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Synthesis and cardiac effects of 3,4-dihydropyrimidin-2-(1*H*)-one-5 carboxylates

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Abstract—A series of 4-(substituted)-3,4-dihydropyrimidinone derivatives have been synthesized by heating 1,3 dicarbonyl compounds, urea, and aromatic aldehydes in acetic acid under microwave irradiation conditions. The cardiovascular effects of 3,4-dihydropyrimidinones were studied on isolated perfused frog heart at different dose levels and compared with the activity of digoxin. The interaction of 3,4-dihydropyrimidinones with β-blocker and calcium channel blocker was also investigated. Compound **4d** emerged as the most interesting compound in this series with potential cardiotonic activity.

Cardiovascular diseases have been the principal cause of death in many developing countries[1] and disability in industrialized nations and is among the syndromes most commonly encountered in clinical practice.[2] The diagnosis of heart failure carries a risk of mortality comparable to that of the major malignancies.[3] Heart failure occurs when cardiac output is insufficient to meet the demands of tissue perfusion and may primarily be due to systolic or diastolic dysfunction.[4] It is frequently, but not always, caused by a defect in myocardial contraction.[5] Myocardial contractility is largely dependent upon the activity of the cardiac sympathetic nerves, but it can also be increased by circulating catecholamines, tachycardia, and inotropic drugs.[6] These drugs induce changes in myoplasmic calcium and this may be responsible for the cardioactive properties. Cardiac glycosides and catecholamines have been used as the main therapeutic drugs in the treatment of congestive cardiac failure.[7] However, the dangers of cardiac glycosides intoxication are well documented[8] and doubts have been expressed about their long-term effectiveness. The use of catecholamines is limited by their insufficient differentiation between positive inotropic and chronotropic actions, their potential arrhythmogenic properties, and tachyphylaxis due to receptor downregulation.[7]

3,4-Dihydropyrimidinones (DHPMs)[9,10] also called Biginelli compounds possess interesting biological applications.[11–13] The apparent structural similarities of DHPMs to the well-known Hantzsch-type dihydropyridines, calcium channel modulators, suggest a good scope for this class of compounds in the field of medicinal chemistry.[14,15] Calcium channel blockers are used in the treatment of angina, hypertension, cardiac arrhythmias,[16] and have a limited role in heart failure. The dihydropyridines are the most potent Ca^{2+} channel blockers. They have little effect on the myocardium and conducting tissue. Used alone, they often cause a reflex tachycardia which can be avoided by concomitant use of a β-blocker. More than 25 years after the introduction of nifedipine, many DHP analogs and numerous second generation products are available in the market (e.g., nicardipine, amlodipine, felodipine, etc.). Advances in the knowledge of the biochemical and physiological changes during cardiac failure as well as the development of new diagnostic and surgical procedures in cardiovascular medicines have been remarkable in the last few decades. Unfortunately no such claim could be made with respect to the development of new pharmacological agents with clinically useful po-

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sitive inotropic properties.[17] This necessitates research for new drugs, which increase cardiac muscle contractility with a broad therapeutic index. It is well-established that only one particular enantiomer is responsible for calcium channel antagonist activity between the existing two enantiomeric DHPMs in the racemic mixture.[11,18] Herein, we present for the first time, cardiotonic activity of DHPMs on an isolated perfused frog heart. The action may be attributed to the presence of the more potent agonistic enantiomer in the racemic mixture. Further we describe the synthesis and cardiovascular activity of DHPMs, which is vital for progress in the medical field.

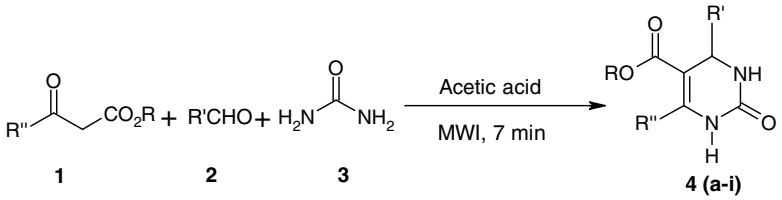
The synthetic pathway employed in the preparation[19] of 4-substituted-3,4-dihydropyrimidinones is outlined in Scheme 1. DHPMs were prepared readily by heating 1,3-dicarbonyl compounds **1**, urea **3**, and aromatic aldehydes **2** in acetic acid under microwave irradiation conditions. The formation of the products was monitored through TLC and irradiation was continued for appropriate time until completion of the reaction.

The structures of the compounds **4a–i** were confirmed by spectral, elemental analyses, and comparison with the available literature data.[20]

The effects of DHPMs were evaluated on an isolated perfused frog heart at various dose levels and compared with the activity of digoxin under identical experimental conditions. To elucidate the mechanism of action, the interaction of selected compounds with β-blocker and calcium channel blocker activities was investigated (Figs. 1–3).

In the present investigation compounds **4a–d** showed dose-dependent increase in force of contraction (positive inotropic action), decrease in rate of contraction (negative chronotropic action), and an increase in cardiac output. Among these compounds **4d** was more potent than digoxin.

This may be contributed by the more potent calcium channel agonistic *S* enantiomer[11] in the racemic mixture of *R* and *S*. Change of 'ethyl' (compound **4a**) by 'phenyl' (compound **4c**) substitution in the ester portion of the DHPM does not have marked effect on cardiovascular activity. Compound **4e** showed negative chronotropic action, increase in cardiac output, positive inotropic action, and was not dose-dependent. Compound **4f** at lower dose levels (at 5 and 50 μg/ml) did not show enhanced activity but at higher dose levels (at 500 μg/ml and 1 mg/ml) showed comparable positive inotropic



4a) R = Et, R' = 3-HOC_6H_4, R'' = CH_3
4b) R = Et, R' = isopropyl, R'' = CH_3
4c) R = Ph, R' = 3-HOC_6H_4, R'' = CH_3
4d) R = Et, R' = 4-HOC_6H_4, R'' = CH_3
4e) R = Et, R' = 4-$MeOC_6H_4$, R'' = CH_3
4f) R = Et, R' = biphenyl, R'' = CH_3
4g) R = Et, R' = 2-$O_2NC_6H_4$, R'' = CH_3
4h) R = Me, R' = 2-ClC_6H_4, R'' = CH_3
4i) R = Et, R' = 2-ClC_6H_4, R'' = CH_3

Scheme 1.

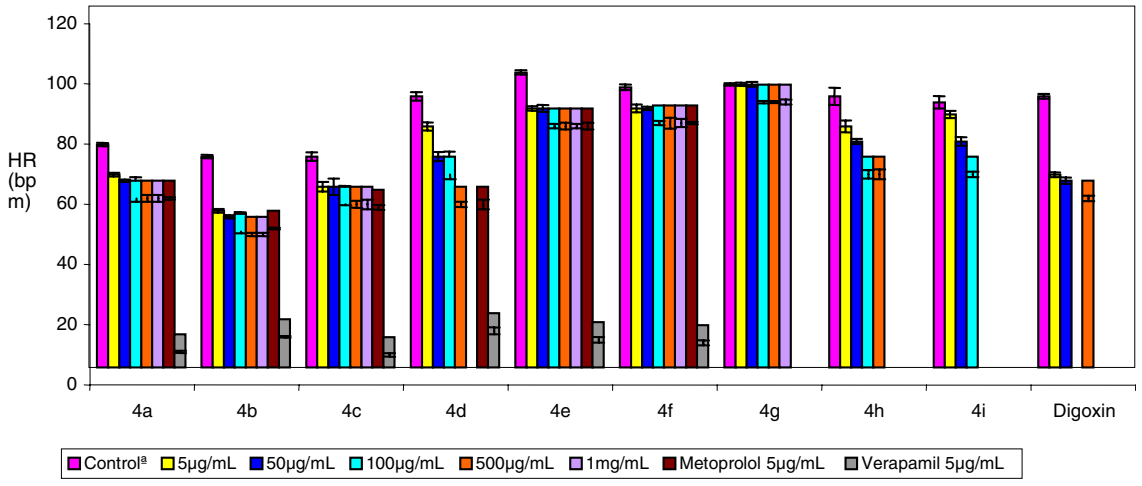


Figure 1. The effects of dihydropyrimidinone derivatives (heart rate) on frog heart rate. [a]1% Sodium carboxy methyl cellulose was used as control. $n = 6$, values are means ± SEM. All compounds showed $p < 0.01$ compared with control (one-way ANOVA) except entry **4g**.

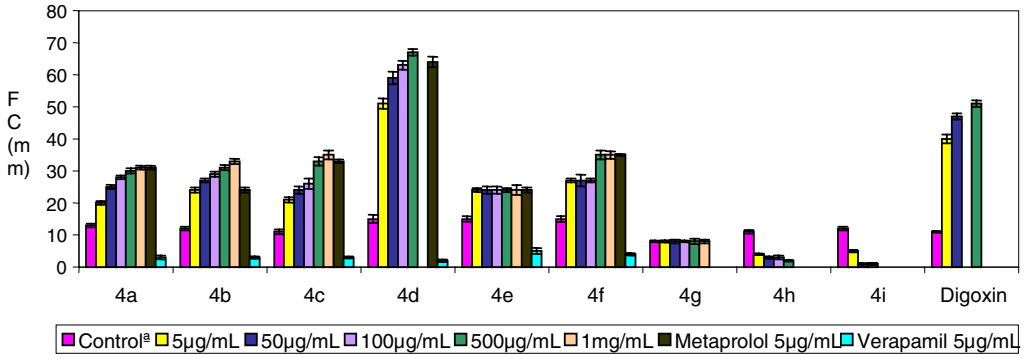


Figure 2. The effects of dihydropyrimidinone derivatives (force of contraction) on frog heart. [a]1% Sodium carboxy methyl cellulose was used as control. $n = 6$, values are means ± SEM. All compounds showed $p < 0.01$ when compared with control (one-way ANOVA) except entry **4g**.

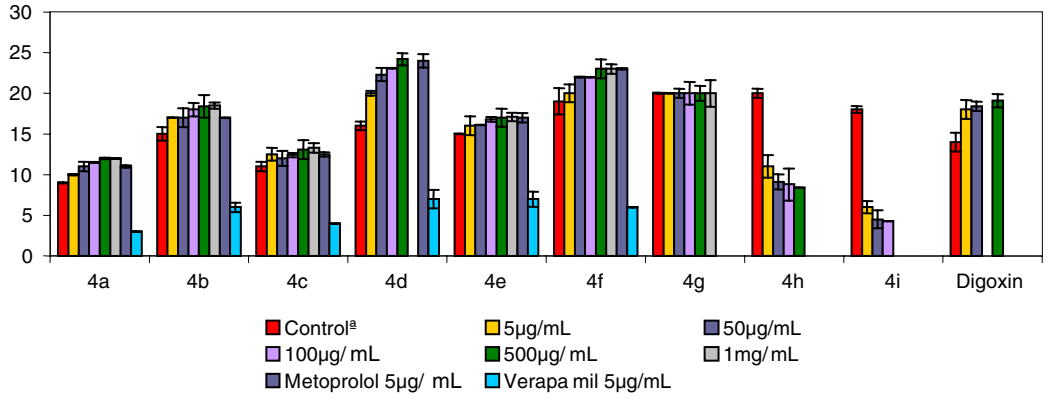


Figure 3. The effects of dihydropyrimidinone derivatives (cardiac output-CO) on frog heart. [a]1% Sodium carboxy methyl cellulose was used as control. $n = 6$, values are means ± SEM. All compounds showed $p < 0.01$ when compared with control (one-way ANOVA) except entry **4g**.

action, and an increase in cardiac output. Compound **4g** showed no change in rate, force of contraction of the heart and cardiac output as expected.[11] Compounds **4h** and **4i** were found to exhibit negative inotropic, chronotropic action, and decrease in cardiac output.[11] Further **4h** and **4i** blocked the effect of adrenalin (5 μg/ml), thereby showing β-adrenergic blocking activity.

The positive inotropic action of the compounds (**4a**–**f**) was not blocked by metoprolol (a cardioselective β-adrenergic blocker) but significantly blocked by the calcium channel blocker—verapamil. Since verapamil blocks the cardiotonic action of the compounds (**4a**–**f**), these compounds might have produced their action by opening the voltage sensitive slow Ca^{2+} channel.

In summary, we have described the synthesis and pharmacological effect of DHPMs on frog heart in this paper. The results obtained clearly indicate that the compounds **4a**–**f** discussed here showed good cardiotonic activity. Compounds **4h** and **4i** evinced β-adrenergic receptor antagonistic activity. Compound **4d** appears to be the most interesting derivative in our series and more potent than the digoxin. It can be a better choice for the existing cardiotonic drugs in the treatment of congestive heart failure and to confirm this, further studies are to be carried out in other laboratory animals. This observation may promote the synthesis of more active DHPMS in future. The development of methods for the enantioselective synthesis of chiral DHPMs using chiral catalysts and enzymes is underway since individual enantiomers of chiral DHPMs have opposing pharmacological effects and the use of enantiomerically pure compounds are a requirement for improving the efficacy of drugs of this type.

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19. ***Experimental: Equipment.*** Mps were determined in open capillary tubes and are uncorrected. IR measurements were obtained as KBr pellets using Perkin-Elmer spectrum RXI FT-IR. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ + DMSO-d_6 with JEOL 400 MHz (model GSX 400) high resolution NMR spectrometer with TMS as internal standard. Mass spectra were obtained using JEOL DX-303 in EI ionization mode at 70 eV. The reaction was carried out in a BPL—SANYO domestic microwave oven operating at 2.45 GHz. TLC was performed on precoated Polygram SIL G/UV_{254} sheets. The elemental analyses of the compounds were recorded using ThermoFinnigan FLASH EA 1112 CHNS analyzer.

Synthesis of 4-(substituted)-3,4-dihydropyrimidinones. A mixture of β-keto ester **1** (1 mmol), aldehyde **2** (1 mmol), and urea **3** (1 mmol) was irradiated in acetic acid medium with few drops of concd HCl. The solution was kept in an alumina bath and irradiated in a domestic microwave oven for 5–7 min with a pulse rate of 40 s and 30% of power. The total consumption of aldehyde as monitored by TLC was an indication of completion of the reaction. After the reaction was over, the mixture was poured into 150 ml of ice-cold water and heated over a water bath for 30 min followed by stirring for 1 h at room temperature. The solid thus obtained **4a**–**i** was collected by filtration and column chromatographed with 1:3 petroleum ether and ethyl acetate mixture. Characterization of the compounds by IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy, elemental analyses, and melting point confirms the formation of products.

Ethyl 4-(3-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4a**): Yield: 84%. Mp: 164–166 °C. IR (KBr): 3518, 3351, 3243, 1722, 1639 cm^{-1}. H NMR δ: 1.07 (t, J = 6.9 Hz, 3H), 2.27 (s, 3H), 3.95 (q, J = 6.9 Hz, 2H), 5.03 (s, 1H, CH(4)), 6.58 (d, J = 8.6 Hz, 1H), 6.63 (d, J = 7.45 Hz, 2H), 7.04 (t, J = 8 Hz, 1H), 7.66 (s,1H, NH(3)), 9.13 (s, 1H, NH(1)), 9.34 (s, 1H, Ar-OH). ^{13}C NMR δ: 14.6, 18.3, 54.3, 59.7, 99.9, 113.6, 114.7, 117.4, 129.8, 146.8, 148.6, 152.8, 157.9, 165.9 MS (EI, *m/z*): 276 (M^+); Anal. Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.81; H, 5.78; N, 10.06.

Ethyl 4-(isopropyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4b**): Yield: 88%. Mp: 192–193 °C. IR (KBr): 3234, 3102, 1692, 1645 cm^{-1}. ^{1}H NMR δ: 0.74 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.9 Hz, 3H), 1.19 (t, J = 7.1 Hz, 3H), 1.68 (m, 1H), 2.18 (s, 3H), 3.96 (t, J = 3.6 Hz, 1H), 4.04 (m, 2H), 7.26 (s, 1H), 8.86 (s, 1H). ^{13}C NMR δ: 14.2, 16.0, 17.7, 18.5, 34.6, 55.5, 58.1, 98.2, 148.4, 153.2, 165.8. MS (EI, *m/z*): 226 (M^+); Anal. Calcd for $C_{11}H_{18}N_2O_3$: C, 58.39; H,8.02; N, 12.38. Found: C, 58.40; H, 8.00; N, 12.45.

Phenyl 4-(3-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4c**): Yield: 85%. Mp: 170–172 °C. IR (KBr): 3365, 2937, 2369, 1716, 1573, 1508 cm^{-1}. ^{1}H NMR δ: 2.28 (s, 3H), 5.22 (s, 1H), 6.79 (s, 1H), 6.85 (d, J = 8.6 Hz,1H), 6.88 (d, J = 7.2 Hz, 1H), 6.96 (t, J = 7.6 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 7.1 (d, J = 6.8 Hz, 1H), 7.56 (s, 1H, NH(3)), 9.05 (s, 1H, NH(1)), 9.25 (s, 1H). ^{13}C NMR δ: 14.5, 52.6, 113.5, 113.9, 116.5,119.7, 121.8, 125.2, 127.6, 130.2, 134.1, 148.0, 153.5, 153.6, 158.2, 170.5.MS (EI, *m/z*): 324 (M^+); Anal. Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H,4.97; N, 8.64. Found: C, 66.82; H, 4.85; N, 8.56.

Ethyl 4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4d**): Yield: 88%. Mp: 228–229 °C. IR (KBr): 3410, 3256, 2990, 1719, 1689 cm^{-1}. ^{1}H NMR: 1.12 (t, 3H, J = 7.5 Hz), 2.22 (s, 3H), 4.03 (q, 2H, J = 7.5 Hz), 5.12 (s, 1H), 6.70 (d, 2H, J = 9 Hz), 7.10 (d, 2H, J = 9.0 Hz), 7.5 (s, 1H, NH(3)), 9.01 (s, 1H, NH(1)), 9.20 (s, 1H, Ar-OH). ^{13}C NMR: 15.04, 17.76, 53.54, 58.76, 99.73, 114.58, 127.19, 136.54, 147.25, 152.30, 156.19 and 165.24. MS (EI, *m/z*): 276 (M^+). Anal. Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.11; H, 5.80; N, 10.42.

Ethy 4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4e**): Yield: 84%. Mp: 199–200 °C. IR (KBr): 3303, 3050, 1710, 1647 cm^{-1}. ^{1}H NMR: 1.08 (t, 3H, J = 7.5 Hz), 2.24 (s, 3H), 3.71 (s, 3H, Ar-OMe), 3.97 (q, 2H, J = 7.5 Hz), 5.23 (d, 1H, J = 2.7 Hz), 6.88 (d, 2H, J = 8.58 Hz, Ar-H), 7.13 (d, 2H,J = 8.58 Hz, Ar-H), 7.67 (s, 1H, NH(3)), 9.15 (s, 1H, NH(1)). ^{13}C NMR: 14.03, 18.32, 53.88, 55.61, 59.73, 100.13, 114.26, 127.96, 137.60, 148.58, 152.73, 159.00, 165.94. MS (EI, *m/z*): 290 (M^+). Anal. Calcd for $C_{15}H_{18}N_2O_4$: C, 62.08; H, 6.25; N, 9.65. Found: C, 61.93; H, 6.19; N, 13.87.

Ethyl 4-(4-(1,1′-biphenyl))-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4f**): Yield: 70%. Mp: 212–214 °C. IR (KBr): 3224, 3105, 1692, 1639 cm^{-1}. ^{1}H NMR: δ 1.04 (t, J = 7.45 Hz, 3H), 2.21 (s, 3H), 3.82 (q, J = 7.45 Hz, 2H), 5.43 (d, 1H, J = 3.5 Hz), 7.26 (m, 3H), 7.38 (t, J = 7.45 Hz, 2H), 7.54 (t, J = 8.1 Hz, 4H), 7.71 (s, 1H, NH(3)), 9.12 (s, 1H, NH(1)). ^{13}C NMR: δ 14.53, 18.27, 54.11, 60.05, 99.94, 127.09, 127.29, 127.41, 128.01, 129.52, 139.83, 140.19, 144.23, 148.88, 152.88, 166.06. MS (EI, *m/z*): 336 (M^+). Anal. Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.33; H, 5.91; N, 8.37.

Ethyl 4-(2-nitrophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4g**): Yield: 90%. Mp: 213–215 °C. IR (KBr): 3112, 1693, 1645, 1524, 1340 cm^{-1}. ^{1}H NMR: 0.93 (t, 3H, J = 7.6 Hz), 2.29 (s, 3H), 3.83 (q, 2H, J = 7.6 Hz), 5.83 (s, 1H), 7.48–7.50 (m, 2H), 7.66 (s, 1H, NH(3)), 7.70 (d, 2H, J = 9 Hz), 9.36 (s, 1H, NH(1)). ^{13}C NMR:13.58, 17.49, 49.20, 58.97, 97.92, 123.67, 128.45, 128.87, 133.85, 139.13, 146.62, 149.42, 150.60, 165.32. MS (EI, *m/z*): 305 (M^+). Anal. Calcd for $C_{14}H_{15}N_3O_5$: C, 55.08; H, 4.95; N, 13.76. Found: C, 55.10; H, 4.91; N, 13.87.

Methyl 4-(2-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (**4h**): Yield: 89%. Mp: 224–226 °C; IR: 3310, 3250, 1701, 1645 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.29 (s, 3H), 3.68 (s, 3H), 5.31 (s, 1H), 7.30–7.52 (m, 4H), 7.53 (s, 1H, NH(3)), 9.31 (s, 1H, NH(1)). ^{13}C NMR δ: 17.85, 54.41, 59.23, 101.83, 126.13, 127.23, 127.51, 128.22, 140.11, 144.54, 150.21, 152.13 and 165.58. MS (EI, *m*/*z*): 280 (M^+) 282 (M+2) Anal. Calcd for $C_{13}H_{13}ClN_2O_3$ C, 55.62; H, 4.67; N, 9.98. Found: C, 55.43; H 4.72; N, 9.97.

Ethyl 4-(2-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1(H)-one-5-carboxylate (**4i**): Yield: 91%. Mp: 213–215 °C. IR (KBr): 3363, 3225, 3100, 1690, 1650 cm^{-1}. ^1H NMR: 1.03 (t, 3H, J = 7.5 Hz), 2.45 (s, 3H), 3.98 (q, 2H, J = 7.5 Hz), 5.64 (s, 1H, NH(1)), 5.88 (d, 1H, J = 2.4 Hz), 7.20–7.24 (m, 3H), 7.36–7.39 (m, 1H), 7.87 (s, 1H, NH(1)). ^{13}C NMR: 15.21, 17.88, 53.17, 58.03, 103.13, 127.23, 128.14, 129.32, 136.54, 140.11, 147.21, 152.37, 156.39 and 165.93. MS (EI, *m*/*z*): 294 (M^+) 296 (M+2) Anal. Calcd for $C_{14}H_{15}ClN_2O_3$: C, 57.05; H, 5.13; N, 9.50. Found: C, 57.02; H, 5.10; N, 9.52;

Pharmacology: Cardiovascular activity. Common Indian adult frogs of *Rana tigerina* species were used in the present study. The great advantage of frog tissues is that they can function as isolated preparations for many hours when handled carefully and to maintain the tissue preparation, no extra supply of oxygen is needed as the frog muscles can directly imbibe oxygen from the atmosphere. The animals were maintained as per the norms of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) and this experiment was cleared by CPCSEA and Institutional Animal Ethics Committee constituted for the purpose. Frogs were dissected from ventral surface to expose the heart and pericardium was removed. The frog heart was isolated and perfusion was done by Bulbring's method as described by Burn.[21] Frog ringer with the following composition was used as the perfusion fluid NaCl, 110.0; KCl, 1.90; $CaCl_2$, 1.10; NaH_2PO_4, 0.06; $NaHCO_3$, 2.40; dextrose, 11.10 μM made up to 1000 ml with distilled water. The apex of ventricle was attached to the sterling heart lever, which in turn was attached by a thread to the muscle forced transducer and this is connected to a 'physiograph'. Normal heart rate, contractile amplitude were recorded. The cardiac output from the isolated frog heart was collected every minute and measured. The various concentrations of compounds to be tested were prepared using 1% sodium carboxy methyl cellulose and administered to the ringer flowing through the cannula and the effects were recorded at different dose level (5 μg, 50 μg, 100 μg, 500 μg, and 1 mg/ml). Only one compound was tested in each preparation. The results are given in Figures 1–3. The responses of the compounds were compared to those of digoxin. During the interaction studies verapamil (5 μg/ml) in frog ringer's solution, metoprolol, a β-adrenergic blocker (5 μg/ml) in frog ringers solution and adrenaline (5 μg/ml) were administered to the ringer solution and recordings were noted. The data presented in figures are means ± SE. One-way ANOVA was used for statistical analysis. P values < 0.01 were considered to be statistically significant.

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