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ORIGINAL ARTICLE

Synthesis and Evaluation of Nitric Oxide Donating Indolizinyl Pyrazoline Derivatives for Antihypertensive Activity

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ABSTRACT

Hypertension, a leading cause of cardiovascular morbidity, necessitates novel therapeutic strategies that target vasodilation and endothelial dysfunction. In this study, four nitric oxide (NO)-donating indolizinyl pyrazoline derivatives (CNPH-1, CNPN-2, CRPH-3, and CRPN-4) were synthesised through a multi-step reaction scheme, integrating indolizine and pyrazoline rings with NO-releasing side chains. The compounds were characterised using melting point, thin-layer chromatography, infrared spectroscopy, and nuclear magnetic resonance spectroscopy to confirm their structural integrity. Pharmacological efficacy was evaluated by experimentally inducing hypertension in rats (Goldblatt method followed by the tail cuff method) and comparing systolic blood pressure (BP) reduction with nifedipine and clonidine. All derivatives significantly lowered BP within 120 min ($p < 0.001$ vs. controls), with CRPN-4 exhibiting sustained effects comparable to those of standard drugs at 240 min (25–30% reduction). At 360 min, the efficacy diminished, mirroring the transient action of nifedipine and clonidine. CRPN-4, which is characterised by moderate molecular weight and stability, has emerged as a lead candidate, likely because of the optimal NO release kinetics from its alkyl side chains. These findings underscore the potential of NO-donating pyrazoline hybrids for hypertension management by combining vasodilatory mechanisms with endothelial repair. Future studies should explore the pharmacokinetics, long-term efficacy, and toxicity of to advance CRPN-4 its clinical development. This study highlights the therapeutic potential of structurally engineered NO donors to address hypertension.

Keywords: Hypertension; Endothelial dysfunction; Nitric oxide; Indolizinyl pyrazoline

INTRODUCTION

Hypertension is a medical condition characterised by elevated blood pressure (BP) that indirectly leads to millions of deaths annually.[1] The majority of hypertension cases have an unknown origin (essential), whereas less than 10% of the cases present as a complication of an existing condition (secondary), such as renal disease, thyroid disease, substance abuse, and severe sleep disruptions.[2] BP is largely controlled by the renin-angiotensin system (RAS) and the sympathetic nervous system.[3] RAS maintains homeostasis of BP through angiotensin (ANG) II, a key regulator of inflammation, thrombosis, vasodilation, and vascular remodelling.[4–6] Endothelial dysfunction arises from reduced ANG II activity, perturbing endothelial-derived nitric oxide (NO) release and enhancing vascular constriction.[6] Sustained elevation of pressure induces remodelling and alters the physical properties (compliance, distensibility, thickness, and stiffness) of the arterial walls.[7] If left untreated, primary hypertension (PH) can increase the risk of cardiovascular diseases, ischaemia (stroke), and damage target organs such as the brain and kidneys.[8,9] Thus, hypertension, often idiopathic and driven by RAS dysregulation, contributes to endothelial dysfunction, vascular remodelling, and severe complications, such as cardiovascular disease and organ damage, if left unmanaged.

Based on the severity of hypertension and pre-existing health conditions, treatment options vary from introducing lifestyle changes to opting for drugs that promote potassium repletion, calcium channel blockers, angiotensin (ANG) II receptor blockers, α/β blockers, or even diuretics.[10] Popular antihypertensive drugs such as nifedipine (a calcium channel blocker) affect endothelial permeability and promote systemic vasodilation.[11] Clonidine, another

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hypotensive drug, lowers blood pressure by acting as an agonist of the α-adrenergic receptors.[12] Both drugs indirectly induced vasodilation. Other drugs such as sodium nitroprusside directly initiate arterial vasodilation.[1,13] PH can also be treated by reversing the endothelial dysfunction.[14–17] Restoration of the disruption of NO using drugs, such as imidapril[18], has led to the reversal of endothelial damage. Overall, existing drugs target various mechanisms, including adrenergic signalling and direct vascular smooth muscle relaxation, to achieve systemic vasodilation and blood pressure control.

However, current treatment options are not as effective in stage 1 hypertension (140-149/90-99 mmHg), as it is dangerous for BP to drop below 110-115/70-75 mmHg.[19] Calcium blockers and diuretics are associated with a higher risk of myocardial infarction.[11,20,21] Beta-blockers have been associated with weight gain and a 10% decrease in metabolic rate[22], increasing the risk of obesity in hypertensive patients. Practitioners have also begun administering combined drug therapies, although no significant reduction in BP has been observed in patients with diabetic hypertension.[23]

The most effective antihypertensive drugs (like nifedipine and clonidine) contain pyridine and imidazole rings, respectively, in their structure. Drugs containing an indolizine or pyrazoline ring in their structure have been proven to have effective antihypertensive activity.[24–27] Another study synthesised derivatives with NO-donating pyrazoline rings.[25] This study aimed to synthesise and characterise novel compounds with a unique indolizinyl pyrazoline that doubles as an NO donor. Four different compounds, which differ in their alkyl groups attached to indolizinyl pyrazoline rings, were screened for hypertensive activity in comparison with standard drugs, such as nifedipine and clonidine.

METHODOLOGY

All chemicals used in this study were of analytical grade. A scheme of 6 step reaction, where the intermediate product of each, was used for obtaining 4 synthetic indolizinyl pyrazoline derivatives as end products was designed. The derivatives differed in their side chains in terms of two alkyl groups (R and R1) and were selected based on their readiness to donate nitric oxide upon reactivity. An overall scheme of the reaction is shown in Figure 1 .

Reaction scheme to obtain NO – donating indolizinyl pyrazoline derivatives

a) Esterification of pyridine

The reaction began with pyridine (10 mmol) and ethyl acetate (60 ml), stirred with chloroacetic acid (100 mmol) at 90°C for 2 h, and refrigerated for 3 h. The solids in the refrigerated solutions were filtered, dried, and recrystallised in hot methanol. This resulted in a yield of 60–80%.

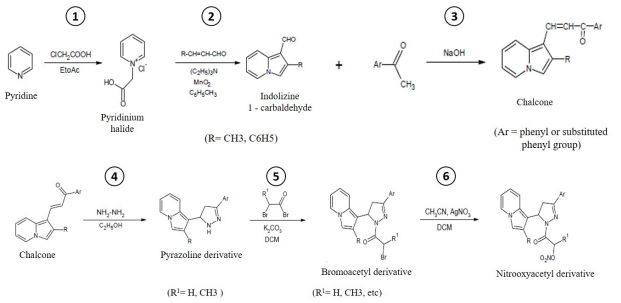

Fig. 1: Scheme of reactions (1 – 6) to obtain nitrooxyacetyl derivatives as the end product

b) Oxidation of pyridinium

Pyridinium crystals (10 mmol) were resuspended in toluene (80 ml) along with aldehyde (50 mmol), triethyl amine (1.5 ml), and manganese dioxide (80 mmol). The reaction mixture was stirred at 90°C for 2 h and then cooled to room temperature. Brown oil (containing indolizine-1-carbaldehyde) was collected by distillation at 130°C, washed with water, and dried on calcium chloride[28,29] to obtain a 57-73% yield of the product.

c) Aldol condensation to form chalcone

Equal amounts of indolizine-1-carbaldehyde and substituted acetophenone (0.04 mol) were dissolved in 4 ml of 95% ethanol. To obtain indolizinyl chalcone solids, 0.5 ml of a 15M NaOH solution was added to obtain indolizinyl chalcone solids.[13] The chalcone solids were filtered, dried, and recrystallised using ethanol. Four chalcone derivatives, differing in their alkyl groups, were obtained (60-70% yield).

d) Cyclization of chalcone derivatives

Each chalcone derivative (10 mmol) and 95% hydrazine monohydrate (20 mmol) were refluxed separately in 50 ml of absolute ethanol for 6–10 h and allowed to cool to room temperature. The pyrazoline-derivative solids were separated and concentrated, as described in the previous steps, and recrystallised in absolute ethanol (55-65% yield).

e) Bromoacetylation of pyrazoline derivatives

The indolizinyl pyrazoline derivatives were dissolved in dichloromethane (DCM) after placing them in an ice bath, and an aqueous solution of K_2CO_3 (6.3 mmol) was added. Bromoacetyl bromide (4.6 mmol) dissolved in DCM (20 ml) was introduced dropwise under continuous stirring for 30 min. The final reaction mixture was stirred at 0°C for 2 h at room temperature for 24 h. This allowed the organic layer to separate from the aqueous layer and the latter was extracted with CH_2Cl_2 (2 × 20 ml). The organic layer containing bromoacetyl derivatives was washed twice with 20 ml of distilled water followed by 1N HCl and distilled water again. The crude bromoacetyl derivatives were dried

over anhydrous sodium sulfate, evaporated under reduced pressure, and purified by recrystallisation from absolute ethanol (50-53% yield).

f) Nucleophilic substitution with $AgNO_3$

Silver nitrate (40 mmol) was allowed to react with solutions of bromoacetyl derivatives (10 mmol) dissolved in acetonitrile (20 ml) by heating at 80°C until precipitates were formed (13-17 hours). Precipitates of silver bromide were filtered, and the filtrate containing nitrooxyacetyl derivatives was evaporated until dry. For purification, the crude products were dissolved in DCM, washed twice with distilled water (20 ml) and brine (20 ml), dried over anhydrous sodium sulfate, and evaporated under reduced pressure. Finally, after recrystallisation in methanol, a 40-55% yield of NO donating indolizinyl pyrazoline derivatives were obtained.[25]

Identification and characterization of novel products

After obtaining the end products, they were identified and characterised using Thiel's tube method for melting point determination, thin layer chromatography (TLC) with hexane: ethyl acetate (4:1 / 7:3 ratio) as the mobile phase to assess the purity of compounds, infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR) for chemical structure determination, and mass spectroscopy for determining atomic and molecular weights. IR spectra of the derivatives were obtained using KBr pellets and recorded in a SHIMADZU FTIR 8400S SPECTROMETER. For NMR, chloroform and DMSO were used as solvents and were carried out using a Bruker spectrospin-400 NMR spectrophotometre at the Indian Institute of Science, Bangalore. Mass spectral analysis was performed using an Electron Spray spectrometer at the IISc, Bangalore.

Pharmacological screening of the novel drug candidates

After characterisation, the obtained compounds were screened for antihypertensive activity in male Sprague-Dawley rats. First, hypertension was experimentally induced in rats using the Goldblatt method.[30] In this study, hypertension in rats (250-300 g) anaesthetised with intraperitoneal injections of pentobarbitone sodium (100 mg/kg during surgery and 30 mg/kg post-surgery) was induced by tying a thread onto the left hilium of the kidney and occluding the renal artery for ~ 4 h. The anaesthetised animals were allowed to recover for 3.5 hours post-surgery, and systolic and diastolic BP were measured (using the tail cuff method) until the reduced BP stabilised. The renal thread was removed and monitored for stable hypertension within 15 min. Rats with successful hypertension induction were selected for the pharmacological screening of the synthesised drugs.

Rats with experimentally induced hypertension were split into seven groups (n=4). Before beginning the experiment, the rats were acclimated to the cages three to four times for 30–60 min. The control group was administered DMSO, whereas the standard groups were administered nifedipine or clonidine. Each of the four novel synthesised drugs was administered by oral gavage to the remaining four groups for a period of 2 days. Systolic BP was measured at the time of administration and several times with at least a 60 min interval using the standard tail-cuff method.[31] The difference in systolic pressure was noted, and the percentage decrease was calculated. Student's t-test was used to assess the significance of differences.

RESULTS

As part of the broader objective of developing novel antihypertensive agents, the initial phase of this study focused on the successful synthesis and characterisation of the target compounds. Four novel indolizinyl pyrazoline derivatives, designated CNPH-1, CNPN-2, CRPH-3, and CRPN-4, were successfully synthesised using an established multi-step reaction scheme. The characterisation of these compounds utilising a range of spectroscopic and analytical techniques revealed distinct physicochemical properties for each derivative.

Table 1: Summary of the characteristic features of four novel indolizinyl pyrazoline derivatives

Compound code	Mol formula	Mol weight	Melting point (°C)	Yield (%)	Rf Value
CNPH-1	$C_{25}H_{20}N_4O_5$	456.45	124	51.3	0.54
CNPN-2	$C_{25}H_{19}N_5O_6$	485.45	147	45.7	0.68
CRPH-3	$C_{20}H_{18}N_4O_5$	394.38	129	53.3	0.61
CRPN-4	$C_{20}H_{17}N_5O_6$	423.38	138	48.6	0.49

Table 1 provides a summary of these characteristic features, including molecular formula, molecular weight, melting point, yield, and Rf value, highlighting the diversity achieved in the final compounds.

All the compounds obtained were in a crystalline state at room temperature, with melting temperatures greater than 124°C. The average yield obtained using this elaborate scheme was 49.73%, with the highest yield obtained for CRPH-3 (53.3%). TLC revealed that all compounds had a non-polar nature with retention factor (Rf) values >0.49, with CNPN-2 being the most non-polar and CRPN-4 the most polar among the four derivatives. NMR, IR, and mass spectrometry revealed the structural and characteristic properties of the novel derivatives (Figure 2) as follows:

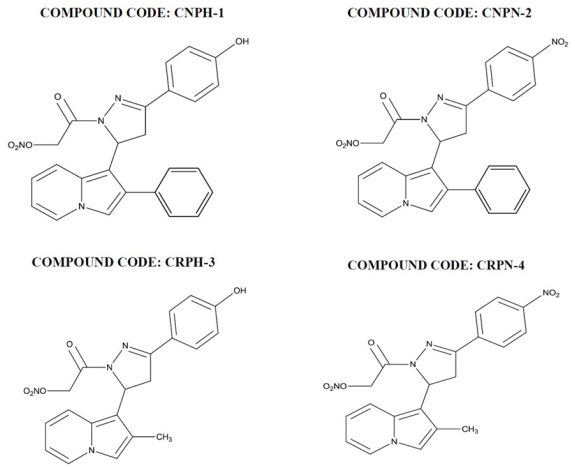

Fig. 2: Structure of the four synthesised indolizinyl pyrazoline derivatives

CNPH-1:

The compound, chemically identified as 2-(3-(4-hydroxyphenyl)-5-(2-phenylindolizin-1-yl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl nitrate, had a molecular formula of $C_{25}H_{20}N_4O_5$ and a corresponding molecular weight of 456.45 g.

Infrared spectroscopy reveals characteristic absorption bands at 3417.46 cm^{-1} (O-H stretch), 1651.30 cm^{-1} (C=O), 1593.78 cm^{-1} (N=O asymmetric stretch), 1501.94 cm^{-1} (C=N aromatic), a series of bands between 1424.20 and 1542.63 cm^{-1} (C=C aromatic stretch), 1378.24 cm^{-1} (N=O symmetric stretch), 1285.79 cm^{-1} (C-O), and 1174.20 cm^{-1} (C-N aromatic).

Proton nuclear magnetic resonance (NMR) spectroscopy revealed a complex aromatic region between δ8.720 and δ6.598 ppm, featuring multiple doublets and triplets indicative of various aryl protons. A singlet at δ5.352 ppm corresponds to a hydroxyl proton, whereas a singlet at δ4.421 ppm and a doublet between δ3.796 and δ3.815 ppm are attributed to aliphatic protons (Ali H). Further structural details are presented in Figure 3.

NMR Peaks: δ8.725-δ8.720 (d, 2H, ArH), δ8.225-δ8.220 (d, 2H, ArH), δ8.203-δ8.198 (d, 2H, ArH), δ7.911-δ7.879 (t, 2H, ArH), δ7.662-δ7.637 (t, 3H, ArH), δ7.240 -(s, 1H, ArH), δ6.896-δ6.874 (d, 2H, ArH), δ6.622-δ6.598 (t, 1H), δ5.352 (s, 1H, OH), δ4.421 (s, 2H, Ali H), δ3.815-δ3.796 (d, 2H).

CNPN-2:

The compound, chemically identified as 2-(3-(4-nitrophenyl)-5-(2-phenylindolizin-1-yl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl nitrate, has a molecular formula of $C_{25}H_{19}N_5O_6$ and a corresponding molecular weight of 485.45 g.

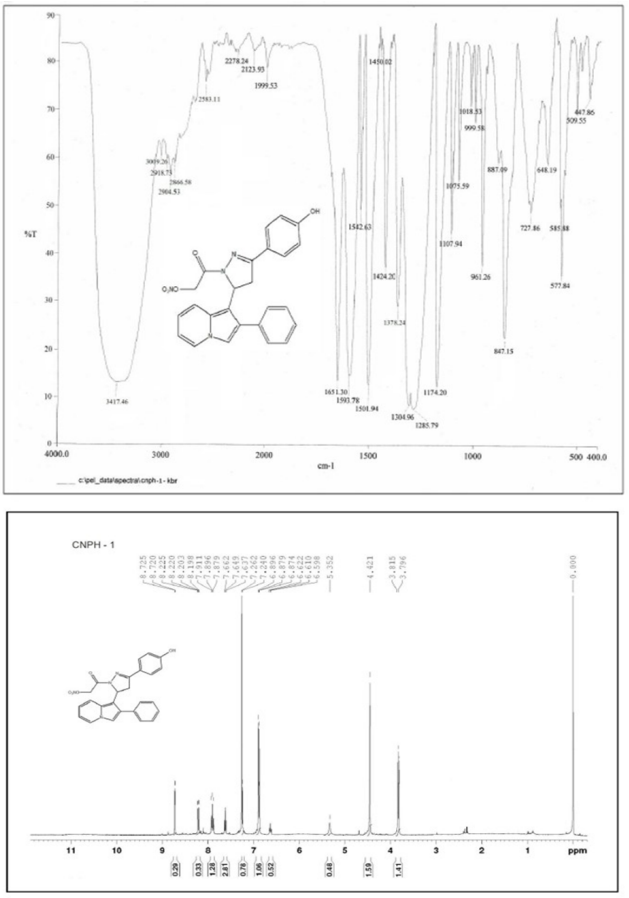

Fig. 3: IR (top) and NMR (bottom) spectra of CNPH-1

Infrared spectroscopy of the compound reveals characteristic absorptions at 3058.32 cm^{-1} (C-H aromatic stretch), a range between 2992.64 and 2968.75 cm^{-1} (C-H aliphatic stretch), 1708.75 cm^{-1} (C=O stretch), 1598.18 cm^{-1} (N=O asymmetric stretch), 1503.81 cm^{-1} (C=N aromatic stretch), 1420.99 cm^{-1} (C=C aromatic stretch), 1358.7 cm^{-1} (N=O symmetric stretch), 1282.45 cm^{-1} (C-O stretch), and 1165.67 cm^{-1} (C-N aromatic stretch).

The proton NMR spectrum exhibited signals in the aromatic region between δ8.332 and δ6.198 ppm, displaying several doublets and triplets, indicative of multiple aryl protons. A singlet at δ4.069 ppm corresponded to aliphatic protons (Ali H), and a doublet between δ3.409 and δ3.431 ppm was also observed. Further structural elucidation is shown in Figure 4.

NMR peaks: δ8.353-δ8.332 (d, 2H, ArH), δ8.028-δ8.006 (d, 2H, ArH), δ7.965-δ7.944 (d, 2H, ArH), δ7.543-δ7.516 (t, 3H, ArH), δ7.365-δ7.341 (t, 2H, ArH), δ7.233 (s, 1H, ArH), δ7.206-δ7.184 (d, 2H, ArH), δ6.225-δ6.198 (t, 1H), δ4.069 (s, 2H, Ali H), δ3.431-δ3.409 (d, 2H).

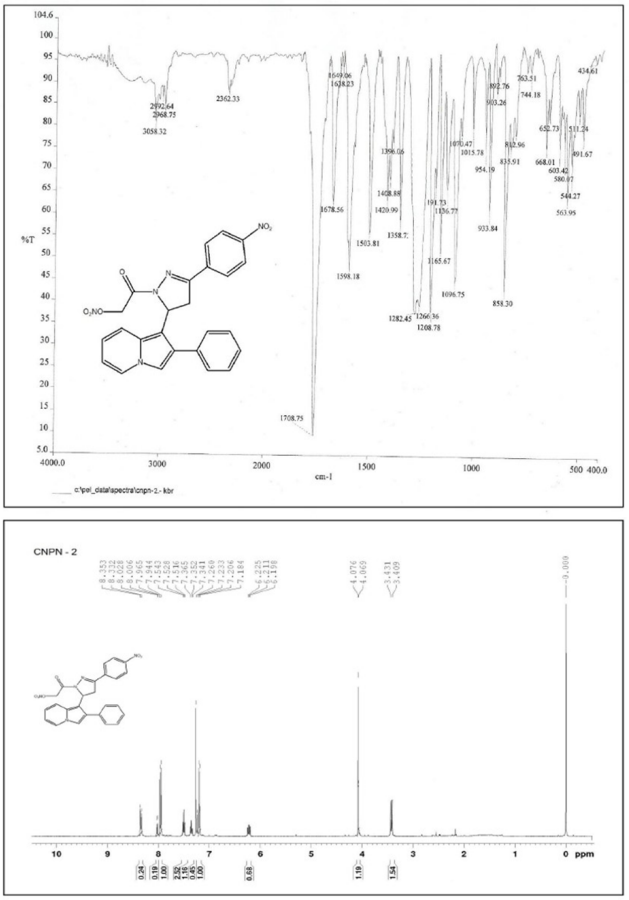

Fig. 4: IR (top) and NMR (bottom) spectra of CNPN-2

CRPH-3:

The compound, chemically identified as 2-(3-(4-hydroxyphenyl)-5-(2-methylindolizin-1-yl)- 4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl nitrate, had a molecular formula of $C_{20}H_{18}N_4O_5$ and a corresponding molecular weight of 394.38 g.

Infrared spectroscopy of the compound reveals key absorption bands at 3404.30 cm^{-1} (O-H stretch), a region between 3190.22 and 3044.54 cm^{-1} (C-H aromatic), 2928.35 cm^{-1} (aliphatic C-H), 1679.46 cm^{-1} (C=O stretch), 1590.41 cm^{-1} (N=O asymmetric stretch), 1505.11 cm^{-1} (C=N aromatic), a range from 1438.46 to 1423.46 cm^{-1} (C=C aromatic stretch), 1362.80 cm^{-1} (N=O symmetric stretch), 1308.98 cm^{-1} (C-O stretch), and 1175.12 cm^{-1} (C-N aromatic stretch).

The proton NMR spectrum exhibited aromatic signals between δ7.689 and δ6.309 ppm, including doublets and a triplet indicative of various aryl protons, as well as a singlet at δ6.547 ppm. A hydroxyl proton was observed as a singlet at δ5.193 ppm, whereas aliphatic protons appeared as a singlet at δ4.681 ppm (Ali H) and a doublet between δ3.561 and δ3.583 ppm. Additionally, a singlet at δ2.556 ppm suggested the presence of another aliphatic group (Ali). Further structural details are presented in Figure 5.

NMR peaks: δ7.711-δ7.689 (d, 2H, ArH), δ7.419-δ7.397 (d, 2H, ArH), δ7.112-δ7.091 (t, 2H, ArH), δ6.733-δ6.711 (d, 2H, ArH), δ6.547 (s, 1H, ArH), δ6.330-δ6.309 (t, 1H), δ5.193 (s, 1H, OH), δ4.681 (s, 2H, Ali H), δ3.583-3.561 (d, 2H), δ2.556 (s, 3H, Ali).

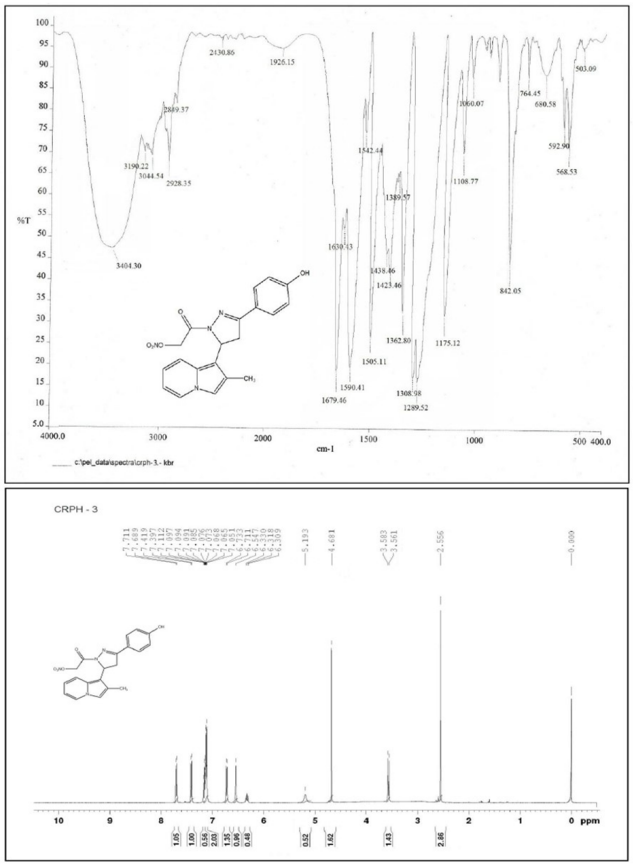

Fig. 5: IR (top) and NMR (bottom) spectra of CRPH-3

CRPN-4:

The compound, chemically identified as 2-(5-(2-methylindolizin-1-yl)-3-(4-nitrophenyl)- 4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl nitrate, has a molecular formula of $C_{20}H_{17}N_5O_6$ and a corresponding molecular weight of 423.38 g.

Infrared spectroscopy of the compound reveals characteristic absorption bands at 3104.50 cm^{-1} (C-H aromatic stretch), 2925.47 cm^{-1} (C-H aliphatic stretch), 1698.08 cm^{-1} (C=O stretch), 1592.26 cm^{-1} (N=O asymmetric stretch), 1514.58 cm^{-1} (C=N aromatic stretch), a range between 1479.13 and 1422.79 cm^{-1} (C=C aromatic stretch), 1363.15 cm^{-1} (N=O symmetric stretch), 1279.42 cm^{-1} (C-O stretch), and 1168.26 cm^{-1} (C-N symmetric stretch).

The proton NMR spectrum displays aromatic signals between δ8.091 and δ6.538 ppm, including doublets, a singlet, and a triplet, indicative of various aryl protons. A singlet at δ4.384 ppm corresponded to aliphatic protons (Ali H), and a doublet between δ3.947 and δ3.968 ppm was also observed. Additionally, a singlet at δ2.407 ppm suggested the presence of another aliphatic group (Ali). Further structural information is presented in Figure 6.

NMR peaks: δ8.112-δ8.091 (d, 2H, ArH), δ7.171-δ7.149 (d, 2H, ArH), 7.018 (s, 1H, ArH), 6.876-6.855 (t, 2H, ArH), δ6.840-6.835 (d, 2H, ArH), δ6.559-6.538 (t, 1H), δ4.384 (s, 2H, Ali H), δ3.968-3.947 (d, 2H), δ2.407 (s, 3H, Ali).

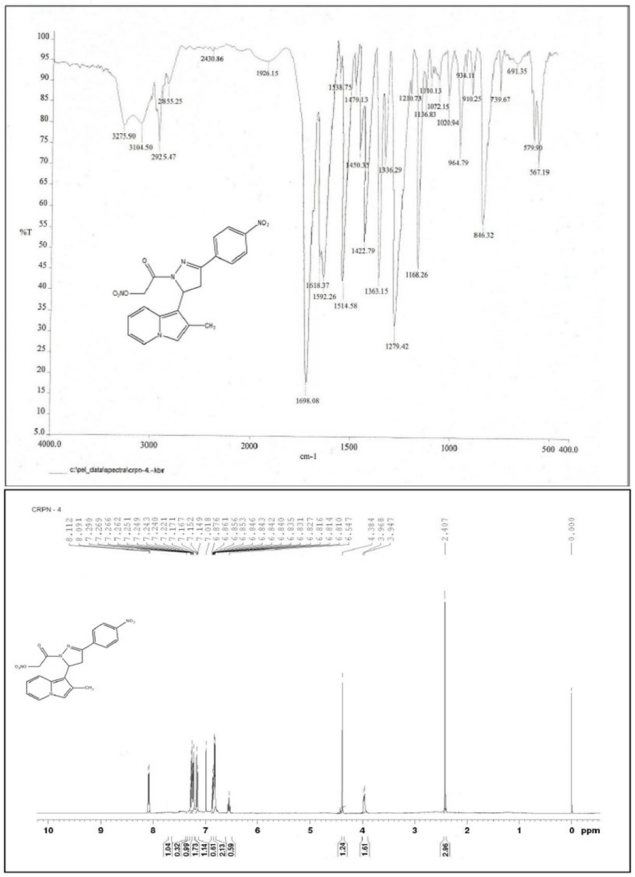

Fig. 6: IR (top) and NMR (bottom) spectra of CRPN-4

Pharmacological screening

The antihypertensive potential of the synthesised compounds was evaluated in male Sprague-Dawley rats. Hypertension was experimentally induced using the Goldblatt method, which involves renal artery occlusion for approximately 4 h in anaesthetised rats. Successfully hypertensive rats were divided into seven groups (n=4) for pharmacological screening. The control group received DMSO, whereas the standard groups were treated with nifedipine or clonidine. The four novel compounds were administered orally for two days, and systolic blood pressure was measured at baseline and at regular intervals thereafter. Changes in systolic blood pressure and percentage decrease were calculated, and statistical significance was determined using the Student's t-test. Systolic BP was measured at an interval of 60 min for each group, and the percent reduction in BP was calculated for 120, 240, and 360 min, in comparison to resting state pressure (time of administration). Both standard drugs, nifedipine and clonidine, drastically reduced systolic pressure 120-240 min after administration compared with controls ($p < 0.001$ and $p < 0.001$, respectively).

A similar reduction in systolic pressure was observed for the test drugs CNPH-1, CNPN-2, CRPH-3, and CRPN-4, not only compared to controls, but also for nifedipine and clonidine 120 min post-exposure. After 240 min, CRPN-4 showed an effect similar to that of nifedipine and clonidine, unlike that of other drug candidates (Figure 7). After 360 min, the effects of the drugs were eliminated (Figure 8).

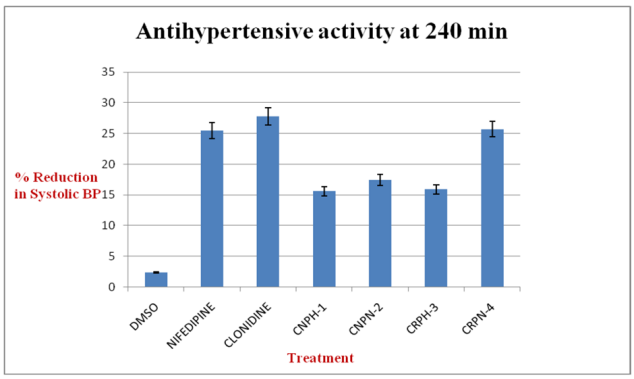

Fig. 7: Bar graph of percentage reduction in systolic BP 240 min after antihypertensive drug administration

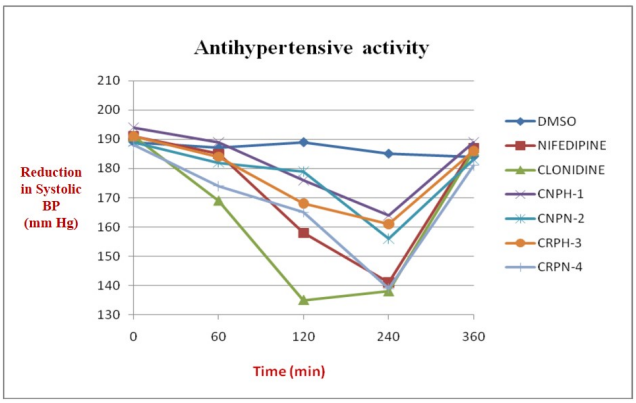

Fig. 8: Line graph of the effect of antihypertensive drugs on systolic blood pressure (BP) at 360 min post-administration. (DMSO = Dimethyl sulfoxide)

CRPN-4 has a moderate molecular weight, remains solid at room temperature, and shows hypertensive activity

similar to that of standard medication for hypertension, nifedipine, and clonidine, making it a potential candidate for drug development.

DISCUSSION

In this study, we successfully synthesised and characterised four novel indolizinyl pyrazoline derivatives as nitric oxide (NO)-donating antihypertensive agents. The multi-step synthetic approach yielded compounds CNPH-1, CNPN-2, CRPH-3, and CRPN-4, which exhibited significant blood pressure-lowering effects in hypertensive rats, comparable to those of the standard drugs nifedipine (a calcium channel blocker) and clonidine (an α2-adrenergic agonist). The rationale behind incorporating a pyrazoline ring is its well-documented vasodilatory effects, primarily through NO release, a key mediator in vascular relaxation and BP regulation.

Nitric oxide plays a crucial role in cardiovascular homeostasis by inducing vasodilation, inhibiting platelet aggregation, and reducing vascular smooth muscle cell proliferation. The synthesised derivatives were designed to release NO upon metabolic activation, which aligns with previous studies demonstrating that NO-donating hybrid drugs exhibit enhanced antihypertensive efficacy.[32–35]

The four indolizinyl pyrazoline derivatives (CNPH-1, CNPN-2, CRPH-3, and CRPN-4) exhibited distinct structure-activity relationships driven by substituent variations. Hydroxyl (-OH) derivatives (CNPH-1, CRPH-3) are more polar (lower Rf values: 0.54–0.61) due to hydrogen bonding, enhancing solubility but reducing thermal stability (melting points: 124-129°C). In contrast, nitro (-NO_2) derivatives (CNPN-2, CRPN-4) displayed higher thermal stability (melting points: 138-147°C) and lower polarity (Rf: 0.49-0.68) due to dipole interactions. The indolizine substituent further modulated the properties: phenyl groups increased the hydrophobicity (CNPH-1, CNPN-2), while methyl groups (CRPH-3 and CRPN-4) improved the synthetic yields (for example, CRPH-3:53.3%) and reduced the steric bulk. CRPN-4 uniquely combined a compact methyl group with a nitro moiety, achieving moderate polarity (Rf = 0.49) and thermal stability (138°C). Its activity parallels that of nifedipine and clonidine due to structural and electronic mimicry; the nitro group mimics the nitroaromatic system of nifedipine, while the methyl group enhances bioavailability, akin to the imidazole ring of clonidine, facilitating receptor binding. The electron-withdrawing nitro group may also enhance affinity for adrenergic or vascular targets. CRPN-4's balanced substituent synergy distinguishes it from hydroxyl derivatives, positioning it as a candidate for cardiovascular or neurological applications; however, further biological validation is required to confirm its efficacy and safety.

Among the synthesised compounds, CRPN-4 showed the most promising activity, maintaining a BP-lowering effect comparable to that of nifedipine and clonidine for up to 240 min post-administration. This suggests that side chain modifications (R and R1 groups) influence the rate and extent of NO release, with CRPN-4 possibly offering a more sustained pharmacological effect. The duration of action of the synthesised drugs was not assessed after 360 minutes. Future studies should include a longer observation period. Although the compounds showed NO-dependent vasodilation, further studies are needed to confirm the exact mechanism and pharmacodynamics of CRPN- 4.

CONCLUSION

While this study demonstrates the successful synthesis and promising antihypertensive effects of NO-donating indolizinyl pyrazoline derivatives, particularly lead candidate CRPN-4, further investigation is critical to advance its therapeutic potential. Future work must prioritise the elucidation of the precise mechanism of action, including NO release kinetics and vascular signalling interactions, alongside rigorous pharmacokinetic and toxicity studies to establish safety and dosing parameters. Additionally, the evaluation of CRPN-4 in combination with standard therapies (for example, ACE inhibitors) could reveal synergistic benefits for resistant hypertension. Given the clinical demand for novel vasodilators, systematic preclinical validation and controlled clinical trials are essential for translating these findings into viable treatments. CRPN-4 can progress from a preclinical point of view to a clinically impactful antihypertensive agent only through comprehensive mechanistic and translational studies.

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