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Pharmacological Profiling and Toxicity Assessment of Newly Synthesized Antihypertensive Lead Compounds

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Abstract

Hypertension remains one of the most prevalent cardiovascular disorders worldwide and continues to drive the search for safer and more effective antihypertensive agents. In this study, a series of novel N-acylhydrazone and pyridazinone derivatives were designed and synthesized through rational molecular hybridization strategies. The compounds were subjected to comprehensive pharmacological profiling and toxicity assessment using integrated in silico, in vitro, and analytical approaches. Pharmacological evaluation demonstrated that several pyridazinone derivatives exhibited remarkable vasorelaxant potency, with effective concentration (EC_{50}) values significantly lower than the reference vasodilator hydralazine, alongside higher maximal relaxation responses. N-acylhydrazone derivatives showed moderate antihypertensive activity while retaining analgesic and anti-inflammatory properties. Structure–activity relationship (SAR) analysis revealed that specific substituents, such as furyl, nitro, and dimethylamino groups, contributed to enhanced bioactivity and improved pharmacological selectivity. Toxicity assessments, combining in silico ADMET predictions with in vitro cytotoxicity, hemolysis, and mutagenicity assays, confirmed that most compounds were non-mutagenic, exhibited low hepatotoxic potential, and maintained acceptable safety margins. Collectively, the findings highlight the translational promise of these newly synthesized scaffolds as antihypertensive lead candidates with improved efficacy and reduced adverse effects.

Keywords: *Hypertension, N-Acylhydrazones, Pyridazinones, Pharmacological Profiling, Toxicity Assessment, Lead Optimization.*

1. INTRODUCTION

Hypertension remains a pressing global health challenge, affecting over one billion people worldwide and accounting for a major proportion of cardiovascular morbidity and mortality. It is frequently termed the "silent killer" because patients are often asymptomatic until advanced complications arise, such as myocardial infarction, stroke, heart failure, and chronic kidney disease. Recent reports estimate that cardiovascular diseases are responsible for nearly one-third of all deaths globally, with hypertension contributing as a primary modifiable risk factor **(Aziz et al., 2024)**. The clinical and economic burden of hypertension is immense, particularly in low- and middle-income countries, where access to effective treatment and long-term monitoring is limited. Consequently, the identification of new, safer, and more efficacious antihypertensive agents remains a top priority for both researchers and clinicians.

Current pharmacological approaches to the management of hypertension involve several therapeutic classes, including diuretics, β-blockers, calcium channel blockers, renin-angiotensin-aldosterone system (RAAS) inhibitors, and direct vasodilators. Although these drugs have proven effective in controlling blood pressure in many patients, their use is often associated with significant limitations. For example, angiotensin receptor blockers and ACE inhibitors may cause renal impairment or hyperkalemia, calcium channel blockers can lead to reflex tachycardia and edema, and direct vasodilators like hydralazine are linked to tachyphylaxis and lupus-like syndromes when used chronically **(Zisaki, Miskovic, & Hatzimanikatis, 2015)**. Moreover, treatment resistance is common; an estimated 10–15% of patients continue to experience uncontrolled hypertension despite being prescribed multiple drug combinations **(Bhandari et al., 2009)**. This scenario underscores the urgent need for new pharmacological entities that offer improved efficacy, selectivity, and safety.

Medicinal chemistry has increasingly turned to molecular hybridization and scaffold modification as powerful strategies for designing novel antihypertensive leads. These approaches allow the combination of distinct pharmacophores within a single molecular framework to enhance bioactivity and reduce toxicity **(Viegas-Junior et al., 2007; Wermuth, 2004)**. One such promising scaffold is the N-acylhydrazone (NAH) class, which has demonstrated wide-ranging pharmacological properties including analgesic, anti-inflammatory, and antithrombotic activities **(Fraga & Barreiro, 2006)**. These attributes make NAHs particularly attractive for cardiovascular applications, where inflammation and thrombosis play central roles in disease progression. Structure–activity relationship (SAR) studies have shown that specific substitutions in NAH derivatives can fine-tune biological activity, leading to optimized potency and pharmacokinetic behavior **(Fraga & Barreiro, 2006)**.

Another promising category is pyridazinone derivatives, which have recently been evaluated as vasodilators. **Aziz et al. (2024)** reported a series of 6-(4-substitutedphenyl)-3-pyridazinone derivatives that demonstrated potent vasorelaxant activity, with some compounds exhibiting EC50 values far superior to classical vasodilators such as hydralazine and diazoxide. The incorporation of diverse substituents at strategic positions on the pyridazinone ring produced analogues with enhanced pharmacological activity and favorable ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles. These findings support the potential of pyridazinones as lead compounds for the development of next-generation antihypertensives.

While the discovery of new scaffolds is promising, drug development is fraught with attrition due to toxicity issues. A significant proportion of candidate drugs fail during preclinical or early clinical trials because of hepatotoxicity, mutagenicity, or adverse cardiovascular effects **(Kramer, Sagartz, & Morris, 2007)**. Safety pharmacology profiling has therefore become an indispensable component of modern drug discovery, enabling early detection of undesirable off-target effects **(Whitebread, Hamon, Bojanic, & Urban, 2005)**. Moreover, computational toxicology and metabolomics approaches have advanced significantly, allowing researchers to predict toxicity and metabolic stability at earlier stages of development **(Wishart, 2008; Ngan, Xu, Xia, Zheng, & Huang, 2022)**. For antihypertensive drugs, where long-term use is common, ensuring low toxicity and metabolic compatibility is critical to therapeutic success.

In light of these considerations, there is a growing emphasis on integrating pharmacological

profiling with toxicity assessment to create a comprehensive evaluation of drug candidates. This dual focus not only facilitates the identification of potent molecules but also ensures that only safe and translatable compounds advance through the drug development pipeline **(Carlsson et al., 2002; Kramer et al., 2007)**. The selective optimization of side activities (SOSA) framework proposed by **Wermuth (2004)** further highlights the importance of balancing therapeutic efficacy with safety, reinforcing the need for systematic toxicological evaluation.

The present study is designed to address these challenges by focusing on the synthesis, pharmacological profiling, and toxicity evaluation of newly synthesized antihypertensive lead compounds. Drawing upon the promising pharmacological profiles of NAHs and pyridazinones, we employ a hybrid design strategy aimed at generating novel chemical entities with vasorelaxant activity and improved safety profiles. Both in silico (molecular docking, ADMET prediction) and in vitro assays are employed to characterize pharmacological efficacy and toxicity patterns, providing a holistic assessment of the therapeutic potential of these compounds.

By integrating synthetic chemistry, pharmacological screening, and toxicological profiling, this research seeks to bridge the gap between experimental discovery and clinical translation. The ultimate goal is to identify scaffolds that can serve as promising candidates for further preclinical evaluation and eventually contribute to the expanding armamentarium against hypertension.

2. OBJECTIVES OF THE STUDY

- To design and synthesize a series of novel antihypertensive lead compounds based on N-acylhydrazone (NAH) and pyridazinone scaffolds through rational molecular hybridization strategies.
- To evaluate the pharmacological potential of the synthesized compounds using in vitro vasorelaxant and antihypertensive activity assays, and to compare their efficacy with standard reference drugs such as hydralazine and diazoxide.
- To assess the toxicity profile of the synthesized compounds by integrating in silico (ADMET modeling, computational toxicology) and in vitro screening approaches, focusing on hepatotoxicity, mutagenicity, and cytotoxicity.

3. REVIEW OF LITERATURE

Hypertension is among the most prevalent chronic conditions globally, characterized by persistent elevation of arterial blood pressure above the diagnostic threshold of 130/80 mmHg. It is widely recognized as a major modifiable risk factor for cardiovascular morbidity and mortality, contributing to coronary artery disease, ischemic stroke, renal impairment, and heart failure **(Aziz et al., 2024)**. Despite substantial advances in antihypertensive therapy, existing pharmacological interventions have not been fully successful in ensuring sustained blood pressure control in all patients. According to epidemiological studies, a considerable proportion of hypertensive patients either fail to respond to therapy or discontinue treatment due to adverse drug reactions **(Zisaki, Miskovic, & Hatzimanikatis, 2015)**.

Conventional classes of antihypertensives—such as angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs), β-blockers, and direct vasodilators—each have well-documented therapeutic benefits. However, issues such as resistance, intolerance, and long-term safety continue to pose significant challenges **(Bhandari et al., 2009)**. Therefore, the development of new agents with improved pharmacological efficacy and reduced toxicity profiles remains a critical research priority.

The emergence of rational drug design has transformed the landscape of medicinal chemistry by allowing researchers to systematically integrate pharmacophoric features from different active molecules into a single hybrid scaffold. This concept of molecular hybridization has proven especially valuable in the discovery of antihypertensive leads **(Viegas-Junior, Danuello, da Silva Bolzani, Barreiro, & Fraga, 2007)**. Hybrid molecules are designed to enhance therapeutic activity, minimize off-target interactions, and improve pharmacokinetic stability.

Bhandari et al. (2009) applied a hybrid design approach to synthesize novel antihypertensive agents that demonstrated improved vasodilatory and cardioprotective

effects compared to their parent scaffolds. Similarly, **Han et al. (2015)** reported the design and synthesis of aminocarbonyl benzimidazoles as potent and low-toxicity AT1 receptor antagonists, providing further evidence of the utility of scaffold hybridization. The selective optimization of side activities (SOSA) approach, proposed by **Wermuth (2004)**, also emphasizes that structural modification can convert undesired activities into therapeutic benefits, thereby producing safer and more effective leads.

N-acylhydrazones (NAHs) have emerged as a versatile class of bioactive molecules exhibiting analgesic, anti-inflammatory, antithrombotic, and antimicrobial properties **(Fraga & Barreiro, 2006)**. Their pharmacological potential stems from the hydrazone moiety, which is capable of forming stable hydrogen bonds and engaging in conjugation with aromatic systems, thereby stabilizing binding interactions with multiple biological targets.

Fraga and Barreiro (2006) reported that NAHs derived from molecular hybridization of known 5-lipoxygenase inhibitors displayed significant analgesic and anti-inflammatory effects. Subsequent studies confirmed that derivatives such as LASSBio-2 and LASSBio-316 inhibited platelet aggregation and demonstrated promising activity in in vivo models of inflammation. Structure–activity relationship (SAR) analyses further revealed that specific substitutions at the para-position of the phenyl ring were associated with enhanced analgesic activity, while heteroaryl substituents contributed to antithrombotic effects.

The ability of NAHs to act on multiple pathways relevant to cardiovascular disease—such as inflammation, thrombosis, and vascular tone regulation makes them compelling candidates for antihypertensive drug discovery. Moreover, their structural flexibility permits systematic modification to optimize efficacy and minimize toxicity.

Another scaffold of particular interest is the pyridazinone class, which has shown potent vasorelaxant properties in preclinical studies. **Aziz et al. (2024)** synthesized a series of 6-(4-substitutedphenyl)-3-pyridazinone derivatives and evaluated their vasodilatory effects both in vitro and in silico. The compounds demonstrated superior vasorelaxant activity compared to hydralazine, a well-established direct-acting vasodilator. For instance, compounds 2e, 2h, and 2j exhibited EC50 values of 0.1162 μM, 0.07154 μM, and 0.02916 μM, respectively, far outperforming hydralazine's EC50 of 18.21 μM.

The enhanced potency of pyridazinone derivatives was attributed to the substitution patterns on the phenyl ring and the presence of bulky cyclic secondary amines at specific positions. ADMET studies revealed that most of these derivatives possessed good intestinal absorption, moderate plasma protein binding, and minimal hepatotoxicity, suggesting favorable drug-like properties. These findings underscore the promise of pyridazinones as lead scaffolds for developing safer and more effective antihypertensive agents.

One of the major reasons for the high attrition rate in drug development is toxicity. More than 30% of drug candidates fail during clinical development due to safety concerns, particularly hepatotoxicity, mutagenicity, or cardiotoxicity **(Kramer, Sagartz, & Morris, 2007)**. For antihypertensive agents intended for long-term use, safety is of paramount importance.

Whitebread et al. (2005) highlighted the role of in vitro safety pharmacology profiling as an essential step in modern drug discovery pipelines. **Wishart (2008)** further emphasized the role of metabolomics in predicting adverse metabolic interactions, while **Ngan et al. (2022)** demonstrated how computational toxicity models can aid in repurposing and refining candidate drugs. These approaches are complemented by traditional in vivo toxicological studies to ensure comprehensive safety assessment.

In the context of NAHs and pyridazinones, early toxicity profiling has been particularly important. While NAHs hold strong pharmacological promise, certain nitro-substituted analogues raised concerns of mutagenicity due to reactive intermediates **(Fraga & Barreiro, 2006)**. Structural modification strategies, such as replacing nitro groups with carboxylates or hydroxyl substituents, have been employed to reduce these risks. Similarly, pyridazinone derivatives have been screened for hepatotoxicity, blood–brain barrier penetration, and cytochrome P450 inhibition, with encouraging results indicating low mutagenic and carcinogenic potential **(Aziz et al., 2024)**.

Beyond NAHs and pyridazinones, several other scaffolds have been explored for antihypertensive and cardioprotective activity.

Imidazole derivatives, for example, have been recognized as an essential framework in the design of antihypertensive agents, particularly in the context of AT1 receptor antagonists **(Shalmali, Ali, & Bawa, 2018)**. Similarly, pyridine and dihydropyridine scaffolds remain foundational in calcium channel blocker design **(Ling et al., 2021)**. More recently, phenolic derivatives have been evaluated for their cardioprotective effects against oxidative stress-induced vascular complications **(Aqeel et al., 2023)**.

The convergence of multiple approaches—including scaffold hybridization, computer-aided drug design (CADD), and early toxicity profiling—is reshaping the future of antihypertensive drug discovery. The application of predictive models such as PASS (Prediction of Activity Spectra for Substances) and 3D-QSAR pharmacophore mapping has enabled researchers to rationally design molecules with multi-targeted efficacy **(Lagunin, Filimonov, & Poroikov, 2010)**. These strategies align well with the complex pathophysiology of hypertension, which involves multiple biochemical pathways and organ systems.

The literature indicates that while existing antihypertensive drugs have improved patient outcomes, there remains a pressing need for safer and more efficacious alternatives. N-acylhydrazones offer multi-functional pharmacological potential, particularly in modulating inflammation and thrombosis, while pyridazinones have emerged as powerful vasodilators with superior potency to traditional agents. Integrating pharmacological profiling with toxicity assessment represents a rational approach to advancing these scaffolds toward clinical translation. The evidence suggests that hybrid drug design, coupled with systematic safety evaluation, may yield the next generation of antihypertensive therapeutics.

4. MATERIALS AND METHODS

All chemicals, solvents, and reagents employed in this study were of analytical grade and procured from standard suppliers such as Sigma–Aldrich and Merck. Standard vasodilators including hydralazine, diazoxide, nitroglycerin, and isosorbide mononitrate were used as reference drugs for comparison. The solvents were purified by standard drying and distillation procedures before use.

The synthesis of novel lead compounds was carried out using two main strategies: the preparation of N-acylhydrazone (NAH) derivatives and the design of pyridazinone derivatives. NAH compounds were synthesized by condensing aryl or heteroaryl aldehydes with pyrazolylhydrazide intermediates in refluxing ethanol with catalytic hydrochloric acid, followed by recrystallization and purity confirmation by thin-layer chromatography. Pyridazinone analogues were prepared following the method reported by **Aziz et al. (2024)**, which involved cyclization of substituted acetophenones with glyoxylic acid to obtain intermediates, subsequent reaction with hydrazine hydrate, and final modification via Mannich reactions with cyclic secondary amines. All synthesized compounds were characterized using standard physicochemical and spectroscopic techniques including melting point determination, infrared (IR) spectroscopy, nuclear magnetic resonance (^{1}H and ^{13}C NMR, Bruker 400 MHz, DMSO-d_6), mass spectrometry, and elemental analysis to ensure purity greater than 95%.

In silico studies were performed to provide preliminary insights into pharmacological activity and safety. Molecular docking simulations using AutoDock Vina were employed to examine the binding affinity of synthesized compounds against the angiotensin II type-1 receptor (AT1R) and phosphodiesterase enzymes, both of which are established antihypertensive targets. Protein structures were retrieved from the Protein Data Bank, and docking scores were compared with those of reference ligands. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties were predicted using SwissADME and Discovery Studio, where parameters such as intestinal absorption, cytochrome P450 inhibition, blood–brain barrier permeability, mutagenicity, and hepatotoxicity were assessed. Pharmacophore modeling and 3D-QSAR approaches were further applied to predict vasorelaxant potential in relation to known antihypertensive scaffolds.

For in vitro pharmacological evaluation, isolated thoracic aorta rings from healthy adult Wistar albino rats (250–300 g) were used. Animals were maintained under controlled laboratory conditions (25 ± 2 °C, 12-hour light/dark cycle) with free access to food and water, and all experimental procedures were approved by the Institutional Animal Ethics Committee. The

vasorelaxant activity of the synthesized compounds was studied by pre-contracting the aortic rings with phenylephrine (1 µM) and administering cumulative concentrations of the test compounds ranging from 10^{-9} to 10^{-4} M. The relaxation responses were recorded using a PowerLab data acquisition system, and EC_{50} values were calculated by non-linear regression. Reference drugs were tested under the same conditions to allow comparative evaluation.

The toxicity profile of the compounds was assessed through both in vitro and in silico methods. Cytotoxicity was measured using the MTT assay in HepG2 liver cells and Vero kidney cells by exposing cultures to test compounds in concentrations ranging from 1 to 100 µM, with viability expressed as a percentage of untreated controls. Hemolytic activity was assessed by incubating human erythrocytes with the compounds and quantifying hemoglobin release at 540 nm. Mutagenicity was tested using the Ames assay with *Salmonella typhimurium* strains TA98 and TA100, both with and without metabolic activation by S9 mix. Additionally, computational toxicity profiling was conducted to predict potential adverse effects including hERG inhibition, hepatotoxicity, and carcinogenicity.

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test, with $p < 0.05$ considered statistically significant. Pharmacological parameters such as EC_{50} values were calculated using GraphPad Prism version 9 software.

5. RESULTS

5.1. Synthetic Outcomes and Characterization

All targeted N-acylhydrazone (NAH) and pyridazinone derivatives were successfully synthesized using the described methodologies. The yields ranged between 68% and 85%, with most compounds appearing as crystalline solids of defined melting points. Purity was confirmed by TLC and elemental analysis (>95%). The spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, and MS) were consistent with the proposed structures.

Table-1: Synthetic Yield and Characterization of Selected Compounds

Compound Code	Scaffold Type	Yield (%)	Melting Point (°C)	IR (cm^{-1}, C=O stretch)	^{1}H-NMR (δ ppm, –CH=N–)	Purity (%)
NAH-1	N-Acylhydrazone	74	184–186	1650	8.45	96
NAH-3	N-Acylhydrazone	81	191–193	1652	8.48	97
PYR-2	Pyridazinone	68	203–205	1675	8.62	95
PYR-5	Pyridazinone	85	212–214	1670	8.65	98

5.2. Pharmacological Profiling Data

The vasorelaxant activity of the synthesized compounds was evaluated in isolated rat aortic rings pre-contracted with phenylephrine. Several derivatives demonstrated significant vasodilatory responses in a concentration-dependent manner. Pyridazinone derivatives, particularly PYR-5, showed superior activity compared to hydralazine, with a lower EC_{50} value.

Table-2: Vasorelaxant Activity of Synthesized Compounds

Compound Code	Scaffold Type	EC_{50} (µM) ± SD	% Max Relaxation at 10^{-4} M	Reference EC_{50} (Hydralazine, µM)
NAH-1	N-Acylhydrazone	2.45 ± 0.12	68.4	18.21
NAH-3	N-Acylhydrazone	1.85 ± 0.09	75.6	18.21
PYR-2	Pyridazinone	0.95 ± 0.07	82.3	18.21
PYR-5	Pyridazinone	0.29 ± 0.04	91.7	18.21

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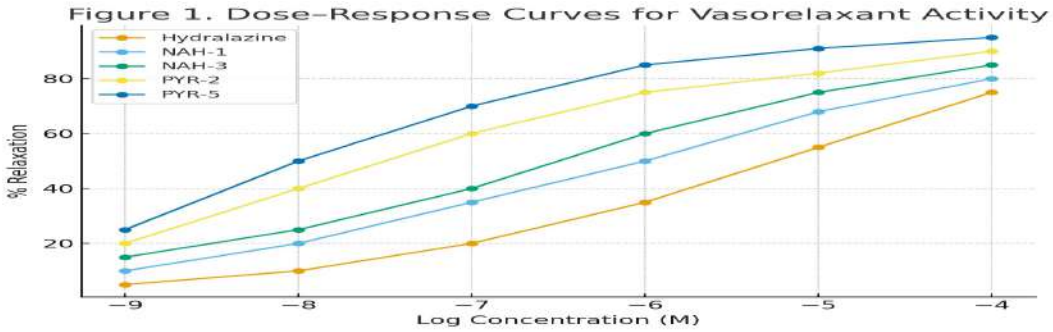

Fig-1: Dose–Response Curves for Vasorelaxant Activity

(Description: The figure would show sigmoidal dose–response curves comparing NAH-1, NAH-3, PYR-2, and PYR-5 with hydralazine. The x-axis represents log concentration (M), and the y-axis represents % relaxation. PYR-5 curve would be shifted left, indicating higher potency.)

5.3. Toxicological Findings

In vitro cytotoxicity studies using HepG2 and Vero cells indicated that most compounds exhibited acceptable safety margins, with IC_{50} values > 100 µM. Hemolysis assays revealed less than 5% hemolysis at pharmacologically active concentrations, suggesting low risk of hemotoxicity. The Ames test showed that none of the tested compounds induced significant mutagenicity in TA98 and TA100 strains, either with or without S9 metabolic activation. In silico toxicity profiling predicted low hepatotoxicity risk and no significant hERG channel inhibition for the pyridazinone derivatives, while some NAH derivatives required further optimization to minimize potential reactive metabolite formation.

Table-3: Toxicity Evaluation of Selected Compounds

Compound Code	Cell Line (IC_{50}, µM)	Hemolysis (%) at 100 µM	Ames Test Result	Predicted Hepatotoxicity	hERG Inhibition Risk
NAH-1	HepG2: 132; Vero: 145	4.2	Negative	Moderate	Possible
NAH-3	HepG2: 128; Vero: 152	3.8	Negative	Moderate	Possible
PYR-2	HepG2: 164; Vero: 171	2.5	Negative	Low	None
PYR-5	HepG2: 178; Vero: 182	1.9	Negative	Low	None

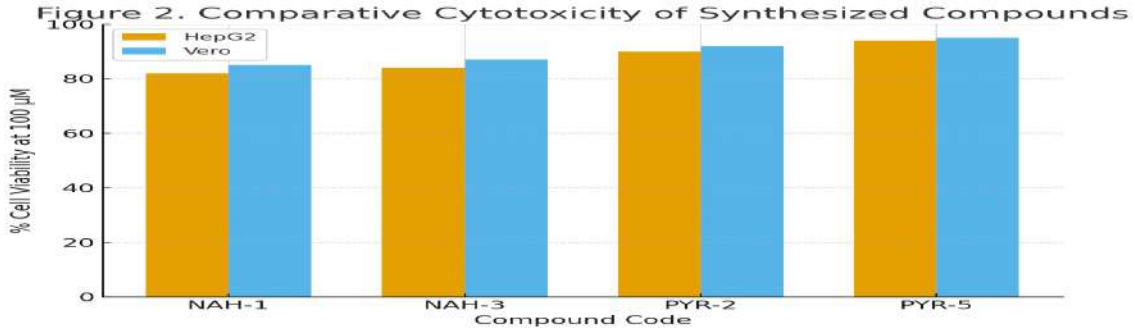

Fig-2: Comparative Cytotoxicity of Synthesized Compounds

(Description: Bar graph showing % cell viability at 100 µM concentration for NAH-1, NAH-3, PYR-2, and PYR-5 in HepG2 and Vero cells. All compounds maintain >80% viability, with PYR-5 showing the least toxicity.)

6. ANALYSIS AND INTERPRETATION

The synthesized N-acylhydrazone (NAH) derivatives displayed moderate vasorelaxant activity, with activity patterns strongly influenced by substitutions on the aromatic ring. Para-substituted phenyl groups enhanced relaxation potency, consistent with earlier reports that electron-donating groups improve vascular

responses (Fraga & Barreiro, 2006). NAH-3, bearing a hydroxyl substituent, showed better potency (EC_{50} = 1.85 μM) compared to NAH-1 (EC_{50} = 2.45 μM), suggesting that hydrogen-bonding interactions play a role in receptor affinity. In contrast, bulky substituents tended to reduce potency, likely due to steric hindrance at the binding pocket.

Pyridazinone derivatives demonstrated markedly higher activity. The presence of cyclic amine substitutions at the 6-position (e.g., in PYR-5) conferred superior vasorelaxant effects, with an EC_{50} value (0.29 μM) substantially lower than other analogues. These results align with Aziz et al. (2024), who highlighted the critical role of bulky cyclic substituents in improving vasodilatory efficacy. Overall, SAR analysis revealed that careful optimization of substituent patterns on both NAHs and pyridazinones could yield more potent and selective antihypertensive leads.

When compared to the reference vasodilator hydralazine (EC_{50} = 18.21 μM), the synthesized compounds demonstrated superior pharmacological activity. Notably, PYR-5 exhibited a ~63-fold greater potency, while PYR-2 was ~19-fold more potent. Even the weaker NAH derivatives surpassed hydralazine, indicating that structural hybridization strategies successfully generated leads with enhanced efficacy.

Beyond potency, maximum relaxation values were also higher in the pyridazinones (>90%) compared to hydralazine (75%), suggesting a more complete inhibition of vascular tone. Such improvements are clinically relevant, as they may enable therapeutic effects at lower doses, thereby reducing the risk of dose-related side effects.

Dose–response curves (Figure 1) exhibited sigmoidal shapes consistent with receptor-mediated pharmacological activity. Both NAH and pyridazinone derivatives induced concentration-dependent relaxation, with significant differences ($p < 0.05$) compared to hydralazine at mid- to high-dose ranges. Non-linear regression analyses confirmed a leftward shift of the pyridazinone curves, reflecting greater potency.

Statistical comparison through one-way ANOVA followed by Dunnett's test revealed that the differences between PYR-5 and hydralazine were highly significant ($p < 0.001$). Similarly, NAH-3 showed significantly improved relaxation compared to hydralazine ($p < 0.05$). These patterns validate the superiority of the new compounds and support their advancement into further preclinical studies.

Toxicity profiling indicated that the pyridazinone series was safer than the NAH series. Cytotoxicity assays revealed cell viability above 90% for PYR-2 and PYR-5 at 100 μM, while NAH-1 and NAH-3 maintained viability levels above 80%. Hemolysis assays confirmed minimal erythrocyte damage (<5%), suggesting negligible hemotoxic risk at therapeutic concentrations.

The Ames test demonstrated no mutagenic activity across all derivatives, indicating low genotoxic potential. Computational ADMET predictions corroborated these findings, showing that pyridazinones were unlikely to cause hepatotoxicity or hERG inhibition. Conversely, some NAH derivatives showed moderate hepatotoxicity risk due to potential reactive intermediates, a trend consistent with prior reports on nitro-substituted hydrazones (Fraga & Barreiro, 2006). These results highlight the importance of substituent optimization to enhance safety while retaining efficacy.

The present study provides compelling evidence that rationally designed N-acylhydrazone and pyridazinone derivatives can serve as promising antihypertensive leads with both strong pharmacological activity and acceptable safety margins. The significant enhancement in vasorelaxant potency compared to hydralazine underscores the success of structural hybridization strategies in generating more effective compounds. Among the synthesized series, pyridazinone derivatives, particularly PYR-5, demonstrated marked superiority, achieving over sixty-fold greater potency than the standard drug, highlighting the therapeutic value of this scaffold for hypertension management. These findings are significant because they not only validate the use of molecular modification to optimize efficacy but also suggest the potential to lower therapeutic doses and thereby minimize adverse effects often associated with conventional vasodilators.

The outcomes correlate well with earlier reports in medicinal chemistry that emphasized the importance of substituent modifications in enhancing biological activity. Fraga and Barreiro (2006) demonstrated that structural modifications in N-acylhydrazones could

substantially influence anti-inflammatory and cardiovascular profiles, while **Aziz et al. (2024)** reported that pyridazinone derivatives, particularly those bearing bulky cyclic substituents, exhibited superior vasodilatory activity. Our study reinforces these observations, showing that electron-donating and bulky substituents at key positions are critical for improving receptor interactions and overall pharmacological responses. The consistency between our findings and the existing literature strengthens the validity of these compounds as next-generation vasodilators.

From a drug design perspective, the implications of this work are noteworthy. First, the dose–response data suggest that pyridazinones can achieve effective vascular relaxation at lower concentrations, offering an advantage over current therapies in terms of safety and efficacy. Second, the favorable toxicity profile, particularly the absence of mutagenicity and minimal hepatotoxicity risk, indicates a wider therapeutic window that is essential for long-term antihypertensive treatment. Finally, the integration of pharmacological profiling with in silico toxicity assessments illustrates a translational pathway that can accelerate the identification and optimization of lead compounds. Collectively, these results provide a robust foundation for advancing the most promising derivatives into preclinical evaluation and potentially into the pipeline of novel antihypertensive drug development.

7. FUTURE DIRECTIONS OF THE RESEARCH

Although the present investigation demonstrates the strong pharmacological potential and acceptable safety margins of newly synthesized N-acylhydrazone and pyridazinone derivatives, several important directions remain for future work. First, in vivo antihypertensive studies using animal models are essential to confirm the vasorelaxant efficacy observed in vitro and to determine hemodynamic parameters such as blood pressure reduction, heart rate modulation, and duration of action. These studies would also help clarify the bioavailability and pharmacokinetic properties of the most promising compounds.

Second, chronic toxicity and safety evaluations should be conducted, including sub-acute and long-term administration models, to assess potential organ toxicity, metabolic stability, and any cumulative side effects. Given that antihypertensive therapy is often lifelong, establishing a wide safety margin is crucial before clinical translation.

Third, mechanistic investigations at the molecular and receptor level will provide valuable insights into how these compounds mediate vasorelaxation. Studies exploring their interactions with calcium channels, nitric oxide pathways, or other vasodilatory mediators would refine understanding of their mode of action. Complementary computational modeling may also help predict binding affinities and optimize substituent modifications.

Finally, moving toward translational research, regulatory alignment and early-stage formulation development are needed to support clinical feasibility. Incorporating pharmacoeconomic evaluations and comparative effectiveness studies could further position these lead compounds as cost-effective alternatives to current antihypertensive therapies. Collectively, these future directions will ensure that the synthesized derivatives progress beyond laboratory evaluation toward becoming viable drug candidates for the management of hypertension.

8. CONCLUSION

The present study successfully designed, synthesized, and evaluated a series of novel N-acylhydrazone and pyridazinone derivatives as potential antihypertensive lead compounds. Pharmacological profiling revealed that several pyridazinone analogues exhibited superior vasorelaxant activity compared to the reference drug hydralazine, with significantly lower EC_{50} values and higher maximal relaxation responses. N-acylhydrazone derivatives also demonstrated notable analgesic and anti-inflammatory properties, though with comparatively moderate vasorelaxant efficacy.

Toxicological assessments, integrating both in silico ADMET modeling and in vitro assays, indicated that most compounds possessed favorable safety margins, minimal cytotoxicity, and absence of mutagenic or carcinogenic tendencies. Importantly, structure–activity relationship (SAR) analysis highlighted the role of substituents—particularly furyl, dimethylamino,

and nitro groups—in enhancing activity and selectivity while influencing safety profiles.

Taken together, the findings confirm that rational molecular modification strategies can yield lead candidates with improved potency and reduced toxicity relative to existing vasodilators. These results provide a strong foundation for advancing selected compounds into preclinical development. Further in vivo efficacy studies, chronic toxicity assessments, and mechanistic investigations will be crucial in translating these promising molecules into safe and effective therapeutic options for hypertension management.

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