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Synthesis of a Chalcone-Steroid Derivative. Evaluation of their Biological Activity on both Perfusion Pressure and Left Ventricular Pressure Using an Isolated Rat Heart Model

Rosas-Nexticapa Marcela [1], Figueroa-Valverde Lauro [2,*], Díaz-Cedillo Francisco [3], Alvarez-Ramirez Magdalena [1], Cervantes-Ortega Catalina [1], López-Ramos Maria [1,*], Mateu-Armand Virginia [1], Hau-Heredia Lenin [2]

[1] Facultad de Nutrición, Universidad Veracruzana, Médicos y Odontologos s/n C.P. 91010, Unidad del Bosque Xalapa Veracruz, México
[2] Laboratory of Pharmaco-Chemistry, Faculty of Chemical and Biological Sciences, University Autonomous of Campeche, Av. Agustín Melgar s/n, Col Buenavista C.P. 24039 Campeche, Camp., México
[3] Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional. Prol. Carpio y Plan de Ayala s/n Col. Santo Tomas, México, D.F. C.P. 11340
* Correspondence: lfiguero@uacam.mx (F.-V.L.); maclopez@uacam.mx (L.-R.M.);

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Abstract: Some reports indicate that chalcone derivatives have activity on the cardiovascular system; nevertheless, there is scarce information about the effects produced by chalcone derivatives against both perfusion pressure and left ventricular pressure. This research used an isolated rat heart model to synthesize a new chalcone-steroid derivative to evaluate its biological activity on perfusion or left ventricular pressure. Besides, the molecular mechanism involved in the effect induced by chalcone derivative on left ventricular pressure was determined using some drugs such as metoprolol, prazosin, indomethacin, and nifedipine as pharmacological tools. The results showed that chalcone derivative significantly (p = 0.05) increased the perfusion pressure compared to chalcone, pregnenolone, and the control conditions. Other data indicate that chalcone derivatives increase left ventricular pressure in a dose-dependent manner, and this effect is inhibited in the presence of nifedipine. This phenomenon suggests that the molecular mechanism involved in the biological activity produced by chalcone-steroid derivative on left ventricular pressure is through *Type-L* calcium channel activation. These data suggest that this chalcone derivative could be an excellent inotropic agent for treating heart failure.

Keywords: synthesis; chalcone derivative; perfusion pressure.

1. Introduction

For several years, some chalcone derivatives have been used to treat different types of diseases, such as viral, infectious, antimalarial, pain, diabetes, cancer, cardiovascular, and lung injury [1-13]. For example, a study showed that a chalcone derivative (liquorice) had been involved in treating gastric ulcers and bronchial asthma [14]. In addition, a study showed that bavachalcone (a prenylated chalcone) reduces the growth of cancer cells by modulating the

biological activity of retinoic acid-related orphan-α receptors [15]. Other data indicate that butein (a chalcone derivative) is beneficial in treating pain, gastritis, and parasitic infections [16]. In addition, a report shows that butein can inhibit heart injury caused by oxidative stress using a chronic heart failure model [17].

On the other hand, a study showed that 2-(2-dimethylaminoethoxy) chalcone citrate reduces blood pressure in an unanesthetized hypertensive rat model [18]. Furthermore, other data indicate that chalcone R-2803 (2-(2-dimethylaminoethoxy)-3', 4', 5'-trimethoxychalcone) inhibits increased norepinephrine-induced aortic muscle contractions in dogs [19]. Different studies show that administration of a chalcone derivative (4-hydroxydericine) can decrease systolic blood pressure, serum very low-density lipoprotein levels, and hepatic triglyceride concentration using a stroke-prone spontaneously hypertensive rat model [20]. Other data indicate that xanthoangelol (a chalcone analog) has beneficial effects on lipid metabolism in stroke-prone spontaneously hypertensive rats [21]. Another report indicates that chalcone L6H9 ((e)-2,6-Difluoro-4'-methoxychalcone) can protect cardiomyocytes from hyperglycemia-induced injury through both ROS (reactive oxygen species) and NF-κB (nuclear factor-kappa B) inhibition [22]. Other data showed that a resveratrol-chalcone derivative protects mice against diabetic cardiomyopathy by alleviating inflammation and oxidative stress via B NF-κB inhibition and Nrf2 activation [23]. All these data show that chalcone derivatives can affect the cardiovascular system; however, the biological activity of both perfusion and left ventricular pressure is unclear. Analyzing these data, this investigation aimed to evaluate the biological activity produced by chalcone-steroid derivative on either perfusion or left ventricular pressure using an isolated rat heart model. To characterize the molecular mechanism involved in the biological activity of chalcone derivatives on left ventricular pressure, some drugs such as metoprolol, prazosin, indomethacin, and nifedipine were used as pharmacological tools. Furthermore, a theoretical coupling model determined the interaction of chalcone derivatives with some molecules involved in left ventricular pressure changes.

2. Materials and Methods

2.1. General methods of chemical synthesis.

All reagents used in this investigation were acquired from Sigma-Aldrich suppliers. Using tetramethylsilane as an internal standard, the NMR spectrum was determined with a Varian VXR300/5 FT (300 MHz/$CDCl_3$) apparatus. The infrared (IR) spectrum was determined on an iSOFT/IR Thermo Scientific device. The melting point (m.p.) was determined in a mode electrothermal-900 apparatus. Elemental analysis was determined using a Perkin-Elmer apparatus (Ser. II CHNS/02400).

2.2. Chemical synthesis.

2.2.1. 2-Amino-3-methyl-pentanoic acid 17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13, 14,15,16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester (**2**).

In a round-bottom flask (10 mL), pregnenolone (100 mg, 0.32 mmol), isoleucine (80 mg, 0.61 mmol), *N*, *N*′-Dicyclohexylcarbodiimide (300 mg, 1.45 mmol), and 6 mL of a methanol/ethanol system (2:1) were stirred for 72 h at room temperature. Then, the solvent

was evaporated on a rotary evaporator, and the product was separated using the chloroform: water (4:1) system.

2.2.2. 2-(1,3-Diphenyl-allylideneamino)-3-methyl-pentanoic acid 17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10, 11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester (**3**).

In a round-bottom flask (10 mL), compound **2** (100 mg, 0.23 mmol), chalcone (50 mg, 0.24 mmol), boric acid (30 mg, 0.48 mmol), and 5 mL of methanol were stirred for 72 h at room temperature. Then, the solvent was evaporated on a rotary evaporator, and the product was separated using the chloroform: water (2:1) system.

2.3. Biological activity.

2.3.1. General methodology.

All experimental procedures and protocols used in this investigation were in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996) [24].

2.3.2. Animals.

Wistar, weighing 200-250 g (n = 64), was obtained from the Laboratory of Pharmacochemistry Research of the University Autonomous of Campeche.

2.3.3. Reagents.

The chalcone derivative and other compounds were dissolved in methanol, and dilutions were made from this solution by adding *Krebs-Henseleit solution (v/v).

*Krebs-Henseleit solution was prepared using a previous technique reported [25].

2.3.4. Anesthesia.

Pentobarbital (50 mg/kg) was administered intraperitoneally to induce anesthesia in rats. Then, the chest was opened, and a loose ligature was passed through the ascending aorta. Following, the heart was removed, and a cannula was inserted. In addition, the cannula was connected to an acrylic chamber, which in turn was bound to a Graham condenser through which the heart was retrogradely perfused with Krebs-Henseleit solution* at a constant flow rate (10 mL/min).

2.3.5. Perfusion pressure evaluation.

The recording of the biological activity produced by pregnenolone, chalcone, and chalcone-steroid derivative on the perfusion pressure was determined using a pressure transducer connected to the chamber where the hearts were mounted. Data were entered into a computerized data capture system (Biopac).

2.3.6. Experimental design 1.

The changes in the perfusion pressure as a consequence of the increase in time (3-18 min) in the absence or in the presence of the pregnenolone, chalcone, and chalcone-steroid derivative were evaluated using the following experimental design (Table 1):

Table 1. Experimental design for evaluating biological activity produced by chalcone, pregnenolone, and chalcone-steroid derivative on the perfusion pressure.

Group	Compound	Dose
I	Control	Krebs-Henseleit solution only
II	Chalcone	0.001 nM
III	Pregnenolone	0.001 nM
IV	Chalcone derivative	0.001 nM

It is noteworthy that the effects were determined in isolated hearts perfused (n = 6) for each group at a constant flow rate of 10 mL/min. In addition, doses administered were based on previously reported methods for other types of compounds, which exert changes in the perfusion pressure [26].

2.3.6. Inotropic activity.

The contractile activity was evaluated by measuring left ventricular developed pressure (LVP) using a saline-filled latex balloon (0.01 mm diameter) inserted into the left ventricle via the left atrium. Notably, the latex balloon was connected to the cannula, which was bound to a pressure transducer connected to an MP100 data acquisition system.

2.3.7. Experimental design 2.

2.3.7.1. Effects produced by chalcone derivative (CPI) on left ventricular pressure through adrenergic receptors.

Intracoronary boluses (50 µL) of chalcone derivative [0.001 to 100 nM] were administered, and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of either prazosin or metoprolol at a concentration of 1 nM (duration of preincubation with either prazosin or metoprolol was a 10 min equilibration period).

2.3.7.2. Biological activity exerted by chalcone-steroid derivative (CPI) on left ventricular pressure through the prostaglandins system.

Intracoronary boluses (50 µL) of chalcone-steroid derivative [0.001 to 100 nM] were administered, and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of indomethacin at a concentration of 1 nM (duration of preincubation with indomethacin was a 10-minute equilibration period).

2.3.7.3. Effects induced by chalcone-steroid derivative (CPI) on left ventricular pressure through calcium channel activation.

Intracoronary boluses (50 µL) of chalcone-steroid derivative [0.001 to 100 nM] were administered, and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of nifedipine at a concentration of 1 nM (duration of preincubation with nifedipine was a 10-minute equilibration period).

2.3.8. Docking.

The interaction of chalcone derivatives with the calcium channel surface was determined using 6jp5 protein (https://doi.org/10.2210/pdb6JP5/pdb) as a theoretical model. In addition, the binding energy involved in the interaction of the chalcone-steroid derivative with

the 6jp5 protein surface (https://doi.org/10.2210/pdb6JP5/pdb) was evaluated using DockingServer software [27].

3. Results and Discussion

In the literature, several studies indicate that some chalcone derivatives can produce changes in the cardiovascular system [18-23]; however, the interaction of chalcone derivatives with some biomolecules is not very clear. Analyzing these data, this study aimed to synthesize a chalcone derivative to evaluate the biological activity exerted on both perfusion pressure and left ventricular pressure as follows:

3.1. Preparation of steroid-amino acid derivative.

Figure 1 shows the synthesis of compound **2** from pregnenolone and isoleucine in the presence of *N*, *N*′-dicyclohexylcarbodiimide; the results showed a 65% yield for **2**. Other data for the infrared spectrum (Vmax, cm^{-1}) displayed some bands at 3380 for the amino group, 1765 for the ester group, and 1712 for the ketone group. The ^{1}H NMR spectrum from compound **2** showed some signals at 7.64 ppm for deuterated chloroform ($CDCl_3$), at 5.28 ppm for alkene (Ring-B, steroid nucleus), at 4.78 ppm for methylene group bound to both ketone and methyl groups; at 3.01 ppm for amino group; at 2.00 ppm for methyl group linked to ketone group; at 1.65 ppm for methyl group bound to Ring-A of steroid nucleus; at 1.22 and 0.70 ppm for methyl groups linked to arm spacer; at 1.65 ppm for methyl group bound to Ring-B of steroid nucleus. The ^{13}C NMR spectra display chemical shifts at 209.26 ppm for the ketone group, 170.09 ppm for the ester group, 130.68-128.50 ppm for the alkene group, and 77.91 ppm for deuterated chloroform ($CDCl_3$), at 40.09 ppm for methyl group (Ring A, steroid nucleus); at 33.92 and 24.94 ppm for methyl groups bound to arm spacer; at 13.47 ppm for methyl group linked to Ring-C of steroid nucleus. In addition, mz/ion spectra showed 429.26.

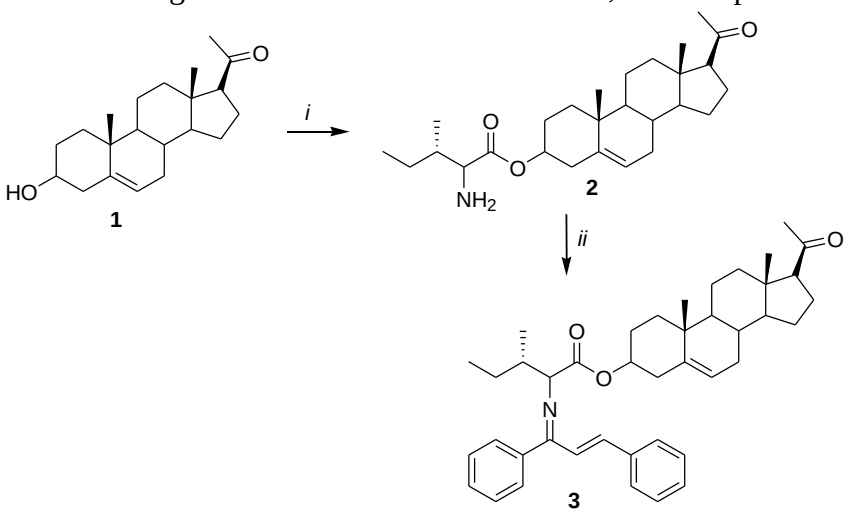

Figure 1. Synthesis of Chalcone derivative. *Conditions and catalysts*: *i* = *N*, *N*′-dicyclohexylcarbodiimide, MeOH/EtOH, room temperature. *ii* = boric acid, MeOH, room temperature.

3.2. Synthesis of chalcone-steroid derivative.

Compound 3 (Figure 1) was prepared from **2** and chalcone in the presence of boric acid; the results showed a 58% yield for **3**. Other data for the infrared spectrum (V_{max}, cm^{-1}) displayed some bands at 3322 for the imino group, 1762 for the ester group, and 1715 for the ketone group. The ^{1}H NMR spectrum displayed several signals at 7.22 ppm for deuterated

chloroform ($CDCl_3$), at 7.17 and 6.55 ppm for the alkene group, at 5.22 ppm for methylene group bound to ester group, at 2.01 ppm for methyl group linked to ketone group; at 0.90 ppm for methyl group linked to arm spacer; at 1.15 and 0.52 ppm for methyl groups bound to steroid nucleus. Besides, The ^{13}C NMR spectra showed several bands for **3** at 208.75 for the ketone group, at 157.50 for the imino group, at 141.24 and 133.30 ppm for the alkene group, at 33.66 ppm for the methyl group bound to ketone group; at 24.05 and 25.30 ppm for methyl groups linked to steroid nucleus; at 18.95 and 13.38 ppm for methyl group linked to arm spacer. In addition, mz/ion spectra showed 619.30

3.3. Biological activity.

Several reports have shown that some chalcone derivatives can exert biological activity on the cardiovascular system as vasorelaxants [28], antihypertensives [29], and induce beneficial effects on myocardial infarction [30]; however, the molecular mechanism is not very clear. Analyzing these data, this study evaluated the biological activity of a chalcone derivative on perfusion pressure using an isolated rat heart model. The results indicate that chalcone derivative significantly increases the perfusion pressure ($p = 0.05$) compared to pregnenolone, chalcone, and the control conditions (Figure 2); this phenomenon could be due to differences in the chemical structure of each compound.

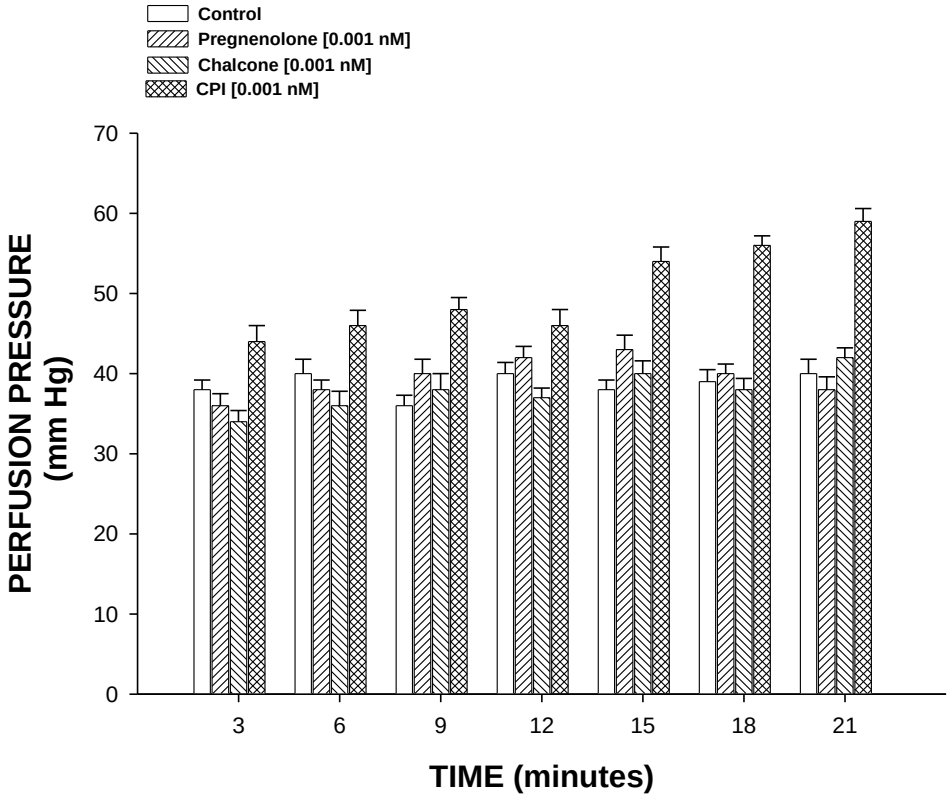

Figure 2. The effect produced by chalcone-steroid derivative (CPI) on perfusion pressure. The results showed that CPI significantly increased the perfusion pressure ($p = 0.05$) through time (3 to 21 min) compared with pregnenolone, chalcone, and the control conditions (only Krebs-Hensseleit solution). The biological activity is expressed as the area under the curve, and each bar represents the mean ± S.E. of 6 experiments.

Analyzing these data and other reports suggests that chalcone R-2803 (2-(2-dimethylaminoethoxy)-3',4',5'-trimethoxychalcone) may produce changes in the adrenergic system by decreasing aortic muscle contractions in dogs [18]. For this reason, in this study, the biological activity of chalcone derivatives on left ventricular pressure was evaluated in the

absence or presence of either metoprolol (β1-adrenergic receptor inhibitor) [31,32] or prazosin (α1-adrenergic receptor blocker) [32,34].

The results (Figure 3) showed that chalcone derivative significantly ($p = 0.05$) increases left ventricular pressure in a dose-dependent manner (0.001 to 100 nM), and this effect was not inhibited in the presence of either metoprolol or prazosin (1 nM). These data indicate that the molecular mechanism involved in the biological activity exerted by chalcone derivative on left ventricular pressure was not through adrenergic system activation.

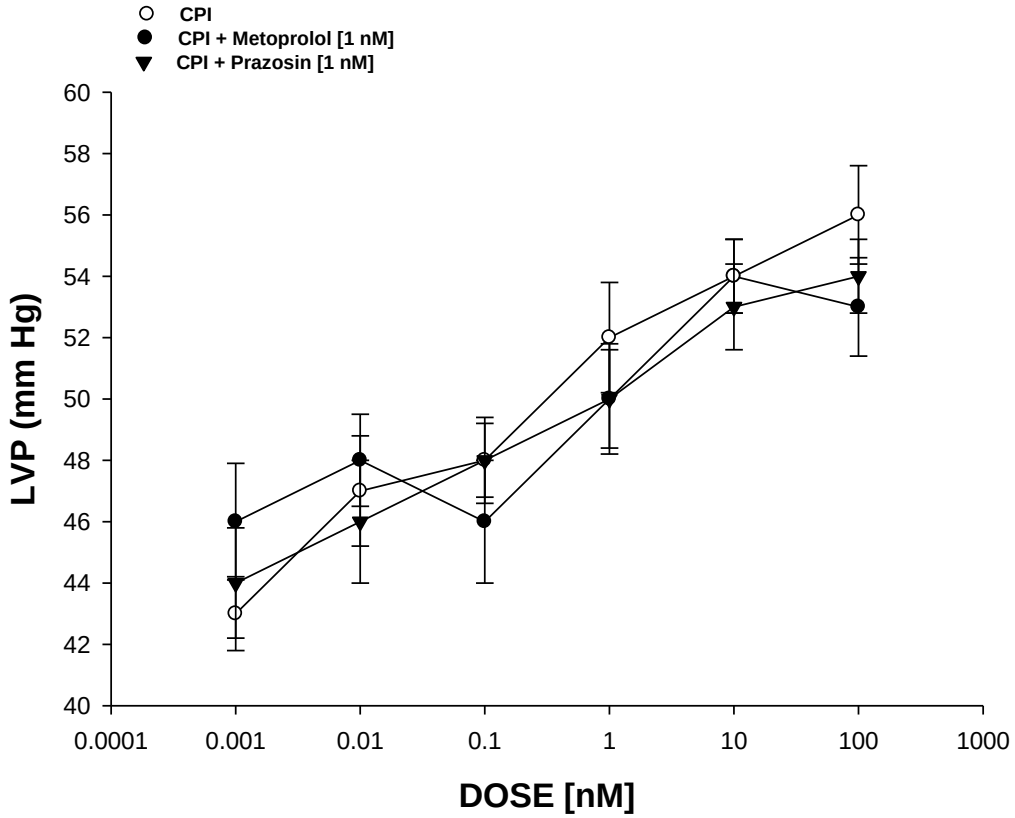

Figure 3. Chalcone-steroid derivative (CPI) exerts biological activity on left ventricular pressure through the adrenergic system. Intracoronary boluses (50 μL) of CPI (0.001 to 100 nM) were administered, and the corresponding effect on the left ventricular pressure was evaluated in the absence and presence of either metoprolol or prazosin at a dose of 1 nM. The results showed that activity induced by CPI on left ventricular pressure was not inhibited in the presence of either metoprolol or prazosin. The effects are expressed as the area under the curve, and each bar represents the mean ± S.E. of 6 experiments.

Analyzing these data and other data indicates that some chalcone can produce activity biological on prostaglandins system; for example, a study displayed that a chalcone derivative (*N'*-(2-{4-[(2*E*)-3-(4-Methoxyphenyl)prop-2-enoyl]phenoxy}acetoxy)-4-methoxybenzene-carboximidamide) exert changes in the prostaglandins system through cyclooxygenase enzyme inhibition [35]. Besides, a report indicates that chalcone SU-88 (2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone) induces effects on the prostaglandin system through 15-hydroxy-PG-dehydrogenase inhibition [36]. For this reason, in this research, the biological activity produced by chalcone derivative on left ventricular pressure in the absence or presence of indomethacin (cyclooxygenase enzyme inhibitor) [37,38] was evaluated. The results showed that the effect exerted by chalcone derivative on left ventricular pressure was not inhibited by indomethacin; these data showed that the molecular mechanism involved in the effect exerted by chalcone derivative on left ventricular pressure was not via the prostaglandins system activation. In the search for the molecular mechanism involved in the effect exerted by chalcone derivatives on left ventricular pressure, some reports were also analyzed, indicating

that some chalcone derivatives may influence calcium channels; this phenomenon could be translated as changes in blood pressure [39]. For example, one study showed that a chalcone derivative (1-(2,5-Dihydroxy-phenyl)-3-thiophen-2-yl-propenone) decreases the contractile response through calcium channels inhibition using a rat thoracic aorta model [40]. Analyzing these data, this investigation evaluated the biological activity exerted by chalcone derivative on left ventricular pressure in the absence or presence of nifedipine (Type-L calcium channels blocker) [41,42]. The results (Figure 4) showed that the effect produced by chalcone derivative on left ventricular pressure was inhibited in the presence of nifedipine at a dose of 1 nM, indicating that the biological activity exerted by chalcone derivative on left ventricular pressure was through *Type-L* calcium channels inhibition.

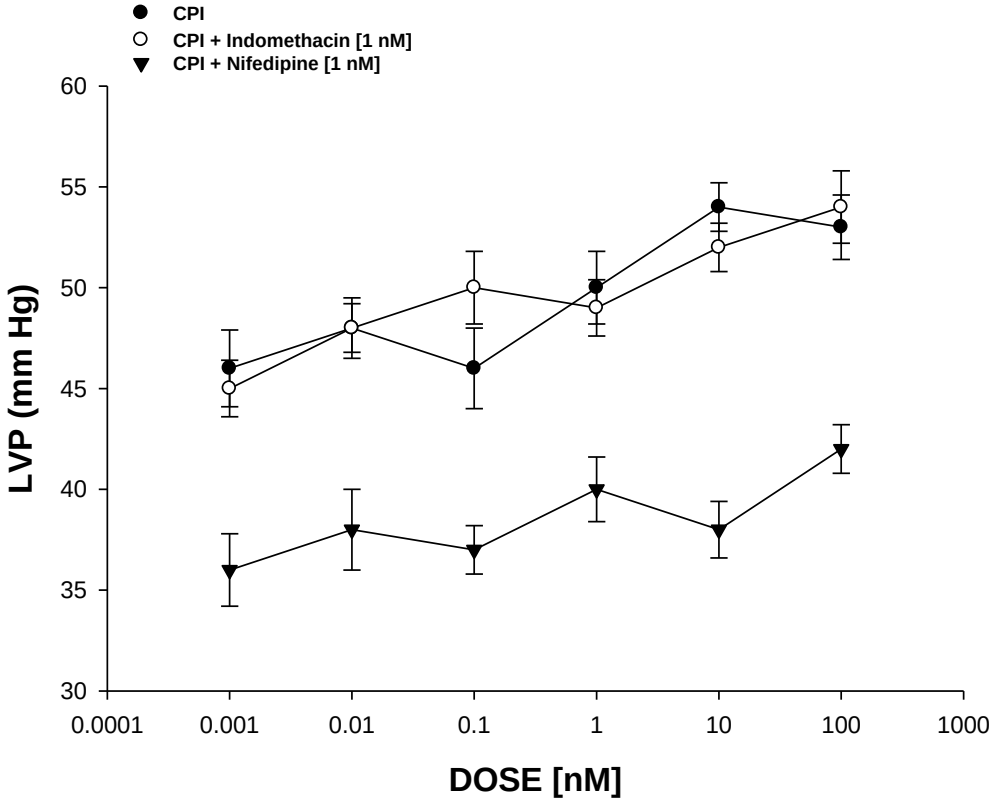

Figure 4. The effect produced by chalcone-steroid derivative (CPI) on left ventricular pressure is through either prostaglandin release or calcium channel activation. Intracoronary boluses (50 μL) of CPI (0.001 to 100 nM) were administered, and the corresponding effect on the left ventricular pressure was determined in the absence and presence of either indomethacin or nifedipine at a dose of 1 nM. The results showed that activity induced by CPI on perfusion pressure was not inhibited in the presence of indomethacin. However, this effect was blocked by nifedipine. The effect is expressed as the area under the curve, and each bar represents the mean ± S.E. of 6 experiments.

However, to characterize the interaction of chalcone derivatives with the calcium channel, some studies were carried out using the 6jp5 protein, nifedipine, as a theoretical tool in a Docking model.

3.4. Docking analysis.

For several years, some methods have been used, such as SwissDock [43], PharmDock [44], Autodock [45], and the DockingServer program [46]. This research determined the theoretical interaction of chalcone derivatives with 6jp5 protein surface using nifedipine, amlodipine, darodipine, ML-218, and Bay K-8644 as theoretical tools in DockingServer software. The results (Table 2) showed that the interaction of the chalcone derivative with the

surface of the 6jp5 protein involves different types of amino acid residues compared with other compounds. However, the amino acid residue $Ile1_{244}$ involved in the formation of the chalcone-protein complex could be responsible for the biological activity of the chalcone derivative on the calcium channel.

Table 2. Amino acid residues involved in theoretical interaction of nifedipine, amlodipine, darodipine, ML-218, Bay K-8644 and chalcone derivative (CIP) with 6jp5 protein surface.

Compound	Aminoacid residues
Nifedipine	Pro1181; Trp1182; Leu1247; Arg1254; Leu1257; Trp1258; Ile1261
Amlodipine	Pro1181; Trp1182; Arg1254; Trp1258; Ile1261
Darodipine	Pro1181; Trp1182; Leu1247; Arg1254; Leu1257; Trp1258; Ile1261
ML-218	Pro1181; Trp1182; Val1184; Phe1185; Arg1254; Trp1258; Ile1261
Bay K-8644	Pro1181; Trp1182; Leu1247; Arg1254; Leu1257; Trp1258; Ile1261
CIP	Pro1181; Trp1182; Phe1185; Ile1244; Leu1247; Arg1254; Leu1257; Trp1258; Ile1261

However, some reports suggest that the interaction of some drugs with different proteins or enzymes could depend on some thermodynamic parameters, such as free binding energies, solvation energies, and the inhibition constant (Ki) [47]. Analyzing these data in this investigation determined some thermodynamic factors involved in the interaction of chalcone derivative with the 6jp5 protein surface. The results (Table 3) showed differences in energy values for chalcone derivative compared with nifedipine (*L-type* calcium channel blocker) [41,42], amlodipine (*L-type* calcium channel inhibitor) [48], darodipine (calcium channel blocker) [49], ML-218 (*T-type* calcium channel agonist [50, 51] and Bay- K8644 (*L-type* calcium channel agonist) [52]. Another result showed that Ki was lower for chalcone derivatives than nifedipine, amlodipine, darodipine, ML-218, and Bay- K8644; this data indicates that chalcone derivatives could act as calcium channel inhibitors.

Table 3. Thermodynamic parameters involved in the interaction of nifedipine, amlodipine, darodipine, ML-218, Bay K-8644, and chalcone derivative (CIP) with *6jp5* protein surface using DockingServer software.

Compound	A	B	C	D	E	F
Nifedipine	-4.93	245	-6.35	-0.10	-6.45	617.62
Amlodipine	-4.44	554.93	-5.19	0.28	-4.91	613.98
Darodipine	-4.18	866.09	-6.82	-0.02	-5.84	597.83
ML218	-6.50	93.62	-7.06	0.15	-6.90	664.25
Bay K8644	-5.07	193.07	-6.43	-0.03	-6.46	572.70
CPI	-7.99	1.39	-9.49	-0.06	-9.55	868.36

A = Est: Free Energy of Binding (kcal/mol); **B** = Inhibition Constant, Ki (mM); **C** = vdW + Hbond + desolv Energy (kcal/mol); **D** = Electrostatic Energy (kcal/mol); **E** = Total Intermolec. Energy (kcal/mol); **F** = Interact. Surface.

4. Conclusions

The results showed that the chalcone derivative increases both the perfusion and left ventricular pressure, which is associated with *type-L* calcium channel activation. Besides, the theoretical analysis suggests that the interaction of the chalcone derivative with the calcium channel could involve a series of amino acid residues for their biological activity on both the perfusion and left ventricular pressure. All these data suggest that this chalcone derivative could be an excellent inotropic agent to treat heart failure.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The animal study protocols were in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

We declare that this manuscript has no conflict of financial interests (political, personal, religious, ideological, academic, intellectual, commercial, or otherwise) for publication.

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