.; Torres-Santos, E. C.; Rossi- Bergmann, B. Synthesis of Chalcone Analogues with Increased Antileishmanial Activity. *Bioorg. Med. Chem.,* **2006**, *14*, 1538-1545.

[13] Loyola, L. A.; Bórquez, J.; Morales, G.; San-Martín, A.; Manríquez, V.; Boys, D.; Darias, J. Yaretol, a Norditerpenoid from *Azorella madreporica. J. Nat. Prod.,* **2002**, *65*, 1678-1680.

[14] Wächter, G. A.; Franzblau, S. G.; Montenegro, G.; Suarez, E.; Fortunato, R. H.; Saavedra, E.; Timmermann, B. N. A New Antitubercular Mulinane Diterpenoid from *Azorella madreporica* Clos. *J. Nat. Prod.,* **1998**, *61*, 965-968.

[15] Molina-Salinas, G. M.; Bórquez, J.; Ardiles, A.; Said-Fernández, S.; Loyola, L. A.; Yam-Puc, A.; Becerril- Montes, P.; Escalante-Erosa, F.; San-Martin, A.; González-Collado, I.; Peña-Rodríguez, L. M. Bioactive Metabolites from the Andean Flora. Antituberculosis Activity of Natural and Semisynthetic Azorellane and Mulinane Diterpenoids. *Phytochem. Rev.,* **2010**, *9*, 271-278.

[16] Loyola, L. A.; Brquez, J.; Morales, G.; San-Martin, A. Diterpenoids from *Azorella compacta.* Phytochemistry, **1997**, 44, 649-651.

[17] Bórquez, J.; Ardiles, A.; Loyola, L. A.; Peña-Rodriguez, L. M.; Molina-Salinas, G. M.; Vallejos, J.; Collado, I.G.; Simirgiotis, M. J. Further Mulinane and Azorellane Diterpenoids Isolated from *Mulinum crassifolium* and Azorella compacta. Molecules, **2014**, 19, 3898-3908.

[18] Bórquez, J.; Kennelly, E. J.; Simirgiotis, M. J. Activity Guided Isolation of Isoflavones and Hyphenated HPLC-PDA-ESI-ToF-MS Metabolome Profiling of *Azorella madreporica* Clos. from Northern Chile. *Food Res. Int.,* **2013**, *52*, 288-297.

[19] Institute, C. and L. S. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard — Eighth Edition, **2009**, 29, 1-65.

[20] Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT - Food Sci. Technol., **1995**, 28, 25-30.

[21] Molyneux, P. The Use of the Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin J. Sci. Technol., **2004**, 50.

[22] Bernstein, F. C.; Koetzle, T. F.; Williams, G. J.; Meyer, E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. The Protein Data Bank. A Computer-Based Archival File for Macromolecular Structures. *Eur. J. Biochem.,* **1977**, *80*, 319-324.

[23] Morris, G. M.; Ruth, H.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. Software News and Updates AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.,* **2009**, *30*, 2785-2791.

[24] Seeliger, D.; de Groot, B. L. Ligand Docking and Binding Site Analysis with PyMOL and Autodock/Vina. J. Comput. Aided. Mol. Des., **2010**, 24, 417-422.

[25] Urzúa, A.; Rezende, M. C.; Mascayano, C.; Vásquez, L. A. Structure-Activity Study of Antibacterial Diterpenoids. *Molecules,* **2008**, *13*, 882-891.

[26] Epand, R. M.; Epand, R. F. Lipid Domains in Bacterial Membranes and the Action of Antimicrobial Agents. Biochim. Biophys. Acta, **2009**, 1788, 289-294.

[27] Molina-Salinas, G. M.; Bórquez, J.; Said-Fernández, S.; Loyola, L. A.; Yam-Puc, A.; Becerril-Montes, P.; Escalante-Erosa, F.; Peña-Rodríguez, L. M. Antituberculosis Activity of Alkylated Mulinane Diterpenoids. *Fitoterapia,* **2010**, *81*, 219-222.

[28] Chukwujekwu, J. C.; Van Heerden, F. R.; Van Staden, J. Antibacterial Activity of Flavonoids from the Stem Bark of Erythrina caffra Thunb. Phytother. Res., **2011**, 25, 46-48.

[29] Quesada, L.; Areche, C.; Astudillo, L.; Gutierrez, M.; Sepúlveda, B.; San-Martín, A. Biological Activity of Isoflavonoids from Azorella madreporica. Nat. Prod. Commun., **2012**, 7, 1187-1188.

[30] Liaras, K.; Geronikaki, A.; Glamočlija, J.; Cirić, A.; Soković, M. Thiazole-Based Chalcones as Potent Antimicrobial Agents. Synthesis and Biological Evaluation. *Bioorg. Med. Chem.,* **2011**, *19*, 3135-3140.

[31] Oldoni, T. L. C.; Cabral, I. S. R.; D'Arce, M. A. B. R.; Rosalen, P. L.; Ikegaki, M.; Nascimento, A. M.; Alencar, S. M. Isolation and Analysis of Bioactive Isoflavonoids and Chalcone from a New Type of Brazilian Propolis. *Sep. Purif. Technol.,* **2011**, *77*, 208-213.

[32] Yin, B.-T.; Yan, C.-Y.; Peng, X.-M.; Zhang, S.-L.; Rasheed, S.; Geng, R.-X.; Zhou, C.-H. Synthesis and Biological Evaluation of A-Triazolyl Chalcones as a New Type of Potential Antimicrobial Agents and Their Interaction with Calf Thymus DNA and Human Serum Albumin. *Eur. J. Med. Chem.,* **2014**, *71*, 148-159.

[33] Konduru, N. K.; Dey, S.; Sajid, M.; Owais, M.; Ahmed, N. Synthesis and Antibacterial and Antifungal Evaluation of Some Chalcone Based Sulfones and Bisulfones. *Eur. J. Med. Chem.,* **2013**, *59*, 23-30.

[34] Siddiqui, Z. N.; Musthafa, T. N. M.; Ahmad, A.; Khan, A. U. Thermal Solvent-Free Synthesis of Novel Pyrazolyl Chalcones and Pyrazolines as Potential Antimicrobial Agents. *Bioorg. Med. Chem. Lett.,* **2011**, *21*, 2860-2865.

[35] Al-anee, R. S. A.; Sulaiman, G. M.; Al-sammarrae, K. W.; Bagnati, R.; Lania, L.; Passoni, A.; Majello, B. *In Vitro* Studies of the Cytotoxicity and Cell Cycle Arrest of *Hymenocrater longiflorus* Plant Extract on Colon Cancer ( RKO ) Cell Line. *Curr. Res. Microbiol. Biotechnol.,* **2014**, *2*, 367-372.

[36] Society, A. cancer. Cancer Facts & Figures. http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2015/index.

[37] Kumar, P. S.; Febriyanti, R. M.; Sofyan, F. F.; Luftimas, D. E.; Abdulah, R. Anticancer Potential of *Syzygium aromaticum* L. in MCF-7 Human Breast Cancer Cell Lines. *Pharmacognosy Res.,* **2014**, *6*, 350-354.

[38] Takuma, K.; Baba, A.; Matsuda, T. Astrocyte Apoptosis: Implications for Neuroprotection. *Prog. Neurobiol.,* **2004**, *72*, 111-127.

**Received: January 01, 2015** **Revised: February 25, 2015** **Accepted: April 03, 2015**