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**Research Article**

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# Synthesis and evaluating cardiac effects of 3,4-dihydropyrimidin-2-one-5-carboxylates in isolated atria of animal model

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**ABSTRACT**

*The synthesis and cardiac activity of four 3,4-dihydropyrimidin-2-one-5 carboxylates are reported. We synthesized 3,4-Dihydropyrimidinones (DHPMs) by Biginelli reaction using classic and microwave methods and the yield of these methods compared with each other. Then cardiotonic activity of four derivatives of DHPMs was investigated on isolated perfused right atria. Compound **2b** had significant chronotropic effect in comparison to negative control at concentration of 0.01M.*

**Key words:** Dihydropyrimidines, Synthesis, Biginelli Compounds, Isolated Atria

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## INTRODUCTION

Heart failure(HF) is a disease in which cardiac function cannot supply enough oxygen to body tissues, despite normal filling pressures (or only at the expense of increased filling pressures).[1, 2] HF affects about 2% of the western population, and the prevalence is increasing sharply from 1% in 40-year-old individuals to 10% above the age of 75 years. It is the most common cause of hospitalization in patients over 65 years [1, 3] .

3,4-Dihydropyrimidinones (DHPMs), also called Biginelli compounds have interesting biological properties. Biginelli compound refers to the reaction that was reported by PietroBiginelli in 1893[4]for first time. The Biginellireaction [5] is a direct and famous method for the synthesis of 3,4-DHPMs by the three-component condensation of an aliphatic or aromatic aldehyde, beta-ketoester and a urea.

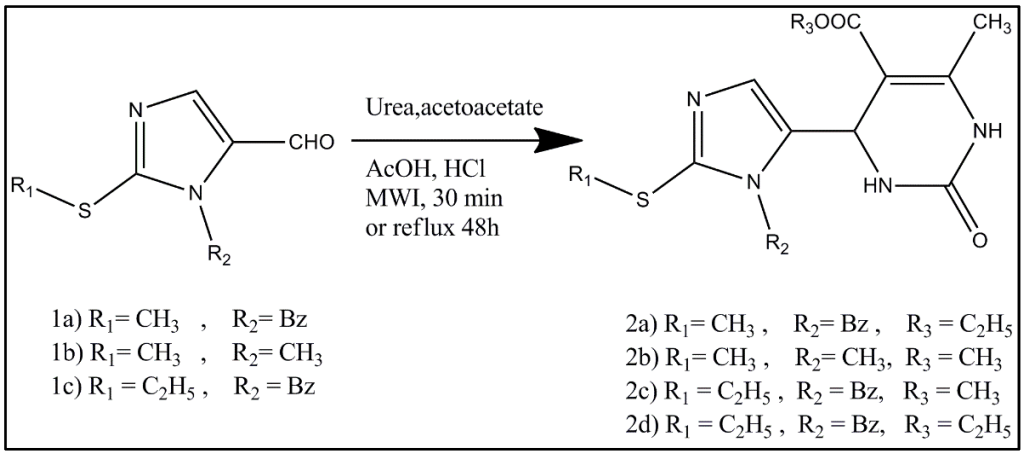
Hantzsch-type dihydropyridines[6], calcium channel modulators, are the evident structural similarities of DHPMs and are discussed in the field of medicinal chemistry. DHPMs also have other pharmacological activities such as antibacterial [7, 8] and antifungal activity[9, 10], anti-inflammatory[7], anticancer[8]and antitubercular[11, 12] in addition to calcium chanel modulation[13].

Interesting problem is that DHPMs synthetized by different method and produced racemic mixture of right and left-hand enantiomers. Right-hand enantiomer has agonist effect on calcium channel and left-hand enantiomer has antagonist effect on this channel, and totally racemic mixture usually shows agonist effect.

In this study, a series of 4-(substituted imidazolyl)-3,4-dihydropyrimidinone-carboxylates were synthesized using Biginelli reaction by two different methods: classical and microwave [14] .

The synthetic pathway employed in the preparation of 4-substituted-3,4-dihydropyrimidinones is outlined in Figure 1.

**Figure 1: synthetic pathway employed in preparation of DHPMs**




The effects of DHPMs were evaluated on an isolated perfused right atria[15] at various dose levels and compared with the activity of theophylline as positive control and DMSO as negative control (Figure 2).

## EXPERIMENTAL SECTION

Melting points were determined using an Electrothermal Capillary apparatus and are uncorrected. $^1$H-NMR spectra were recorded using Bruker AC-80 NMR spectrometer. The chemical shift values (δ) are in ppm relative to tetramethylsilane as internal standard. Errors of elemental analyses were within ±0.4% of theoretical values.

In classic method, a mixture of 10 mmol aldehyde,15 mmol urea ,10 mmolbetaketoesters (ethyl or methyl acetoacetate) and %5 mol I2 in 15 ml toluene was stirred at reflux for 48h. After completion of the reaction a solid precipitant was produced and it was filtered and washed with cold methanol to remove excess iodine. The solid powder was collected by filtration and purified by thin layer chromatography . A mixture of 95ml chloroform and 5ml methanol as the mobile phase were used. Compounds were characterized by 1H NMR methods and melting point confirmed the formation of products.

In microwave method, a mixture of 10 mmol aldehyde,10 mmolurea ,10mmol of beta-ketoesters (ethyl or methyl acetoacetate) was irradiated in 25ml of acetic acid medium with few drops of concentrated HCl. The solution was irradiated in microwave (Milestone, Italy ) for 30 min at 100oC. Then a mixture of water and chloroform (30:30) was added to the reaction medium and the organic phase was separated under a vacuum condition. The residue was purified by thin layer chromatography as described above.

***Methyl 4-[1-benzyl-2-(ethylthio)-1H-imidazol-5-yl)- 1,2,3,4- tetrahydro- 6- methyl- 2-oxopyrimidin-5-carboxylate (2a).***

This compound was obtained in 45.5% yield; MP: 141-142o C; $^1$HNMR(DMSO-$d_6$): δ9.15(s,1H,NH),7.24-6.71 (m, 6H, arom, H4- imidazole), 6.61(s, 1H, NH),5.26 (s, 2H, -CH2N),5.01 (s,1H, H4-dihydropyrimidine) ,3.21 (s, 3H, $OCH_3$),2.95-2.65(q, 2H, -$CH_2$S), 2.16 (s, 3H, $CH_3$-dihydropyrimidine) , 1.11ppm (t, 3H, $CH_3CH_2$S). Anal.calcd for $C_{19}H_{22}N_4O_3S$: C, 59.05; H, 5.74; N, 14.50. Found: C, 59.04; H, 5.71; N, 14.53.

***Methyl 1,2,3,4- tetrahydro- 6- methyl -4-[1-methyl-2-(methylthio)-1H-imidazol-5-yl)- 2-oxopyrimidin-5-carboxylate (2b).***

This compound was obtained in 15% yield; MP: 198-199$^o$C; $^1$HNMR(DMSO-$d_6$): δ9.1(s,1H,NH), 6.54(s,1H,$H_4$-imidazole) ,4.71 (s,1H, $H_4$-dihydropyrimidine) ,4.0-3.50(m, 6H,$NCH_3$, $OCH_3$),2.23 ppm(s,6H, $SCH_3$ ,$CH_3$-dihydropyridine). Anal.calcd for $C_{12}H_{16}N_4O_3S$: C, 48.64; H, 5.44; N, 18.91. Found: C, 48.61; H, 5.46; N, 18.89.

***Ethyl 4-[1-benzyl-2-(methylthio)-1H-imidazol-5-yl)- 1,2,3,4- tetrahydro- 6- methyl- 2-oxopyrimidin-5-carboxylate (2c).***
This compound was obtained in 39.5% yield; MP: 193-194$^{o}$C; $^{1}$HNMR(DMSO-$d_6$):δ9.06 (s,1H,NH),7.21-6.79 (m,6H,arom,$H_4$- imidazole) ,6.60 (s,1H, NH),5.20 (s,2H,-$CH_2$N),5.01 (s,1H,$H_4$-dihydropyrimidine) ,4.05-3.65(q,2H,$CH_2$O) , 2.29 (s,3H,$CH_3$-dihydropyrimidine) ,2.08 (s,3H,$SCH_3$) ,1.05(t,3H,$CH_3CH_2$O). Anal.calcd for $C_{19}H_{22}N_4O_3S$: C, 59.05; H, 5.74; N, 14.50. Found: C, 59.02; H, 5.76; N, 14.49.

***Ethyl 4-[1-benzyl-2-(ethylthio)-1H-imidazol-5-yl)- 1,2,3,4- tetrahydro-6-methyl-2-oxopyrimidin-5-carboxylate (2d).***
This compound was obtained in 73.5% yield; MP: 164-165$^{o}$C; $^{1}$HNMR(DMSO-$d_6$): δ9.04(s,1H,NH),7.24-6.77 (m, 6H, arom, $H_4$-imidazole), 6.61(s, 1H, NH), 5.22 (s, 2H, -$CH_2$N), 5.02 (s, 1H, $H_4$-dihydropyrimidine) , 4.05-3.65(q, 2H, $CH_2$O), ,3.95-2.50(q, 2H, $CH_2$S), 2.10 (s, 3H, $CH_3$-dihydropyrimidine) ,1.06ppm(t, 6H, $CH_3CH_2$O, $CH_3CH_2$S). Anal.calcd for $C_{20}H_{24}N_4O_3S$: C, 59.98; H, 6.04; N, 13.99. Found: C, 59.95; H, 6.02; N, 13.11.

***Isolated right atria***
30 Male Sprague-dawley rats (250 - 300 g) were kept in plastic cage in a controlled environment and were given free access to food and water until they were killed by decapitation. Animals were cared according to institutional guidelines. Hearts were removed and placed in a modified Krebs-Ringer bicarbonate buffer (KRBB) solution. The KRBB contained: NaCl (78mM), KCl (4.7mM), $CaCl_2.2H_2O$ (25mM), $NaH_2PO_4$(1.36mM), $MgCl_2.6H_2O$ (1.16mM) $NaHCO_3$ (25mM), HEPES (20mM), Glutamate (2mM), Sodium fumarate (4mM), Sodium lactate (2mM), Glucose (11.6mM). The pH was adjusted with 1N NaOH so that the final pH at 30 C was 7.4. The dissection of the procedure was as follows. A PE-160 cannula (id 1.14 mm), approximately 30 mm in length, was inserted in the inferior vena cava and out of the superior vena cava. The cannula had an opening midway between the inferior and superior vena cava was positioned in a way that the opening faced the inside of the right atria chamber. The cannula was fixed to each vena cava by a silk suture. The coronary sinus was ligated, the left auricle was removed, and the aorta and pulmonary vein were severed immediately above the ventricles. The atrium was cleaned of connective tissue and fat. The lower ¾ of the ventricles were removed, and a rigid heat-curved cannula (PE-10), the end of which was heated to form a lip, was inserted in the right atrium via the tricuspid valve; this cannula was held in place by tying a silk suture around the atrioventricular junction. The right atrium dissected in this manner was fixed to a plastic discs by inserting the inferior vena cava and outflow cannulas onto tubing-covered syringe needle ends that passed through the disc. The disc was then tightly inserted in the opening of an inverted heat (30$^{o}$C) organ chamber. The superior vena cava was attached to a cannula from which KRBB (preheated to 30oC and gassed with 95% O2 /5% CO2) was infused at a rate of 3 ml/min by peristaltic pomp. The inferior vena cava was connected to a pressure transducer that was linked to a physiograph. The organ chamber contained 50 ml of KRBB, was replenished at a rate of 3 ml/min, and gassed with 95% $O_2$ /5% $CO_2$ throughout the experiment. We waited for a short time in order to reach its steady state and atrial rate is regulated. Then, we injected the desired compounds separately with the concentrations of 0.001, 0.005 and 0.01M and reviewed the tissue reaction from the point of contractile power and compared it with the negative control (DMSO) and positive control (theophylline, 0.0001M).

## RESULTS AND DISCUSSION

The purpose of designed and synthesized four derivatives 3,4-dihydropyrimidines-2-one-5-carboxylate, achievement of compounds that racemic mixture of them have agonists effect on the calcium channels. Purity of all compounds was determined by thin layer chromatography and structure elucidation was performed by nuclear magnetic resonance spectroscopy. Biginelli synthesis yield was between 15 - 70 percent. There was no significant difference between classic and microwave synthesis yields and only the reaction time reduced from 48h (in classic method) to 30 min (in microwave method) (Table 1) .

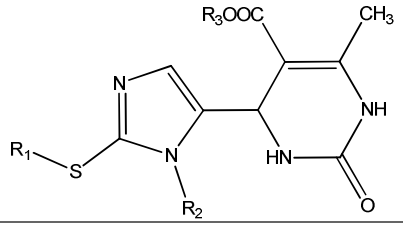
In the present investigation we study the chronotropic and inotropic effects of compounds **2a, 2b, 2c** and **2d**.

According to the results compound **2b** (in concentration of 0.01M) showed increase in rate of atrial contractions (positive choronotropic effect) as well as theophyline (positive control in concentration of 0.0001M). Other compound didn't show chronotropic action.

The chronotropic action of compound **2b** ($R_1$=$R_2$=$R_3$ = Me) may be contributed by chemical structure of this compound. According to structure, in the other compounds **2a** ($R_1$= Me, $R_2$=Bz ,$R_3$=Et **), 2c** ($R_1$=Et, $R_2$=Bz, $R_3$=Me)

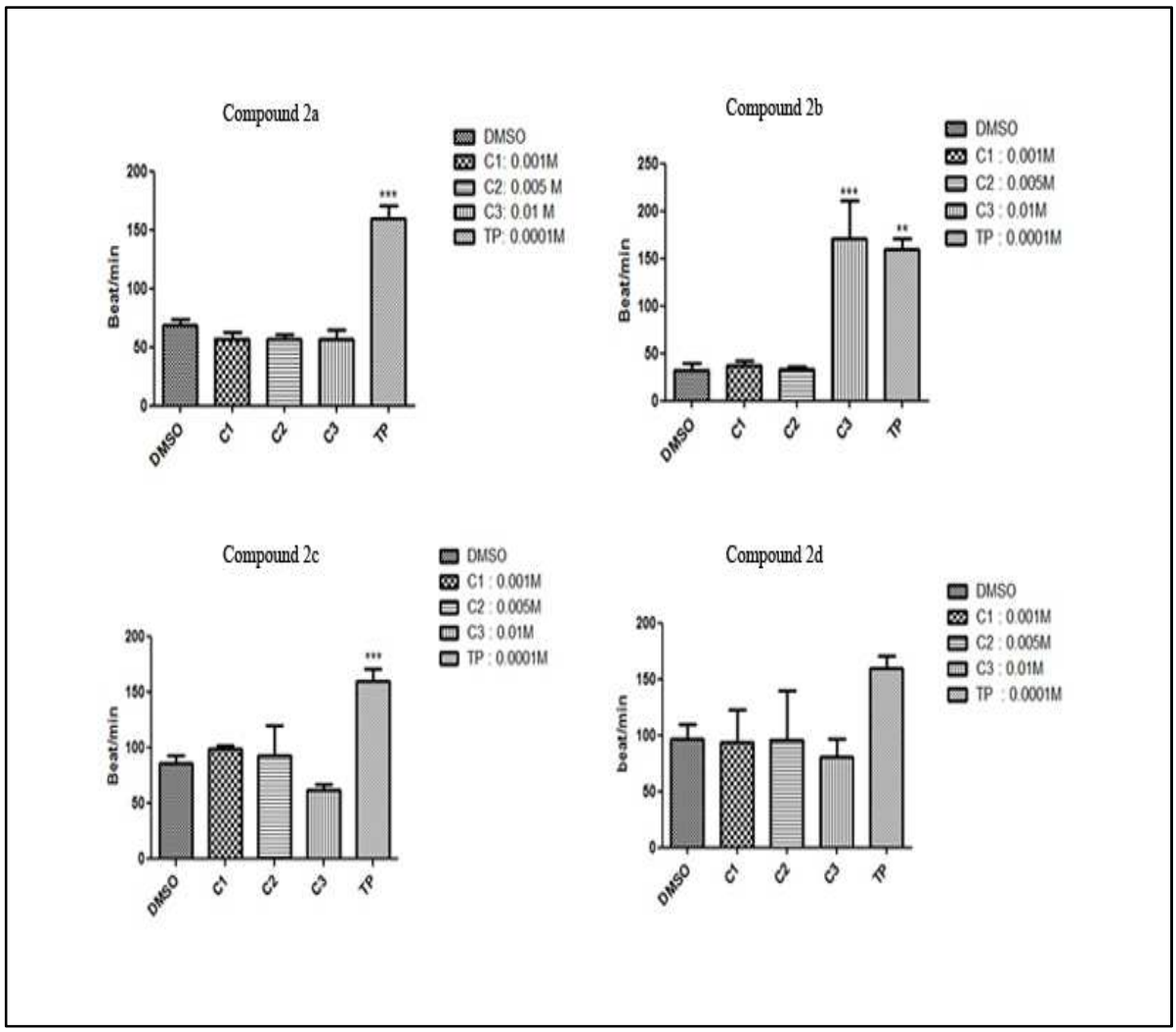
and **2d** ($R_1$, $R_3$ =Et, $R_2$=Bz) , R2 is Bz, so in these compound R2 is very bigger in comparison with Me in compound **2b** and carboxylate group in compound **2b** can bind and react better to calcium channel in compared other compounds.

**Table 1: Biginelli synthesis yield in classic and microwave method**




| Product | Substituent | Yield (classic) | Yield (microwave) |
|---|---|---|---|
| **2a** | $R_1$:Me, $R_2$: Bz , $R_3$: Et | 45.5% | 67.5% |
| **2b** | $R_1$, $R_2$, $R_3$ : Me | 15% | 15% |
| **2c** | $R_1$:Et , $R_2$: Bz , $R_3$:Me | 39.5% | 25% |
| **2d** | $R_1$, $R_3$ :Et, $R_2$:Bz | 73.5% | 60% |

**Figure 2:The effects of dihydropyrimidinones( 2a, b, c, d) on atria rate. DMSO used as negative control. Theophylline (TP) was used as positive control, n = 5, values are means ± SEM. All compounds compared with negative control (one-way ANOVA) in Tukey-Kramer test : ***p<0.001, **p<0.01**



About the inotropic action of this compound, because our positive control (theophylline) also failed to cause a significant increase in atrial contraction force, it seems that this method (isolated perfused right atria) is not a suitable method for studying the inotropic effects of agents. This is probably due to poor contractile force of atrial tissue in comparison to muscular ventricles. However, in the past, this method has not been used to investigate the chronotropic and inotropic effects of different materials andone of the objects of the present study was to evaluate effectiveness of this new method in studying inotropic and chronotropic effects of compounds. It seems that the isolated perfused right atria method in evaluation of chronotropic effects is suitable but this method is not for investigating inotropic effects.

## CONCLUSION

This study suggests that the isolated perfused right atria method, which we first examined for study of the chronotropic effects of different materials on atrial tissue, is a suitable method for this work.Also compound **2b** among the compound synthesized, had significant chronotropic effect in comparison to the negative control, this may be due to the agonist action of this compound on calcium channel.

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