Asian Journal of Chemistry; Vol. 23, No. 4 (2011), 1599-1604

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

# Synthesis of a New Inotropic Steroid Derivative and its Relationship with log P, π, $R_m$, $V_m$, $P_c$ and $S_t$

FIGUEROA-VALVERDE LAURO[1,*], DÍAZ-CEDILLO FRANCISCO[2], LOPEZ-RAMOS MARÍA[1], GARCÍA-CERVERA ELODIA[1], K.A. QUIJANO-ASCENCIO[1] and J.C. CORDOBA-VAZQUEZ[1]

[1]Laboratorio de Investigación de la Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Campeche, Av. Agustín Melgar, Col Buenavista C.P. 24039 Campeche Cam., México
[2]Laboratorio de Química Orgánica de la Esc. Nal. de Ciencias Biológicas del Instituto Politécnico Nacional. Prol. Carpio y Plan de Ayala s/n Col. Santo Tomas, D.F. C.P. 11340, México

*Corresponding author: Fax: +981 8119800 Ext. 73099; Tel: +981 8119800 Ext. 73006; E-mail: lauro_1999@yahoo.com

(*Received*: 14 June 2010; *Accepted*: 7 December 2010) AJC-9367

In this work, a new inotropic steroid derivative was synthesized. The route involved preparation of furosemide-pregnenolone derivative by the reaction of furosemide with pregnenolone-succinate-ethylenediamine conjugate using a carbodiimide as catalyst. The biological activity of furosemide-pregnenolone and pregnenolone-succinate-ethylenediamine was evaluated in isolated rat hearts using Langendonff model. To delineate the structural chemical requirements of furosemide-pregnenolone as inotropic agent, other parameters such as the physicochemical descriptors log P, π, $R_m$, $V_m$, $P_c$ and $S_t$ were calculated. The results showed that the furosemide-pregnenolone derivative significantly increase the perfusion pressure ($p = 0.006$), coronary resistance ($p = 0.005$) and intracellular calcium ($1.35 \times 10^{-3}$ to $2.70 \times 10^{-3}$ mM) in isolated heart. Additionally, other data indicate that values of log P, π, $R_m$, $V_m$, $P_c$ and $S_t$ are high in the furosemide-pregnenolone in comparison with pregnenolone-succinate-ethylenediamine. These data suggest that functional groups involved in the structure of furosemide-pregnenolone are specific for their positive inotropic activity.

**Key Words: Pregnenolone, Langendonff model, Physicochemical, Descriptors.**

## INTRODUCTION

There are reports which indicate that congestive heart failure (CHF) is a mainly cause of death in patients with heart disease[1-3]. Several drugs have been used for the treatment of CHF such as the digitalis glycosides. Unfortunately the use of these agents is limited by their narrow therapeutic window and their propensity to cause life-threatening arrhythmias[4,5]. In this sense, there has been a resurgence of interest in cardiotonic steroids derivatives. It is important to mention that these molecules exert a large number of effects in cardiac tissue[6,7]. For example, low concentrations of strophanthidin (steroid derivative) induce negative inotropic activity by diminish the intracellular calcium ($[Ca^{2+}]i$) in a cardiac action potential model, nevertheless a positive inotropic effect occurred with higher doses[8]. There are studies that show the synthesis of other steroid derivative (F90927) which exerts a positive inotropic activity in cardiac muscle *via* activation of the L-type $Ca^{2+}$ channel[9]. Additionally, a series of steroid derivatives[10,11] were synthesized and showed a positive inotropic effect, mainly by inhibition of $Na^+$, $K^+$-ATPase. Other reports indicate that 14β-hydroxyprogesterone[12] induces increase the contractility of isolated cardiac tissue *via* glycoside receptor.

To delineate the structural chemical requirements of some inotropic steroid derivatives has been used the QSAR (quantitative structure-activity) study, for example a series of 17β-(hydrazonomethyl)-5β-androstane-3β,14β-diol were synthesized and the molecular orbital package model was used to evaluate the relation of some physicochemical parameters with changes in the inotropic activity induced by the steroid derivatives[13]. Other reports[14] show the inotropic effect of a series of steroid derivatives, in addition of the chemical features involved in the biological activity using the comparative molecular field analysis (CoMFA). All these data, show several protocols to evaluate the inotropic activity of several steroid derivatives. Nevertheless some physicochemical descriptors such as log P, π, $M_V$ (molar volume), $M_R$ (molar refractivity), parachor ($P_c$) and surface tension ($S_t$) has not been evaluated with clarity in the inotropic activity induced by some steroids. Therefore, in this work we report a straightforward route for the synthesis of furosemide-pregnenolone derivative and characterize its inotropic effect in an isolated rat heart model. Additionally, the physicochemical parameters such as log P, π, $M_V$, $M_R$, $P_c$ and $S_t$ were evaluated to characterize its relationship with the inotropic activity induced by the steroid derivative.

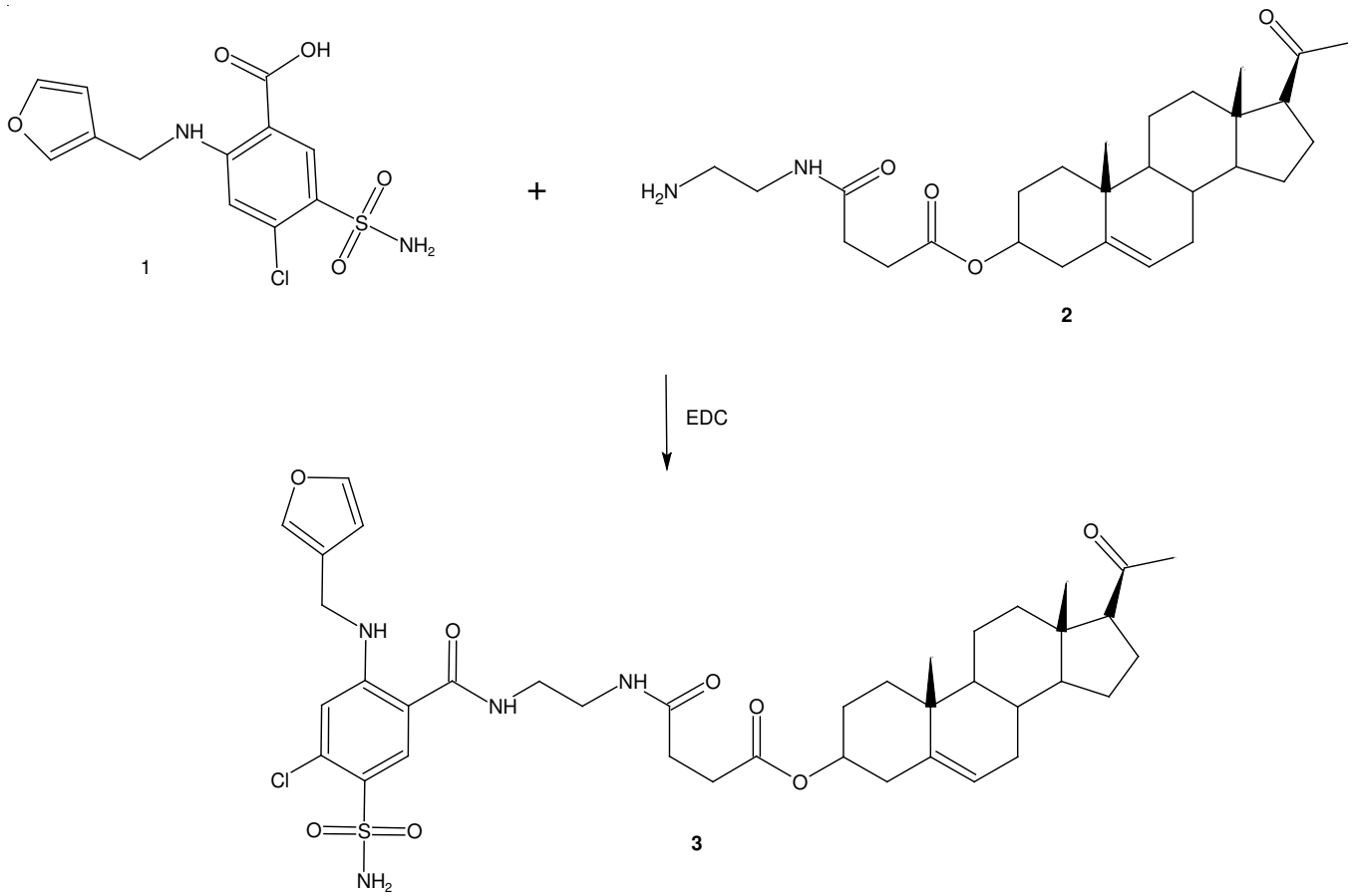
## EXPERIMENTAL

Pregnenolone-succinate-ethylendiamine (**2**) was prepared according to reported method[15]. The other compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting points for the different compounds were determined on an Electrothermal (900 model). Infrared spectra (IR) was recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

**N-[2-{4-Chloro-2-[(furan-3-ylmethyl)amino]-5-sulfamoyl-benzoylamino}-ethyl)-succinamic acid 17-isopropenyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15, 16, 17-tetra-decahydro-1*H*-cyclopenta[a]phenanthren-3-yl ester (3):** A solution of furosemide (**1**) [100 mg, 0.30 mmol], pregnenolone-succinate-ethylendiamine (**2**) [200 mg, 0.42 mmol] and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide 120 mg (0.62 mmol) in acetonitile:water 10 mL (2:1) was stirring by 48 h at room temperature (**Scheme-I**). After the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1) yielding 75 mg of product; m.p. 198-200 ºC; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3328, 1738, 1642. $^{1}H$ NMR (300 MHz, $CDCl_3$) $\delta_H$: 0.64 (s, 3H), 1.04 (s, 3H), 1.04-1.16 (m, 2H), 1.25-1.49 (m, 5H), 1.57-1.77 (m, 5H), 1.86-1.99 (m, 2H), 2.05-2.08 (m, 2H), 2.10 (s, 3H), 2.21 (m, 1H), 2.30-2.38 (m, 2H), 2.42 (t, 2H, *J* = 6.4 Hz), 2.50 (s, 1H), 2.54 (t, 2H), 3.42 (t, 2H, *J* = 6.3 Hz), 3.52 (t, 2H), 4.53 (m, 1H), 4.60 (s, 2H), 5.46 (m, 1H), 6.20 (d, 1H, *J* = 2.0 Hz), 7.03-7.25 (s, 2H), 7.83 (s, 5H), 8.07 (s, 1H) ppm. $^{13}C$ NMR (75.4 MHZ, $CDCl_3$) $\delta_C$: 13.17 (C-50), 19.48 (C-49), 21.90 (C-41), 22.86 (C-46), 23.61 (C-53), 27.80 (C-33), 29.55 (C-28), 31.40 (C-47), 31.81 (C-37), 31.87 (C-45), 32.03 (C-27), 37.03 (C-34), 37.75 (C-22), 38.22(C-35), 38.80 (C-42), 38.86 (C-40), 39.44 (C-6), 43.50 (C-23), 43.90 (C-39), 50.03 (C-36), 56.78 (C-38), 63.60 (C-48), 73.90 (C-32), 108.45 (C-4), 122.51 (C-9), 122.65 (C-44), 128.02 (C-13), 128.57 (C-3), 130.78 (C-12), 134.43 (C-11), 136.66 (C-10), 139.90 (C-2), 140.07 (C-43), 140.36 (C-5), 143.90 (C-8), 170.08 (C-17), 171.38 (C-25), 171.90 (C-29), 209.20 (C-51) ppm. EI-MS, m/s 770.10 ($M^+$, 11), 298.29(45), 265.20 (55), 91.16 (100). Anal. calcd. (%) for $C_{39}H_{51}N_4O_8SCl$; C, 60.73, H, 6.66, Cl, 4.60, N, 7.26, O, 16.59, S, 4.16. Found (%): C, 60.67, H, 6.98.

**Biological method:** All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of Universidad Autonoma de Campeche (UAC) and were in accordance with the Guide for the Care and Use of Laboratory Animals[16]. Male rats (Wisstar; weighing 200-250 g) were obtained from UAC.

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




**Scheme-I:** Synthesis of furosemide-pregnenolone derivative (**3**). Reaction of furosemide (**1**) with pregnenolone-succinate-ethylendiamine (**2**) to form **3** using 1-ethyl-3-(3-dimethylamino- propyl)carbodiimide (EDC) as catalyst in acetonitrile/water

**Langendorff method:** In brief, 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 non-cardiac tissue and retrograde perfused *via* a non-circulating perfusion system at a constant flow rate. It is important to mention that perfusion medium was the Krebs-Henseleit solution (pH 7.4, 37 ºC) composed of (mM); 117.8 NaCl; 6 KCl; 1.75 $CaCl_2$; 1.2 $NaH_2PO_4$; 1.2 $MgSO_4$; 24.2 $NaHCO_3$; 5 glucose and 5 sodium pyruvate. The solution was actively bubbled with a mixture of $O_2/CO_2$ (95:5). 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 25 min equilibration period at a perfusion rate of 10 mL/min. All experimental measurements were done after this equilibration period.

**Perfusion pressure:** Evaluations of measurements of perfusion pressure changes induced by drugs application in this study were 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).

**Biological evaluation**

**Effect induced by the compounds 2 and 3 on perfusion pressure:** Changes in perfusion pressure as a consequence of increases in time (3-18 min) in absence (control) or presence of **2** and **3** at a concentration of $1 \times 10^{-9}$ mM were determined. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 mL/min.

**Effect induced by 2 and 3 on the concentration of intracellular calcium:** All mixtures used to evaluation of calcium were obtained with the perfused solution in several lapses of time (3-18 min) in absence (control) or presence of **2** and **3** at a concentration of $1 \times 10^{-9}$ mM. It is important to mention that the intracellular calcium was evaluated with the colorimetric method reported by Rhoe and Khan[17].

**Statistical analysis:** The obtained values are expressed as average ± SE, using each heart as its own control. The data obtained were put under an analysis of variance (ANOVA) using the Bonferroni correction factor[18]. The differences were considered significant when $p$ was equal or smaller than 0.05.

**QSAR study:** In study, physicochemical descriptors such as log P, $\pi$, $R_m$, $V_m$, $P_c$ and $S_t$ were evaluated using the methods reported by Mannhold, Waterbeemd, Petrauskas, Kolovanov[19,20].

## RESULTS AND DISCUSSION

**Chemical evaluation:** In this work we report a straightforward route for the synthesis of pregnenolone-furosemide derivative (**3**). This procedure was achieved by reacting furosemide (**1**) with pregnenolone-succinate-ethylendiamine (**2**) to form **3**, since the nature of functional groups contained in the chemical structure of this compound involves an amide group in the spacer arm between the furosemide freagment and the steroid nucleus of **3** (**Scheme-I**). It is important to mention that many procedures for the formation of amide groups are known in the literature. The most widely practiced method employs carboxylic acid chlorides as the electrophiles[21] which react with the amino group in the presence of an acid scavenger. Despite its wide scope, the former protocol suffers from several drawbacks; most notable are the limited stability of many acid chlorides and the need for hazardous reagents[22] for their preparation (thionyl chloride), therefore, in this study a derivative of carbodiimide[23] was used to form **3**.

$^1$H NMR spectra of **3** showed chemical shifts at 0.64, 1.04 and 2.10 ppm corresponding to methyls presents in the steroid nucleus. Additionally, other signals at 2.42-3.52 for methylenes involved in spacer arm between steroid nucleus and furosemide fragment were found. A signal at 4.60 for methylene bound to furan-ring was displayed. Finally, other signal at 7.83 ppm for both amine and amide groups was found. The $^{13}$C NMR spectra display chemical shifts at 13.17, 19.48 and 23.61 ppm for the carbons of methyls presents in the steroid nucleus of compound **3**. Another chemical shifts at 21.90-73.90 ppm for carbons of methylenes involved in the steroid nucleus were exhibited. Several signals at 29.55, 30.07, 38.86 and 39.44 ppm for carbons corresponding to methylenes involved in the spacer arm involved between furosemide fragment and pregnenolone nucleus were found. Additionally, other chemical shifts at 122.51-143.90 ppm for phenyl ring; at 108.45-140.36 ppm for furan-ring were display. Finally, other signals at 170.08 and 171.38 ppm for amide groups; at 171.90 ppm for ester group and at 209.20 for ketone group were exhibited. The presence of **3** was further confirmed from mass spectrum which showed a molecular ion at m/z 770.10.

**Biological analyses:** In this study, the effect induced by pregnenolone-succinate-ethylendiamine (**2**) and pregnenolone-furosemide derivative (**3**) on the blood vessel capacity and coronary resistance translated as changes in perfusion pressure and inotropic activity in isolated rat heart (Langendorff model) were evaluated. The results obtained from changes in perfusion pressure and inotropic activity as a consequence of increases in the time (3-18 min) in absence (control) or in presence of **2** and **3** (Fig. 1), showed that **3** [$1 \times 10^{-9}$ mM] significantly increase the perfusion pressure ($p = 0.006$) in comparison with the control conditions and **2** [$10^{-9}$ mM]. It is important to mention here that the concentration applied [$1 \times 10^{-9}$ mM] is the minimal dose to produce a biological effect by the compound studied as happens to another type of steroids[24-26]. Those experimental data indicate that **3** compound exert effects on perfusion pressure and induce changes in the inotropic activity, which could consequently bring modifications in vascular tone and coronary resistance. In order to verify this hypothesis, the effects induced by both **2** and **3** on coronary resistance were evaluated.

The results showed that coronary resistance, calculated as the ratio of perfusion pressure at coronary flow assayed (10 mL/min) was higher in the presence of **3** ($p = 0.005$) at a concentration of $1 \times 10^{-9}$ mM (Fig. 2) in comparison with **2** and control conditions. These experimental data suggest that changes in the chemical structure of **2** to form **3** induce a greater effect on the vascular tone and inotropic activity. In order to characterize the molecular mechanism of this phenomenon and analyzing the effect induced by some steroids on blood pressure *via* calcium channel[27], in this study was evaluated the possibility that inotropic activity exerted by **3** could involve increase of calcium levels. Therefore, the effect induced by **3** at concentrations of $1 \times 10^{-9}$-$1 \times 10^{-4}$ mM, in absence or

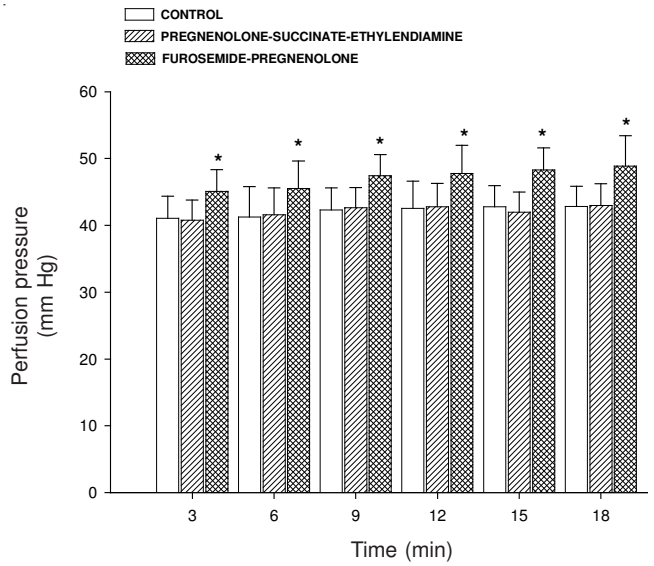



Fig. 1. Effect induced by progesterone and furosemide-pregnenolone derivative on perfusion pressure. The results showed that furosemide-pregnenolone [$1 \times 10^{-9}$ mM] significantly increase the perfusion pressure ($p = 0.006$) through of time (3-18 min) in comparison with the control conditions and pregnenolone-succinate-ethylendiamine [$1 \times 10^{-9}$ mM]. Each bar represents the mean ± SE of 9 experiments

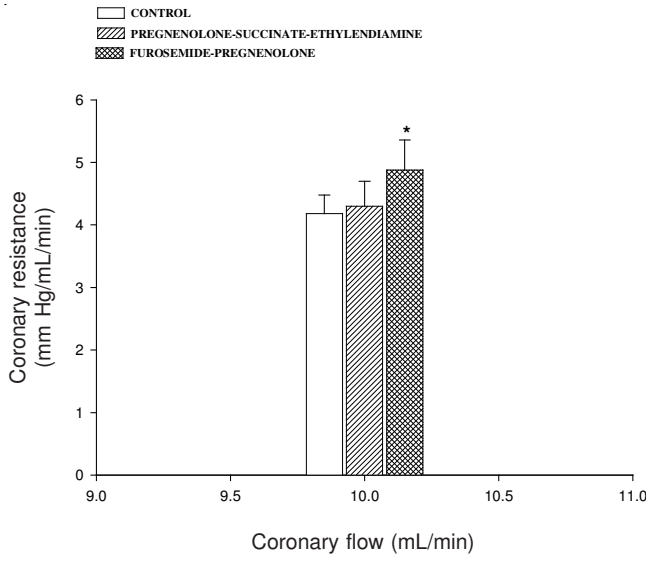



Fig. 2. Activity induced by furosemide-pregnenolone derivative on coronary resistance. The results showed that coronary resistance was higher ($p = 0.005$) in presence of furosemide-pregnenolone [$1 \times 10^{-9}$ mM] in comparison with the control conditions and pregnenolone-succinate-ethylendiamine [$1 \times 10^{-9}$ mM]. Each bar represents the mean ± SE of 9 experiments

presence of nifedipine [$1 \times 10^{-6}$ mM] on perfusion pressure was evaluated. The results showed that activity of **3** in presence of nifedipine (Fig. 3) was blocked significantly ($p = 0.005$). These results indicate that inotropic activity exerted by steroid-derivate on perfusion pressure could involve increase of calcium trough of activation L-type calcium channel. In order to evaluate this premise, in this study the intracellular calcium was determinate by means of the method reported by Rhoe and Khan[17]. It is important to mention here that intracellular calcium was evaluated in presence or absence of the compounds **2** and **3** at

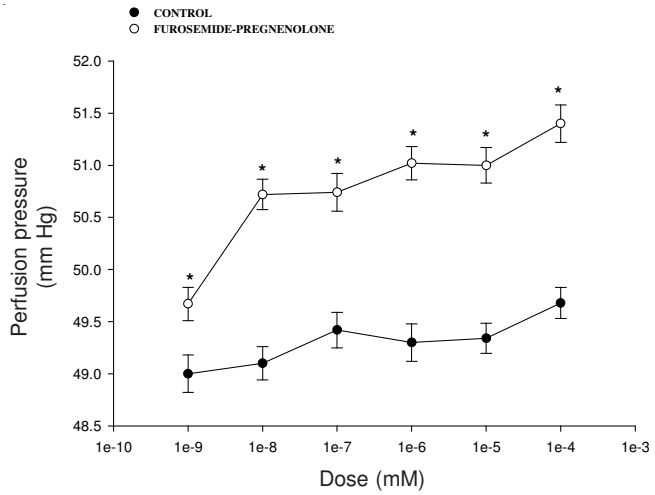



Fig. 3. Effects induced by furosemide-pregnenolone derivative on perfusion pressure through L-type calcium channel. The boluses (50 µL) of furosemide-pregnenolone [$1 \times 10^{-9}$-$1 \times 10^{-4}$ mM] were administered in absence and presence of nifedipine [$1 \times 10^{-6}$ mM]. The results showed that effect induced by furosemide-pregnenolone on perfusion pressure in presence of nifedipine was significantly inhibited ($p = 0.005$). Each bar represents the mean ± SE of 9 experiments

a dose of $1 \times 10^{-9}$ mM (minimal dose) as a consequence of increases in the time (3-18 min). The results (Table-1) indicate that the intracellular calcium was high in presence of **3** in comparison with **2** and conditions control. All data suggest that the biological activity of **3** increase the perfusion pressure and induce a positive inotropic activity by increase the concentration of intracellular calcium. These phenomenon depends of the chemical structure of **3**.

TABLE-1
INTRACELLULAR CALCIUM LEVELS ($[Ca_i^{2+}]$) OF THE **2** AND **3** COMPOUNDS AND CONTROL CONDITIONS. THE OBTAINED VALUES ARE EXPRESSED AS MEAN ± SE

| Time (min) | $[Ca_i^{2+}]$ (mM) | | |
|---|---|---|---|
| | Control | **2** | **3** |
| 3 | $6.75 \times 10^{-4} \pm 2.3$ | $6.75 \times 10^{-4} \pm 1.4$ | $1.35 \times 10^{-3} \pm 2.1$ |
| 6 | $6.75 \times 10^{-4} \pm 1.8$ | $6.75 \times 10^{-4} \pm 1.6$ | $1.35 \times 10^{-3} \pm 2.4$ |
| 9 | $6.75 \times 10^{-4} \pm 2.5$ | $6.75 \times 10^{-4} \pm 1.3$ | $2.03 \times 10^{-3} \pm 1.3$ |
| 12 | $6.75 \times 10^{-4} \pm 1.3$ | $6.75 \times 10^{-4} \pm 2.4$ | $2.03 \times 10^{-3} \pm 2.2$ |
| 15 | $6.75 \times 10^{-4} \pm 3.1$ | $6.75 \times 10^{-4} \pm 1.6$ | $2.70 \times 10^{-3} \pm 1.5$ |
| 18 | $6.75 \times 10^{-4} \pm 1.4$ | $6.75 \times 10^{-4} \pm 2.1$ | $2.70 \times 10^{-3} \pm 2.3$ |

**QSAR analyses:** It is important to note that positive inotropic activity could have a relation with some physico-chemical parameters involved in the chemical structure of **3**. This presumption is supported by some reports which indicate that biological activity induced for another type of steroid derivatives can have a relationship with several molecular descriptors[28] such as log P, π, $M_V$ (molar volume), $M_R$ (molar refractivity), parachor ($P_c$) and surface tension ($S_t$). Therefore, in this study these physicochemical parameters involved in the chemical structure of **3** were evaluated. With respect to log P and π, it is important to mention that log P describes the logarithmic octanol-water partition coefficient. Therefore, it represents the lipophilic effects of a molecule that includes the sum of the lipophilic contributions of the parent molecule and its substituents[29]. The difference between the substituted

and unsubstituted log P values is conditioned by the π value for a particular substituent. Hammett showed that π values measure the free energy change caused by a particular substituent to relate to biological activity[30]. Therefore, in this work, the log P and π parameters were calculated by the method reported by Mannhold and Waterbeemd[20]. The results (Tables 2 and 3) showed an increase in log P and π values in compounds **3** with respect to **2**. This phenomenon is conditioned mainly by the contribution of all substituent atoms involved in the chemical structure of the different compounds. These results showed that aliphatic carbon, aromatic carbon and aromatic chlorine in compound **3** contribute to the high lipophilicity in comparison with **2**. All data indicate that an increase in the degree of lipophilicity could affect the inotropic activity of **3**. Nevertheless, it is important to mention that exist other steric constants such as the molar volume ($V_m$) and molar refractivity ($R_m$) that can affect the inotropic activity of **3**. These options are useful tool for the correlation of different properties that depend on characteristics of substituents attached to a constant reaction center[31,32]. Therefore in present study, both $V_m$ and $R_m$ descriptors were evaluated using the ACDLabs program[19,20]. The results showed an increase in both $R_m$ and $V_m$ values for **3** in comparison with **2** (Table-4). These data indicate that steric impediment, conformational preferences and internal rotation of **3** could influence the degree of lipophilicity and the inotropic activity of this compound.

TABLE-2
PHYSICOCHEMICAL PARAMETERS log Kow AND π OF PREGNENOLONE-SUCCINATE-ETHYLEN-DIAMINE (**2**)

| Compound | log Kow fragment | Contributions |
|---|---|---|
| **2** | $-CH_3$ [aliphatic carbon] | 1.6419 |
| | $-CH_2-$ [aliphatic carbon] | 5.8932 |
| | -CH [aliphatic carbon] | 1.8070 |
| | =CH- or =C< [olefinc carbon] | 0.7672 |
| | $-NH_2$ [aliphatic attach] | -1.4148 |
| | -NH- [aliphatic attach] | -1.4962 |
| | -C(=O)- [carbonyl, aliphatic attach] | -1.5586 |
| | -C(=O)O [ester, aliphatic attach] | -0.9505 |
| | -C(=O)N [aliphatic attach] | -0.5236 |
| | *-tert* Carbon [3 or more carbon attach] | 0.5352 |
| | Fused aliphatic ring unit correction | -2.0526 |
| | Equation constant | 0.2290 |
| | log Kow | 2.8772 |
| | π | -1.7629 |

TABLE-3
PHYSICOCHEMICAL PARAMETERS log Kow AND π OF FURESEMIDE-PREGNENOLONE (**3**)

| Compound | log Kow fragment | Contributions |
|---|---|---|
| **2** | $-CH_3$ [aliphatic carbon] | 1.6419 |
| | $-CH_2-$ [aliphatic carbon] | 6.3843 |
| | -CH [aliphatic carbon] | 1.8070 |
| | =CH- or =C< [olefinc carbon] | 0.7672 |
| | $-NH_2$ [aliphatic attach] | -1.4148 |
| | -NH- [aliphatic attach] | -2.9924 |
| | Aromatic carbon | 2.9400 |
| | -Cl [chlorine, aromatic attach] | 0.6445 |
| | -N [aliphatic N, one aromatic attach] | -0.9170 |
| | -C(=O)- [carbonyl, aliphatic attach] | -1.5586 |
| | -C(=O)O [ester, aliphatic attach] | -0.9505 |
| | -C(=O)N [aliphatic attach] | -0.5236 |
| | -C(=O)N [aromatic attach] | 0.1599 |
| | $-SO_2-N$ [aromatic attach] | -0.2079 |
| | Aromatic oxygen | -0.0423 |
| | *-tert* Carbon [3 or more carbon attach] | 0.5352 |
| | Fused aliphatic ring unit correction | -2.0526 |
| | Ring reaction -> ortho amino-type/-C(=O)N | 0.6194 |
| | Aromatic-C-N-aromatic correction | -0.3000 |
| | Equation constant | 0.2290 |
| | log Kow | 4.7687 |
| | π | 0.1286 |

TABLE-4
*PHYSICOCHEMICAL PARAMETERS OF BOTH **2** AND **3** COMPOUNDS

| Compound | $R_m$ ($cm^3$) | $V_m$ ($cm^3$) | $P_c$ ($cm^3$) | $S_t$ (dyne/cm) |
|---|---|---|---|---|
| **2** | 128.65 ± 0.4 | 400.3 ± 5.0 | 1053 ± 6.0 | 48.0 ± 5.0 |
| **3** | 200.07 ± 0.4 | 571.1 ± 5.0 | 1611 ± 6.0 | 63.4 ± 5.0 |

*$R_m$ (molar refractivity), $V_m$ (molar volume), $P_c$ (parachor) and $S_t$ (surface tension).

It is important to mention that there are reports which suggest that $V_m$ is directly related to parachor ($P_c$) and surface tension ($S_t$) which are cumulative effects of the different intra- and intermolecular forces involved in the structural chemistry of some compounds[33,34]. Therefore, in this study these physicochemical descriptors were evaluated using the same ACDLabs program. The results indicate that both values of $P_c$ and $S_t$ for **3** were high in comparison with **2** (Table-4). These data indicate that this parameters can also conditioned the degree of lipophilicity and consequently the inotropic activity of **3**.

## Conclusion

We report an easy methodology to synthesize a furosemide-pregnenolone derivative (**3**). The nature of functional groups contained in the chemical structure of this compound involves an amide group in the spacer arm between the furosemide fragment and the steroid nucleus of **3**. Additionally, **3** showed a positive inotropic activity through of increase the calcium levels. It is important to mention that inotropic activity induced by **3** showed a relationship with physicochemical descriptors such as log P, π, $R_m$, $V_m$, $P_c$ and $S_t$.

## REFERENCES


1. S. Katz, M.L. Hediger, B.S. Zemel and J.S. Parks, *Hypertension*, **8**, 277 (1986).
2. H. Schunkert, W. Hense, T. Andus, A. Riegger and R. Straub, *Am. J. Hyperten.*, **12**, 1140 (1999).
3. E. Braunwald and M.R. Bristow, *Circulation*, **102**, 14 (2000).
4. J.R. Kersten, M.W. Montgomery, P.S. Pagel and D.C. Warltier, *Anesth. Analg.*, **90**, 5 (2000).
5. D. Silverberg, *J. Am. Coll. Cardiol.*, **35**, 1737 (2000).
6. H. Lullman and J. Peters, *Prog. Pharmacol.*, **2**, 1 (1979).
7. D. Noble, *Cardiovasc. Res.*, **9**, 495 (1980).
8. G. Hart, D. Noble and Y. Shimoni, *J. Physiol.*, **334**, 103 (1983).
9. C. Pignier, M. Keller, B. Vié, B. Vacher, M. Santelli, E. Niggli, M. Egger and B. Le Grand, *Br. J. Pharmacol.*, **147**, 772 (2006).
10. M. Gobbini, P. Barassi, A. Cerri, S. De Munari, G. Fedrizzi, M. Santagostino, A. Schiavone, M. Torri and P. Melloni, *J. Med. Chem.,* **44**, 3821 (2001).
11. S. De Munari, A. Cerri, M. Gobbini, N. Almirante, L. Banfi, G. Carzana, P. Ferrari, G. Carazzi, R. Micheletti, A. Schiavone, S. Sputore, M. Torri, M. Zappavigna and P. Melloni, *J. Med. Chem.*, **46**, 3644 (2003).
12. J.F. Templeton, V.P. Kumar, D. Cote, D. Bose, D. Elliott, R.S. Kim and F.S. LaBella, *J. Med. Chem.*, **30**, 1502 (1987).

13. L. Quadri, A. Cerri, P. Ferrari, E. Folpini, M. Mabilia and M.P. Melloni, *J. Med. Chem*., **39**, 3385 (1996).
14. D.C. Farr, C. Burd, M.R. Tabet, X. Wang, W. Welsh and W. Ball, *Biochemistry*, **41**, 1137 (2002).
15. L. Figueroa-Valverde, F. Díaz-Cedillo, L. Tolosa, G. Maldonado and G. Ceballos-Reyes, *J. Mex. Chem. Soc*., **50**, 42 (2006).
16. K. Bayne, *Physiologist*, **39**, 208 (1996).
17. H.J. Roe and S.B. Khan, *J. Biol. Chem*., **81**, 1 (1928).
18. C. Hocht, L. Opezzo, S. Gorzalczany, G. Bramuglia and C. Tiara, Rev. *Argent. Cardiol*., **67**, 769 (1999).
19. A. Petrauskas and A. Kolovanov, *Persp. Drug Discov. Design*, **19**, 99 (2000).
20. R. Mannhold and H. Waterbeemd, *J. Comput. Aided Mol. Design*, **15**, 337 (2001).
21. A. Medvedeva, M. Andreev, L. Safronova, G. Sarapulova and A. Afonin, *Arkivoc*, 143 (2001).
22. D. Levin, *Org. Process. Res. Dev*., **1**, 182 (1997).
23. N.S. DeSilva, I. Ofek and E. Crouch, *Am. J. Respir. Cell. Mol. Biol*., **29**, 757 (2003).
24. L. Figueroa, F. Díaz, A. Camacho, E. Díaz and R. Marvin, *Biomédica*, **29**, 625 (2009).
25. L. Figueroa-Valverde, G. Ceballos-Reyes, F. Díaz-Cedillo, A. Camacho-Luis, M. López Ramos and G. Maldonado-Velazquez, *Afr. J. Pharm. Pharmacol*., **4**, 170 (2010).
26. L. Figueroa-Valverde, E. Diaz-Ku, F. Díaz-Cedillo, C. Baqueiro-Bricaire and A. Camacho-Luis, *Acta Bioquím. Clín. Latinoam*, **44**, 37 (2010).
27. L. Figueroa-Valverde, F. Díaz-Cedillo, E. Diaz-Ku and A. Camacho-Luis, *Afr. J. Pharm. Pharmacol.*, **3**, 234 (2009).
28. L. Figueroa-Valverde, F. Díaz-Cedillo, A. Camacho-Luis, M. López Ramos and E. García- Cervera, *Monatsh Chem*., **141**, 373 (2010).
29. A. Leo, P.Y. Jow, C. Silipo and C. Hansch, *J. Med. Chem*., **18**, 865 (1975).
30. C. Hansch, A. Leo and R.W. Taft, *Chem. Rev*., **91**, 165 (1991).
31. K. Yoshida, T. Shigeoka and F. Yamauchi, *Ecotox. Environ. Safety*, **7**, 558 (1983).
32. K.L. Schnackenberg and D.R. Beger, *J. Chem. Inf. Model*., **45**, 360 (2005).
33. A. Thakur, *Arkivoc*, 49 (2005).
34. V. Dimova and N. Perišic-Janjic, *Macedonian J. Chem. Chem. Engin*., **28**, 79 (2009).