

Inotropic and Chronotropic Effects of 6-Hydroxy-4-Methylquinolin-2(1H)-One Derivatives in Isolated Rat Atria

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ABSTRACT

Background: Selective phosphodiesterase (PDE3) inhibitors improve cardiac contractility and may use in congestive heart failure. However, their proarrhythmic potential is the most important side effect. **Methods:** In this research, we evaluated the potential cardiotoxic activity of six new synthesized selective PDE3 inhibitors (6-hydroxy-4-methylquinolin-2(1H)-one derivatives) using the spontaneously beating atria model. In each experiment, atrium of reserpine-treated rat was isolated and the contractile and chronotropic effects of a synthesized compound were assessed. The 3-isobutyl-1-methylxanthine, a non-selective PDE inhibitor, was used for comparison. **Results:** The results showed that, among new compounds, the best pharmacological profile was obtained with the compound 6-[4-(4-methylpiperazine-1-yl)-4-oxobutoxy]-4-methylquinolin-2(1H)-one, C6, which displayed selectivity for increasing the force of contraction ($168 \pm 5\%$ change over the control) rather than the frequency rate ($138 \pm 5\%$ change over the control) at 300 μM . However, C6 at concentrations of 10 and 100 μM produced left and upward shift in the positive inotropic concentration-response curve of isoprenaline. The $-\log EC_{50}$ of isoprenaline was 8.843 ± 0.171 in the absence, 9.448 ± 0.138 and 9.456 ± 0.107 in the presence of 10, 100 μM of C6, respectively ($P < 0.001$, $n = 6$). Also, amrinone, a selective PDE3 inhibitor, shifted the isoprenaline concentration-response curve to the left and upward. The concentration of 10 and 100 μM amrinone decreased $-\log EC_{50}$ of isoprenaline to 9.527 ± 0.287 and 9.423 ± 0.243 , respectively ($P < 0.001$, $n = 6$). Moreover, the positive chronotropic effect of isoprenaline was not affected by amrinone or C6. **Conclusion:** This study provides functional evidence for the positive inotropic effect of C6. Considering the augmentation of isoprenaline positive inotropic concentration-response with C6 and amrinone, we conclude that C6 produces its effect via potentiation of cAMP-dependent signaling system and possibly by inhibition of PDE3 activity. *Iran. Biomed. J. 12 (2): 77-84, 2008*

Keywords: Phosphodiesterase inhibitor, 4-methylquinolinone derivatives, Inotropic activity, Rat atria, Isoprenaline

INTRODUCTION

Congestive heart failure (CHF) is a major cause of death in patients with heart disease. For many years, digitalis glycosides have been used for the treatment of CHF. However, the application of these agents is limited because of their narrow therapeutic window and their propensity to cause life-threatening arrhythmias (arrhythmogenic liability). The search for orally active 'non-glycoside'

cardiotonic drugs displaying a greater safety profile and improving efficacy on patient survival resulted in establishing the selective inhibitors of cyclic nucleotide phosphodiesterase (PDE) enzymes as a new class of cardiotonic agents [4].

The relation between cAMP and myocardial contractility has been well documented [10]. The agents that increase the synthesis of cAMP, e.g. beta-receptor stimulants, as well as agents that inhibit the degradation of cAMP, PDE inhibitors, all

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increase myocardial contractility [11].

On the basis of structure, kinetic properties substrate specificity and the ability to regulate PDE enzymes can be grouped into eleven different families, PDE1-PDE11 [1, 8]. At least four of these families present in the heart, namely PDE 1-4, which are classified based on protein and cDNA sequences. However, in mammalian heart, PDE 1 or 2 have not predominant action in cardiac activity, whereas PDE 3 and 4 play an important functional role in cardiac contractility [2, 12].

Major therapeutic actions of PDE3 inhibitors include positive inotropic effects in heart, dilation of vascular smooth muscle and inhibition of platelet aggregation [16]. Some studies indicate that local increases in cAMP in intracellular compartments may be important for the positive inotropic responses to specific PDE3 inhibitors [17].

Amrinone and milrinone are the prototypes of selective PDE3 inhibitors, proposed as a replacement for digitalis [3]. In particular, milrinone, which has been approved by the FDA and is used in the clinical treatment of patients with severe heart failure [2], improves the hemodynamic status of heart failure via inotropic/vasodilatory effects attributable to the increase in intracellular cAMP level [4, 5].

The aim of the present study was to assess the cardiotoxic compound with low side effect. So, we designed and synthesized a series of new cardiotoxic agents structurally similar to cilostamide, using a computerized molecular modeling of PDE3B. These compounds are 6-hydroxy-4-methylquinolin-2(1H)-one derivatives. Then, we characterized the cardiac activity of these compounds in the rat isolated atrial preparations.

MATERIALS AND METHODS

Drugs and chemicals. The test compounds were synthesized by Department of Organic Chemistry, University of Ferdowsi, Mashhad, Iran. Reserpine, tyramine HCl, (-) isoprenaline HCl and amrinone, were purchased from Sigma Chemical Co. (St. Louis, MO, USA), as well as 3-isobutyl-1-methylxanthine (IBMX), DMSO were provided from Fluka Chemical Co. All other chemicals obtained from Merck (Germany).

Animals. Adult male Wistar rats (250-350 g), obtained from animal house of Mashhad Medical School, were kept in controlled environmental

conditions (temperature: $23 \pm 2^\circ\text{C}$; light-dark cycle: 7 am to 7 pm). Animals had free access to a standard laboratory diet and water. In order to obtain atrial preparation, depleted in endogenous catecholamine, the animals were treated intraperitoneally with reserpine (5 mg/kg body weight) 24 h before euthanasia [6]. In each experiment, the animals were anaesthetized with intraperitoneal injection of thiopental 80 mg/kg, and after midline thoracotomy, the heart was rapidly excised and placed in a dissection dish filled with oxygenated Krebs-Henseleit solution.

Assessment of inotropic and chronotropic activities on isolated atria preparations. The whole atrium was separated from ventricle and mounted vertically in a 50-mL organ bath containing Krebs-Henseleit solution constantly gassed by 95% O₂ and 5% CO₂ at 31°C, pH 7.35-7.45. The bathing solution contained (in mM): NaCl, 118; KCl, 4.5; CaCl₂, 1.36; MgSO₄, 1.21; NaH₂PO₄, 1.22; NaHCO₃, 25 and glucose, 11 [7]. Resting tension was adjusted to about 0.5 g in the whole atria [8]. The developed tension was recorded isometrically by means of a high-sensitivity force transducer (type E. Zimmermann, Eipzig, Berlin) connected to an ink writing oscillograph (George Washington LTD., Sheerness Kent, England).

The initial equilibration period was 40-50 minutes for each preparation. Since the atria were isolated from reserpine-treated animals, before the beginning of the experiment, depletion of catecholamine was verified by lack of any positive inotropic effect induced by tyramine (1.5 μM) [7]. Experiments were performed only in preparations that did not response to tyramine. IBMX, a non-selective PDE inhibitor, and the test compounds were added cumulatively (1-1000 μM) to the organ bath. The responses caused by each drug concentration were recorded up to the maximum response before a higher concentration was added.

To assess the potential synergism between isoprenaline and PDE inhibition, preparations were first treated with C6 (10 and 100 μM) or amrinone (10 and 100 μM) for 20 min, and after reaching steady state, isoprenaline (0.1-100 nM) was cumulatively added. The effects of each compound on the force of contraction and the frequency were expressed as the percentage of controls.

All the test compounds, amrinone and IBMX were dissolved in DMSO. The final concentration of DMSO in the medium was not exceeding 1% which did not affect the basal activity of the atria. In each

Table 1. Effects of the test compounds upon contractile force of whole atria from reserpine-treated rats: comparison with control.

Conc.	1 μ M	3 μ M	10 μ M	30 μ M	100 μ M	300 μ M	1 mM
IBMX	134 \pm 16	157 \pm 18*	159 \pm 8***	148 \pm 8**	147 \pm 7**	-	-
C1	106 \pm 17	113 \pm 15	117 \pm 10	122 \pm 10	129 \pm 10	129 \pm 8	126 \pm 4
C2	112 \pm 18	123 \pm 19	126 \pm 18	130 \pm 15	130 \pm 10	131 \pm 9*	133 \pm 5
C3	112 \pm 7	114 \pm 9	116 \pm 12	119 \pm 17	127 \pm 13	132 \pm 12*	-
C4	119 \pm 10	119 \pm 17	125 \pm 16	129 \pm 15	132 \pm 13*	-	-
C5	108 \pm 12	116 \pm 13	124 \pm 11	137 \pm 11*	144 \pm 8*	143 \pm 7**	-
C6	110 \pm 5	-	126 \pm 4*	145 \pm 4**	165 \pm 5***	168 \pm 5***	-

The effect of each concentration of a compound was defined by the difference between the forces of contraction before and after its addition to the bathing fluid, and was expressed as a percent variation in developed tension of the controls. The value of basal force of contraction was 14.14 ± 1.17 mN. Results are means \pm S.E.M from six atria. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ indicate significantly different from controls by one-way analysis of variance followed by *Tukey's t*-test. -, Not determined.

experiment, isoprenaline was dissolved in distilled water containing 0.1% vitamin C and diluted with Krebs-H solution to final concentration desired. Reserpine was dissolved in glacial acetic acid and, after neutralization with NaOH, diluted in distilled water to 5 mg/2 ml.

Statistical analysis. Data were expressed as mean \pm standard error of mean (S.E.M). Student's unpaired *t*-test was used to evaluate the effect of an intervention between two experimental groups. In the case of examine more than two groups, we used One-way analysis of variance (ANOVA) and the *Tukey's post hoc* test. Differences among means were considered significant if $P < 0.05$. All data passed a normality test.

The EC_{50} values were calculated by means of Graph Pad Prism 3.03 software from the concentration-response data using non-linear sigmoidal curve fitting [9].

RESULTS

All the newly synthesized analogues C1-C6, and IBMX, as standard, were tested for their effects on the force of contraction and frequency rate of rat spontaneously beating atria. To avoid a catecholamine release which affects experiments, we used preparations obtained from reserpine-pretreated animals. In comparison with control, all the test compounds increased contractility in a concentration-dependent manner, except for C1. Also, they significantly increased contractility in maximal dose used (Table 1).

The effect of C6 on contractility was more predominant (Fig. 1). None of these compounds significantly affected the atrial frequency even at 100 μ M concentration (Table 2). Washout of

myocardial preparations completely retained the contractility to the pre-drug state. This shows the reversibility for the effect of all the new compounds.

The inotropic effect of the highest concentration of C6 (300 μ M), was comparable with the maximum effect of IBMX. However, the potency of C6 was 30 times lower than that of IBMX (Table 1). During experiment C6 did not cause tachyarrhythmias (Table 2). IBMX at a concentration of 100 μ M increased heart rate 64% but C6 increased 15% which was not significant (Table 2). Amrinone increased heart rate 20% at 100 μ M (Fig. 2).

In similar conditions, isoprenaline, a β_1 -adrenoceptor agonist, produced maximum effect at a concentration of 0.3 μ M which caused an increase in

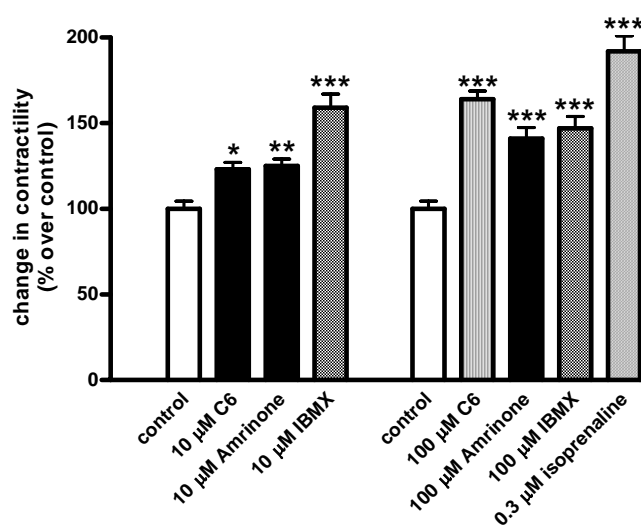


Fig. 1. Concentration-response inotropic effects of different concentration of amrinone, IBMX and C6 in isolated whole atria from reserpine-pretreated rats. Data was expressed as a percent variation in atrial force of contraction in respect to the controls. Results are means \pm SEM from six atria. ** $P < 0.01$ and *** $P < 0.001$ indicate significantly difference from controls by one-way analysis of variance followed by *Tukey's t*-test. Data are expressed as percent of the control.

Table 2. Effects of the test compounds upon frequency rate of whole atria from reserpine-treated rats: comparison with control.

Conc.	1 μ M	3 μ M	10 μ M	30 μ M	100 μ M	300 μ M	1 mM
IBMX	123 \pm 11	159 \pm 8***	166 \pm 18***	166 \pm 18***	164 \pm 15***	-	-
C1	106 \pm 16	111 \pm 15	116 \pm 13	126 \pm 12	126 \pm 13	126 \pm 12	131 \pm 13
C2	111 \pm 12	118 \pm 13	119 \pm 13	132 \pm 11	132 \pm 11	147 \pm 13**	131 \pm 16
C3	106 \pm 10	106 \pm 9	108 \pm 9	101 \pm 12	98 \pm 10	66 \pm 18	-
C4	109 \pm 5	109 \pm 3	112 \pm 2	109 \pm 2	109 \pm 2	-	-
C5	104 \pm 8	104 \pm 6	108 \pm 7	109 \pm 7	113 \pm 6	123 \pm 5	-
C6	100 \pm 8	-	104 \pm 10	112 \pm 9	115 \pm 13	138 \pm 5	-

The effect of each concentration of a compound was defined by the difference between the frequency before and after its addition to the bathing fluid, and was expressed as a percent variation in frequency of the controls. Results are means \pm S.E.M from six atria. ** P <0.01 and *** P <0.001 indicate significantly different from controls by one-way analysis of variance followed by *Tukey's t*-test. -, Not determined.

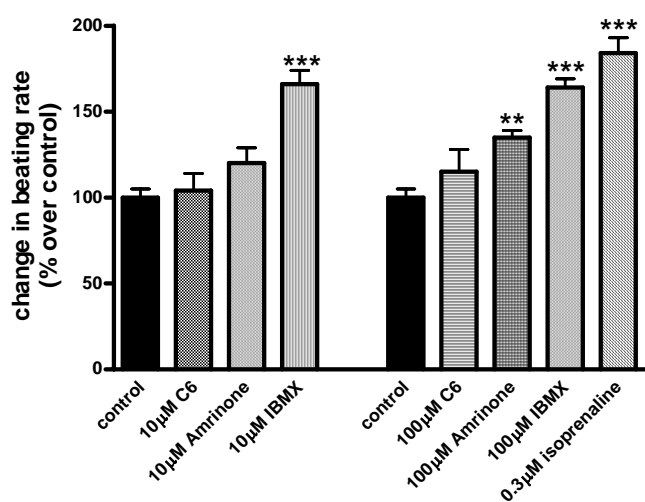


Fig. 2. Concentration-response chronotropic effects of different concentration of amrinone, IBMX or C6 in isolated whole atria from reserpine-pretreated rats. Data was expressed as a percent variation in beating rate in respect to the controls. There was no significant difference between all groups of rats. Results are means \pm S.E.M from six atria. ** P <0.01 and *** P <0.001 indicate significantly different from controls by one-way analysis of variance followed by *Tukey's t*-test. Data are expressed as percent of the control.

the force of contraction $191.7 \pm 9.2\%$ and an increase in heart rate $84 \pm 9\%$ over the control (Figs. 1 and 2).

Effects of amrinone or C6 on the positive inotropic and chronotropic function of isoprenaline. For investigating the mechanism of action of the new-synthesized compounds, we chose C6. Since, among the tested compounds, its effect was more predominant. In assumption that these compounds are PDE inhibitors and exert their effect via cAMP accumulation, we tested the potential synergism effect between C6 and isoprenaline on contractility and heart rate. As shown in Figure 3A and 3B isoprenaline (0.1-100 nM) produced a concentration-dependent positive inotropic effect in

the rat isolated atrium which was rapidly reversed after washout. In conjunction with C6 at concentrations of 10 and 100 μ M, there was a leftward and upward shift in the isoprenaline positive inotropic concentration-response curve (Fig. 3A). The $-\log EC_{50}$ of isoprenaline was 8.843 ± 0.171 in the absence, and 9.448 ± 0.138 and 9.456 ± 0.107 in the presence of 10 and 100 μ M of C6, respectively (P <0.001, $n = 6$).

Similar findings were observed with amrinone, a selective PDE3 inhibitor. When amrinone was applied in conjunction with isoprenaline, it shifted the isoprenaline positive inotropic concentration-response curve to the left and upward (Fig. 3B) and significantly increased the $-\log EC_{50}$ of isoprenaline from 8.843 ± 0.171 to 9.527 ± 0.287 and 9.423 ± 0.243 at concentrations of 10 and 100 μ M, respectively (P <0.001, $n = 6$). Moreover, both of amrinone, and C6 had no significant effects on positive chronotropic dose-response curve of isoprenaline (Fig. 4, P >0.05). The presence of DMSO in organ bath used for dilution of the compounds did not produce significant changes in the cardiac effects of isoprenaline or PDE inhibitors.

DISCUSSION

In the current study, we present new inotropic agents, 6-hydroxy-4-methylquinolin-2(1H)-one derivatives. Considering the structure-activity relationship with their cardiotoxic activity in computerized molecular modeling, we used these compounds as probable selective PDE3 inhibitors. For prevention of endogenous catecholamine release which affects the experiments, we used the spontaneously beating atria model from reserpine-treated rats. To block all PDE activity we applied IBMX, a non-selective PDE inhibitor.

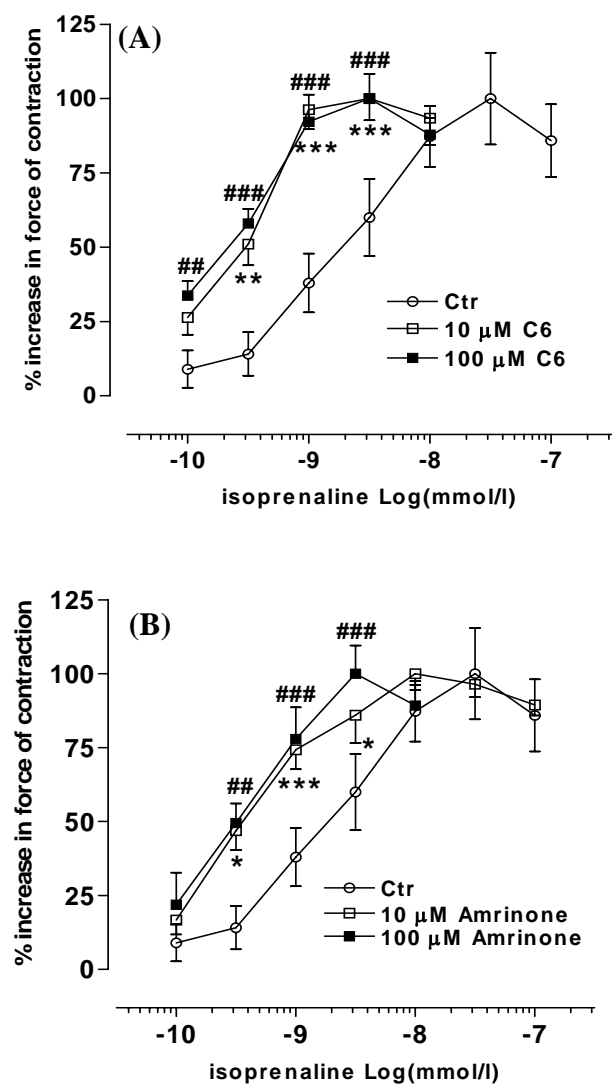


Fig. 3. Cumulative concentration-response curves for the inotropic effect of isoprenaline (Ctr). (A) In the absence (○) and in the presence of test compound C6 (□10 μM, ■100 μM). C6 increased the maximum inotropic response of isoprenaline 69% or 75% in 10 μM and 100 μM, respectively; (B) in the absence (○) and in the presence of amrinone (□10 μM, ■100 μM). Amrinone increased the maximum inotropic response of isoprenaline 61% or 46% in 10 μM and 100 μM, respectively. Each point represents the mean value ± S.E.M of 6. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ### $P < 0.01$, #### $P < 0.001$, compared with control values. Statistical significance was calculated by Student's *t*-test.

Similar to IBMX, all of the tested compounds dose-dependently increased the contractility of rat atria. Moreover, C6 was the best of them and the maximal positive inotropic effect of C6 is comparable to that of IBMX. The cardiac activity of this active compound does not involve catecholamine release because the compounds were tested in atria isolated from reserpine-treated rats not

responding to the tyramine test [18].

So, increasing the force of contraction by these drugs is in agreement with evidences for activity of PDE3 in the rat heart. Among these agents, C6 [4-(4-methylpiperazine-1-yl)-4-oxobutoxy]-4-methylquinolin-2(1H)-one] (Fig. 5) was the most effective one. Therefore, we chose this compound as a novel cardiotoxic agent for more investigation in the mechanisms of action. In this way, we evaluated the possible synergism between the inotropic and chronotropic effects of isoprenaline in combination with C6 or amrinone, a selective PDE3 inhibitor.

Isoprenaline produced a positive inotropic response in atrial tissue. This effect was dose-dependent.

Previous studies showed that isoprenaline activates adenylate cyclase [19] and accumulation of cAMP in the myocytes results increasing in contractile force of heart. Therefore, when isoprenaline is used in combination with a PDE inhibitor, a synergistic effect is expected. In this experiment, C6 and amrinone at low concentration potentiated the effect of isoprenaline and significantly shifted the positive inotropic concentration-response curve for isoprenaline to the left and upward. In this combination, the effect of C6 at a concentration of 10 μM seems to be the maximum effect, because C6

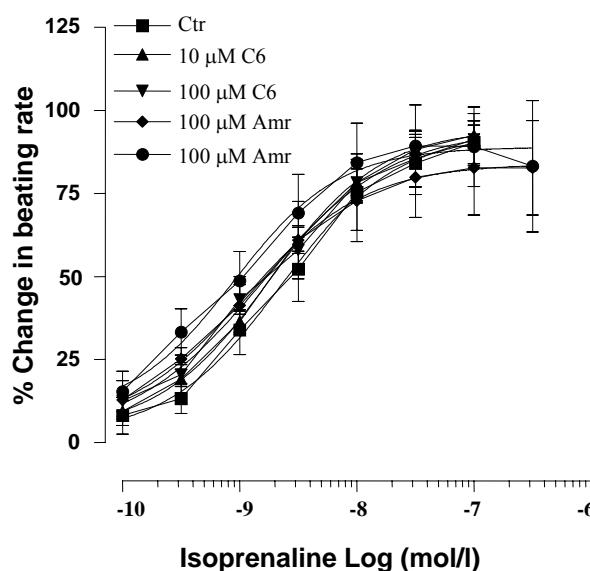


Fig. 4. Cumulative concentration-response curve for the chronotropic effect of isoprenaline in the absence (■) and in the presence of the synthetic compound C6 (▲10 μM, ▼100 μM) and in the presence of amrinone (◆10 μM, ●100 μM). Each point represents the mean value ± S.E.M of 6. The difference between the maximum beating rate produced by the agents and the basal beating rate was taken as 100%, and the percent changes in the beating rate produced by various concentrations of the agents were plotted.

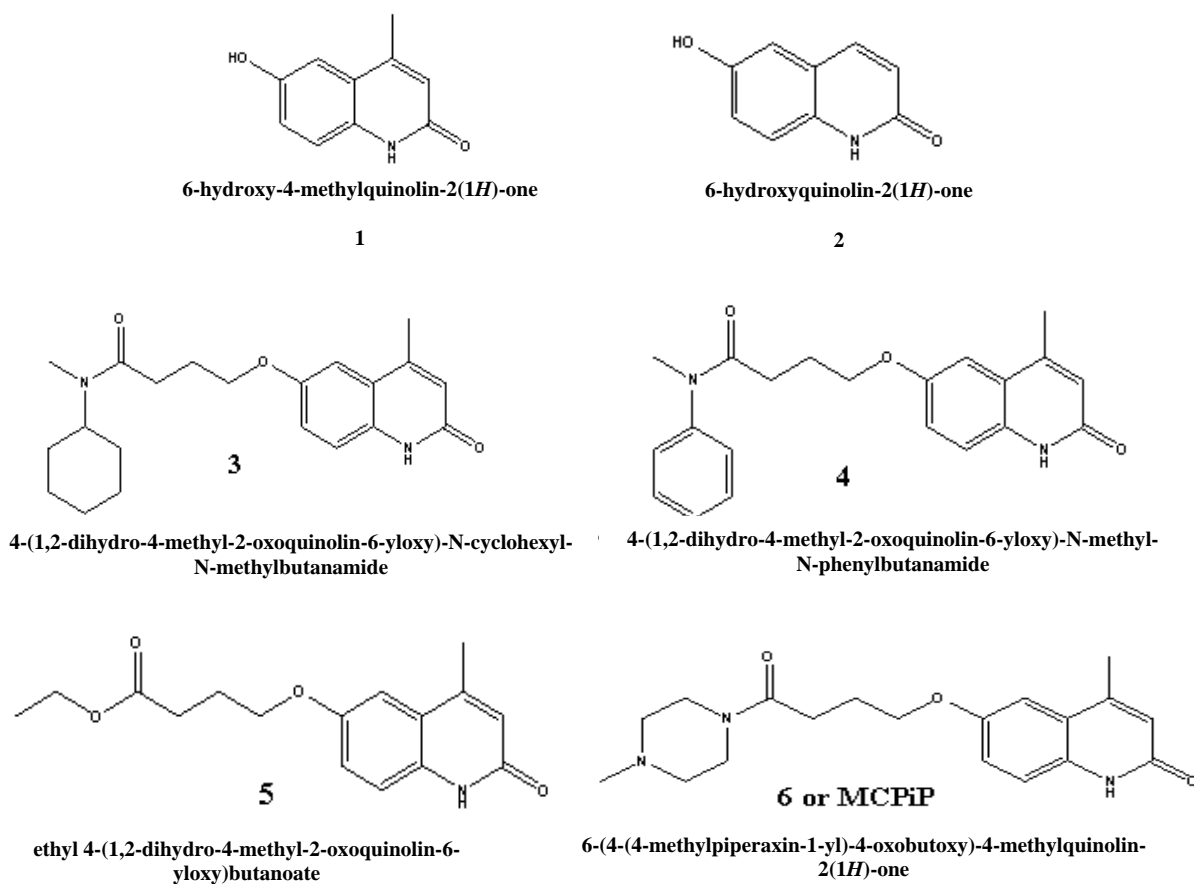


Fig 5. Structure of the test compounds (C1-C6).

at higher concentration (100 μ M) did not produce further significant augmentation in atrial contractility. The effects of C6 and amrinone in potentiation of isoprenaline were similar. Considering the mechanism of action of amrinone as a selective PDE3 inhibitor, we may conclude that the effect of C6 is mediated by PDE inhibition.

Different isoforms of cyclic nucleotide PDE are present in the heart. Although, PDE3 is the most important functional PDE in the heart which its inhibition increases heart contractility, the inhibition of other PDE, especially PDE4, may be involved. So, at high concentrations, C6 may produce a non-selective inhibition. The synergistic interaction of C6 with isoprenaline seems to be consequence of PDE inhibition, but our present experiment can not distinguish this relative selectivity of C6 and needs further investigations. This is in agreement with previous results demonstrating a potentiation of the functional effects of isoprenaline through PDE inhibition [20]. It is well known that isoprenaline increases cardiac contractility by enhancing cAMP production through a β -adrenoceptor-mediated

mechanism. This response is amplified by the presence of PDE inhibitors [21].

In spite of increasing contractility force, C6 did not produce a predominant positive chronotropic effect on the isolated atria. This was shown in the screening of the new compounds. The mechanism of this effect remains to be established, and may involve different pathways similar to that of vesnarinone, a selective PDE3 inhibitor. In the presence of vesnarinone, the cAMP-mediated positive chronotropic effect is counteracted by its effect to decrease the K^+ conductance in myocardial cell membrane [22]. In addition, the positive chronotropic dose-response curve of isoprenaline was not affected by amrinone, selective PDE3 inhibitor, or C6. So, we may conclude that this is a special property for some of the selective PDE3 inhibitor as shown in the previous work in which amrinone potentiated inotropic effect of isoprenaline without affecting chronotropic function [23].

Although the exact mechanism involved in the action of C6 remains to be clear, it may be an advantage for its clinical application that this

compound produces inotropic effect without any obvious changing heart rate. This prevents unnecessary increase in heart oxygen consumption that is an important determinant to exacerbate the heart failure syndrome [24].

Finally, C6 may be a good candidate for treatment of congestive heart failure since; in spite of having high efficacy, it may have less proarrhythmic side effect. The exact mechanism of C6 action is a subject for further investigations. However, the possibility for involvement of a cyclic AMP-independent mechanism can not be excluded.

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