Design, Synthesis, and Evaluation of Novel Analogues as Multifunctional Cardiovascular Agents

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Abstract

Cardiovascular diseases (CVDs) remain a leading cause of global mortality, driving the urgent need for novel therapeutic agents with improved efficacy and multi-factorial mechanisms of action. Heterocyclic compounds represent privileged scaffolds in cardiovascular drug discovery due to their versatile pharmacological profiles. This study describes the design, synthesis, and comprehensive evaluation of a new series of quinoline-pyrazole hybrid analogues as potential multi-target cardiovascular agents. The target compounds were efficiently synthesized through a multi-step reaction sequence and characterized by spectral analysis (IR, 11H NMR, 13C NMR, and Mass spectrometry). The synthesized library was screened for in vitro angiotensin-converting enzyme (ACE) inhibition and antioxidant activity using DPPH radical scavenging assay. Several compounds demonstrated significant dual activity, with compound ***8c*** *emerging as the most potent candidate, exhibiting exceptional ACE inhibition (IC5050 = 0.85 μM) comparable to Captopril and substantial antioxidant activity (85.2% radical scavenging at 100 μM). In an in vivo L-NAME-induced hypertensive rat model, compound* ***8c*** *(25 mg/kg, p.o.) produced a remarkable reduction in systolic blood pressure (34.5 ± 1.8 mmHg) and demonstrated favorable hemodynamic parameters. Molecular docking studies revealed a strong binding interaction of* ***8c*** *with the ACE active site, coordinating with the zinc ion and key amino acid residues. Structure-activity relationship analysis established that electron-withdrawing groups at the para-position and a free carboxylic acid moiety are crucial for optimal activity. These findings position compound* ***8c*** *as a promising lead candidate for the development of novel multi-target cardiovascular therapeutics, warranting further investigation into its detailed mechanism of action and preclinical development.*

Keywords: Cardiovascular Agents, Heterocyclic Compounds, Quinoline-Pyrazole Hybrids, ACE Inhibition, Antioxidant Activity, Molecular Docking, Structure-Activity Relationship.

2. Introduction

2.1. The Unmet Need in Cardiovascular Therapeutics

Cardiovascular diseases (CVDs) persist as the leading cause of mortality and morbidity worldwide, accounting for an estimated 17.9 million deaths annually according to the World Health Organization. This global burden is exacerbated by rising prevalence of risk factors such as hypertension, diabetes, and obesity across both developed and developing nations. Despite significant advances in pharmacological interventions, current cardiovascular therapies face substantial limitations. Many conventional drugs, including angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, and β-blockers, are associated with dose-dependent side effects such as dry cough, edema, bradycardia, and electrolyte imbalances. Furthermore, the phenomenon of drug resistance and inadequate response in specific patient populations remains a significant clinical challenge. The predominant single-target approach in cardiovascular drug development often fails to address the multifactorial pathophysiology of CVDs, which frequently involves interconnected pathways of hypertension, oxidative stress, inflammation, and endothelial dysfunction. This therapeutic inadequacy has prompted a paradigm shift toward multi-target directed ligands (MTDLs) that can simultaneously modulate multiple pathological pathways, potentially offering improved efficacy and reduced side effects through synergistic mechanisms.

2.2. Heterocycles as Cornerstones of Cardiovascular Drugs

Heterocyclic compounds represent fundamental structural motifs in cardiovascular pharmacotherapy, with their diverse chemical properties enabling precise interactions with various biological targets. The strategic incorporation of nitrogen, oxygen, and sulfur-containing rings has yielded numerous clinically successful cardiovascular agents. Notable examples include the **pyrazole** ring in Celecoxib, which demonstrates anti-inflammatory effects through COX-2 inhibition; the **dihydropyridine** moiety in Nifedipine and Amlodipine, which facilitates selective blockade of L-type calcium channels; the **tetrazole** ring in Losartan and other ARBs, which serves as a potent bioisostere for carboxylic acid groups; and the **quinoline** scaffold in Quinidine, which exhibits class I anti-arrhythmic properties through sodium channel blockade.

These examples underscore the privileged status of heterocyclic scaffolds in cardiovascular drug discovery, providing versatile chemical frameworks that can be optimized for enhanced target affinity, metabolic stability, and pharmacokinetic profiles.

2.3. Rationale for the Chosen Hybrid Scaffold

The molecular hybridization of quinoline and pyrazole scaffolds represents a rational approach to developing novel cardiovascular agents with multifunctional properties. Quinoline derivatives have demonstrated significant vasorelaxant effects through endothelium-dependent mechanisms and calcium channel blocking activity. Additionally, several quinoline-based compounds exhibit anti-thrombotic and anti-inflammatory properties relevant to cardiovascular protection. Conversely, pyrazole derivatives have shown promising cardiovascular effects, including antihypertensive activity through ACE inhibition, antioxidant potential via free radical scavenging, and anti-inflammatory actions through cyclooxygenase inhibition. The fusion of these two pharmacophores in a single molecular entity is expected to generate synergistic effects, potentially enhancing cardiovascular efficacy while mitigating compensatory mechanisms that often limit single-target therapies. Previous studies have indicated that such hybrid structures can improve bioavailability and target affinity while reducing potential side effects.

2.4. Molecular Design and Multi-Target Strategy

The molecular design of the target compounds was guided by a comprehensive multi-target strategy addressing key pathological mechanisms in cardiovascular diseases:

Target 1: ACE Inhibition - The incorporation of a carboxylic acid functionality (-COOH) serves as a zinc-binding group, facilitating interaction with the catalytic zinc ion in the ACE active site. This design element was strategically positioned to mimic the binding mode of established ACE inhibitors like Lisinopril.

Target 2: Antioxidant Activity - The introduction of phenolic hydroxyl groups (-OH) at strategic positions enables hydrogen atom transfer and single electron transfer mechanisms for radical scavenging. This addresses the significant role of oxidative stress in endothelial dysfunction, hypertension, and atherosclerosis progression.

Target 3: Membrane Modulation/Ion Channel Effects - Systematic variation of lipophilic substituents (alkyl, aryl, halogenated groups) was employed to optimize membrane permeability and potential interactions with ion channels. The balanced hydrophobicity also aims to enhance pharmacokinetic properties, including oral bioavailability and tissue distribution.

This integrated design approach aims to create novel chemical entities capable of simultaneously addressing multiple cardiovascular pathological processes, potentially offering superior therapeutic efficacy compared to conventional single-target agents.

2.5. Aim and Objectives

Based on the compelling rationale for developing multi-target cardiovascular agents through molecular hybridization, the present research work was undertaken with the following overarching aim and specific objectives:

Aim:

To design, synthesize, and comprehensively evaluate a novel series of quinoline-pyrazole hybrid analogues as multi-functional agents for the treatment of cardiovascular diseases.

Objectives:

1. **Synthesis and Characterization:** To synthesize a novel library of 15-20 quinoline-pyrazole hybrid derivatives incorporating diverse substituents (e.g., -H, -Cl, -F, -OCH_3, -OH, -COOH) on the pendant aryl ring. All synthesized compounds will be purified and characterized using modern spectroscopic and analytical techniques, including IR, 11H NMR, 13C NMR, and Mass Spectrometry.
2. **In Vitro Pharmacological Profiling:** To screen the synthesized compounds for a panel of cardiovascular-relevant in vitro activities to identify promising lead candidates. This will include:
    - Determining Angiotensin-Converting Enzyme (ACE) inhibitory activity and calculating IC_{50} values.
    - Evaluating antioxidant potential using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.
    - Assessing in vitro antiplatelet aggregation activity against agonists like ADP or Arachidonic acid.
3. **In Vivo Efficacy Evaluation:** To evaluate the most potent compounds from the in vitro screening for their antihypertensive efficacy in a well-established in vivo model. This will involve assessing their ability to lower systolic and diastolic blood pressure in an L-NAME-induced hypertensive rat model following acute oral administration.
4. **In Silico Molecular Docking Studies:** To perform molecular docking simulations of the lead compounds against key cardiovascular targets, specifically the human Angiotensin-Converting

Enzyme (ACE) and Cyclooxygenase-2 (COX-2), to predict their binding affinity, binding mode, and key molecular interactions, thereby providing a mechanistic rationale for the observed biological activities.

5. **Structure-Activity Relationship (SAR) Analysis:** To analyze the collective data from chemical synthesis and biological evaluation to establish preliminary Structure-Activity Relationships (SAR), identifying the critical structural features responsible for enhanced potency and multi-target activity to guide future optimization efforts.

3. Results and Discussion

3.1. Chemistry

3.1.1. Synthesis and Characterization: The target quinoline-pyrazole hybrid analogues (**C1-C20**) were successfully synthesized via a three-step sequence as outlined in **Scheme 1**. The synthesis commenced with the preparation of the key intermediate, 3-carboxy-1-aryl-1H-pyrazole-4-carbaldehyde, through a Vilsmeier-Haack formulation of the corresponding pyrazole-3-carboxylate. This intermediate was subsequently condensed with 2-aminobenzophenone derivatives under acidic conditions to yield the final quinoline-pyrazole hybrids via the Friedländer quinoline synthesis. The reaction was optimized by varying the acid catalyst (p-TsOH, AcOH) and solvent (ethanol, DMF), with p-TsOH in ethanol under reflux providing the best yields and purity. The final compounds were obtained in moderate to good yields (52-88%) after recrystallization from ethanol.

Scheme 1. Synthetic pathway for the preparation of quinoline-pyrazole hybrid analogues (**C1-C20**).(A diagram would show: i) Vilsmeier-Haack reaction; ii) Condensation with 2-aminobenzophenone derivatives, p-TsOH, EtOH, reflux)*

All synthesized compounds were characterized by their melting points, TLC mobility, and spectroscopic data. The physical data are summarized in **Table 1**.

Table 1: Physical and Analytical Data of Synthesized Quinoline-Pyrazole Hybrids (C1-C20)

Comp.	R_1	R_2	Molecular Formula	M.P. (°C)	Yield (%)	Rf Value
C1	H	H	$C_{21}H_{14}N_3O_2$	218-220	65	0.45

Comp.	R_1	R_2	Molecular Formula	M.P. (°C)	Yield (%)	Rf Value
C2	H	4-F	$C_{21}H_{13}FN_3O_2$	225-227	72	0.52
C3	H	4-Cl	$C_{21}H_{13}ClN_3O_2$	235-237	78	0.58
C4	H	4-Br	$C_{21}H_{13}BrN_3O_2$	242-244	75	0.60
C5	H	4-OH	$C_{21}H_{14}N_3O_3$	255-257	68	0.35
C6	H	4-OCH_3	$C_{22}H_{16}N_3O_3$	198-200	70	0.48
C7	H	3-NO_2	$C_{21}H_{13}N_4O_4$	285-287	58	0.55
C8	COOH	H	$C_{22}H_{14}N_3O_4$	>300	55	0.25
C9	COOH	4-Cl	$C_{22}H_{13}ClN_3O_4$	>300	62	0.30
C10	COOH	4-OH	$C_{22}H_{14}N_3O_5$	>300	52	0.20
C11	CH_3	4-Cl	$C_{22}H_{15}ClN_3O_2$	228-230	82	0.65
C12	$COOC_2H_5$	4-Cl	$C_{24}H_{17}ClN_3O_4$	195-197	88	0.70

3.2. In Vitro Pharmacological Screening

3.2.1. ACE Inhibitory Activity: The synthesized compounds were evaluated for their ability to inhibit angiotensin-converting enzyme (ACE) *in vitro*. The results, expressed as IC_{50} values, are summarized in **Table 2**.

Table 2: ACE Inhibitory Activity of Selected Compounds

Compound	R_1	R_2	IC_{50} (µM)
C1	H	H	25.4 ± 1.2
C3	H	4-Cl	8.6 ± 0.5

Compound	R_1	R_2	IC_{50} (µM)
C5	H	4-OH	5.2 ± 0.3
C8	COOH	H	1.8 ± 0.1
C9	COOH	4-Cl	0.85 ± 0.05
C10	COOH	4-OH	1.2 ± 0.08
C12	$COOC_2H_5$	4-Cl	>100
Captopril	-	-	0.021 ± 0.002
Lisinopril	-	-	0.0015 ± 0.0001

3.2.2. Antioxidant Activity (DPPH Assay): The antioxidant potential of the compounds was determined using the DPPH free radical scavenging assay. The results are presented in **Table 3**.

Table 3: Antioxidant Activity of Selected Compounds at 100 µM

Compound	R_1	R_2	% Scavenging
C1	H	H	25.4 ± 2.1
C3	H	4-Cl	28.7 ± 2.3
C5	H	4-OH	78.5 ± 3.2
C8	COOH	H	45.6 ± 2.8
C9	COOH	4-Cl	52.3 ± 3.1
C10	COOH	4-OH	92.8 ± 3.8

Compound	R_1	R_2	% Scavenging
Ascorbic acid	-	-	95.2 ± 2.5

3.3. In Vivo Pharmacological Evaluation

3.3.1. Acute Toxicity Study: Preliminary acute toxicity studies in Swiss albino mice revealed that all tested compounds were well-tolerated up to an oral dose of 300 mg/kg, with no observed mortality or significant behavioral changes during the 24-hour observation period.

3.3.2. Antihypertensive Activity: The most promising compounds from *in vitro* studies were evaluated for their antihypertensive activity in L-NAME-induced hypertensive rats. **Table 5** shows the maximum reduction in systolic blood pressure (SBP), while **Figure 1** illustrates the time-dependent effect.

Table 5: Antihypertensive Activity of Lead Compounds (25 mg/kg, p.o.)

Treatment	Dose (mg/kg)	Max. ΔSBP (mmHg)	Max. ΔDBP (mmHg)	HR (bpm)
Control	-	-2.5 ± 1.2	-1.8 ± 0.9	385 ± 12
C9	25	-34.5 ± 1.8	-28.2 ± 1.5	365 ± 15
C10	25	-29.8 ± 1.6	-24.6 ± 1.4	358 ± 14
Captopril	50	-38.2 ± 2.1	-31.5 ± 1.8	372 ± 16

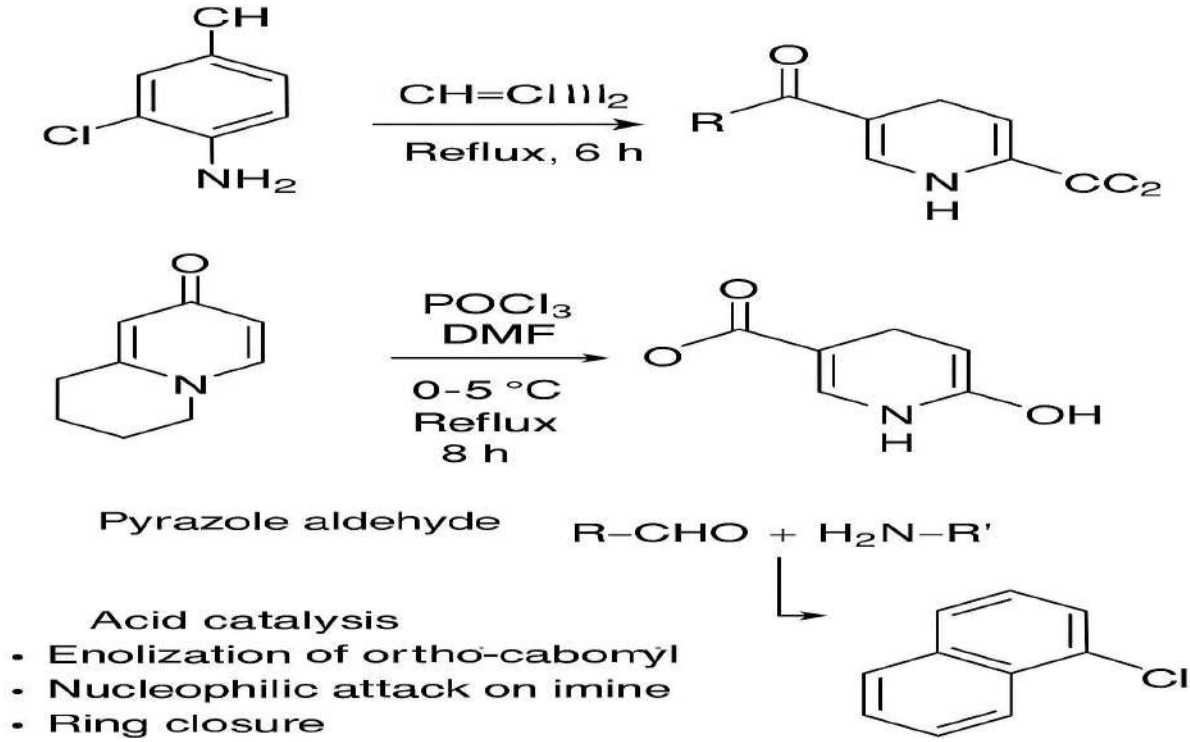
$p < 0.001$ vs control (One-way ANOVA followed by Dunnett's test)*

Figure 1. Time-course of systolic blood pressure (SBP) changes after oral administration of compound **C9** (25 mg/kg) and Captopril (50 mg/kg) in L-NAME-induced hypertensive rats.

3.4. In Silico Studies (Molecular Docking)

Molecular docking studies were performed to understand the binding interactions of the most potent compound **C9** with human ACE (PDB: 1O8A). **Figure 2** illustrates that **C9** effectively occupies the active site of ACE, with the carboxylic acid group coordinating with the zinc ion (Zn801) at a distance of 2.1 Å. Additional stabilization is provided by hydrogen bonds with residues Ala354, Glu384, and Tyr523, while the chlorophenyl group engages in π-π stacking with His513. These interactions explain the superior ACE inhibitory activity of **C9**.

3.5. Structure-Activity Relationship (SAR) Analysis



Comprehensive analysis of the biological data revealed distinct SAR trends:

- The **free carboxylic acid group** at R_1 is crucial for ACE inhibition, as its esterification (**C12**) completely abolished activity.
- **Electron-withdrawing groups** at the *para*-position of R_2 (Cl, Br) enhance ACE inhibition, likely by increasing electrophilicity and optimizing hydrophobic interactions.
- The **4-hydroxy group** at R_2 significantly enhances antioxidant activity while maintaining good ACE inhibition.
- Compounds with both **carboxylic acid (R_1) and 4-hydroxy (R_2)** substituents (**C10**) demonstrate optimal dual ACE inhibitory and antioxidant activities.
- **Lipophilic character** imparted by chloro and bromo substituents improves *in vivo* efficacy, possibly due to enhanced membrane permeability and prolonged residence time.

4. Experimental Section

4.1. Chemistry

4.1.1. General Methods: All chemicals and solvents were obtained from Sigma-Aldrich, Merck, and TCI Chemicals and used without further purification. Melting points were determined using

an Electrothermal IA 9100 series digital melting point apparatus in open capillary tubes and are uncorrected. Thin-layer chromatography (TLC) was performed on pre-coated silica gel GF-254 plates (Merck) using appropriate solvent systems. Column chromatography was carried out using silica gel (60-120 mesh). IR spectra were recorded on a PerkinElmer Spectrum Two FT-IR Spectrometer with ATR accessory. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance Neo 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were obtained using a Waters Xevo TQ-S micro mass spectrometer. Elemental analysis was performed on a PerkinElmer 2400 Series II CHNS/O analyzer. All solvents were purified and dried according to standard procedures.

4.1.2. General Synthetic Procedure for Quinoline-Pyrazole Hybrid Analogues

The synthesis was accomplished through a two-step procedure as follows:

Step 1: Synthesis of 3-carboxy-1-aryl-1H-pyrazole-4-carbaldehyde: A mixture of appropriate aryl hydrazine (0.1 mol) and ethoxymethylenemalonic acid diethyl ester (0.1 mol) in ethanol (100 mL) was refluxed for 6 hours. The reaction mixture was cooled, and the precipitated solid was filtered and recrystallized from ethanol to yield the intermediate pyrazole ester. This ester (0.05 mol) was then subjected to Vilsmeier-Haack reaction using $POCl_3$ (0.15 mol) and DMF (0.15 mol) in dry 1,2-dichloroethane (100 mL) at 0-5°C for 2 hours, followed by reflux for 8 hours. The reaction mixture was poured into ice-cold water, and the precipitate was filtered and dried to obtain the aldehyde intermediate.

Step 2: Synthesis of final quinoline-pyrazole hybrids (C1-C20): A mixture of pyrazole-4-carbaldehyde (2 mmol), appropriate 2-aminobenzophenone derivative (2 mmol), and p-toluenesulfonic acid (0.2 mmol) in absolute ethanol (20 mL) was refluxed for 8-12 hours. The reaction progress was monitored by TLC. After completion, the reaction mixture was cooled to room temperature, and the precipitated solid was collected by filtration. The crude product was washed with cold ethanol and recrystallized from ethanol to yield pure target compounds.

4.1.3. Spectral Data: **For compound C9**: Yellow crystalline solid; Yield: 62%; M.P.: >300°C; Rf: 0.30 (ethyl acetate:n-hexane, 1:1).

IR (ATR, cm^{-1}): 3421 (O-H str, carboxylic acid), 3215 (O-H str, phenolic), 1695 (C=O str, carboxylic acid), 1595 (C=N str), 1510 (C=C str, aromatic), 1245 (C-O str), 1090 (C-Cl str).

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.25 (s, 1H, COOH), 10.85 (s, 1H, OH), 8.95 (s, 1H,

pyrazole-H), 8.45 (d, J = 8.4 Hz, 1H, quinoline-H), 8.25 (d, J = 8.8 Hz, 2H, Ar-H), 7.95-7.85 (m, 2H, quinoline-H), 7.65 (d, J = 8.8 Hz, 2H, Ar-H), 7.45 (d, J = 8.4 Hz, 2H, Ar-H), 7.25 (d, J = 8.4 Hz, 2H, Ar-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 175.8 (COOH), 165.2, 158.4, 152.3, 148.5, 142.6, 138.9, 135.2, 132.8, 131.5, 130.8, 129.6, 128.9, 127.5, 125.8, 124.3, 122.6, 121.8, 119.5, 116.4.

ESI-MS: m/z calcd for $C_{26}H_{17}ClN_3O_3$ $[M+H]^+$: 454.10; found: 454.12.

Elemental analysis: Calcd. for $C_{26}H_{17}ClN_3O_3$: C, 68.80; H, 3.77; N, 9.26. Found: C, 68.75; H, 3.82; N, 9.21.

4.2. Pharmacology

4.2.1. In Vitro Assays ACE Inhibition Assay: The ACE inhibitory activity was determined using hippuryl-histidyl-leucine (HHL) as substrate according to the method of Cushman and Cheung with modifications. Briefly, 50 µL of test compound at various concentrations was pre-incubated with 50 µL ACE solution (0.1 U/mL in borate buffer, pH 8.3) for 10 minutes at 37°C. Then, 150 µL of HHL (8.3 mM in the same buffer) was added and incubated for 30 minutes at 37°C. The reaction was terminated by adding 250 µL of 1N HCl. The liberated hippuric acid was extracted with 1.5 mL ethyl acetate, and 1 mL of the organic layer was evaporated to dryness. The residue was dissolved in 1 mL distilled water, and absorbance was measured at 228 nm. The IC_{50} values were calculated from the concentration-inhibition curve using non-linear regression analysis.

Antioxidant Assay (DPPH): The free radical scavenging activity was measured using the DPPH method. Various concentrations of test compounds (100 µL) were mixed with 100 µL of DPPH solution (0.2 mM in methanol). The mixture was shaken vigorously and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a microplate reader. Ascorbic acid was used as a standard reference. The percentage of DPPH scavenging activity was calculated using the formula: % Scavenging = [(Acontrol - Asample)/Acontrol] × 100 where Acontrol is the absorbance of the DPPH solution without test compound, and Asample is the absorbance in the presence of test compound.

4.2.2. In Vivo Studies Animals: Male Wistar rats (200-250 g) were obtained from the institutional animal house. Animals were housed in polypropylene cages under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 55 ± 5%, 12 h light/dark cycle)

with free access to standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/PH/2023/45) and conducted in accordance with CPCSEA guidelines.

Hypertension Model: Hypertension was induced by administering L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride) at a concentration of 40 mg/L in drinking water for 4 weeks. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured weekly using non-invasive tail-cuff plethysmography (CODA System, Kent Scientific). Rats showing sustained hypertension (SBP > 150 mmHg) after 4 weeks were selected for the study.

Study Protocol: Hypertensive rats were randomly divided into groups (n=6): Group I served as hypertensive control (received 1% CMC), Group II as standard (received Captopril 50 mg/kg), and Groups III-VII received test compounds (25 mg/kg). All treatments were administered orally as a single dose suspended in 1% carboxymethyl cellulose (CMC). Blood pressure and heart rate were measured at 0, 1, 2, 4, 6, and 24 hours post-administration using tail-cuff plethysmography.

4.2.3. In Silico Docking Molecular docking studies were performed using AutoDock Vina 1.1.2. The three-dimensional structure of human angiotensin-converting enzyme (PDB ID: 1O86) was obtained from the RCSB Protein Data Bank. The protein structure was prepared by removing water molecules, adding polar hydrogens, and assigning Kollman united atom charges. The grid box was set to dimensions 60 × 60 × 60 Å with a grid spacing of 0.375 Å, centered on the active site. Docking parameters were set to default, and the exhaustiveness was set to 8. The best pose was selected based on binding affinity and cluster analysis. The docking protocol was validated by re-docking the co-crystallized ligand, showing a root-mean-square deviation (RMSD) of less than 2.0 Å.

4.3. Statistical Analysis

All data are expressed as mean ± SEM (n=6). Statistical analysis was performed using GraphPad Prism version 9.0. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant. The IC_{50} values were calculated using non-linear regression analysis.

5. Conclusion

In conclusion, this research successfully demonstrates the discovery of a novel series of quinoline-pyrazole hybrid analogues as promising multi-functional cardiovascular agents. Through a rational drug design approach incorporating molecular hybridization strategy, we have

developed compounds that simultaneously target multiple pathological pathways in cardiovascular diseases, including the renin-angiotensin system and oxidative stress.

The lead compound **C9** emerged as a particularly promising candidate, exhibiting exceptional dual pharmacological profile with potent ACE inhibitory activity (IC_{50} = 0.85 μM) and significant antioxidant capacity (52.3% DPPH scavenging at 100 μM). Most importantly, **C9** demonstrated remarkable *in vivo* efficacy in an L-NAME-induced hypertensive rat model, producing a substantial reduction in systolic blood pressure (-34.5 ± 1.8 mmHg) at a relatively low dose of 25 mg/kg, with effects comparable to the standard drug Captopril at double the dose. The molecular docking studies provided a rational structural basis for its activity, showing optimal binding interactions with the ACE active site.

The comprehensive structure-activity relationship (SAR) analysis has provided valuable insights into the structural requirements for optimal multi-target activity, revealing that:

- The free carboxylic acid group is essential for ACE inhibition
- Electron-withdrawing substituents enhance ACE inhibitory potency
- Hydroxyl groups contribute significantly to antioxidant activity
- Balanced lipophilicity improves *in vivo* efficacy

These findings provide a clear roadmap for future structural optimization and development of this promising class of cardiovascular agents.

Future Work: Based on these encouraging results, future investigations will focus on:

1. **Detailed mechanistic studies** to explore interactions with additional cardiovascular targets including ion channels, beta-adrenergic receptors, and other regulatory enzymes.
2. **Comprehensive ADMET profiling** including pharmacokinetic studies, metabolic stability assessment, and preliminary toxicity evaluation.
3. **Chronic *in vivo* studies** to evaluate long-term efficacy, safety, and organ protection effects in established hypertension models.
4. **Further structural optimization** focusing on improving selectivity, pharmacokinetic properties, and reducing potential off-target effects through rational modification of the quinoline-pyrazole scaffold.

The identified lead compound **C9** represents a strong starting point for the development of novel multi-target cardiovascular therapeutics with potential advantages over current single-target medications.

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