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SYNTHESIS AND *IN-VIVO* ACTIVITY OF NOVEL ANTIHYPERTENSIVE AGENT BASED ON PYRIDAZINE SCAFFOLD

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Keywords:

β-Aroyl propionic acid, Pyridazine, Antihypertensive activity, Non-invasive method, *in-vivo*

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ABSTRACT: The main objective of the present research work to synthesize, characterization, and *in-vivo* evaluation of Pyridazine derivatives. To study the different synthesized derivatives by using different analytical parameters like IR, Mass, and NMR analysis. And also find out the antihypertensive activity. The studies on the hydralazine group drugs led to the synthesis of many Pyridazine derivatives with a wide activity spectrum on the cardiovascular system. Pyridazine derivatives, a class of compounds containing the N-N bond, exhibit a wide range of pharmacological activities such as antidepressant, antihypertensive, and cardiotonic, *etc*. Some 6-(substituted phenyl)-2-(substituted methyl)-4,5-dihydropyridazin-3(2H)-one derivative was synthesized by reacting 6-Phenyl substituted 2,3,4,5-Tetrahydro pyridazin-3-one with cyclic secondary amine under Mannich reaction conditions. A total of twenty compounds (vj1-vj20) were synthesized under Mannich reaction conditions. Out of twenty compounds, around six derivatives were selected for evaluation of antihypertensive activities by a non-invasive method using the Tail Cuff method. Most of the compounds showed good antihypertensive activity. Few compounds like vj3, vj6, vj9, vj14, vj19, and vj20 were found to show a highly significant reduction in mean arterial blood pressure but at a higher dose in comparison to standard drugs like propanolol and hydralazine. The substituted pyridazine derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of hypertension.

INTRODUCTION: In the present scenario, a large number of medications acting through different mechanisms for the treatment of hyper-tension are available.

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Even one has to admit that the blood pressure of the majority of hypertensive patients is inadequately controlled, partly because the treatment is not conducted intensively enough, but partly also because the medication(s) are not taken as prescribed. Lowering blood pressure in hyper-tensive patients requires, therefore, not only a broad choice of effective and well-tolerated medications, but also skills to motivate them to comply lifelong with the treatment. Hypertension is the most common cardiovascular disease.

The definition of hypertension, therefore using any specific cut off point, is arbitrary. Pyridazine derivatives were reported to exhibit diverse pharmacological activities such as antidepressant [1], antihypertensive [2, 3], antithrombotic [4], anti-convulsant [5], cardiotonic [6], antibacterial [7], diuretics [8], anti-HIV [9] and anticancer [10]. Some pyridazinone derivatives like indolidan [11], bemoradan [12], primobendan [13], levosimendan [14] (antihypertensive), minaprine [15] (antidepressant), emorfazone [16] (anti-inflammatory), and azanrinone [17] (cardiotonic), already appeared in the clinical market. In continuation to the work on pyridazine/ pyridazinone ring system in our lab, we have synthesized some pyridazinone derivatives and evaluated them for antihypertensive activity by non invasive method.

The main objective of present research study to synthesis, characterization and in-vivo evaluation of Pyridazine derivatives. All the synthesized compounds were obtained in good yield by optimizing various synthetic procedures. The structures of the compounds were established by elemental analysis, IR, 1H-NMR and Mass spectral data analysis. To study the different synthesized derivative by using different analytical parameters. And also to find out the antihypertensive activity of active synthesized compounds.

MATERIALS AND METHODS:

Chemistry for Basic Compound: Some 6-(substituted phenyl)-2-(substituted methyl)-4,5-dihydropyridazin-3(2H)-one derivative were synthesized according to scheme. The Friedel Craft acylation of aromatic hydrocarbon with succinic anhydride afforded the β-substituted benzoyl propionic acid in presence of lewis acid, aluminium chloride. The resulting β-benzoyl propionic acids were on hydrazinolysis gave the Pyridazine. The Pyridazine was subjected to Mannich reaction with cyclic secondary amine and formaldehyde to get the final compounds (vj1-vj20).

General procedure for the synthesis of substituted *β*-aroyl propionic acids was synthesized from respective aromatic hydrocarbon and characterized on the basis of spectral data as per the reported procedure. The appropriate substituted *β*-aroyl propionic acid was reacted with hydrazine hydrate to get the corresponding pyradazinone and characterized on the basis of spectral basis as per earlier reported procedure [18-21].

Two series of pyridazinone derivatives, 6-(aryl)-2-(substituted methyl)-4,5-dihydro (2H) pyridazin-3-one derivatives (3a-8a and 3b-8b) were synthesized by reacting 6-phenyl substituted 2,3,4,5-Tetrahydro pyridazin-3-one (2a and 2b) with cyclic secondary amine under Mannich reaction conditions.

On the basis of the literature report, nitrogen-containing heterocyclic compounds showed diverse pharmacological activities. In this series, pyridazinone derivatives were reported to exhibit diverse pharmacological activities. During recent years substituted pyridazinones have been a subject of demanding research due to their wide range of pharmacological actions [22-24].

Differently substituted pyridazinone derivatives were exhibited diverse potential pharmacological activities like an antidepressant, antihypertensive, antithrombotic, anticonvulsant, cardiotonic, analgesic, anti-inflammatory, diuretics, antibacterial, anti-fungal, antiviral, anticancer, hypotensive, antiulcer and other biological activities [25-29].

General procedure for the preparation of 6-(substituted phenyl)-2-(substituted methyl)-4,5-dihydropyridazin-3(2H)-one (vj1-vj20): To a solution of 6-substitued phenyl 2,3,4,5-tetra-hydropyridazine-3-one (0.001 mole) in absolute ethanol (30 ml), formaldehyde (37-41%) (1.5 ml) and cyclic secondary amine (0.001 mole) were added and the contents refluxed for 24 h. After completion of the reaction, ethanol was distilled off, and the residue poured into crushed ice and kept in the refrigerator overnight to separate out the compound.

The solid which separated out was filtered and recrystallized from ethanol. A total of around twenty different derivatives was synthesized by using the below scheme and characterized by IR, NMR, and Mass spectroscopy.

Pharmacology of Experimental Pharmacology Protocol for Synthesized Derivatives:

Procurement, Identification, and Housing of Animals: Albino rats were selected (bodyweight 200-250 g) were supplied by Central Animal House facility of Registration number 173/CPCSEA.

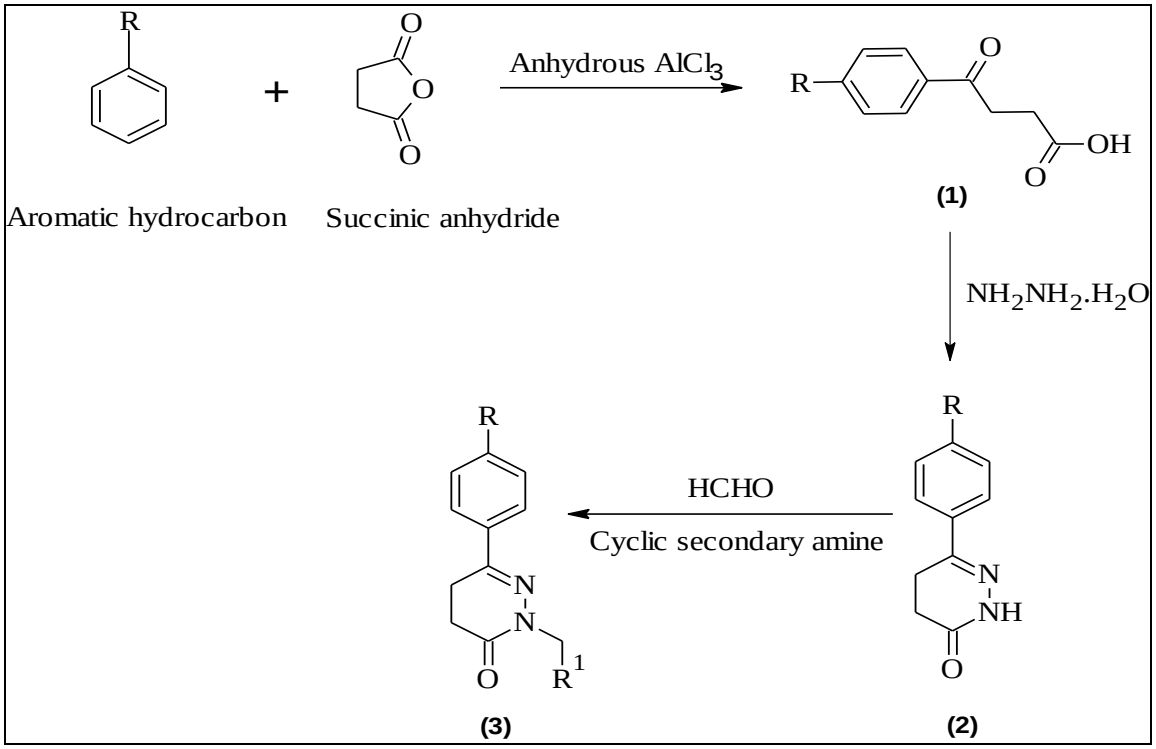

FIG. 1: SCHEMATIC REPRESENTATION OF 6-(SUBSTITUTED-PHENYL)-2-(SUBSTITUTED METHYL)-4, 5-DIHYDROPYRIDAZIN- 3(2*H*)-ONE DERIVATIVES

Conditioning / Training of Animals: For conducting the BP measurement studies, the animals were kept in a restrainer for 10 min every day for one week. This exercise was done to avoid the fluctuation in blood pressure due to the aggressive behavior of animals while keeping into the restrainer for measuring the activity.

Induction of Hypertension in Normotensive Rats: After recording the initial BP of rats, the animals were divided into groups of 5 animals each. One group was taken as control.

Measurement of Mean Blood Pressure of Rats: Mean arterial blood pressure was measured in conscious rats using CODA Non-Invasive Blood Pressure Recorder by Tail-Cuff method (Kent Scientific Corporation, USA).

RESULTS AND DISCUSSION: Antihypertensive activities of the compounds were tested by using Tail Cuff method. The results were compared with standard drug hydralazine [30]. Compound numbers vj3, vj6, vj9, vj14, vj19, and vj20 were found to show **a** highly significant reduction in mean arterial blood pressure but at higher dose in comparison to hydralazine. On this basis, it can be concluded that small electron releasing groups like p-CH_3, p-ethyl in phenyl ring at 6- position increases the activity.

Experimental Protocols:

Chemistry: Melting points were determined by the open tube capillary method and are uncorrected. The purity of the compounds was checked by thin-layer chromatography (TLC) plates (silica gel G) which were visualized by exposing them to iodine vapors and UV light. The FT-IR spectra were recorded on Bio-rad FTS-135 spectrophotometer using KBr pellets; υ_{max} values are given in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker Spectrospin DPX 300 MHz using $CDCl_3$ as a solvent and trimethylsilane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) scale, and coupling constants (J values) are expressed in Hz. The FAB Mass spectra were obtained on JEOL-JMS-DX 303 system, equipped with a direct inlet probe system. Elemental analysis was carried out on CHNS Elementar (Vario EL III) using sulphanilic acid as a standard and tugsten (VI) oxide as a combusting agent, and analyses for C, H, N were within ±0.4% of the theoretical values.

General Procedure for the Preparation of 6-(substituted phenyl)-2-(substituted methyl)-4,5-dihydropyridazin-3(2H)-one (vj1-vj20): To a solution of 6-substituted phenyl 2,3,4,5-tetrahydro-pyridazine-3-one (0.001 mole) in absolute ethanol (30 ml), formaldehyde (37-41%) (1.5 ml) and

cyclic secondary amine (0.001 mole) were added and the contents refluxed for 24 h. After completion of the reaction, ethanol was distilled off, and the residue poured into crushed ice and kept in the refrigerator for overnight to separate out the compound. The solid which separated out was filtered and recrystallized from ethanol. A total of around twenty different derivatives was synthesized and characterized by IR, NMR, and Mass spectroscopy. The interpretation results of different parameters like IR, NMR, and Mass of synthesized derivatives were mentioned below.

6-Anisyl-2-(morpholin-4-ylmethyl)-4, 5-dihydro-pyridazin-3(2H)-one(vj1): Morpholine was used as cyclic secondary amine for Mannich reaction. Yield: 53%; MP.135-136 °C; IR (KBr) υ_{max} (cm^{-1}): 2970 (CH), 1672 (C=O), 1452 (C=C); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.48 (t, 2H, CH_2), 2.73 (t, 2H, CH_2), 2.9 (m, 4H, 2xCH_2), 3.6 (m, 4H, 2xCH_2), 3.85 (s, 3H, CH_3O), 4.76 (s, 2H, -N-CH_2-N-), 6.91(dd, 2H, J= 8.7, H-3', H-5'), 7.68 (dd, 2H, J=8.7, H-2', H-6'); Ms (m/z): 304 (M^++1). Anal. Calc. for $C_{16}H_{21}N_3O_3$: C: 63.35, H: 6.98, N: 13.85. Found: C: 63.10, H: 6.88, N: 13.66.

6-Anisyl-2-(piperazin-1-ylmethyl)- 4, 5-dihydro-pyridazin-3(2H)-one(vj2): Piperazine was used as cyclic secondary amine for Mannich reaction. Yield: 46%; MP.127-128 °C; IR (KBr) υ_{max} (cm^{-1}): 2972 (CH), 1678 (C=O), 1530 (C=C); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.60 (t, 2H, CH_2), 2.9 (t, 2H, CH_2), 3.0 (m, 8H, 4xCH_2), 3.8 (s, 3H, CH_3O), 4.74 (s, 2H, -N-CH_2-N-), 7.32 (dd, J=8.4, 2H, H-3', H-5'), 7.74 (dd, J=8.4, 2H, H-2', H-6'), 9.3 (brs, 1H, NH); Ms (m/z): 303 (M^++1). Anal. Calc. for $C_{16}H_{22}N_4O_2$: C: 63.55, H: 7.33, N: 18.53. Fond: C: 63.38, H: 7.12, N: 18.44.

6-Anisyl-2-(piperidin-1-ylmethyl)- 4, 5-dihydro-pyridazin-3(2H)-one(vj3): Piperidine was used as cyclic secondary amine for Mannich reaction. Yield: 50%; MP. 132-134 °C; IR (KBr) υ_{max} (cm^{-1}): 2998 (CH), 1688 (C=O), 1455 (C=C); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.61 (t, 2H, CH_2), 2.65 (m, 6H, 3xCH_2), 2.82 (t, 2H, CH_2), 2.98 (m, 4H, 2xCH_2), 3.86 (s, 3H, CH_3O), 5.2 (s, 2H, -N- CH_2-N-), 7.42 (dd, J=8.2, 2H, H-3', H-5'), 7.78 (dd, J=8.2, 2H, H-2', H-6'); Ms (m/z): 302 (M^++1). Anal. Calc. for $C_{17}H_{23}N_3O_2$: C: 67.75, H: 7.69, N: 13.94. Found: C: 67.55, H: 7.48, N: 13.76.

6-Anisyl-2-[(4-methylpiperazin-1-yl)methyl]-4,5-dihydropyridazin- 3(2H)- one (vj4): 1- Methyl-piperazine was used as cyclic secondary amine for Mannich reaction. Yield: 56%; MP. 135-137 °C; IR (KBr) υ_{max} (cm^{-1}): 2980 (CH), 1685 (C=O), 1590 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.2 (s, 1H, N-CH_3), 2.55 (t, 2H, CH_2), 2.91 (t, 2H, CH_2), 3.01 (m, 4H, 2xCH_2), 3.3 (m, 4H, 2xCH_2), 3.86 (s, 3H, CH_3O), 5.24 (s, 2H, -N-CH_2-N-), 7.35 (dd, J=8.4, 2H, H-3', H-5'), 7.76 (dd, J=8.4, 2H, H-2', H-6'); Ms (m/z): 317 (M^++1). 287 (M^++1), 187, 99. Anal. Calc. for $C_{17}H_{24}N_4O_2$: C: 64.53, H: 7.65, N: 17.71. Found: C: 64.42, H: 7.53, N: 17.54.

6-Anisyl-2-(1, 2-dihydro-10H-phenothiazin-10-yl methyl)-4,5-dihydropyridazin-3(2H)-one (vj5): Phenothiazine was used as cyclic secondary amine for Mannich reaction. Yield: 60%; MP. 108-110 °C; IR (KBr) υ_{max} (cm^{-1}): 2986 (CH), 1664 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.62 (t, 2H, CH_2), 2.99 (t, 2H, CH_2), 3.82 (s, 3H, CH_3O), 5.40 (s, 2H, -N-CH_2-N-), 6.90-7.78 (m, 12H, Ar-H); Ms (m/z): 416 (M^++1). Anal. Calc. for $C_{24}H_{21}N_3O_2S$: C: 69.37, H: 5.09, N: 10.11. Found: C: 69.18, H: 4.88, N: 9.92.

6-Anisyl-2- (1H-indol-1-ylmethyl)- 4, 5-dihydro-pyridazin-3(2H)-one(vj6): Indole was used as cyclic secondary amine for Mannich reaction. Yield: 46%; MP.116-118 °C; IR (KBr) υ_{max} (cm^{-1}): 3005 (CH), 1680 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.63 (t, 2H, CH_2), 2.97 (t, 2H, CH_2), 3.8 (s, 3H, CH_3O), 5.28 (s, 2H, -N-CH_2-N-), 7.32-7.67 (m, 10H, Ar-H); Ms (m/z): 323 (M^++1). Anal. Calc. for $C_{20}H_{19}N_3O_2$: C: 72.05, H: 5.74, N: 12.60. Found: C: 71.92, H: 5.54, N: 12.46.

6-Anisyl-2-(pyrrolidin-1-ylmethyl)-4, 5-dihydro-pyridazin-3(2H)-one(vj7): Pyrrolidine was used as cyclic secondary amine for Mannich reaction. Yield: 41%; MP. 128-130 °C; IR (KBr) υ_{max} (cm^{-1}): 3001 (CH), 1685 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.61 (t, 2H, CH_2), 2.92 (t, 2H, CH_2), 3.04 (m, 8H, 4xCH_2), 3.9 (s, 3H, CH_3O), 5.26 (s, 2H, -N-CH_2-N-), 7.41 (dd, J=8.4, H-3', H-5'), 7.79 (dd, J=8.4, H-2', H-6'); Ms (m/z): 288 (M^++1). Anal. Calc. for $C_{16}H_{21}N_3O_2$: C: 66.88, H: 7.37, N: 14.62. Found: C: 66.64, H: 7.14, N: 14.56.

6-Anisyl- 2- (1, 2, 4-triazolin-1-ylmethyl)-4, 5-dihydropyridazin-3(2H)-one (vj8): 1,2,4-triazole was used as cyclic secondary amine for Mannich

reaction. Yield: 58%; MP. 130-132 °C; IR (KBr) υ_{max} (cm^{-1}): 3005 (CH), 1680 (C=O), 1580 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 2.62 (t, 2H, CH_2), 3.02 (t, 2H, CH_2), 3.76 (s, 3H, CH_3O), 5.34 (s, 2H, -N-CH_2-N-), 7.36-7.86 (m, 6H, Ar-H); Ms (m/z): 286 (M^++1). Anal. Calc. for $C_{14}H_{15}N_5O_2$: C: 58.94, H: 5.30, N: 24.55. Found: C: 58.72, H: 5.16, N: 24.36.

6-(p-Ethylphenyl)-2-(morpholin-4-ylmethyl)-4,5-dihydropyridazin- 3(2H)- one (vj9): Morpholine was used as cyclic secondary amine for Mannich reaction. Yield: 62%; MP.133-135 °C; IR (KBr) υ_{max} (cm^{-1}): 2954 (CH), 1658 (C=O), 1448 (C=C); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.92 (t, 2H, CH_3), 2.54 (q, 2H, CH_2), 2.60 (t, 2H, CH_2), 2.74 (t, 2H, CH_2), 2.96 (m, 4H, 2xCH_2), 3.68 (m, 4H, CH_2-O-CH_2), 5.16 (s, 2H, -N-CH_2-N-), 7.42 (dd, J=8.2, 2H, H-3', H-5'), 7.78 (dd, J=8.2, 2H, H-2', H-6'); Ms (m/z): 302 (M^++1). Anal. Calc. for $C_{17}H_{23}N_3O_2$: C: 67.75, H: 7.69, N: 13.94. Found: C: 67.54, H: 7.46, N: 13.82.

6-(p-Ethylphenyl)-2-(piperazin-1-ylmethyl)-4, 5-dihydropyridazin- 3(2H)-one (vj10): Piperazine was used as cyclic secondary amine for Mannich reaction. Yield: 50%; MP. 137-138 °C; IR (KBr) υ_{max} (cm^{-1}): 3338 (NH), 2968 (CH), 1668 (C=O); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.92 (t, 2H, CH_3), 2.54 (q, 2H, CH_2), 2.60 (t, 2H, CH_2), 2.80-2.86 (m, 8H, 4xCH_2), 2.96 (t, 2H, CH_2), 5.24 (s, 2H, -N-CH_2-N-), 7.32 (dd, J=8.5, 2H, H-3', H-5'), 7.78 (dd, J=8.5, 2H, H-2', H-6'), 9.6 (s, 1H, NH) ; Ms (m/z): 301 (M^++1). Anal. Calc. for $C_{17}H_{24}N_4O$: C: 67.97, H: 8.05, N: 18.65. Fond: C: 67.84, H: 7.88, N: 18.43.

6- (p-Ethylphenyl)- 2- [(4-methylpiperazin-1-yl) methyl]-4, 5-dihydropyridazin-3(2H)-one (vj12): 1-Methylpiperazine was used as cyclic secondary amine for Mannich reaction. Yield: 52%; MP. 139-140 °C; IR (KBr) υ_{max} (cm^{-1}): 2970 (CH), 1680 (C=O), 1595 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.96 (t, 2H, CH_3), 1.18 (q, 2H, CH_3), 2.2 (s, 1H, N-CH_3), 2.50 (q, 2H, CH_2), 2.62 (t, 2H, CH_2), 2.91 (t, 2H, CH_2), 3.01 (m, 4H, 2xCH_2), 3.3 (m, 4H, 2xCH_2), 5.2 (s, 2H, -N-CH_2-N-), 7.39 (dd, J=8.2, 2H, H-3', H-5'), 7.7 (dd, 2H, H-2', H-6'); Ms (m/z): 315 (M^++1). Anal. Calc. for $C_{18}H_{26}N_4O$: C: 68.76, H: 8.33, N: 17.82. Found: C: 68.66, H: 8.14, N: 17.76.

6- (p-Ethylphenyl)-2-(1, 2- dihydro-10H-phenothiazin-10-ylmethyl)- 4, 5- dihydropyridazin-3(2H)-one(vj13): Phenothiazine was used as cyclic secondary amine for Mannich reaction. Yield: 60%; MP. 126-128 °C; IR (KBr) υ_{max} (cm^{-1}): 2968 (CH), 1664 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 1.02 (t, 2H, CH_2), 2.54 (q, 2H, CH_2), 2.60 (t, 2H, CH_2), 2.96 (t, 2H, CH_2), 5.32 (s, 2H, -N-CH_2-N-), 6.96-7.82 (m, 12H, Ar-H); Ms (m/z): 414 (M^++1). Anal. Calc. for $C_{25}H_{23}N_3OS$: C: 72.16, H: 5.61, N: 10.16. Found: C: 71.92, H: 5.48, N: 9.98.

6-(p-Ethylphenyl)- 2- (1H-indol-1-ylmethyl)-4, 5-dihydropyridazin-3(2H)-one (vj14): Indole was used as cyclic secondary amine for Mannich reaction. Yield: 42%; MP. 125-127 °C; IR (KBr) υ_{max} (cm^{-1}): 3001 (CH), 1680 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.88 (t, 2H, CH_3), 2.52 (q, 2H, CH_2), 2.602 (t, 2H, CH_2), 2.96 (t, 2H, CH_2), 5.28 (s, 2H, -N-CH_2-N-), 7.38-7.78 (m, 10H, Ar-H); Ms (m/z): 332 (M^++1). Anal. Calc. for $C_{21}H_{21}N_3O$: C: 67.11, H: 6.39, N: 12.68. Found: C: 66.92, H: 6.12, N: 12.51.

6-(p-Ethylphenyl)-2-(pyrrolidin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)- one (vj15): Pyrrolidine was used as cyclic secondary amine for Mannich reaction. Yield: 42%; MP.140-142 °C; IR (KBr) υ_{max} (cm^{-1}): 3000 (CH), 1680 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 1.01 (t, 2H, CH_3), 2.50 (t, 2H, CH_2), 2.60 (t, 2H, CH_2), 2.94 (t, 2H, CH_2), 3.0 (m, 8H, 4xCH_2), 5.16 (s, 2H, -N-CH_2-N-), 7.40 (m, 2H, Ar-H), 7.82 (m, 2H, Ar-H); Ms (m/z): 286 (M^++1). Anal. Calc. for $C_{17}H_{23}N_3O$: C: 76.11, H: 6.39, N: 12.68. Found: C: 75.88, H: 6.28, N: 12.56.

6-(p-Ethylphenyl)-2-(1,2,4-triazolin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)- one (vj16): 1,2,4-triazole was used as cyclic secondary amine for Mannich reaction. Yield: 51%; MP. 141-143 °C; IR (KBr) υ_{max} (cm^{-1}): 3002 (CH), 1680 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 1.18 (t, 2H, CH_3), 2.59 (t, 2H, CH_2), 2.66 (t, 2H, CH_2), 3.01 (t, 2H, CH_2), 5.32 (s, 2H, -N-CH_2-N-), 7.36-7.86 (m, 6H, Ar-H); Ms (m/z): 284 (M^++1). Anal. Calc. for $C_{15}H_{17}N_5O$: C: 63.59, H: 6.05, N: 24.72. Found: C: 63.52, H: 5.82, N: 24.58.

6-(p-Isobutylphenyl)-2-(morpholin-4-ylmethyl)-4,5-dihydropyridazin-3(2H)-one (vj17): Morpholine was used as cyclic secondary amine for Mannich reaction. Yield: 38%; MP. 152-154 °C; IR (KBr) υ_{max} (cm^{-1}): 2998 (CH), 1675 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.9 (d, 6H, 2xCH_3), 1.8 (m, H, -CH), 2.69 (m, 4H, 2xCH_2), 2.92 (t, 2H, CH_2), 3.0 (m, 4H, 2xCH_2), 3.2 (m, 4H, 2xCH_2), 4.78 (s, 2H, -N-CH_2-N-), 7.34 (dd, J=8.2, 2H, H-3', H-5'), 7.42 (dd, J=8.2, 2H, H-2', H-6'); Ms (m/z): 330 (M^++1). Anal. Calc. for $C_{19}H_{27}N_3O_2$: C: 69.27, H: 8.26, N: 12.76. Found: C: 69.12, H: 8.12, N: 12.58.

6-(p-Isobutylphenyl)-2-(piperazin-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-one (vj18): Piperazine was used as cyclic secondary amine for Mannich reaction. Yield: 52%; MP. 147-148 °C; IR (KBr) υ_{max} (cm^{-1}): 3000 (CH), 1680 (C=O), 1590 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.89 (d, 6H, 2xCH_3), 1.8 (m, H, -CH), 2.62 (m, 4H, 2xCH_2), 2.79 (m, 8H, 4xCH_2), 2.92 (t, 2H, CH_2), 4.79 (s, 2H, -N-CH_2-N-), 7.42 (dd, J=8.3, 2H, H-3', H-5'), 7.80 (dd, J=8.2, 2H, H-2', H-6'), 8.1 (s, 1H, NH); Ms (m/z): 329 (M^++1). Anal. Calc. for $C_{19}H_{28}N_4O$: C: 69.48, H: 8.59, N: 17.06. Fond: C: 69.24, H: 8.36, N: 16.86.

6-(p-Isobutylphenyl)-2-(piperidin-1-ylmethyl)-4,5- dihydropyridazin-3(2H)-one(vj19): Piperidine was used as cyclic secondary amine for Mannich reaction. Yield: 48%; MP. 137-138 °C; IR (KBr) υ_{max} (cm^{-1}): 3010 (CH), 1685 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.8 (d, 6H, 2xCH_3), 1.85 (m, H, -CH), 2.65 (m, 4H, 2xCH_2), 2.9 (t, 2H, CH_2), 3.0-3.4 (m, 10H, 5xCH_2), 5.02 (s, 2H, -N-CH_2-N-), 7.42 (dd, J=8.4, 2H, H-3', H-5'), 7.8 (dd, J=8.4, 2H, H-2', H-6'); Ms (m/z): 328 (M^++1). Anal. Calc. for $C_{20}H_{29}N_3O$: C: 73.36, H: 8.93, N: 12.83. Found: C: 73.23, H: 8.86, N: 12.76.

6-(p-Isobutylphenyl)-2-[(4-methylpiperazin-1-yl) methyl]-4,5-dihydropyridazin-3(2H)- one (vj20): 1-Methylpiperazine was used as cyclic secondary amine for Mannich reaction. Yield: 51%; MP. 129-131 °C; IR (KBr) υ_{max} (cm^{-1}): 2995 (CH), 1680 (C=O), 1600 (C=N); ^{1}H-NMR ($CDCl_3$-d_6) δ (ppm): 0.78 (d, 6H, 2xCH_3), 1.82 (m, H, -CH), 2.5 (s, 3H, CH_3), 2.65 (m, 4H, 2xCH_2), 2.85 (t, 2H, CH_2), 3.0-3.3 (m, 8H, 4xCH_2), 4.78 (s, 2H, -N-CH_2-N-), 7.35 (dd, J=8.5, 2H, H-3', H-5'), 7.80 (dd, J=8.5, 2H, H-2', H-6'); Ms (m/z): 343 (M^++1). Anal. Calc. for $C_{20}H_{30}N_4O$: C: 70.14, H: 8.83, N: 16.36. Found: C: 69.88, H: 8.67, N: 16.18.

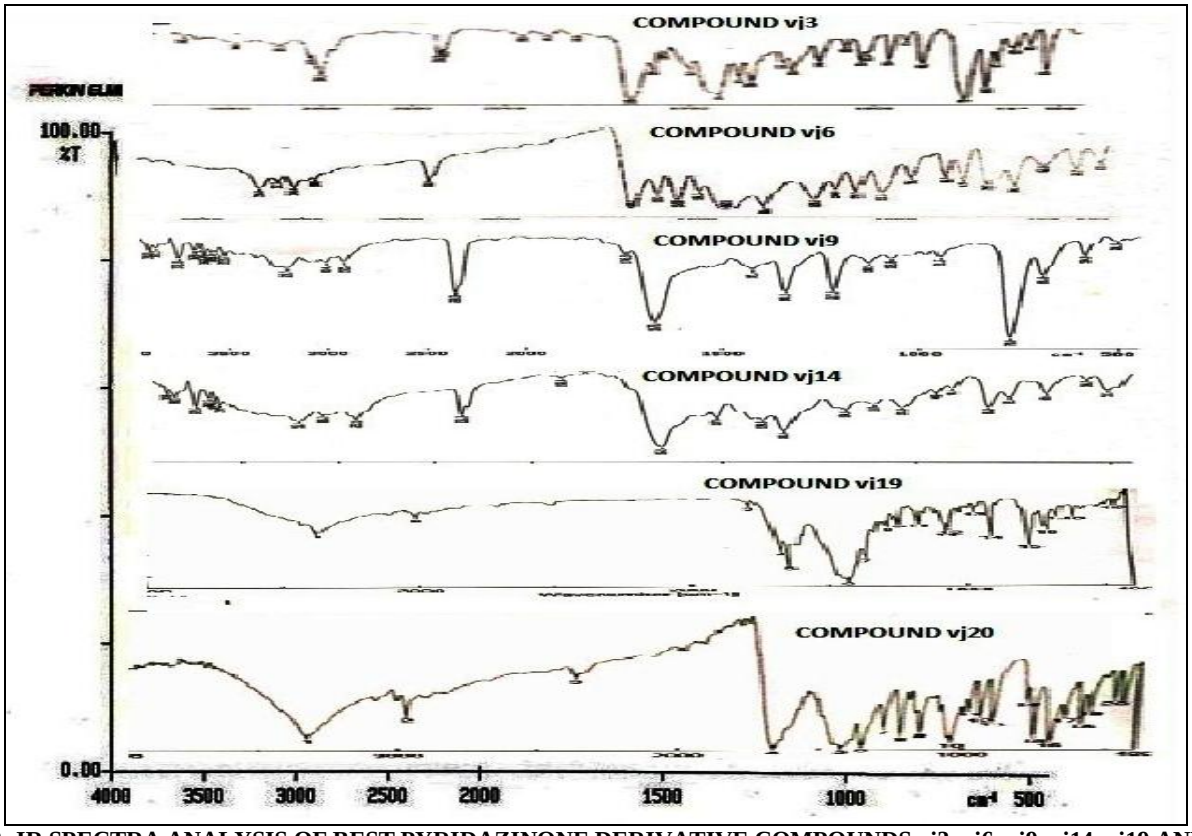

FIG. 2: IR SPECTRA ANALYSIS OF BEST PYRIDAZINONE DERIVATIVE COMPOUNDS vj3, vj6, vj9, vj14, vj19 AND vj20

A total of Twenty compounds (vj1-vj20) was synthesized and analyzed for IR, NMR, and Mass analysis. The six derivatives were selected as best compounds (vj3, vj6, vj9, vj14, vj19, and vj20) were evaluated for antihypertensive activities by a non-invasive method using Tail Cuff method. The IR, NMR, and Mass spectra of optimized compounds were shown below **Fig. 2** to **4**.

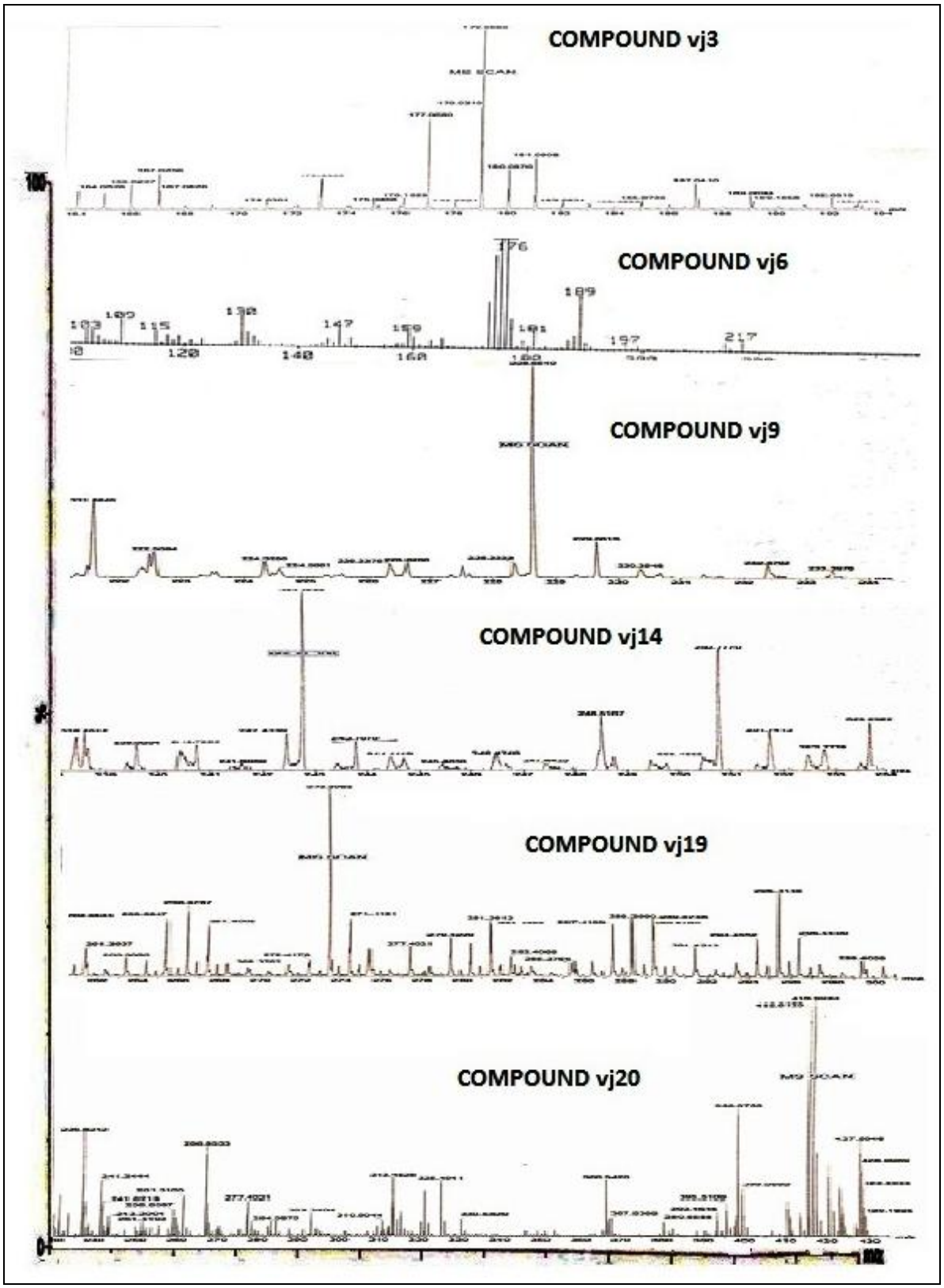

FIG. 3: MASS SPECTRAL ANALYSIS OF BEST PYRIDAZINONE DERIVATIVE COMPOUNDS vj3, vj6, vj9, vj14, vj19 AND vj20

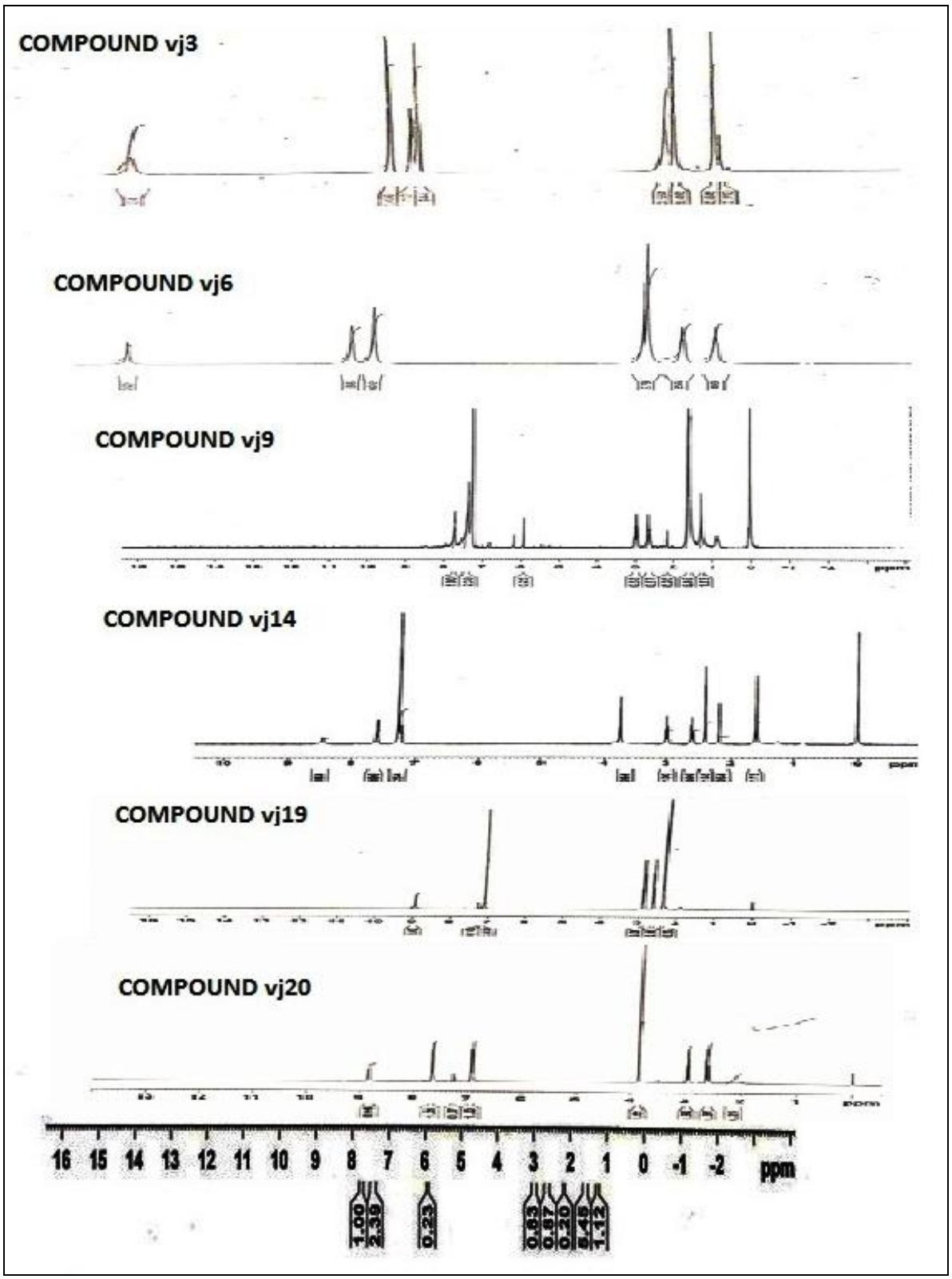

FIG. 4: NMR SPECTRAL ANALYSIS OF BEST PYRIDAZINONE DERIVATIVE COMPOUNDS vj3, vj6, vj9, vj14, vj19 AND vj20

Pharmacology:

Procurement, Identification, and Housing of Animals: Albino rats (bodyweight 200-250 g) were supplied by Central Animal House facility Registration number 173/CPCSEA and kept under standard laboratory conditions in 12-hour light/dark cycle at 25 °C ± 2 °C. Animals were provided with a pellet diet (Lipton, Calcutta, India) and water *ad libitum*. They were marked for easy identification.

Conditioning / Training of Animals: For conducting the BP measurement studies, the animals were kept in a restrainer for 10 min every day for one week. This exercise was done to avoid the fluctuation in blood pressure due to the aggressive behavior of animals while keeping into the restrainer for measuring the activity.

Induction of Hypertension in Normotensive Rats: After recording the initial BP of rats, the animals were divided into groups of 5 animals each. One group was taken as control. Hypertension was induced in the remaining groups by subcutaneous injection of methylprednisolone acetate (20 mg/kg/wk) for 2 weeks as per the method reported by Krakoff *et al*. [31]

Measurement of Mean Blood Pressure of Rats: [31-34] Mean arterial blood pressure was measured in conscious rats using CODA Non-Invasive Blood Pressure Recorder by Tail-Cuff method (Kent Scientific Corporation, USA). The restrainer carrying the rat was placed in the BP instrument with the tail protruding out. The tail was gently placed in contact with a transducer membrane, which was connected to the digital BP display panel. The instrument was then turned on and allowed to stabilize until a steady pulse rate was observed. Once the "pulse level ready" signal appeared, the BP recording button was pressed, and the mean arterial BP was recorded. Albino rats (bodyweight 200-250 g) were used in the present study. Rats were assigned to groups of five animals in each. Each compound was suspended in 1% carboxymethylcellulose (CMC) solution at the dose level of 20mg/kg body weight was injected intraperitoneally then mean arterial blood pressure was recorded after one hour.

The percent reduction in mean arterial blood pressure (MABP) of compounds at a dose of 20 mg/kg after 1 h is depicted in the below shown **Fig. 5-6**.

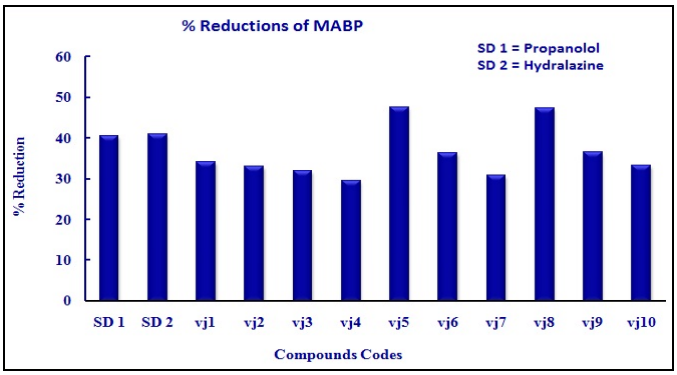

FIG. 5: PERCENT REDUCTION IN MEAN ARTERIAL BLOOD PRESSURE (MABP) BY THE COMPOUNDS Vj1-vj10 AT A DOSE OF 20 mg/kg AFTER 1 h

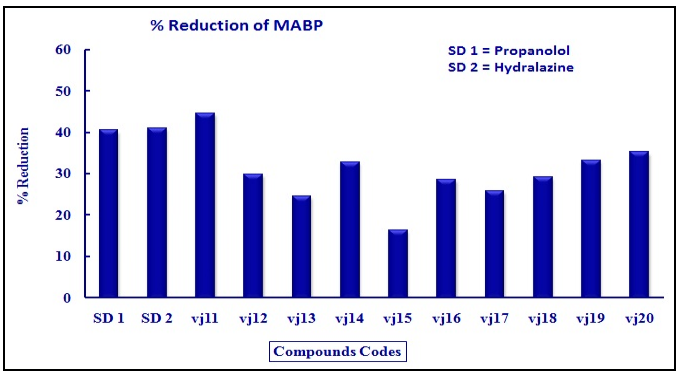

FIG. 6: PERCENT REDUCTION IN MEAN ARTERIAL BLOOD PRESSURE (MABP) BY THE COMPOUNDS vj11 - vj20 AT A DOSE OF 20 mg/kg AFTER 1 h

Statistical Analysis of Data: The statistical analysis was performed using GraphPad INSTAT 3 software (Graph Pad Software Inc, San Diego, CA). Data obtained from animal experiments were expressed as arithmetic mean ± SEM. The comparison between various groups was performed by one-way analysis of variance (ANOVA), and the effect in treatment groups was compared with the toxic control group by Dunnet multiple comparison test. $p<0.05$ was considered to be significant [$^{*}p<0.05$; $^{**}p<0.01$]. The percentage reduction in BP for all the treatment groups was also calculated and compared.

CONCLUSION: From the above research study concluded that different Pyridazinone derivatives compounds were synthesized by using different analytical parameters. And also to find out the antihypertensive activity of synthesized derivative compounds. All the synthesized derivatives were obtained in good yield by optimizing various synthetic procedures. The structures of the compounds were established by elemental analysis, IR, ^{1}H-NMR, and Mass spectral data analysis.

The final all the twenty compounds (vj1-vj20) were evaluated for antihypertensive activity by a non-invasive method using the Tail Cuff method. Few compounds like vj3, vj6, vj9, vj14, vj19, and vj20 were found to show a highly significant reduction in mean arterial blood pressure but at a higher dose in comparisons to standard drugs like propanolol

and hydralazine. The substituted pyridazine derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of hypertension.

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CONFLICTS OF INTEREST: Nil

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