Volume 10, Issue 2, 2020, 5145 - 5155 ISSN 2069-5837
Biointerface Research in Applied Chemistry
www.BiointerfaceResearch.com
https://doi.org/10.33263/BRIAC102.145155
Original Research Article Open Access Journal
Received: 07.12.2019 / Revised: 20.01.2020 / Accepted: 25.01.2020 / Published on-line: 28.01.2020

New chloroamide-steroid derivative with biological activity on a casein kinase 2 (CK2)

Figueroa-Valverde Lauro [2,*], Camacho-Luis Abelardo [6], Garcimarrero E. Alejandara [1], Rosas-Nexticapa Marcela [3,*], Mateu-Armad Maria Virginia [3], Garcia-Martinez Rolando [1], Díaz-Cedillo Francisco[4], López-Ramos Maria[5], Cauich-Carrillo Regina[1], Marin-Parra A [3], Estrella-Barron Raquel [5]

[1]Laboratory of Pharmaco-Chemistry, Faculty of Chemical Biological Sciences, University Autonomous of Campeche, Av. Agustín Melgar s/n, Col Buenavista C.P. 24039 Campeche, Camp., México
[2]Facultad de Medicina, Universidad Veracruzana, Médicos y Odontologos s/n C.P. 91010, Unidad del Bosque Xalapa Veracruz, México
[3]Facultad de Nutrición, Universidad Veracruzana, Médicos y Odontologos s/n C.P. 91010, Unidad del Bosque Xalapa Veracruz, México
[4]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
[5]Universidad Autónoma del Carmen, Facultad de Ciencias de la Salud Campus III, Av. Central s/n Esq. Fracc. Mundo Maya, C.P. .24153 Ciudad del Carmen, Campeche
*corresponding author e-mail address: ***lfiguero@uacam.mx; camachoabelardo@gmail.com*** | Scopus ID 55995915500; 56928399200

ABSTRACT

Several casein kinase 2 inhibitors have been prepared using some protocols which require dangerous reagents and specific conditions. The aim of this study was to synthesize a new chloroacetamide-steroid derivative using some chemical tools. In addition, the effect exerted by chloroacetamide-steroid derivative on casein kinase 2 (CK2) was determinate in an ischemia-reperfusion injury model using quinalizarin and N-tertbuthyl-2-chloroacetamide as controls. The results showed that 1) the chloroacetamide-steroid derivative significantly decrease the ischemia-reperfusion injury translated as infarct area compared with quinalizarin and N-tertbuthyl-2-chloroacetamide. In conclusion, in this study, is reported a facile synthesis of a new chloroacetamide-steroid derivative with biological activity against CK2.

Keywords: *Synthesis; steroid; derivative; casein kinase 2.*

1. INTRODUCTION

Myocardial infarction is a major cause of health worldwide; this clinical pathology can be conditioned by the cardiac myocyte cell death caused by prolonged myocardial ischemia [1, 2]. It is important to mention, that acute myocardial infarction can produce alterations in the topography of both the infarcted and non-infarcted regions of the ventricles [3]. There are some studies that indicate that the most effective method of limiting necrosis is through restoration of the blood flow; however, the effects of reperfusion may be associated with tissue injury [4]. It is noteworthy that several vasoactive substances can be involved in the ischemia/reperfusion injury such as the generation of reactive oxygen species and protein phosphorylation [5, 6]. In this way, casein kinase (CK2) plays an important role in the phosphorylation of some proteins [7-9] such as cAMP-dependent protein kinase [10], NF-κB (nuclear factor kappa B), STAT-1 (signal transducer and activator of transcription-1) [11] and CREB (cAMP response element-binding) [12], which may be associated with an inflammatory response produced by ischemia/reperfusion injury [13-15]. It is important to mention that to characterize the molecular mechanism involved in the effect exerted by CK2 in different tissues, several studies have been carried out using some drugs such as benzimidazole [16], TBB (4,5,6,7-tetrabromo-2-azabenzimidazole) [17], heparin [18], emodin (6-methyl-1,3,8-trihydroxyanthraquinone) [19], quinalizarin [20] that can modify their biological activity. There are studies that indicate that these drugs can act as CK2 inhibitors using some biological experimental models; for example, a study showed that TBB inhibits the biological activity of CK2 in a model of isolated cardiomyocytes [21]. Another report indicates that the emodin can exert cardioprotective effects against acute myocardial infarction via inhibition of inflammation and apoptosis in local ischemic myocardium [22].

These data indicate that some drugs can inhibit the effect of CK2; however, the biological activity of these drugs on CK2 is very confusing, possibly this phenomenon is due to; 1) differences in the chemical structure of each drug; or 2) to different protocols used in each experiment. Therefore, the aim of this study was to synthesize a new chloroacetamide-steroid derivative to evaluate their biological activity against ischemia-reperfusion injury. In addition, the effect exerted by chloroacetamide-steroid analog on left ventricular pressure was evaluated.

2. MATERIALS AND METHODS

2.1. General methods.

Compounds **1** and **2** were prepared by a previously reported method [23]. In addition, all the reagents used in this study were purchased from Sigma-Aldrich Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were determined using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded on a Varian VXR300/5 FT NMR spectrometer at 300 MHz (megahertz) in $CDCl_3$ (deuterated chloroform) using TMS

(tetramethylsilane) as an internal standard. EIMS (electron impact mass spectroscopy) spectra were determined using a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis data were determined from a Perkin Elmer Ser. II CHNS/02400 elemental analyzer.

2.2. Chemical Synthesis.

Preparation of two nitro-azahexacyclo-carbonitrile derivatives.

A solution of compounds **1** or **2** (0.50 mmol), (4-nitro-phenyl)-acetonitrile (90 mg; 0.55 mmol), sodium hydroxide (20 mg, 0.50 mmol), and 5 ml of ethanol was stirring for 72 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:water:hexane (4:1:1) system.

12,21-dihydroxy-22-methyl-7-nitro-10-azahexacyclo[12.11.0.$0^{2,11}$, $0^{4,9}$.$0^{17,25}$.$0^{18,22}$]pentacosa-1(14),2,4(9),5,7,10,12-hepta-ene-3-carbo-nitrile (3)

yielding 56 %; IR (V_{max}, cm^{-1}) 3400, 3430, 2220, and 1540: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.82-3.64 (m, 16H), 5.70 (broad, 2H), 7.10-9.26 (m, 4H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 15.82, 24.26, 25.72, 27.74, 29.62, 32.80, 33.71, 37.19, 43.01, 44.39, 50.76, 82.44, 104.20, 117.12, 118.40, 120.50, 123.30, 134.65, 135.32, 136.70, 137.82, 137.90, 137.94, 138.60, 147.92, 154.80 ppm. EI-MS m/z: 443.18. Anal. Calcd. for $C_{26}H_{25}N_3O_4$: C, 70.41; H, 5.68; N, 9.47; O, 14.43. Found: C, 70.36; H, 5.60, 9.44.

12-hydroxy-22-methyl-7-nitro-21-oxo-10-zahexacyclo[12.11.0.$0^{2,11}$.$0^{4,9}$.$0^{17,25}$.$0^{18,22}$]pentacosa-1(14),2,4(9),5,7,10,12-hepta- ene-3-carbonitrile (4)

yielding 44 %; IR (V_{max}, cm^{-1}) 2222, 1712 and 1540: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.92 (s, 3H), 1.20-3.14 (m, 15H), 5.00 (broad, 1H), 7.10 (m, 1H), 8.30-9.26 (m, 4H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 13.80, 21.70, 26.18, 27.12, 29.62, 31.37, 35.30, 37.27, 45.89, 48.16, 50.22, 104.16, 117.10, 118.41, 118.52, 123.30, 134.64, 135.33, 136.32, 137.42, 137.94, 137.96, 138.64, 147.94, 154.86, 220.36 ppm. EI-MS m/z: 441.16. Anal. Calcd. for $C_{26}H_{25}N_3O_4$: C, 70.73; H, 5.25; N, 9.52; O, 14.50; Found: C, 70.68; H, 5.20; N, 9.50.

Reduction of nitro group.

A solution of compounds **3** or **4** (0.50 mmol), sodium borohydride (50 mg, 1.32 mmol), zinc dust (30 mg) and 5 ml of ethanol was stirring for 72 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:water:hexane (4:1:1) system.

7-amino-12,21-dihydroxy-22-methyl-10-azahexacyclo[12.11.0.$0^{2,11}$,$0^{4,9}$.$0^{17,25}$.$0^{18,22}$]pentacosa-1(14),2,4(9),5,7,10,12-hepta- ene-3-carbonitrile (5)

80 %; IR (V_{max}, cm^{-1}) 3400, 3325 and 2220: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.82-3.64 (m, 16H), 5.10 (broad, 4H), 7.10 (m, 1H), 7.16-8.10 (m, 4H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 15.82, 24.26, 25.72, 27.74, 29.62, 32.78, 33.71, 37.19, 43.00, 44.39, 50.76, 82.44, 103.50, 116.42, 117.10, 118.38, 118.44, 120.52, 126.30, 135.02, 136.70, 136.92, 137.80, 140.24, 145.00, 147.92, 155.80. ppm. EI-MS m/z: 413.21. Anal. Calcd. for $C_{26}H_{27}N_3O_2$: C, 75.52; H, 6.58; N, 10.16; O, 7.74. Found: C, 75.48; H, 6.50, N, 10.12.

Protection of hydroxyl group.

A solution of compound **5** (0.50 mmol), terbuthyldimethylsylane chloride (tert-butyldimethylsilyl chloride (200 µl, 1.07 mmol) in 3 ml chloroform was stirring for 24 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:ketone (3:1) system.

7-amino-12,21-bis[(tert-butyldimethylsilyl)oxy]-22-methyl-10-aza- hexa-cyclo[12.11.0.$0^{2,11}$.$0^{4,9}$.$0^{17,25}$.$0^{18,22}$]pentacosa-1(14),2, 4(9),5,7, 10,12-hep- taene-3-carbonitrile (6)

yielding 85 %; IR (V_{max}, cm^{-1}) 3328, 2222, and 1080: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.08 (s, 6H), 0.24 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 0.99 (s, 9H), 1.00-3.54 (m, 16H), 4.62 (broad, 2H), 7.10 (m, 1H), 7.18-8.10 (m, 4H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: -4.60, -4.54, 15.22, 17.82, 18.10, 25.52, 25.62, 25.70, 25.74, 27.76, 29.64, 32.94, 35.10, 37.22, 43.00, 43.74, 51.52, 82.60, 103.18, 116.34, 118.40, 118.66, 120.52, 120.94, 126.32, 135.04, 136.32, 136.71, 140.22, 140.72, 145.00, 156.80 ppm. EI-MS m/z: 641.38. Anal. Calcd. for $C_{38}H_{55}N_3O_2Si_2$: C, 71.09; H, 8.63; N, 6.54; O, 4.98; Si, 8.75. Found: C, 71.00; H, 8.58; N, 6.52.

Synthesis of a chloroamide derivative.

A solution of compound **6** (0.50 mmol), chloroacetyl chloride (50 µl, 0.63 mmol), triethylamine (100 µl, 0.71 mmol) in 3 ml chloroform was stirring for 24 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (3:1:1) system.

N-{12,21-bis[(tert-butyldimethylsilyl)oxy]-3-cyano-22-methyl-10-aza-hexacyclo[12.11.0.$0^{2,11}$.$0^{4,9}$.$0^{17,25}$.$0^{18,22}$]pentacosa-1(14),2,4(9), 5,7,10,12-heptaen-7-yl}-2-chloroacetamide (7)

yielding 45 %; IR (V_{max}, cm^{-1}) 2222, 1680 and 1080: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.08 (s, 6H), 0.24 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 0.99 (s, 9H), 1.00-3.54 (m, 16H), 4.10 (m, 2H), 7.10 (m, 1H), 7.62-9.10 (m, 3H), 10.10 (broad, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: -4.60, -4.54, 15.22, 17.82, 18.02, 25.52, 25.62, 25.70, 25.74, 27.76, 29.62, 32.94, 35.03, 37.20, 43.00, 43.02, 43.72, 51.52, 82.60, 105.94, 118.46, 118.64, 120.52, 120.94, 125.70, 127.72, 134.00, 136.38, 136.72, 138.22, 138.40, 139.44, 155.42, 164.62 ppm. EI-MS m/z: 717.35. Anal. Calcd. for $C_{40}H_{56}ClN_3O_3Si_2$: C, 66.86; H, 7.86; Cl, 4.93; N, 5.85; O, 6.68; Si, 7.82. Found: C, 66.80; H, 7.80; N, 5.82.

Preparation of 3-phenyloxirane-2-carboxamide derivative.

A solution of compound **7** (0.50 mmol), benzaldehyde (80 µl, 0.79 mmol), sodium hydroxide (20 mg, 0.50 mmol), and 5 ml of ethanol was stirring for 24 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (3:1:2) system.

N-{12,21-bis[(tert-butyldimethylsilyl)oxy]-3-cyano-22-thyl-10-aza-hexacyclo[12.11.0.$0^{2,11}$.$0^{4,9}$.$0^{17,25}$.$0^{18,22}$]pentacosa-1(14),2,4(9),5,7, 10,12-heptaen-7-yl}-3-phenyloxirane-2-carboxamide (8)

yielding 65 %; IR (V_{max}, cm^{-1}) 2220, 1680 and 1080: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.08 (s, 6H), 0.24 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 0.99 (s, 9H), 1.00-3.54 (m, 16H), 3.74-3.92 (m, 2H), 6.92 (m, 2H), 7.10 (m, 1H), 7.26-9.10 (m, 6H), 9.72 (broad, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: -4.60, -4.54, 15.22, 17.82, 18.02, 25.52, 25.62, 25.70, 25.74, 27.76, 29.62, 32.94, 35.03, 37.20, 43.00, 43.72, 51.52, 55.26, 57.10, 82.60, 105.94, 118.46, 118.64, 119.40, 120.92, 124.64, 125.52, 127.72, 128.22, 128.52, 133.00, 136.32, 136.40, 136.74, 138.22, 138.26, 138.86, 139.92.155.42, 174.44 ppm. EI-MS m/z: 787.42. Anal.

Calcd. for $C_{47}H_{61}N_3O_3Si_2$: C, 71.62; H, 7.80; N, 5.33; O, 8.12; Si, 7.13; . Found: C, 71.56; H, 7.74; N, 5.30.

Preparation of a 3-(naphthalen-1-yl)oxirane-2-carboxamide derivative.

A solution of compound **7** (0.50 mmol), 2-hydroxy-naphthalene-1-carbaldehyde (90 mg, 0.52 mmol), sodium hydroxide (20 mg, 0.50 mmol), and 5 ml of ethanol was stirring for 24 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (3:1:1) system.

N-[(22S)-12,21-bis[(tert-butyldimethylsilyl)oxy]-3-cyano-22-methyl-10-azahexacyclo[12.11.0.0^{2,11}.0^{4,9}.0^{17,25}.0^{18,22}]pentacosa-1(14),2,4,6,8,10,12-heptaen-7-yl]-3-(2-hydroxynaphthalen-1-yl) oxirane-2-carboxamide (9)

yielding 48 %; IR (V_{max}, cm^{-1}) 2220, 1680 and 1080: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.08 (s, 6H), 0.24 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 0.99 (s, 9H), 1.00-3.54 (m, 16H), 4.00-4.36 (m, 2H), 7.10 (m, 1H), 7.20-9.10 (m, 9H), 9.42 (broad, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: -4.60, -4.54, 15.22, 17.82, 18.02, 25.52, 25.62, 25.70, 25.74, 27.76, 29.62, 32.94, 35.03, 37.20, 43.00, 43.72, 51.52, 53.26, 55.78, 82.60, 105.94, 118.46, 118.64, 118.80, 119.40, 120.92, 121.42, 122.64, 123.44, 124.62, 126.82, 127.70, 120.13, 129.24, 130.34, 133.00, 134.30, 136.32, 136.74, 138.22, 138.88, 139.92, 152.52, 155.40, 174.44 ppm. EI-MS m/z: 853.43. Anal. Calcd. for $C_{51}H_{63}N_3O_6Si_2$: C, 71.71; H, 7.43; N, 4.92; O, 9.36; Si, 6.70. Found: C, 71.67; H, 7.40; N, 4.90.

Preparation of a 3-phenylprop-2-en-1-yl]oxirane-2-carboxamide derivative.

A solution of compound **7** (0.50 mmol), cinnamaldehyde (90 µl, 0.71 mmol), sodium hydroxide (20 mg, 0.50 mmol), and 5 ml of ethanol was stirring for 24 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (3:1:1) system.

N-[(22S)-12,21-bis[(tert-butyldimethylsilyl)oxy]-3-cyano-22-me-thyl-10-azahexacyclo[12.11.0.0^{2,11}.0^{4,9}.0^{17,25}.0^{18,22}]pentacosa-1(14),2,4,6,8,10,12-heptaen-7-yl]-3-[(1Z)-2-phenylethenyl]oxi ra ne-2-car-boxamide (10)

yielding 42 %; IR (V_{max}, cm^{-1}) 2220,1725, 1682 and 1080: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.08 (s, 6H), 0.24 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 0.99 (s, 9H), 1.00-2.02 (m, 12H), 2.84-3.06 (m, 3H), 3.48 (m, 1H), 3.54 (m, 1H), 4.00 (m, 1H), 6.00-7.00 (m 2H), 7.10 (m, 1H), 7.12-9.10 (m, 8H), 9.92 (broad, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: -4.60, -4.54, 15.22, 17.82, 18.02, 25.52, 25.62, 25.70, 25.74, 27.76, 29.62, 32.94, 35.03, 37.20, 43.00, 43.72, 51.52, 57.26, 58.56, 82.60, 105.94, 118.46, 118.64, 119.62, 120.92, 124.82, 126.32, 127.70, 128.32, 128.85, 129.78, 130.66, 133.00, 136.32, 136.76, 138.22, 138.88, 139.72, 155.42, 174.90 ppm. EI-MS m/z: 813.43. Anal. Calcd. for $C_{49}H_{63}N_3O_4Si_2$: C, 72.28; H, 7.80; N, 5.16; O, 7.86; Si, 6.90. Found: C, 72.20; H, 7.76; N, 5.12.

Physicochemical parameters evaluation.

Some electronic parameters such as HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital) energy, orbital coefficients distribution, molecular dipole moment, HBD (hydrogen bond donor groups), HBA (hydrogen bond acceptor groups) and PSA (polar surface area) were evaluated using the SPARTAN'06 software [24].

Pharmacophore evaluation.

The 3D pharmacophore model for the compounds **2-4** was determinate using LigandScout 4.08 software [25].

Biological Method.

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal care and use Committee of University Autonomous of Campeche (no. PI-420/12) and were in accordance with the Guide for the Care and Use of Laboratory Animals [26]. Male Wistar rats, weighing 200-250 g, were obtained from University Autonomous of Campeche.

Reagents.

All drugs were dissolved in methanol and different dilutions were obtained using Krebs-Henseleit solution (≤0.01%, v/v).

Experimental Design.

Briefly, the male rat (200–250 g) was anesthetized by injecting them with pentobarbital at a dose rate of 50 mg/Kg body weight. Then the chest was opened, and a loose ligature passed through the ascending aorta. The heart was then rapidly removed and immersed in ice-cold physiologic saline solution. The heart was trimmed of noncardiac tissue and retrograde perfused via a non-circulating perfusion system at a constant flow rate. The perfusion medium was the Krebs-Henseleit solution (pH = 7.4, 37 ^{o}C) composed of (mmol) 117.8, NaCl; 6, KCl; 1.75, $CaCl_2$; 1.2, $NaHPO_4$; 1.2, $MgSO_4$; 24.2, $NaHCO_3$; 5, glucose; 7 and 5, sodium pyruvate. The solution was actively bubbled with a mixture of O_2/CO_2 (95: 5/5%). The coronary flow was adjusted with a variable speed peristaltic pump. An initial perfusion rate of 15 mL/min for 5 min was followed by a 15 min equilibration period at a perfusion rate of 10 mL/min. All experimental measurements were done after this equilibration period.

Perfusion Pressure.

Evaluation of measurements of perfusion pressure changes induced by drugs administration in this study was assessed using a pressure transducer connected to the chamber where the hearts were mounted, and the results entered into a computerized data capture system (Biopac).

Table 2. Design experimental. Hearts were subjected to ischemia/reperfusion and treated with in absence (received vehicle only; Krebs-Henseleit solution) or presence of the compounds C-1 to C-10 (at a dose of 0.001 nM) before ischemia period (for 10 minutes) and during the entire period of reperfusion (n = 9).

Group	Treatment
I	Control (without treatment)
II	C-1 (0.001 nM)
III	C-2 (0.001 nM)
IV	C-3 (0.001 nM)
V	C-4 (0.001 nM)
VI	C-5 (0.001 nM)
VII	C-6 (0.001 nM)
VIII	C-7 (0.001 nM)
IX	C-8 (0.001 nM)
X	C-9 (0.001 nM)
XI	C-10 (0.001 nM)

First Stage.

Biological activity induced by the chloroacetamide-steroid derivative using an ischemia/reperfusion model. After 15 minutes of equilibration time, the hearts were subjected to ischemia for 40 minutes by turning off the perfusion system [27]. After this period,

the system was restarted, and the hearts were re-perfused by 40 minutes with Krebs-Henseleit solution. The hearts were randomly divided into 11 major treatment groups (Table 2) with n = 9. The areas of the normal left ventricle no-risk region, area at risk, and infarct region were determined using a previous method reported [27]. The total area at risk was expressed as the percentage of the left ventricle.

Second Stage.

Effects exerted by the chloroacetamide-steroid derivative on left ventricular pressure through the calcium channel. Intracoronary boluses (50 µL) of the chloroacetamide-steroid derivative [0.001 to 100 nM] were administered and the corresponding effect on the left ventricular pressure was evaluated. The dose-response curve (control) was repeated in the presence of nifedipine* at a concentration of 1 nM (duration of the preincubation with nifedipine was for a period of 10 min). *Dose of nifedipine has been with a previous method reported [28].

Statistical Analysis.

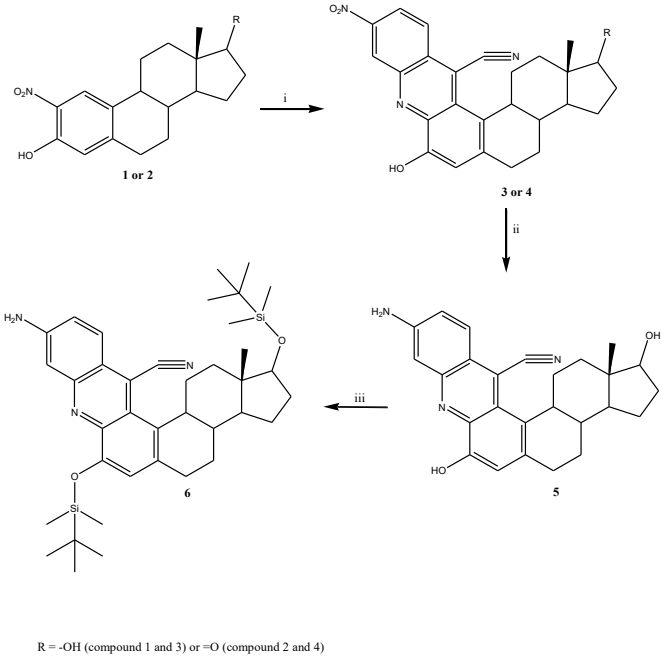
The obtained values are expressed as average ± SE. The data obtained were put under the analysis of variance (ANOVA) with the Bonferroni correction factor using the SPSS 12.0 program [29]. The differences were considered significant when was equal to or smaller than 0.05.

3. RESULTS

There is little information on the biological activity of steroid derivatives against casein kinase 2 in the ischemia/reperfusion injury; therefore, in this study, the effect exerted by a chloroacetamide-steroid derivative as casein kinase-2 inhibitor was evaluated using an ischemia/reperfusion model. The first stage was achieved by the synthesis of some steroid derivatives as follows:

Preparation of two nitro-azahexacyclo-carbonitrile derivatives.

Several azahexacyclo analogs have been using different methods which use some reagents such an oligoether derivative [30], 1,3-diphenyl-2H-cyclopenta[l]phenanthren-2-one [31], 1,2-phenyl-enediamine [32], benzyl amine [33], thiourea/hydrochloric acid [34], α-cyanocinnamonitriles [35] and others. However, some of these reagents are expensive and require special conditions for their management. In this study, two nitro-azahexacyclo-carbonitrile derivatives from 2-nitroestradiol, 2-nitroestrone and (4-nitro-phenyl)-acetonitrile in basic conditions were prepared (Figure 1).



R = -OH (compound 1 and 3) or =O (compound 2 and 4)

Figure 1. Preparation of an amino-azahexacyclo-terbuthyldimethylsylane-steroid-carbonitrile derivative (**6**). R eaction of either 2-nitroestradiol (**1**) or 3-nitroestrone (**2**) derivatives with (4-nitro-phenyl)-acetonitrile to form 12,21-dihydroxy-steroid-3-carbonitrile (**3**) or 12-hydroxy-steroid-3-carbonitrile (**4**). Then, a 7-amino-steroid-carbonitrile analog (**5**) was prepared via reduction of either **3** or **4**. Finally, hydroxyl group of **5** was protected to form **6**. Reagents and conditions; i = NaOH/EtOH/rt; ii = sodium borohydride/Zn/EtOh/rt; iii = terbuthyldime thylsylane chloride/$CHCl_3$/rt.

The ^{1}H NMR spectrum of **3** shows signals at 0.76 ppm for methyl group bound to steroid nucleus; at 0.82-3.64 ppm for steroid moiety; at 5.80 ppm for both hydroxyl groups; at 7.10-9.20 ppm for phenyl group (Figure 2). ^{13}C NMR spectra showed chemical shifts at 15.82 for methyl group bound to steroid nucleus; at 24.26-82.44, 117.12, 120.50, 136.70-137.82 and 154.80 ppm for steroid moiety; at 104.20, 137.94 and 138.60 ppm for pyridine ring; at 118.40 ppm for nitrile group; at 123.30-135.30, 137.90 and 147.92 ppm for phenyl group. In addition, the mass spectrum from **3** showed a molecular ion (m/z) 443.18.

Other data showed (Figure 3) several signals of ^{1}H NMR spectrum for **4** at 0.92 ppm for methyl group bound to steroid nucleus; at 1.20-3.14 and 7.10 ppm for steroid moiety; at 5.00 ppm for hydroxyl group; at 8.30-9.26 ppm for phenyl group.

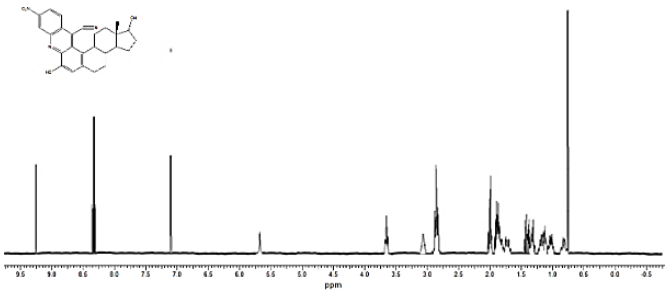


Figure 2. The scheme showed ^{1}HNMR spectrum from compound **3**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

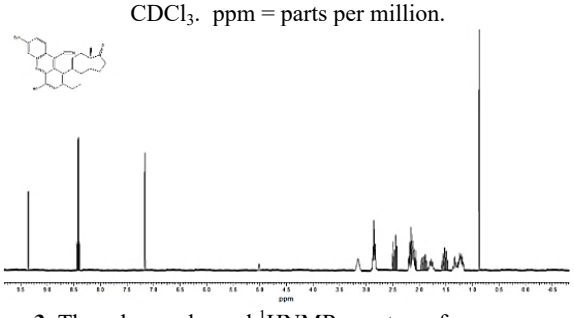


Figure 3. The scheme showed ^{1}HNMR spectrum from compound **4**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

^{13}C NMR spectra showed chemical shifts at 13.80 ppm for methyl group bound to steroid nucleus; at 21.70-50.22, 117.10, 118.52, 136.32-137.42 and 158.86 ppm for steroid moiety; at 104.16, 137.96-138.64 ppm for pyridine ring; at 118.41 ppm for nitrile group; at 123.30-135.33, 137.94 and 147.94 ppm for phenyl

group; at 220.36 ppm for ketone group. In addition, the mass spectrum from **4** showed a molecular ion (m/z) 220.36.

Reduction of both nitro and ketone groups.

There are several methods for nitro group reduction using iron oxide [36], Ferric(III) acetylacetonate [37], zinc/hydrazine glyoxylate [38], $SnCl_2$ [39], triethylphosphite [40] and others. However, some of these reactive protocols require special conditions for their management. Therefore, in this study, the nitro groups involved in the chemical structure of compounds **3** or **4** were reduced in the presence of sodium borohydride /Zn (Figure 1). The ^{1}H NMR spectrum of **5** shows signals at 0.76 ppm for methyl group bound to steroid nucleus; at 0.82-3.64 and 7.10 ppm for steroid moiety; at 5.10 ppm for both amino and hydroxyl groups; at 7.16-8.10 ppm for phenyl group (Figure 4).

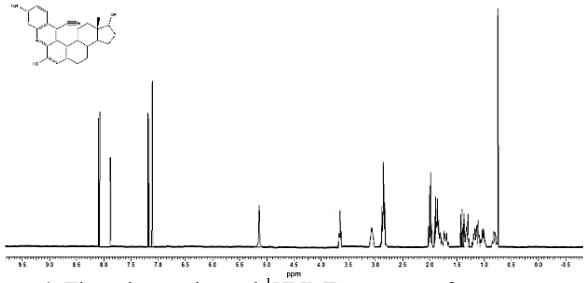


Figure 4. The scheme showed ^{1}HNMR spectrum from compound **5**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

^{13}C NMR spectra showed chemical shifts at 15.80 ppm for methyl group bound to steroid nucleus; at 24.26-82.44, 117.10-118.38, 136.70, 137.80 and 155.80 ppm for steroid moiety; at 103.50 and 136.92 ppm for pyridine ring; at 118.44 ppm for nitrile group; at 116.42, 120.52-135.02 and 140.23-145.00 ppm for phenyl group. In addition, the mass spectrum from **5** showed a molecular ion (m/z) 413.21.

Protection of hydroxy groups.

Some tert-butyldimethylsilyl derivatives have been used to protect hydroxyl groups in organic synthesis [41] (Corey and venkateswarlu, 1972). Therefore, in this study the compound **5** reacted with tert-butyldimethylsilyl chloride (Figure 1) to form the amino-azahexacyclo-terbuthyldimethylsylane-steroid-carbonitrile derivative (**6**). The ^{1}H NMR spectrum of **6** shows signals at 0.08-0.24, 0.88 and 0.99 ppm terbuthyldimethylsylane fragment; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.00-3.54 and 7.10 ppm for steroid moiety; at 4.26 ppm for amino group; at 7.18-8.10 ppm for phenyl group (Figure 5).

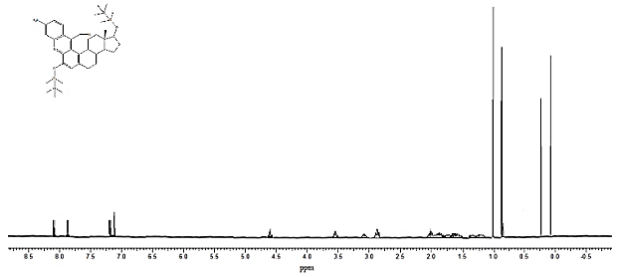


Figure 5. The scheme showed ^{1}HNMR spectrum from compound **6**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in CDC_{l3}. ppm = parts per million.

^{13}C NMR spectra showed chemical shifts at -4.60, -4.54, 17.82-25.52 and 25.74 ppm for terbuthyldimethylsylane fragment; at 15.22 ppm for methyl group bound to steroid nucleus; at 25.62-25.70, 27.76-82.60.116.66, 136.32-140.22 and 158.80 ppm for steroid moiety; at 103.18 and 120.94-126.32 ppm for pyridine ring; at 118.40 ppm for nitrile group; at 116.34, 120.55, 135.04 and 140.72-145.00 ppm for phenyl group. In addition, the mass spectrum from **6** showed a molecular ion (m/z) 641.38.

Preparation of a chloroamide derivative.

There are many procedures for the formation of chloroamides; for example, the reaction of an amino derivative with trichloroisocyanuric acid [42] or an amide secondary with N-chlorobenzotriazole to form a chloroamide derivative [43]. Additionally, some chloroamides have been prepared using chloroacetyl chloride [44]. In this study, the chloroamide derivative (**7)** was synthesized from the compound **6** and chloroacetyl chloride/triethylamine (Figure 6).

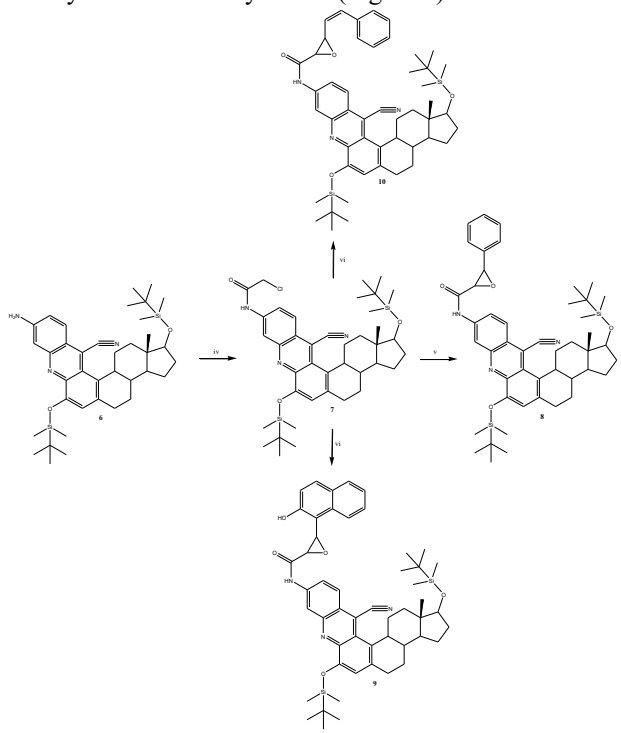


Figure 6. Acetilation of an amino-steroid-carbonitrile derivative (**6**) with chloroacetyl chloride (iv) to form a chloroacetamide-steroid derivative (**7**). Then, **7** reacted with benzaldehyde (v) to form a 3-phenyloxirane-2-carboxamide (**8**). After, a 3-(naphthalen-1-yl)oxirane-2-carboxamide derivative (**9**) was prepared via reaction of **7** with 2-hydroxy-naphthalene-1-carbaldehyde (vi). Finally, **7** reacted with cinnamaldehyde (vii) to form a 3-phenyl- prop-2-en-1-yl]oxirane-2-carboxamide analog **10**. Conditions; iv = $Et_3N/CHCl_3$/rt; v, vi, vii =NaOH/EtOH/rt.

The ^{1}H NMR spectrum of **7** shows signals at 0.08-0.24, 0.88 and 0.99 ppm terbuthyldimethylsylane fragment; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.00-3.54 and 7.10 ppm for steroid moiety; at 4.10 ppm for methylene bound to chloroamide group; at 7.62-9.10 ppm for phenyl group; at 10.10 ppm for amide group (Figure 7). ^{13}C NMR spectra showed chemical shifts at -4.60, -4.54, 17.82-25.52 and 25.74 ppm for terbuthyldimethyl-sylane fragment; at 15.22 ppm for methyl group bound to steroid nucleus; at 25.62-25.70, 27.76-43.00, 43.72-86.60, 118.64, 136.38-138.22 and 158.42 ppm for steroid moiety; at 105.94, 120.94 and 138.40 ppm for pyridine ring; at 118.46 ppm for nitrile group; at 120.52, 125.70-134.00 and 139.44 ppm for phenyl group; at 164.62 ppm for amide group. In addition, the mass spectrum from **7** showed a molecular ion (m/z) 717.35.

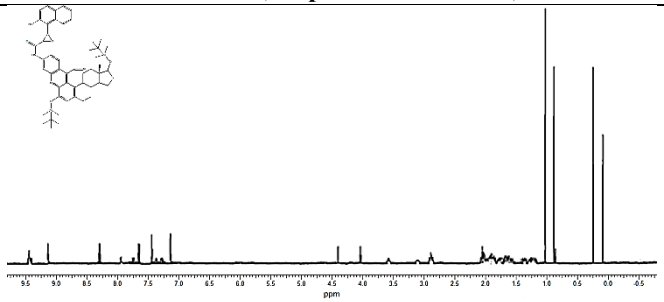


Figure 7. The scheme showed [1]HNMR spectrum from compound **7**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

Preparation of epoxide derivatives.

It is noteworthy that several epoxides have been prepared using different protocols; nevertheless, expensive reagents and special conditions are required; the most widely practiced methods employ some reagents such as Co(III) [45] and Cr(III) [46]. In this study, three epoxide derivatives (compounds **8**, **9** and **10**) were prepared by the reaction of **7** with aldehyde derivatives in mild conditions (Figure 6). The ^{1}H NMR spectrum of **8** showed several chemical shifts at 0.08-0.24, 0.88 and 0.99 ppm terbuthyldimethylsylane fragment; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.00-3.54 and 7.10 ppm for steroid moiety; at 3.74-3.92 ppm for oxirane ring; at 6.92 and 7.26-9.10 ppm for phenyl groups; at 9.72 ppm for amide group (Figure 8).

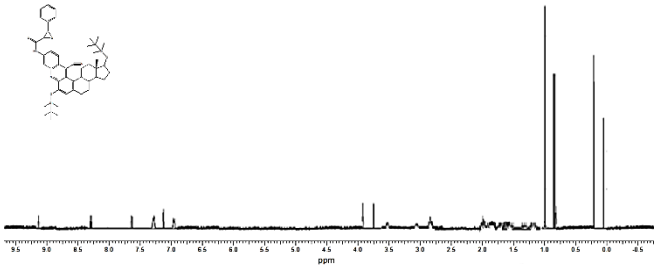


Figure 8. The scheme showed [1]HNMR spectrum from compound **8**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in CDCl3. ppm = parts per million.

^{13}C NMR spectra showed chemical shifts at -4.60, -4.54, 17.82-25.52 and 25.74 ppm for terbuthyldimethylsylane fragment; at 15.22 ppm for methyl group bound to steroid nucleus; at 25.62-25.70, 27.76-51.52, 82.60, 118.64, 136.32 and 155.42 ppm for steroid moiety; at 55.26-57.10 ppm for oxirane ring; at 105.94, 120.92 and 138.22-138.86 ppm for pyridine ring; at 118.46 ppm for nitrile group; at 119.40, 124.64-133.00 and 139.92 ppm for phenyl group; at 174.44 ppm for amide group. In addition, the mass spectrum from **8** showed a molecular ion (m/z) 787.42.

On the other hand, the ^{1}H NMR spectrum of **9** shows signals at 0.08-0.24, 0.88-0.99 ppm terbuthyl-dimethylsylane fragment; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.00-3.54 and 7.10 ppm for steroid moiety; at 4.00-4.36 ppm for oxirane ring; at 7.20-9.10 ppm for phenyl groups; at 9.42 ppm for both hydroxyl and amide groups (Figure 9). ^{13}C NMR spectra showed chemical shifts at -4.60, -4.54, 17.82-25.52 and 25.74 ppm for terbuthyldimethylsylane fragment; at 15.22 ppm for methyl group bound to steroid nucleus; at 25.62-25.70, 27.76-51.52, 82.60-110.64, 118.64, 136.32-138.22 and 155.40 ppm for steroid moiety; at 53.26-57.78 ppm for oxirane ring; at 105.94, 120.92, 121.45, 124.62, 127.70, 133.00 and 138.88-139.92 ppm for pyridine ring; at 118.46 pm for nitrile group; at 118.80, 119.40, 122.64, 126.82, 129.13-130.34, 134.30 and 152.52 ppm for phenyl group; at 174.44 ppm for amide group. In addition, the mass spectrum from **9** showed a molecular ion (m/z) 853.43.

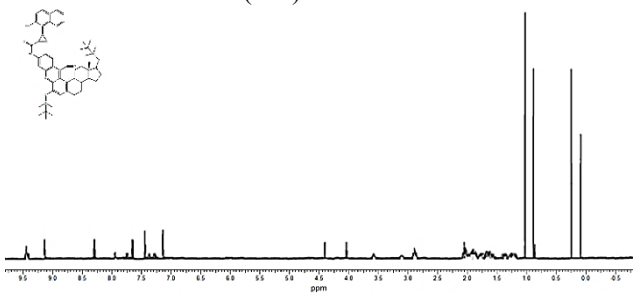


Figure 9. The scheme showed [1]HNMR spectrum from compound **9**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in CDCl3. ppm = parts per million.

Finally, the ^{1}H NMR spectrum of **10** shows signals at 0.08-0.24, 0.88-0.99 ppm terbuthyldimethylsylane fragments; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.00-3.06, 3.54 and 7.10 ppm for steroid moiety; at 3.48 and 4.00 ppm for oxirane ring; at 3.48 and 4.00 ppm for oxirane ring; at 6.00-7.00 ppm for alkene group; at 7.12-9.10 ppm for phenyl groups; at 9.92 ppm for amide group (Figure 10).

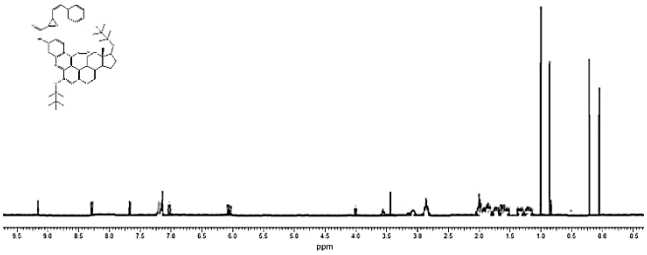


Figure 10. The scheme showed [1]HNMR spectrum from compound **10**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

^{13}C NMR spectra showed chemical shifts at -4.60, -4.54, 17.82-25.52 and 25.74 ppm for terbuthyldimethylsylane fragment; at 15.22 ppm for methyl group bound to steroid nucleus; at 25.62, 25.76-51.52, 82.60, 118.64, 136.32-138.25 and 155.42 ppm for steroid moiety; at 55.26-58.56 ppm for oxirane ring; at 105.94 and 138.88 ppm for pyridine ring; at 118.46 ppm for nitrile group; at 119.62, 128.85, 133.00 and 139.72 ppm for phenyl group; at 129.78 and 130.66 for alkene group; at 174.90 ppm for amide group. In addition, the mass spectrum from **10** showed a molecular ion (m/z) 813.43.

Electronic parameters.

Several studies have shown molecular orbitals and frontier electron density is used to predict the most reactive position in some electron system on several types of reactions [47, 48]. In this way, the values of the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) and their energy gap reflect the chemical activity of a molecule [49]. It is noteworthy, that several protocols have been used to evaluate the relation between HOMO and LUMO with the biological activity of some compounds [50, 51]**.**

Table 1. Physicochemical parameters involved in the structure of compounds **8**, **9** and **10**.

Parameters	C-7	C-8	C-9	C-10
Polarizability (cm^3)	101.12	108.75	113.42	111.31
PSA	56.77	68.47	83.97	68.76

Parameters	C-7	C-8	C-9	C-10
LogP	12.04	13.62	14.32	14.39
Dipole moment (debyte)	3.83	4.73	3.77	-4.99
HBD	1	1	2	1
HBA	6	7	8	7
HOMO (eV)	-7.61	-7.63	-7.59	-7.61
LUMO (eV)	-0.15	-0.119	-0.13	-0.15

HBD (hydrogen bond donors); HBA (hydrogen bond acceptors); PSA (polar surface area).

In this study, the Hartee-Fock method (method of approximation for the determination of the wave function and the energy of a quantum many-body system in a stationary state) was used to determine either HOMO and LUMO orbitals (Figure 11 and Table 1) in Spartan'06 V112 program [24].

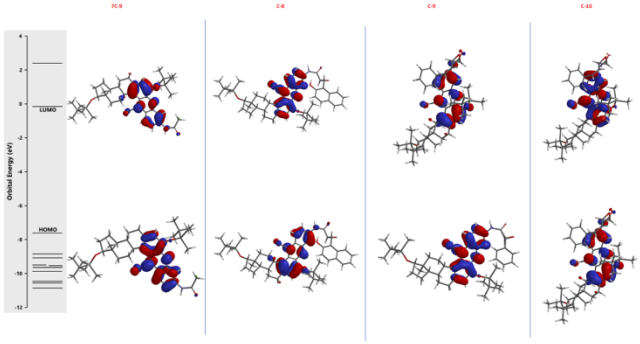


Figure 11. Molecular orbitals (HOMO and LUMO) involved in the compounds **7** (C-7), **8** (C-8), **9** (C-9) and **10** (C-10) (II). Visualized with SPARTAN'06 software.

The results showed that both HOMO and LUMO values for the compounds **7** and **10** were similar; however these values were different compared with results for **8** and **9**; it is noteworthy that both HOMO and LUMO of for both compounds **7** and **10** are delocalized over steroid nucleus, whereas, for either compounds **8** and **9** the LUMO molecular orbitals excluding the steroid nucleus. This phenomenon could be conditioned by the difference in π orbitals density that is involved in the chemical structure of these compounds.

Pharmacophore ligand model.

Several chemical models have been used to determine the three-dimensional orientation adopted by the functional groups of a molecule to predict its interaction with several biomolecules [52]; for example, the use of a pharmacophore model which can furnish a new insight to design novel molecules that can enhance or inhibit the function of a biological target which can be useful in new drug discovery. Analyzing this premise in this study, the LigandScout software [53] was used to develop a pharmacophore model for compounds **7** to **10** (Figure 12). The results showed that functional groups involved in the compounds **7** to **10** could interact via hydrophobic contacts or as hydrogen bond acceptors or as hydrogen bond donor with some biomolecules.

Biological activity.

In this study, the biological activity of compounds **1** to **10** on myocardial injury was evaluated using an ischemia/reperfusion model. Hearts were subjected to ischemia/reperfusion and treated in the absence (received vehicle only; Krebs-Henseleit solution) or presence of following compounds C-1 to C-10 (at a dose of 0.001 nM) before ischemia period (for 10 minutes) and during the entire period of reperfusion (30 minutes). The results showed that only the chloroamide-steroid derivative (compound **7**) significantly reduced infarct size (expressed as a percentage of the area at risk) compared with the compounds **1-6** and **8-10** and vehicle-treated hearts (Figure 13).

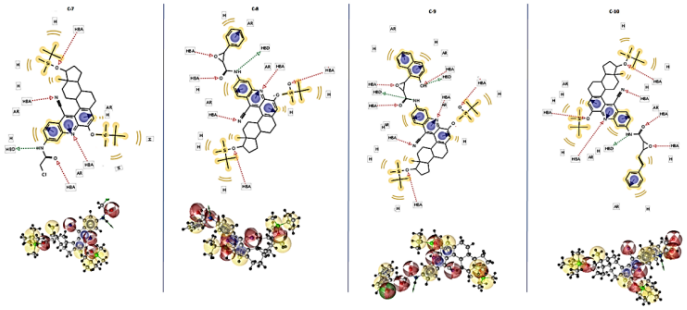


Figure 12. Theorethical pharmacophore from both compounds **7 to 10** using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red) and hydrogen bond donor (HBD, green).

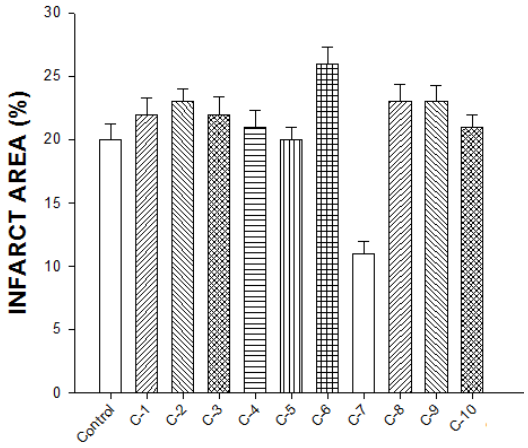


Figure 13. Biological activity induced by compounds **1** to **10** against ischemia/reperfusion injury translated as infarct area. The results showed that compound **7** decreased significantly ($p = 0.05$) the area de infarct compared with compounds **1-6**, **8-10** and control conditions. Each bar represents n = 9.

In addition, other experiments were carried out to evaluate whether higher doses of chloroamide exert the same effect on ischemia/reperfusion injury. The results showed that chloroamide derivative decreases the myocardial injury in a dose-dependent manner (Figure 14); this phenomenon may be conditioned by the interaction of the different functional groups involved in the chemical structure of the chloroamide derivative with some biomolecules.

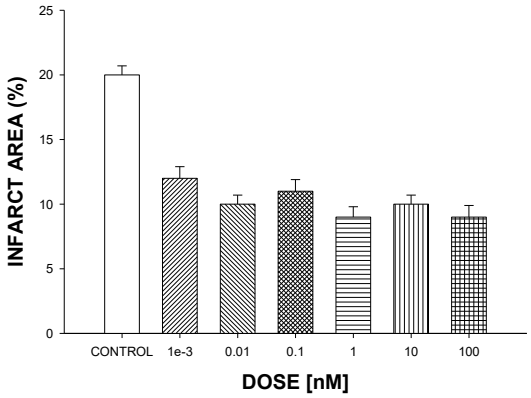


Figure 14. Effect exerted by compound **7** against ischemia/reperfusion injury translated as infarct area. The results showed that compound **7** decreased the area de infarct in a dose-dependent manner compared with control conditions. Each bar represents n = 9.

Analyzing these data and other reports which indicate that some biomolecules such as CK2 (casein kinase 2) could be involved in the regulation of some biochemical phenomena produced in the ischemia/reperfusion injury [54-58]. Therefore, in this study the biological activity of quinalizarin (CK2 inhibitor, Figure 15) on ischemia/reperfusion injury was evaluated.

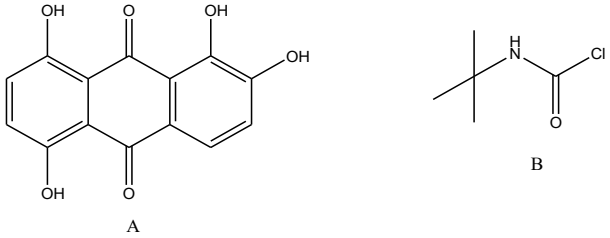


Figure 15. Chemical structure of quinalizarin (**A**) and N-tertbuthyl-2-chloroacetamide (**B**).

The results showed (Figures 15 and 16) that quinalizarin decrease the infarction area in a dose-dependent manner and this effect was similar to the effect produced by compound **7** on ischemia/reperfusion injury.

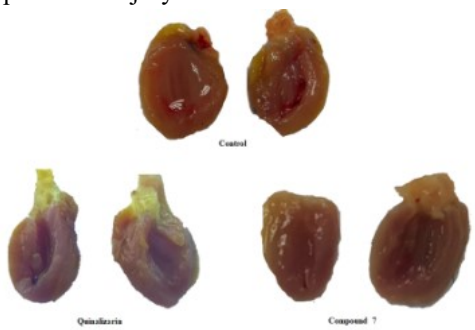


Figure 16. Comparison of the cardioprotective effect of chloroacetamide-steroid derivative [0.001 nM) with quinalizarin [0.001 nM] and the control conditions on the functional recovery of rat hearts subjected to ischemia and reperfusion.

These data indicate that compound **7** can act as a CK2 inhibitor and this effect could depend on the functional groups involved in the chemical structure of chloroamide derivative. To asses this hypothesis, in this study the effect exerted by a chloroamide derivative (N-tertbuthyl-2-chloroacetamide; Figure 15) against ischemia/reperfusion injury was evaluated. The results showed that compound N-tertbuthyl-2-chloroacetamide increases the infarction at a dose of 100 nM (Figure 16). These data suggest that compound **7** exerts a cardioprotective effect on ischemia/reperfusion injury via CK2 inhibition which could consequently bring changes in myocardial pressure, as is the case with other types of steroid derivatives [28, 59].

To validate this hypothesis, the biological activity exerted by compound **7** on left ventricular pressure was evaluated using an isolated rat heart model. The experimental results showed that compound **7** significantly increases the left ventricular pressure in manner dose-dependent (Figure 17).

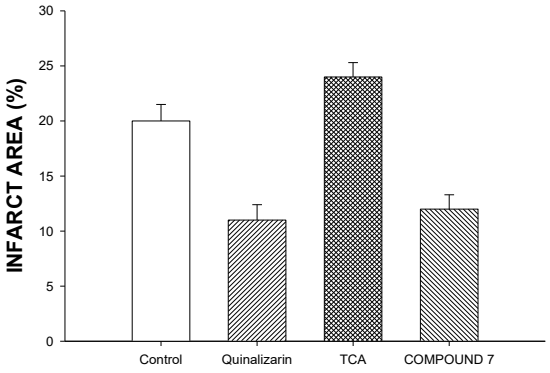


Figure 16. Biological activity exerted by quinalizarin, *N*-tertbuthyl-2-chloroacetamide (TCA) and compound **7** against ischemia/reperfusion injury translated as infarct area. The results indicate that the compound **7** significantly decreased ($p = 0.05$) the infarct area in a similar manner to activity exerted by quinalizarin; however, TCA increases the infarct area. Each bar represents n = 9.

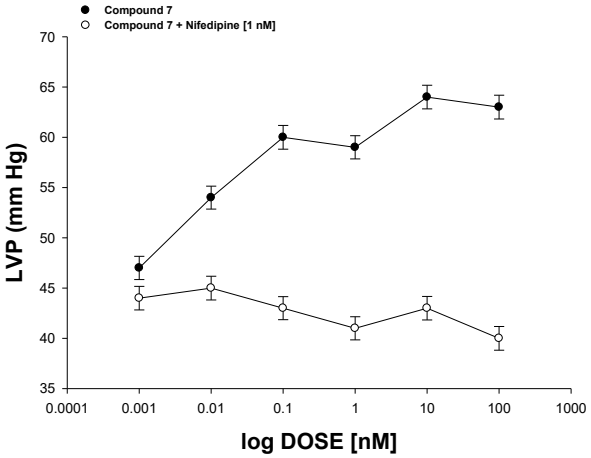


Figure 17. Effects induced by compound **7** in absence or presence of nifedipine on LVP. Intracoronary boluses (50 µl) of **7** [0.001 to 100 nM] were administered and the corresponding effect on the LVP was determined. The results showed that compound **7** increases the LVP in a dependent dose manner and this effect was t inhibited in the presence of nifedipine. Each bar represents the mean ± SE of 9 experiments. LVP = left ventricular pressure.

Analyzing these data and other reports which suggest that calcium levels can condition changes in the left ventricular pressure; in this study, the effect exerted by compound **7** against left ventricular pressure was evaluated in presence of nifedipine (Type-L calcium channel inhibitor) [60]. The results indicated that effects exerted by compound **7** on left ventricular pressure were inhibited in the presence of nifedipine (Figure 17). This data suggest that biological activity exerted by compound **7** on left ventricular pressure was via calcium channel activation.

4. CONCLUSIONS

In this study, facile synthesis of the new chloroacetamide-steroid derivative is reported. In addition, the biological activity exerted by this compound on ischemia/reperfusion injury indicated that compound **7** might regulate the biological activity of CK2 translated as a cardioprotective effect by increase the left ventricular pressure via *type-L* calcium channels. This phenomenon depends on functional groups of **7** compared with the other compounds involved in this study.

5. REFERENCES

1. Cheng, W.; Li, B.; Kajstura, J.; Li, P.; Wolin, M.; Sonnenblick, E.; Anversa, P. Stretch-induced programmed

myocyte cell death. *Journal of Clinical Investigation* **1995**, *96*, 2247-2259, https://doi.org/10.1172/JCI118280.
2. Thygesen, K.; Alpert, S.; White, D. Third universal definition of myocardial infarction. *J. Am. Coll. Cardiol*. **2012**, *50*, 2173-2195, https://doi.org/10.1161/CIR.0b013e31826e1058.
3. Pfeffer, M.A. Left ventricular remodeling after acute myocardial infarction. *Annuual Review of. Medicine* **1995**, *46*, 455-466, https://doi.org/10.1146/annurev.med.46.1.455
4. Kloner, R.; Przyklener, K.; Whittaker, P. Left ventricular remodeling after acute myocardial infarction. *Circulation* **1989**, *80*, 1115-1127.
5. Yu, W.; Beaudry, S.; Negoro, H.; Boucher, I.; Tran, M.; Kong, T. H2O2 activates G protein, α 12 to disrupt the junctional complex and enhance ischemia reperfusion injury. *Procedins of the National Academy of Sciences of the United States of America* **2012**, *109*, 6680-6685, https://doi.org/10.1073/pnas.1116800109.
6. Bae, U.; Do-Yang, J.; Ka, S.; Koo, J.; Lee, Y. SPA0355 attenuates ischemia/reperfusion-induced liver injury in mice. *Experimental and Molecular and Medicine* **2014**, *46*, 1-8, https://doi.org/10.1038/emm.2014.48.
7. Maulik, N.; Watanabe, M.; Zu, Y.; Huang, C.; Cordis, G.; Schley, J. Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts. *FEBS Lett*. **1996**, *396,* 233-237, https://doi.org/10.1016/0014-5793(96)01109-X.
8. Kim, S.; Baines, C.; Critz, S.; Pelech, S.; Katz, S.; Downey, J. Ischemia induced activation of heat shock protein 27 kinases and casein kinase 2 in the preconditioned rabbit heart. *Biochemistry and Cell Biology* **1999**, *77,* 559-567, https://doi.org/10.1139/o99-065.
9. Schulz, R.; Cohen, M.; Behrends, M.; Downey, J.; Heusch, G. Signal transduction of ischemic preconditioning. *Cardiovascular Research* **2001**, *52*, 181-198, https://doi.org/10.1016/S0008-6363(01)00384-4.
10. Carmichael, D.; Geahlen, R.; Allen, S.; Krebs, E. Type II regulatory subunit of cAMP-dependent protein kinase. Phosphorylation by casein kinase II at a site that is also phosphorylated in vivo. *Journal of Biology and Chemistry.* **1982**, *257*, 10440-10445.
11. Valen, G. Signal transduction through nuclear factor kappa B in ischemia-reperfusion and heart failure. *Basic Research in Cardiology*. **2004**, *99,* 1-7, https://doi.org/10.1007/s00395-003-0442-7.
12. Singh, N.; Ramji, D. Protein kinase CK2, an important regulator of the inflammatory response? *Journal Molecular of Medicine* **2008**, *86*, 887, https://doi.org/10.1007/s00109-008-0352-0.
13. Stephanou, A.; Scarabelli, T.; Brar, B.; Nakanishi, Y.; Matsumura, M.; Knight, R. Induction of apoptosis and Fas receptor/Fas ligand expression by ischemia/reperfusion in cardiac myocytes requires serine 727 of the STAT-1 transcription factor but not tyrosine 701. *Journal of Biological Chemical* **2001**, *276,* 28340-28347, https://doi.org/10.1074/jbc.M101177200.
14. Das, S.; Cordis, G.; Maulik, N.; Das, D. Pharmacological preconditioning with resveratrol: role of CREB-dependent Bcl-2 signaling via adenosine A3 receptor activation. *American Journal Physiology Heart Circulatpry Physiology*. **2005**, *288,* H328-H335, https://doi.org/10.1152/ajpheart.00453.2004.
15. Oka, T.; Hikoso, S.; Yamaguchi, O.; Taneike, M.; Takeda, T.; Tamai, T.; Akira, S. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature* **2012**, *485*, 251, https://doi.org/10.1038/nature10992
16. Litchfield, D. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *Biochemistry Journal* **2003**, *369*, 1-15, https://doi.org/10.1042/bj20021469.
17. Ka, S.; Hwang, H.; Jang, J.; Bang, I.; Bae, U.; Yu, H. The protein kinase 2 inhibitor tetrabromobenzotriazole protects against renal ischemia reperfusion injury. *Science Reports* **2015**, *5*, 14816, https://doi.org/10.1038/srep14816.
18. Hathaway, G.; Lubben, T.; Traugh, J. Inhibition of casein kinase II by heparin. *Journal of Biology Chemistryl*. **1980**, *255,* 8038-8041.
19. Yim, H.; Lee, Y.; Lee, C.; Lee, S. Emodin, an Anthraquinone Derivative Isolated from the Rhizomes of *Rheum palmatum*, Selectively Inhibits the Activity of Casein Kinase II as a Competitive Inhibitor. *Planta Medica* **1999**, *65*, 9-13, https://doi.org/10.1055/s-1999-13953.
20. Cozza, G.; Mazzorana, M.; Papinutto, E.; Bain, J.; Elliott, M.; Di-Maira, G.; Battistutta, R. Quinalizarin as a potent, selective and cell-permeable inhibitor of protein kinase CK2. *Biochemistry Journal* **2009**, *421*, 387-395.
21. Prudent, R.; Moucadel, V.; Nguyen, C.; Barette, C.; Schmidt, F.; Florent, J.; Filhol, O. Antitumor activity of pyridocarbazole and benzopyridoindole derivatives that inhibit protein kinase CK2. *Cancer Research* **2010**, *70,* 9865-9874. https://doi.org/10.1158/0008-5472.CAN-10-0917.
22. Wu, Y.; Tu, X.; Lin, G.; Xia, H.; Huang, H.; Wan, J. Emodin-mediated protection from acute myocardial infarction via inhibition of inflammation and apoptosis in local ischemic myocardium**.** *Life Science* **2010**, *81,* 1332-1338, https://doi.org/10.1016/j.lfs.2007.08.040
23. Kraychy, S. Synthesis of Potential Metabolites of Estradiol. *Journal of American Chemical Society* **1959**, *81,* 1702-1704, https://doi.org/10.1021/ja01516a045
24. Obot, I.; Obi-Egbedi, N. Adsorption properties and inhibition of mild steel corrosion in sulphuric acid solution by ketoconazole: experimental and theoretical investigation. *Corrosion Science* **2010**, *52,* 657-660, https://doi.org/10.1016/j.corsci.2009.09.002.
25. Kirchweger, B.; Kratz, J.; Ladurner, A.; Grienke, U.; Langer, T.; Dirsch, V.; Rollinger, J. In Silico Workflow for the Discovery of Natural Products Activating the G Protein-Coupled Bile Acid Receptor 1. *Frontiers in Chemistry* **2018**, *6*, 1-14, https://doi.org/10.3389/fchem.2018.00242.
26. Figueroa, L.; Diaz, F.; Camacho, A.; Garcia, E.; Pool, E.; Rosas, M. Activity induced by a progesterone derivative on injury by ischemia-reperfusion in an isolated heart model. *African Journal of Pharmacy and Pharmacology* **2013**, *7*, 1957-1968, https://doi.org/10.5897/AJPP12.1137.
27. Sarabia, B.; Figueroa, L.; Díaz, F.; Hau, L.; Rosas, M.; García, E. Activity induced by a naphthalene-prazosin derivative on ischemia/reperfusion injury in rats. *Pharmacology and Pharmacy* **2014**, *5,* 1130-1142, https://doi.org/10.4236/pp.2014.512123.
28. Figueroa, L.; Diaz, F.; Rosas, M.; Pool, E.; Lopez, M.; Sarabia, B. Evaluation of activity exerted by a steroid derivative on injury by ischaemia/reperfusion. *African Journal of Pharmacy and Pharmacology* **2014**, *8*, 157-167, https://doi.org/10.5897/AJPP2013.3908.
29. Hocht, C.; Opezzo, L.; Gorzalczany, S.; Bramuglia, G.; Tiara, C. Una aproximación cinética y dinámica de metildopa en ratas con coartación aórtica mediante microdiálisis. *Revista Argentina de Cardiologia* **1999**, *67*, 769-773.
30. Levov, A.; Le-An, T.; Komarova, A.; Strokina, V.; Soldatenkov, A.; Khrustalev, V. Synthesis and molecular structure of bis (areno) piperidinoaza-14 (17)-crowns-4 (5). *Russian Journal of Organic Chemistry* **2008**, *44*, 456-461, https://doi.org/10.1134/S1070428008030238.
31. Szulczyk, D.; Bielenica, A.; Dobrowolski, M.; Dobrzycki, L.; Krawiecka, M.; Kuran, B. Synthesis and structure evaluation of new complex butylarylpiperazin-1-yl derivatives. *Medicinal Chemistry Research* **2014**, *23*, 1519-1536,

https://doi.org/10.1007/s00044-013-0740-z.
32. Meyer, S.; Hoehner, M.; Lynch, V.; Sessler, J. Synthesis and Characterization of New Non-aromatic Texaphyrin-type Expanded Porphyrins. *Journal of Porphyrines and Phthalocyanines* **1999**, *3,* 148-158, https://doi.org/10.1002/(SICI)1099-1409(199902)3:2<148::AID-JPP111>3.0.CO;2-O.
33. Sharma, R.; Joubert, J.; Malan, S. Synthesis and biological evaluations of NO-donating oxa-and aza-pentacycloundecane derivatives as potential neuroprotective candidates. *Molecules* **2018**, *23*, 308, https://doi.org/10.3390/molecules23020308.
34. Tret'yakova, E.; Flekhter, O.; Galin, F.; Shul'ts, E.; Tolstikov, G. Synthesis of 5-Hydroxy-1, 3-benzoxathiol-2-one and 2-Amino-1, 3-benzothiazol-6-ol Derivatives from Chrysenequinonecarboxylic Acid. *Russian Journal of Organic Chemistry* **2005**, *41*, 828-831, https://doi.org/10.1007/s11178-005-0252-5.
35. Radini, I.; El-Wahab, A. Heteroaromatization with 4-phenyldiazenyl-1-naphthol. Part I: Synthesis of some new naphthopyrans and naphthopyranopyrimidines. *European Journal of Chemistry* **2016**, *7*, 230-237, https://doi.org/10.5155/eurjchem.7.2.230-237.1432.
36. Lauwiner, M.; Roth, R.; Rys, P. Reduction of aromatic nitro compounds with hydrazine hydrate in the presence of an iron oxide/hydroxide catalyst. III. The selective reduction of nitro groups in aromatic azo compounds. *Applied Catalyses A. General* **1999**, *177*, 9-14, https://doi.org/10.1016/S0926-860X(98)00110-0.
37. Pehlivan, L.; Métay, E.; Laval, S.; Dayoub, W.; Demonchaux, P.; Mignani, G. Iron-catalyzed selective reduction of nitro compounds to amines. *Tetrahedron Letters* **2010**, *51*, 1939-1941, https://doi.org/10.1016/j.tetlet.2010.01.067.
38. Raju, B.; Ragul, R.; Sivasankar, B. A new reagent for selective reduction of nitro group. *Indian Journal of Chemistry* **2009**, *48B,* 1315-1318.
39. Bellamy, F.; Ou, K. Selective reduction of aromatic nitro compounds with stannous chloride in non acidic and non aqueous medium. *Tetrahedron Letters* **1984**, *25,* 839-842, https://doi.org/10.1016/S0040-4039(01)80041-1.
40. Cadogan, J.; Cameron, M.; Mackie, R.; Searle, R. The reactivity of organophosphorus compounds. Part XIX. Reduction of nitro-compounds by triethyl phosphite: a convenient new route to carbazoles, indoles, indazoles, triazoles, and related compounds. *Journal Chemistry Society* **1965**, 4831-4837.
41. Corey, E.; Venkateswarlu, A. Protection of hydroxyl groups as tert-butyldimethylsilyl derivatives. *Journal of American. Chemistry Society* **1972**, *94,* 6190-6191, https://doi.org/10.1021/ja00772a043.
42. Hiegel, G.; Hogenauer, T.; Lewis, J. Preparation of N-chloroamides using trichloroisocyanuric acid. *Synthesis Communications*. **2005**, *35,* 2099-2105, https://doi.org/10.1081/SCC-200066703.
43. Katritzky, A.; Majumder, S.; Jain, R. Direct nitration of five membered heterocycles. *ARKIVOC* **2003**, *12*, 74-79.
44. Figueroa, L.; Diaz, F.; Garcia, E. Design and Synthesis of Some Estradiol Derivatives Using an Estradiol dicarbaldehyde as Chemical Tool. *Letters in Organic Chemistry* **2005**, *12,* 699-707, https://doi.org/10.2174/157017861266615091022224 5.
45. Schaus, S.; Brandes, B.; Larrow, J.; Tokunaga, M.; Hansen, K.; Gould, A. Highly selective hydrolytic kinetic resolution of terminal epoxides catalyzed by chiral (salen) CoIII complexes. Practical synthesis of enantioenriched terminal epoxides and 1, 2-diols. *Journal of American Chemistry Society* **2002**, *124*, 1307-1315, https://doi.org/10.1021/ja016737l.
46. Martinez, L.; Leighton, J.; Carsten, D.; Jacobsen, E. Highly enantioselective ring opening of epoxides catalyzed by (salen) Cr (III) complexes. *Journal of American Chemistry Society* **1985**, *117*, 5897-5898, https://doi.org/10.1021/ja00126a048.
47. Walter, W.; Ruback, W. 2, 2, 4-Trisubstituted 2H-1, 3-Oxazetes A New Type of Heterocycles-A Reinvestigation. *Liebigs Annual of Chemistry* **1982**, *2*, 231-239, https://doi.org/10.1002/jlac.198219820206.
48. Houk, K. Frontier molecular orbital theory of cycloaddition reactions. *Account Chemical Research* **1975**, *8,* 361-369, https://doi.org/10.1021/ar50095a001.
49. Ess, D.; Houk, K. Theory of 1, 3-dipolar cycloadditions: distortion/interaction and frontier molecular orbital models. *Journal of American Chemistry Society* **2008**, *130,* 10187-10198, https://doi.org/10.1021/ja800009z.
50. Latosińska, J.; Kasprzak, J.; Kazimierczuk, Z. Effects of chlorination and deoxiribose substitution on electron density distribution in indazole molecule studied by 35Cl NQR spectroscopy and ab initio calculations. *Journal Mololecular Struc-THEOCHEM* **2000**, *530,* 217-222, https://doi.org/10.1016/S0166-1280(00)00338-9.
51. Prasa, Y.; Kumar, P.; Smiles, D.; Babub, P. QSAR studies on chalcone derivatives as antibacterial agents against *Bacillus pumilis* (08-2695KP). *ARKIVOK* **2008**, *2008,* 266-276, http://dx.doi.org/10.3998/ark.5550190.0009.b26.
52. Deng, Z.; Chuaqui, C.; Singh, J. Structural interaction fingerprint (SIFt): a novel method for analyzing three-dimensional protein− ligand binding interactions. *Journal of Medicine. Chemistry* **2004**, *47,* 337-344, https://doi.org/10.1021/jm030331x.
53. Sakkiah, S.; Thangapandian, S.; John, S.; Lee, K. 3D QSAR pharmacophore based virtual screening and molecular docking for identification of potential HSP90 inhibitors. *European Journal of Medicine Chemistry* **2011**, *46,* 2937-2947, https://doi.org/10.1016/j.ejmech.2010.01.016.
54. Sarno, S.; Reddy, H.; Meggio, F.; Ruzzene, M.; Davies, S. Selectivity of 4, 5, 6, 7-tetrabromobenzotriazole, an ATP sitedirected inhibitor of protein kinase CK2 (casein kinase 2). Donella-Deana, *FEBS Lett*. **2001**, *496*, 44-48, https://doi.org/10.1016/S0014-5793(01)02404-8.
55. Ampofo, E.; Widmaier, D.; Montenarh, M.; Menger, M.; Laschke, M. Protein kinase CK2 regulates Leukocyte-endothelial cell interactions during ischemia and reperfusion in striated skin muscle. *European Sergey Research*. **2016**, *57,* 111-124, https://doi.org/10.1159/000446367.
56. Zhou, J.; Zhang, Y.; Song, H.; Ji, H.; Wang, X.; Wang, L. 5d, a novel analogue of 3-n-butylphthalide, decreases NADPH oxidase activity through the positive regulation of CK2 after ischemia/reperfusion injury. *Oncotarget*. **2016**, *7*, 39444-39457, https://doi.org/10.18632/oncotarget.8548.
57. Baltan, S.; Bastian, C.; Quinn, J.; Aquila, D.; McCray, A.; Brunet, S. CK2 inhibition protects white matter from ischemic injury. *Neuroscience Letters* **2018**, *687*, 37-42, https://doi.org/10.1016/j.neulet.2018.08.021.
58. Bitirim, C.; Tuncay, E.; Turan, B. Demonstration of subcellular migration of CK2α localization from nucleus to sarco (endo) plasmic reticulum in mammalian cardiomyocytes under hyperglycemia. *Molecular Cell. Biochemistry* **2018**, *443,* 25-36, https://doi.org/10.1007/s11010-017-3207-6.
59. Figueroa, L.; Diaz, F.; Rosas, M.; Mateu, V.; Pool, E.; Lopez, M.; Hau, L.; Alfonso, A.; Cabrera, J. Preparation of a steroid-oxazole-1,2′-[1,3]oxazete] derivative: biological and theoretical evaluation of its interaction with a kinase protein (CK2). *SN Applied Sciences* **2019**, *1*, https://doi.org/10.1007/s42452-019-0378-7.
60. Motro, M.; Shemesh, J. Calcium channel blocker nifedipine slows down progression of coronary calcification in

hypertensive patients compared with diuretics. *Hypertension* | **2001**, *37*, 1410-1413, https://doi.org/10.1161/01.hyp.37.6.1410.

6. ACKNOWLEDGEMENTS

To Benjamin Valverde and Raquel Anzurez, for your unconditional support on this manuscript. We declare that this manuscript does not have any conflict of financial interests (political, personal, religious, ideological, academic, intellectual, commercial or otherwise) for its publication.