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Design and Synthesis of New Pirrol-Indol Derivative with Positive Inotropic Activity

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ABSTRACT

There are studies which indicate that some heterocyclic derivatives have inotropic effect; nevertheless, its inotropic activity is very confusing perhaps to difference in the chemical structure. In order, to clarify these phenomena in this study, some pirrol-indol derivatives (compounds 3, 4, 5 and 6) were synthesized with the objective of to evaluate its biological activity using a biological model. The results indicate that only the compound 4 induce a positive inotropic activity in comparison with the compound 3, 5 and 6. This datasuggest that the different groups involved in the chemical structure of 4 arethe responsible of its positive inotropic activity. In addition, this phenomenon its conditioned by some physicochemical parameters such as LogP, σ, V_m, R_m, P_c and S_t,which are cumulative effects of the different intra-and intermolecularforces involved in the structural chemistry of the compound 4.

Key words: Synthesis, pirrol, índol, derivative.

INTRODUCTION

Since several years ago a series of positive inotropic drugs have been developed; for example the synthesis of compound (-)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl) amino]-ethanol (TA-064) which exerts a positive inotropic activity in isolated guinea pig heart[1]. Other

studies indicate that the compounds MDL 17,043 [1,3-dihydro-4-methyl-5-[4-(methylthio)-benzoyl]-2H-imidazol-2-one] and AR-L 115 BS [sulmazole, 2-[(2-methoxy-4-methylsulfinyl)phenyl]-1H-imidazo[4,5-b] pyridine] exerts positive inotropic effects in an isolated canine ventricular trabeculae model[2]. In addition, other data indicate that a dihydropyridine derivative (Bay k 8644) induce positive inotropic activity in cells myocardial through of calcium channels activation[3].

On the other hand, a series of heterociclyc derivatives have been prepared with inotropic activity; for example, the compounds 3-alkyl-4-aryl-l,5-dihydro-W-pyrrol-2-ones were prepared as potential inhibitors of cardiac cAMP phosphodiesterase[4]. Other data showed the compound DPI 201-106 (piperazinyl-indol derivative) induce positive inotropic effects on left atria of rat using an isolated rat heart model[5]. In addition, other compound (1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2H-indol-2-one) was syn- thesized and its inotropic effect on an *in vivo* animal model was evaluated[6]. Other results showed the synthesis of 4,5-dihydro-6-(IH-ind01-5-yl)pyridazin-3(2H)-ones which induces a positivity inotropic activity by changes of blood pressure and heart rate in an animal model[7]. Also a series of indol derivatives (5H-Pyridazino[4,5-b]indole) were prepared and its positive inotropic activity was evaluated on the enzymatic activity of phosphodiesterase-IV which was isolated from dog heart[8]. Other data indicate the synthesis of the compound 3,9-dioxopyrrolo[1,2-a]indole derivatives and the evaluating of its cardiotonic activity using isolated spontaneously contracting right atria from guinea pigs[9]. All these data show that some heterocyclic derivatives induce inotropic effects in the cardiovascular system; nevertheless, the cellular site and molecular mechanism involved in its inotropic activity are very confusing, perhaps this phenomenon is due to differences in the chemical structure of both pirrol andindolderivatives or to the different pharmacological approaches used. Therefore, more data are needed to characterize the activity induced by theseheterocyclic derivatives at cardiovascular level. To provide this information, the present study was designed to investigate the effects of a pirrol-indol derivative on perfusion pressure in isolated rat hearts using the Langendorff technique. In addition, to evaluate the chemical characteristic involved in the activity induced by the pirrol-indol derivative on perfusion pressure were used some physicochemical parameters such as LogP, ð, V_m, R_m, P_c and S_t.

EXPERIMENTAL

The 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) were 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.

Synthesis of pyrrolo[3,2-*f*]indole-1,7-diol (3)

A solution of 3,5-dinitrobenzoic acid (100 mg, 0.47mmol), 1-hexyne(70 µl, 0.55mmol), potassium carbonate anhydride (25 mg, 0.18 mmol) in 10 ml of toluenewas stirring for 48 h to reflux. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (4:1) yielding 66 % of product, m.p. 88-90°C; IR (V_{max}, cm^{-1}):3380 and 3200;^{1}H NMR (300 MHz, $CDCl_3$) d_H: 6.10 (m, 2H), 7.16 (m, 1H), 7.34 (m, 1H), 7.70 (m, 2H), 10.24 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) d_C: 103.82 (C-9, C-11), 104.08(C-12), 110.20(C-4), 125.12(C-3, C-5), 131.30(C-2, C-6), 133.14(C-8, C-10) ppm.EI-MS *m/z:* 188.05 (M^+ 11). Anal. Calcd. for $C_{10}H_8N_2O_2$: C, 63.82; H, 4.28; N, 14.89; O, 17.00. Found: C, 63.78; H, 4.24.

Synthesis of5,5´(pyrrolo[3,2-*f*]indole-1,7-diylbis(oxy))bis(3-nitrobenzoic acid (4)

A solution of **3** (100 mg, 0.53mmol), 3,5-dinitrobenzoic acid (115 mg, 0.54mmol), potassium carbonate anhydride (25 mg, 0.18 mmol) in 10 ml of DMSO was stirring for 48 h at reflux. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water-hexane (4:2:1)

yielding 43 % of product, m.p. 104-106°C; IR (V_{max}, cm^{-1}):3204, 1710, 1485 and 1142;^{1}H NMR (300 MHz, $CDCl_3$) d_H: 6.20 (m, 2H), 7.34 (m, 1H), 7.78 (m,1H), 7.90 (m, 2H), 7.94 (m, 2H), 8.30 (m, 2H), 8.34 (m, 2H), 11.60 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) d_C: 104.58(C-9, C-11), 105.66(C-12), 108.70 (C-19, C-26), 110.90 (C-4), 118.20(C-15, C-22), 120.10(C-17, C-24), 123.78 (C-2, C-6), 125.80(C-3, C-5), 136.98 (C-8, C-10), 137.40(C-16, C-23), 149.30 (C-18, C-25), 164.57 (C-27, C-31), 166.12(C-14, C-21) ppm.EI-MS *m/z:* 518.07 (M^+ 10). Anal. Calcd. for $C_{24}H_{14}N_4O_{10}$: C, 55.61; H, 2.72; N, 10.81; O, 30.86. Found: C, 55.56; H, 2.68.

Synthesis of 5,5´-(pyrrolo[3,2-*f*]indole-1,7-diylbis(oxy))bis(*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-3-nitrobenzamide) (5)

A solution of **4** (200 mg, 0.39mmol), 4-aminoantipyrine (80 mg, 0.39 mmol), boric acid (37 mg, 0.60mmol) in 10 ml of methanol was stirring for 48 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (5:2) yielding 78 % of product, m.p. 158-160°C; IR (V_{max}, cm^{-1}):3202, 1640, 1482 and 1144; ^{1}H NMR (300 MHz, $CDCl_3$) d_H: 2.20 (m, 6H), 2.90 (m, 6H), 6.22 (m, 2H), 7.16 (m, 2H), 7.30 (m, 4H), 7.39 (m, 1H), 7.56 (m, 4H), 7.82 (m, 1H), 7.86 (m, 2H), 7.94 (m, 2H), 8.20 m, 2H), 8.26 (m, 2H), 9.50 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) d_C:13.78(C-57, C-66), 32.50(C-56, C-65), 104.60(C-9, C-11), 104.86(C-37, C-43), 105.70(C-12), 110.97 (C-4), 113.56 (C-15, C-26), 116.42 (C-17, C-24), 120.40 (C-19, C-22), 121.88 (C-2, C-6), 125.17 (C-51, C-55, C-60, C-64), 125.80(C-3, C-5), 127.21 (C-52, C-54, C-61, C-63), 128.3 (C-53, C-62), 133.30(C-50, C-59), 136.40 (C-18, C-23), 136.97 (C-8, C-10), 142.50(C-41 C-47), 150.44 (C-16, C-25), 162.00 (C-29, C-31), 162.35(C-38, C-44), 164.54 (C-14, C-21) ppm.EI-MS *m/z:* 888.26 (M^+ 12). Anal. Calcd. for $C_{46}H_{36}N_{10}O_{10}$: C, 62.16; H, 4.08; N, 15.76; O, 18.00. Found: C, 62.10; H, 4.00.

Synthesis of 1,7-bis(3-((5*Z*,9*E*)-2,3-dimethyl-1-phenyl-2,4,7,8-tetrahydro-1*H*-pyrazolo[4,3-*d*][1,3,6]triazocin-5-yl)-5-nitrophenoxy)-1,7-dihydropyrrolo[3,2,*f*]indole (6)

A solution of **5** (200 mg, 0.22mmol), ethylenediamine (78µl, 1.16mmol), boric acid (37 mg, 0.60 mmol) in 5 ml of methanol was stirring for 48 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (4:1) yielding 66 % of product, m.p. 212-214°C; IR (V_{max}, cm^{-1}):3204, 1484 and 1140; ^{1}H NMR (300 MHz, $CDCl_3$) d_H: 2.04 (m, 6H), 3.12 (m, 6H), 3.90 (m, 4H), 4.68 (m, 4H), 6.22 (m, 2H), 6.96-7.10 (m, 6H), 7.22 (broad 2H), 7.38 (m, 1H), 7.40 (m, 4H), 7.64 (m, 2H), 7.80 (m, 1H), 7.90 (m, 2H), 8.20-8.22 (m, 4H) ppm.^{13}C NMR (75.4 Hz, $CDCl_3$) d_C: 14.30 (C-61, C-69), 34.38(C-60, C-68), 49.46 (C-33, C-43), 52.26 (C-42, C-43),103.62 (C-49), 104.59 (C-9, C-11), 105.74 (C-12), 110.07 (C-4, 15, C-26), 110.94 (C-4), 113.60(C-17, C-24), 114.80 (C-19, C-22), 123.50(C-55, 59, C-63, C-67), 125.17 (C-2, C-6),125.84 (C-3, C-5), 126.80(C-57, C-65), 128.76(C-37, C-48), 131.80(C-56, C-58,C-64,C-66), 136.93 (C-8, C-10), 136.97 (C-8, C-10), 139.66 (C-18, C-23), 140.42 (C-44, C-45), 143.30 (C-54, C-62), 152.75 (C-16, C-25), 160.16(C-29, C-40), 164.59 (C-14, C-21) ppm.EI-MS *m/z:* 936.35 (M^+ 12). Anal. Calcd. for $C_{50}H_{44}N_{14}O_6$: C, 64.09; H, 4.73; N, 20.93; O, 10.25. Found: C, 64.00; H, 4.66.

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[14]. 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 (dŠ 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 non-cardiac 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°C) composed of (mmol); 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 (95:5/5 %). 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 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).

Inotropic activity

Contractile function was assessed by measuring left ventricular developed pressure (LV/dP), using a saline-filled latex balloon (0.01 mm, diameter) inserted into the left ventricle via the left atrium. The latex balloon was bound to cannula which was linked to pressure transducer that was connected with the MP100 data acquisition system.

Biological evaluation

Effects induced by the compound 3, 4, 5 and 6 on perfusion pressure

Changes in perfusion pressure as a consequence of increases in time (3 to 18 min) in absence (control) and presence of the compound 3, 4, 5 and 6 at a concentration of 0.001 nM were determined. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min.

Effect exerted by the compound 4 on left ventricular pressure through synthesis of prostanglandins

The boluses (50 ìl) of the compound **4** [0.001 to 100 nM] were administered and the corresponding effect on the left ventricular pressure was evaluated. The bolus injection administered was done in the point of cannulation. The dose response curve (control) was repeated in the presence of indomethacin at a concentration of 1 nM (duration of the pre-incubation with indomethacin was for a period of 10 min).

Effects of the compound 4 on left ventricular pressure through the calcium channel activation

Intracoronary boluses (50 ìl) of the compound **4**[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 pre-incubation with nifedipine was for a period of 10 min).

Statistical analysis

The obtained values are expressed as average ± SE, using each heart (n = 9) as its own control. The data obtained were put under Analysis of Variance (ANOVA) with the Bonferroni correction factor[15] using the SPSS 12.0 program. The differences were considered significant when *p* was equal or smaller than 0.05.

RESULTS AND DISCUSSION

Chemical Synthesis

There are several procedures for the synthesis of both pyrrol and indole derivatives[16-20]; nevertheless, expensive reagents and special conditions are required; therefore, in this study is reported a straight forward route for the preparation of some pyrrole-indole derivatives (Figure 1 and 2). The first stage was achieved by the synthesis of pyrrolo[3,2-*f*]indole-1,7-diol (**3**) by the reaction of 3,5-dinitrobenzoic acid with 1-hexyne in basic medium. The ^{1}H NMR spectrum of **3** shows signals at 6.10 and 7.70 ppm for both pyrrole rings; at 7.16 and 7.34 for phenyl group; at 10.24 ppm for both hydroxyl groups. The ^{13}C NMR spectrum of **3** contains peaks at 103.82 and 133.14 ppm for both pyrrol rings; at 104.08-130.30 ppm for phenyl group. Finally, the presence of compound **3**was further confirmed from mass spectrum which showed a molecular ion at *m/z* 188.05.

The second stage involved the preparation of 5,5´(pyrrolo[3,2-*f*]indole-1,7-diylbis(oxy))bis(3-nitrobenzoic acid (**4**) via displacement of nitro group from 3,5-dinitrobenzoic

Table 1. Physicochemical parameters [log P (log Kow), and ?] of compounds 3, 4, 5 and 6

Compounds	LogKow Fragment	Contributions
3	Aromatic	Carbon2.94
	OH [hydroxy, aromatic attach]	-0.9604
	Aromatic Nitrogen [5-member ring]	-1.0524
	Equation Constant	0.229
	σ	-0.354
	Log Kow	1.1562
4	Aromatic Carbon	6.468
	-O- [aliphatic O, two aromatic attach]	0.5846
	-NO2 [nitro, aromatic attach]	-0.3646
	-COOH [acid, aromatic attach]	-0.2372
	Aromatic Nitrogen [5-member ring]	-1.0524
	Equation Constant	0.229
	σ	4.4712
	Log Kow	5.6274
5	-CH3 [aliphatic carbon]	2.1892
	=CH- or =C< [olefinc carbon]	1.5344
	-NH- [aliphatic attach]	-2.9924
	-N< [aliphatic attach]	-3.6646
	Aromatic Carbon	9.996
	N [aliphatic N, one aromatic attach]	-1.834
	-O- [aliphatic O, two aromatic attach	] 0.5846
	-NO2 [nitro, aromatic attach]	-0.3646
	-C(=O)N [aliphatic attach]	-1.0472
	-C(=O)N [aromatic attach]0.3198	
	Aromatic Nitrogen [5-member ring]	-1.0524
	Di-N urea/acetamide aromatic correction	-1.4406
	>N-N<- structure correction	1.4612
	-C=C(-N-)-C(=O)- correction	1.5
	Equation Constant	0.229
6	σ	-0.21556
	Log Kow	5.4184
	-CH3 [aliphatic carbon]	2.1892
	-CH2- [aliphatic carbon]	1.9644
	-C [aliphatic carbon - No H, not tert]	3.8892
	=CH- or =C< [olefinc carbon]	1.5344
	-NH- [aliphatic attach]	-2.9924
	-N< [aliphatic attach]	-3.6646
	Aromatic Carbon	9.996
	-N [aliphatic N, one aromatic attach]	-1.834
	-O- [aliphatic O, two aromatic attach]	0.5846
	-NO2 [nitro, aromatic attach]	-0.3646
	Aromatic Nitrogen [5-member ring]	-1.0524
	-N=C [aliphatic attach]	-0.004
	>N-N<- structure correction	1.4612
	Fused aliphatic ring unit correction	-0.6842
	Equation Constant	0.229
	σ	5.8334
	Log Kow	11.2518

acid. It is important to mention that there are several methods for displacement of nitro groups, for example the synthesis of bis(2-bromo-4-methoxyphenyl)methanone by the reaction of 2,2´dibromo-4,4´dinitrobenzophenone with methoxide using a dipolar aprotic solvent. In general, dipolar solvents are used to attain high yield of ether groups[21]. In this study, the compound 4was synthetized by the reaction of the compound 3 with3,5-dinitro benzoic acid in presence of dimetyhylsulfoxide at mild conditions.The^{1}H NMR spectrum of 4 shows signals at 6.20 and 7.94 ppm for both pyrrol rings; at 7.34-7.78 ppm for phenyl group involved in thepyrrol-indol derivative; at 7.90, 8.30-8.34 ppm for both phenyl groups bound to both nitro and carboxyl groups; at 11.60 ppm for both carboxyl groups. The ^{13}C NMR spectrum of 4 contains peaks at 104.58 and 136.98 ppm for both pyrrol rings; at 105.66, 110.90, 123.78-125.80 ppm for phenyl group involved in the pyrrol-indolderivative; at 108.70, 118.20-120.10, 137.40-149.30 and 166.12 ppm for both phenyl groups bound to both nitro and carboxyl groups; at 164.57 ppm for both carboxyl groups. Finally, the presence of compound 4was further confirmed from mass spectrum which showed a molecular ion at *m/z* 518.07.

The third stagethe compound5 was synthesized by the reaction of 4 with 4-aminoantipyrine to formation of an amide group. It is important to mention that many procedures for the formation of amide groups are known in the literature[22], the most widely practiced method employs carboxylic acid chlorides as the electrophiles which react with the amino group in the presence of an acid scavenger[23]. 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 for their preparation (thionyl chloride)[24]. Other data indicate that boric acid catalyzed amidation of carboxylic acids and amines[25]; therefore, in this study the compound 5 was synthetized by the reaction of 4 with 4-aminoantipyrine using boric acid as catalyst. The^{1}H NMR spectrum of 5 shows signals at 2.20-2.90 ppm for methyl groups; at 6.22 and 7.94 ppm for protons of both pyrrol groups; at 7.16-7.30 and 7.56 ppm forboth phenyl groups bound to both pyrazole ring;

Table 2: Physicochemical parameters of compounds 3, 4, 5, and 6

Compound	R_m (cm^3)	V_m (cm^3)	P_c(cm^3)	I_r(cm^3)	S_t(cm^3)	Density (g/cm^3)	Polarizability (10^{-24} cm^3)
3	50.59 ± 0.50	124.10 ±7.00	355.60 ± 8.00	1.750 ±0.05	67.30 ±7.00	1.51 ± 0.10	20.05 ± 0.50
4	125.82 ± 0.50	310.10 ±7.00	927.10 ± 8.00	1.745 ±0.05	79.80 ±7.00	1.67 ± 0.10	49.88 ± 0.50
5	238.22 ± 0.50	596.90 ±7.00	1703.00 ± 8.00	1.729 ±0.05	66.30 ±7.00	1.48 ± 0.10	94.43 ± 0.50
6	260.36 ± 0.50	633.50 ±7.00	1804.60 ± 8.00	1.758 ±0.05	65.80 ±7.00	1.47 ± 0.10	103.21 ± 0.50

R_m = molar refractivity; V_m = molar volume; P_c = Parachor; I_r = Index of refraction; S_t = surface tension

at 7.39 and 7.82 ppm for phenyl group involved in the pyrrol-indol derivatives; at 7.82-8.26 ppm for both phenyl groups bound to both amide groups; at 9.50 ppm for both carboxyl groups. The ^{13}C NMR spectrum of 5 contains peaks at 13.76-32.50 ppm for methyl groups; at 104.60, 121.88-125.80 ppm for both pyrrol groups; at 104.86, 142.50 and 162.35 ppm for carbons of both pyrazole rings; at 105.70-110.97 ppm for phenyl group involved in the pyrrol-indol derivative; at 113.56-120.40, 136.40, 150.44 and 164.54 ppm for both phenyl group bound to both amide groups; at 125.17 and 127.21-133.30 ppm for both phenyl groups bound to both pyrazole rings; at 16200 for both amide groups. Finally, the presence of compound 5was further confirmed from mass spectrum which showed a molecular ion at *m/z* 162.35.

The fourth stage was achieved by the reaction of 5 with ethylenediamine using boric acid

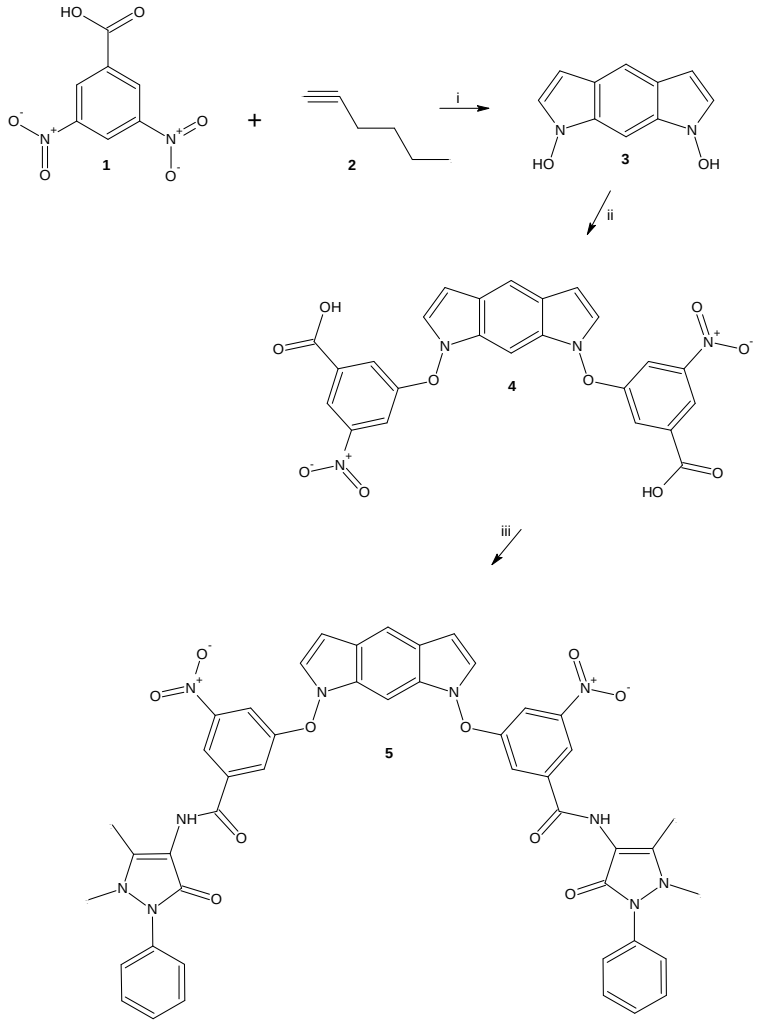

Fig. 1: Synthesis of 5,5´-(pyrrolo[3,2-*f*]indole-1,7-diylbis(oxy))bis(*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-3-nitrobenzamide) (5). The first stage was achieved by preparation of pyrrolo[3,2-*f*]indole-1,7-diol (3) by the reaction of 3,5-dinitrobenzoic acid (2) with 1-hexyne (1). The second stage involved the reaction of 3with2to synthesis of5,5´(pyrrolo[3,2-*f*]indole-1,7-diylbis(oxy))bis(3-nitrobenzoic acid (4). Finally, 4 was made reactioned with 4-aminoantipyrine in presence of boric acid (iii) to form 5. i = Na_2CO_3/toluene; ii = Na_2CO_3/DMSO

as catalyst. The ^{1}H NMR spectrum of 6 shows signals at2.04-3.12 ppm for methyl groups; at 3.90-4.68 ppm for methylene groups involved in the triazocine ring; at 6.22 and 7.90 ppm for both pyrrol rings; at 6.96-7.10 and 7.40 ppm for phenyl bound to triazole ring; at 7.22 ppm for both amino groups; at 7.38 and 7.80 ppm for phenyl group involved in the indole group; at 7.64, 8.20-8.22 ppm for both phenyl groups bound to both nitro groups.The ^{13}C NMR spectrum of 6 contains peaks at 14.30-34.38 ppm for methyl groups; at 49.46-52.26 ppm for methylene groups involved in the triazocine ring; at 103.62, 128.76 and 140.42 ppm for carbons of both triazole ring; at 104.59 and 136.93 ppm for both pyrrol rings; at 105.74, 110.94 and 125.84 ppm for methylene of indol ring; at 110.07, 113.60, 114.80, 139.66 and 152.75 ppm for both phenyl groups bound to both nitro groups; at 125.17, 126.80, 131.80 and 143.30

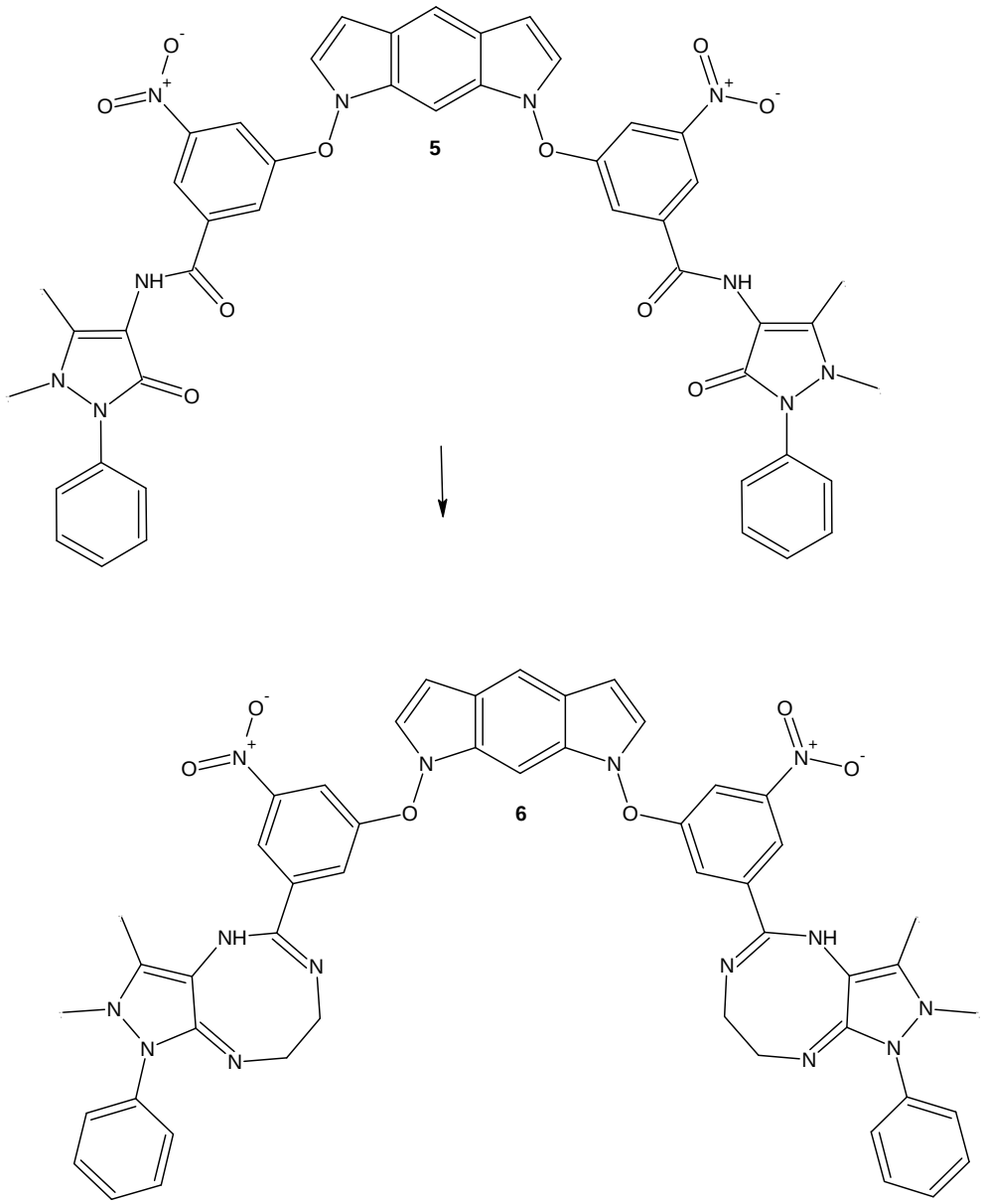

Fig. 2: Synthesis of 1,7-bis(3-((5*Z*,9*E*)-2,3-dimethyl-1-phenyl-2,4,7,8-tetrahydro-1*H*-pyrazolo[4,3-*d*][1,3,6]triazocin-5-yl)-5-nitrophenoxy)-1,7-dihydropyrrolo [3,2,*f*]indole (6). Reaction of 5 with ethylenediamine (iv)

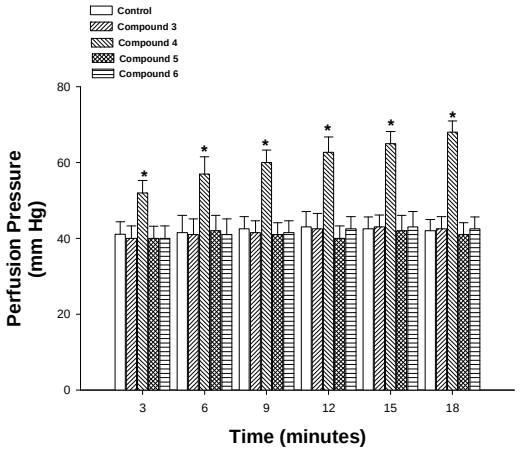

Fig. 3: Effect induced by the compound 4on perfusion pressure. The results show that the compound 4 significantlyincrease perfusion pressure (p = 0.05) through time in comparison with the compounds 3, 5,6 and the control conditions.Each bar representsthe mean ± S.E. of 9 experiments

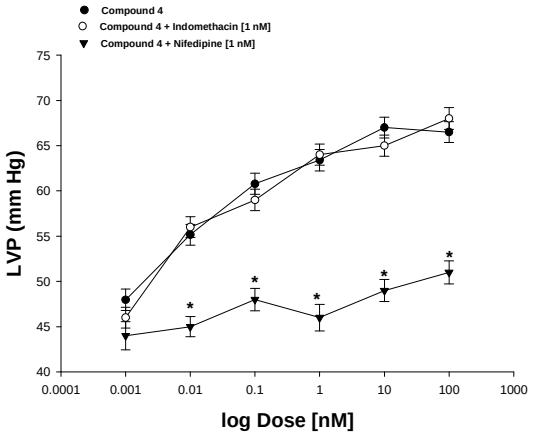

Fig. 4: *Effects induced by the compound4on LVP through prostaglandins synthesis or calcium channel activation*.Intracoronary boluses (50 µl) of the compound 4 [0.001 to 100 nM] wereadministered and the corresponding effect on the LVP was determined. Theresults showed that compound 4increases the LVP in a dependent dosemanner and this effect was not inhibited in the presence of indomethacin ornifedipine at a dose of 1 nM. Each bar represents the mean ± S.E. of 9experiments. LVP = left ventricular pressure.

ppm for both phenyl groups bound to both triazole rings.Finally, the presence of compound **6**was further confirmed from mass spectrum which showed a molecular ion at *m/z* 936.35.

Biological evaluation

There are several reports which indicate that somepyrrol and indol derivatives exert effects on cardiovascular system[4-9]; however there are not insufficient data on the activity exerted by these compounds. Therefore, in this study the effect exerted by new pyrrol-indol derivatives (compounds 3and4) on perfusion pressure were evaluated in an isolated rat heart model. The results obtained (Figure 3)show that the compound 4 significantly increases the perfusion pressure over time compared with the compounds 3and the control conditions. These data indicatedthatpossibly thenitrobenzoic acid fragments involved in the chemical structure of 4 could be responsible of the activity exerted by 4 on perfusion pressure.

In order to asses this hypothesis, also the effect induced by compounds 5and6on perfusion pressure was evaluated. The results showed that these compounds have not activity on perfusion pressure; this results indicates that the only the nitrobenzene fragment is the responsible of activity of the compound 4.

In the search of molecular mechanism involved in the activity exerted by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure, we considered validating the activity induced by the compound 4 on stimulation and secretion of prostaglandins such happening with other type of substances [25]. In this sense, in this experimental study, the activity exerted by the compound 4on left ventricular pressure in the absence or presence of indomethacin was evaluated. The results showed that effect induced by the thecompound 4on left ventricular pressure was not blocked by indomethacin. These data indicate that activity exerted by this steroid derivative on left ventricular pressure was not viaprostanoids synthesis and secretion.

Therefore, analyzing the possibility of that compound 4could induce its activity on left ventricular pressure through of activation of other

molecular system that involved increase in the intracellular calcium and consequently bring a positive inotropic effect such as happening with other type of substances[26]. In this study, the activity induced by the compound 4on left ventricular pressure was evaluated in the absence or presence of nifedipine. The results showed that effect exerted by the compound 4was inhibited in the presence of nifedipine which indicate that activity exerted by this compound involves activation calcium channel, this phenomenon could be conditioned by interaction between the pirrol-indol derivative and some endogenous substances involved in the heart or by the degree of lipophilicity exerted by the heterocyclic derivative; this effect may depend of some physicochemical parameters involved in its structure chemical such as happening with other type of compounds[27].

Physicochemical parameters

In order to delineate the structural chemical requirements involved in the degree of lipophilicity of the compounds 3, 4, 5 and 6, some parameters such as the descriptors[28] log P and ð were calculated. Is important to mention that, the descriptor logP estimates the logarithmic octanol-water partition coefficient; therefore, log P represents the lipophilic effects of a molecule which includes the sum of the lipophilic contributions of the parent molecule and its substituent[29]. The difference between the substituted and unsubstituted log P values is conditionated by the ð value for a particular substituent. Several years ago, Hammett showed that ð values measure the free energy change caused by particular substituent to relate to biological activity[30]. Therefore, in this study, the logP and ð parameters were calculated by previously methods reported[31]. The results (Table 2) showed an increase in logP and ð values in the compound 4 with respect to the compounds 3 and 5, this phenomenon is conditioned mainly, by the contribution of all substituent atoms involved in the chemical structure of compounds.However the logP was low in relationship to the compound 6; these data indicate that aliphatic carbons involved in thethe compound 6 contributes to increase the degree of lipophilicity in comparison with the compound 4. All these resultssuggest that different functional groups involved in thechemical structure of 6 induce changes inthe higher degree of lipophilicityin comparison with 4; however, there are other studies which indicate that other type of physicochemical parameters such as the molar volume (V_m) and molar refractivity (R_m) that are steric constantmayinducechanges in some biological activities.

In order to asses this hypothesis,in this work V_m and R_m werecalculated using ACD/Chem Sketch algorithms[32]. The resultsshowed an increase in bothR_m and V_m values for 6in comparisonwith 3, 4and 5. These data indicate that steric impediment,conformational preferences and internal rotation of 6 couldinfluence its biological activity in comparison with 4.

On the other hand, it isimportant to mention that there are reports which suggest thatV_m is directly related to parachor (P_c) and surface tension (S_t),which are cumulative effects of the different intra- and intermolecularforces involved in the structural chemistry of somecompounds[33]. Therefore, in this study P_c and S_t were alsoevaluated. The results indicate that both values of P_c and S_t for6were high in comparison with 3, 4and 5(Table 2). These data indicate that these physicochemical parameters can also modifythe biological activityof 6with respect to 4.

In conclusion, the experimental and theoretical data suggest that:(1) The compound 4 induces a positive inotropic effect via activation of calcium channels and this effect depends of its chemical structure; 2) The compound 6 exert higher lipophilicityand steric impediment in comparison with the compound 4 which consequently brings low activity on perfusion pressure of 6 in relationship to 4.

Confiicts of interest

The authors declare that they have no conûict of interest.

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