Antihypertensive activity of novel 1,4-dihydropyridine derivatives

Riazuddin Mohammed1, Asma Samaunnisa A2*, C.H.S. Venkataramana2 and V Madhavan3

1Department of Medical biosciences, Linkoping University, Linkoping, Sweden.
2Department of Pharmaceutical chemistry, M.S. Ramaiah College of Pharmacy, Bangalore, India.
3Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore, India.

*Correspondence Info:
A. Asma Samaunnisa, Department of Pharmaceutical Chemistry, M.S. Ramaiah College of Pharmacy, Bangalore, India.
E-mail: asmasamaunnisa@gmail.com

Abstract
In the present work, screening for antihypertensive activity was carried out on a series of sixteen novel 2, 6-dimethyl-N1, N2-diphenyl-1, 4-dihydropyridine-3, 5-dicarboxylic acids (2A-2D') and 2,6-dimethyl-1,4-dihydropyridine-3,5-yl-bis(carbonyl-2- ( phenyl) pyrazolidine-3,5-diones) (3A-3D') derivatives. Structures of all the derivatives were previously confirmed by IR, 1HNMR, mass and elemental analysis data. These derivatives contain central 1,4-dihydropyridine ring which is renowned for its calcium channel blocking activity. Antihypertensive activity was evaluated using tail cuff method in rats. Nifedipine was used as standard and 1% w/v sodium carboxymethyl cellulose suspension as control. Most of the compounds exhibited appreciable antihypertensive activity and are significant when compared with the standard. The Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial Blood Pressure (MABP) were measured in both normotensive and hypertensive rats. Keywords: Antihypertensive, 1, 4-Dihydropyridine, Dicarboxylic acid, pyrazolidine-3,5-dione, tail cuff method.

1. Introduction
Hypertension is a consequence of many diseases. Hemodynamically, blood pressure is a function of the amount of blood pumped by the heart and ease with which the blood flows through the peripheral vasculature. Therapy using antihypertensive agents evolved rapidly between 1950 and 1960. During that time, a number of drugs for the treatment and control of hypertensive disease were discovered. Despite the many years of experience, treatment remains empiric because the etiology of the principal form of hypertension is unknown. Since then, numbers of molecules are being developed to treat hypertension.

1,4-Dihydropyridines are well known as calcium channel blockers, have emerged as one of the important class of drugs for the treatment of hypertension. They act by inhibiting the entry of Ca2+ ions into the cardiac cells and vascular muscle through the voltage dependent calcium channels. Recently reported studies have shown that compounds with 1,4-Dihydropyridine (1, 4-DHP) nucleus possess variety of biological activities including antinocicribal and neuroprotectant. They have been extensively utilized to study the mechanism and synthetic potential of various NAD(P)H mediated redox processes. Calcium entry blockers of Hantzsch 1, 4-DHP type are rapidly emerging as one of the most important classes of drugs for the treatment of cardiovascular disease. In addition, DHP nucleus is common to numerous bioactive compounds which include vasodilator, antihypertensive, bronchodilator, antitumor and antidiabetic agents. Most of the existing antihypertensives cause adverse effects like dizziness, ankle swelling, headache, fatigue, chest discomfort and cough. The aim of this study was to evaluate the calcium channel blocking efficacy of novel 1,4-dihydropyridines. Two sets of eight derivatives in each, 2, 6-dimethyl-N1, N2-diphenyl-1, 4-dihydropyridine-3, 5-dicarboxylic acids (2A-2D') and 2,6-dimethyl-1,4-dihydropyridine-3,5-yl-bis(carbonyl-2- (phenyl) pyrazolidine-3,5-diones) (3A-3D') were subjected to the antihypertensive evaluation.

2. Materials and Methods
2.1 Experimental
Chemicals and solvents used were of reagent grade and used without further purification, were procured from SpectroChem, Hi-Media, Merck, Sigma Aldrich and Ranbaxy. The purity of the synthesized compounds was determined by melting point using open capillary method and is uncorrected. IR (infra-red) was performed using SHIMADZU FTIR- 8400S. The compounds 2A-2D' were identified by HNMR (proton nuclear magnetic resonance) using amx-400 NMR. Mass using LC-MS 2010A and elemental analysis using Flash EA 1112 Thermo finnigan. TLC was performed using Solvent system Ethyl acetate: n-hexane: Stationary phase- Silica Gel-G.

2.2 Animals
Albino Wistar rats of either sex with body weight 150-250g were used for the screening of antihypertensive activity. Animals were retained at standard conditions of temperature (24±2°C) and relative humidity (30-70%) and exposed to 12hr light and dark cycle. Animals were given standard diet and water ad libitum. All the procedures involving animals were carried out as per OECD guidelines and under the institutional animal ethical committee approval.

2.3 Synthesis of 1,4-dihydropyridines
2.3.1 Synthesis of Diethyl-2, 6-dimethyl- 1, 4-dihydropyridine-3, 5-dicarboxylate (1A) and diethyl-4-(4-hydroxyphenyl)-2, 6- dimethyl-1, 4- dihydropyridine-3, 5- dicarboxylate (1A')
2.3.2 Synthesis of 2, 6-dimethyl-N3, N5- diphenyl-1, 4-dihydropyridine-3, 5- dicarbohydrazide (2A-2D')

\[
\text{2A-2D'}
\]

2.3.3 Synthesis of 2, 6-dimethyl-1, 4-dihydropyridine-3, 5-yl-bis [carbonyl-2-(phenyl)] pyrazolidine-3, 5- diones] (3A-3D')

Substitutions for the derivatives are given in table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(R^1)</th>
<th>(R^2)</th>
<th>(R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>-</td>
<td>-</td>
<td>H</td>
</tr>
<tr>
<td>1A'</td>
<td>-</td>
<td>-</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2A</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>2A'</td>
<td>H</td>
<td>H</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2B</td>
<td>NO(_2)</td>
<td>NO(_2)</td>
<td>H</td>
</tr>
<tr>
<td>2B'</td>
<td>NO(_2)</td>
<td>NO(_2)</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2C</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
</tr>
<tr>
<td>2C'</td>
<td>H</td>
<td>Cl</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2D</td>
<td>H</td>
<td>NO(_2)</td>
<td>H</td>
</tr>
<tr>
<td>2D'</td>
<td>H</td>
<td>NO(_2)</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2A''</td>
<td>H</td>
<td>H</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2B''</td>
<td>NO(_2)</td>
<td>NO(_2)</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2C''</td>
<td>H</td>
<td>Cl</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>2D''</td>
<td>H</td>
<td>NO(_2)</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>3A</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>3A'</td>
<td>H</td>
<td>H</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>3B</td>
<td>NO(_2)</td>
<td>NO(_2)</td>
<td>H</td>
</tr>
<tr>
<td>3B'</td>
<td>NO(_2)</td>
<td>NO(_2)</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>3C</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
</tr>
<tr>
<td>3C'</td>
<td>H</td>
<td>Cl</td>
<td>CH(_2)OH</td>
</tr>
<tr>
<td>3D</td>
<td>H</td>
<td>NO(_2)</td>
<td>H</td>
</tr>
<tr>
<td>3D'</td>
<td>H</td>
<td>NO(_2)</td>
<td>CH(_2)OH</td>
</tr>
</tbody>
</table>

2.4 Biological activity

Albino rats weighing 200-250 gm were used to evaluate antihypertensive activity of the novel 1,4-dihydropyridine derivatives. Suspension of the synthesized compounds was prepared in 1% w/v sodium carboxy methyl cellulose and administered at a dose level of 60 mg/kg body weight to different groups with five rats in each group. Control group received an equal quantity of 1% w/v sodium carboxy methyl cellulose suspension. After administration of specified dose to the animals, blood pressure was measured by Non-invasive Tail cuff Method using pressure meter. Measurements were carried out after 1 hour and 3 hour time intervals in a stepwise manner. One hour after administration of drug samples, animal was shifted to the restrainers, which restricts the movement of animals. The tail was cleaned with moist cotton in order to remove dirty matter and talcum powder was sprayed on tail to make its surface smooth. A tail cuff and pulse transducer was fixed around the tail.

Initially the normal range of pulse rate was measured. Later SBP (systolic blood pressure), DBP (Diastolic blood pressure) and MABP (mean arterial blood pressure) was recorded using a recorder and it is displayed on the monitor, the pressure can be read from the pre-calibrated monitor.

2.5 Statistical Analysis

The experimental data were expressed as mean ± SEM. The data were analyzed using ANOVA and Tukey-Kramer multiple comparison test. The results were considered statistically significant if P<0.05.

3. Results and Discussion

Two sets of eighteen novel 1,4-dihydropyridine derivatives 2A-2D' and 3A-3D' were synthesized as provided in the literature, the structures of the derivatives were characterized using IR, NMR, mass and elemental analysis data. All the derivatives were subjected to the evaluation of antihypertensive activity by tail cuff method in rats using nifedipine as standard and 1% w/v sodium carboxy methyl cellulose suspension as control, derivatives were administered at a dose of 60 mg/kg body weight. Systolic, diastolic and mean arterial blood pressure was significantly increased in hypertension induced rats. Administration of these derivatives produced blood pressure lowering effects in hypertensive rats, which was measured as fall in blood pressure after stabilization.

In the current study, all the novel 1,4-dihydropyridine derivatives 2A-2D' and 3A-3D' exhibited noticeable fall in the arterial blood pressure of hypertensive rats. Reduction in both systolic and diastolic blood pressure was observed in the rats treated with the above mentioned derivatives. Almost all of the derivatives displayed fall in the blood pressure and the activity is comparable to that of the standard drug.

Derivatives 2A-2D and 3A-3D which does not contain phenol group substituted at 4\(_\phi\) position on dihydropyridine ring showed relatively less activity when compared to their counter parts containing phenol group at 4\(_\phi\)position, 2A'-2D' and 3A'-3D'. On the other hand, derivatives with electron withdrawing groups at para position on the two phenyl rings connected on either sides of central 1,4-dihydropyridine ring exhibited significantly good antihypertensive activity. Derivatives 2B,3B,2B',3B' with dinitro groups at ortho, para positions (R\(^1\)R\(^2\), NO\(_2\)), 2C,3C,2C',3C' with chloro group at para position (R\(^2\)-Cl), 2D,3D,2D',3D' with nitro group at para position (R\(^2\)-NO\(_2\)) displayed significantly better activity providing more active compounds. The comparable activities of the tested derivatives against a standard and control are given in figures 1-6.
The results are expressed as Mean ± SEM. Control-1% sodium carboxy methyl cellulose suspension, Standard- nifedipine 30 mg/kg, n=5, *P < 0.001, † P < 0.01, ‡ P < 0.05, in comparison with the control group.

**Figure 1: Antihypertensive activity using tail cuff method in rats (SBP of Hypertension induced normotensive rats)**

![Graph showing antihypertensive activity using tail cuff method in rats (SBP of Hypertension induced normotensive rats).](image1)

**Figure 2: Antihypertensive activity using tail cuff method in rats (DBP of Hypertension induced normotensive rats)**

![Graph showing antihypertensive activity using tail cuff method in rats (DBP of Hypertension induced normotensive rats).](image2)

**Figure 3: Antihypertensive activity using tail cuff method in rats (MABP of Hypertension induced normotensive rats)**

![Graph showing antihypertensive activity using tail cuff method in rats (MABP of Hypertension induced normotensive rats).](image3)
The results are expressed as Mean ± SEM. Control-1% sodium carboxy methyl cellulose suspension, Standard- nifedipine 30 mg/kg, n=5, \(^{a}P < 0.001, ^{b}P < 0.01, ^{c}P < 0.05\), in comparison with the control group.

**Figure 4:** Antihypertensive activity using tail cuff method in rats (Derivatives effect on Hypertension induced rats- SBP)

**Figure 5:** Antihypertensive activity using tail cuff method in rats (Derivatives effect on Hypertension induced rats- DBP)

**Figure 6:** Antihypertensive activity using tail cuff method in rats (Derivatives effect on Hypertension induced rats- MABP)
4. Conclusion
In the present study, pharmacological screening of 2, 6-dimethyl-N\(^2\), N\(^3\)-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (2A-2D\(^2\)) and 2,6-dimethyl-1,4-dihydropyridine-3,5-yl-bis[carbonyl-2- (phenyl)]pyrazolidine-3,5-diiones (3A-3D\(^3\)) derivatives for their antihypertensive activity was reported. All the tested derivatives exhibited significantly good antihypertensive activity. Further exploration of synthesized derivatives, employing structural modification might result in therapeutically better candidates.

Acknowledgement
I am grateful to express my sincere thanks to the Gokula Education Foundation (GEF medical), the Management and staff of M.S.Ramaiah College of Pharmacy, Bangalore for providing all the facilities and encouragement for carrying out the work. I am also thankful to Indian Institute of Science (IISC), Bangalore for their analytical services (Spectral data).

References