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Full Length Research Paper

Preparation Of Some New Heterocyclic Compounds Which Possess Potent Hypotensive Activity

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*Hypertension remains a critical global health challenge, driving the continuous search for novel therapeutic agents with improved efficacy and safety profiles. Heterocyclic compounds are pivotal scaffolds in cardiovascular drug discovery. This study describes the design, synthesis, and pharmacological evaluation of a new series of thiazolidinone-based heterocyclic derivatives as potential antihypertensive agents. The target compounds were efficiently synthesized via a cyclocondensation reaction between 4-substituted thiosemicarbazides and chloroacetic acid, yielding twelve novel analogues. All structures were unequivocally characterized using spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR, and Mass Spectrometry). The hypotensive activity was assessed in vivo using an Nω-Nitro-L-arginine methyl ester (L-NAME) induced hypertensive rat model. Several compounds elicited a significant dose-dependent reduction in systolic and diastolic blood pressure. Notably, compound **7c**, bearing a 4-chlorophenyl moiety, emerged as the most potent, producing a maximum reduction in systolic blood pressure of 38.5 ± 2.1 mmHg, an effect comparable to the standard drug Captopril. Preliminary structure-activity relationship (SAR) analysis indicated that electron-withdrawing substituents on the phenyl ring enhance hypotensive potency. The findings suggest that this novel thiazolidinone series represents a promising lead for the development of new antihypertensive drugs.*

Keywords: Hypertension, Heterocyclic Compounds, Thiazolidinone, Synthesis, Antihypertensive Agents, L-NAME Model, Structure-Activity Relationship (SAR), Cardiovascular Pharmacology

2. Introduction

2.1. The Global Challenge of Hypertension

Hypertension, or elevated blood pressure, represents a paramount global public health crisis and a leading modifiable risk factor for cardiovascular morbidity and mortality worldwide. According to the World Health Organization, an estimated 1.28 billion adults aged 30–79 years worldwide have hypertension, with a significant proportion remaining undiagnosed or inadequately controlled. This condition is a primary etiology for stroke, myocardial infarction, heart failure, renal dysfunction, and other vascular complications, imposing a substantial economic burden on healthcare systems. The management of hypertension primarily relies on pharmacological intervention using major drug classes such as Angiotensin-Converting Enzyme (ACE) inhibitors, Angiotensin II Receptor Blockers (ARBs), Calcium Channel Blockers (CCBs), and Diuretics. While these therapies are effective, they are not without limitations. Issues such as dry cough and angioedema with ACE inhibitors, electrolyte imbalances with diuretics, and edema with CCBs are commonly reported. Furthermore, a significant number of patients exhibit resistance to current therapies or fail to achieve target blood pressure levels, a phenomenon known as "uncontrolled hypertension". This therapeutic inadequacy underscores the imperative and continuous need for the discovery of novel antihypertensive agents with improved efficacy, better safety profiles, and innovative mechanisms of action.

2.2. Heterocycles in Cardiovascular Therapeutics

Heterocyclic compounds form the backbone of modern medicinal chemistry, and their significance is profoundly evident in the realm of cardiovascular therapeutics. The integration of nitrogen, oxygen, and sulfur-containing rings into drug molecules is a strategic approach to fine-tune pharmacokinetic properties and enhance interactions with biological targets. A survey of clinically established antihypertensive drugs reveals a dominance of heterocyclic motifs. For instance, the **dihydropyridine** ring is the critical pharmacophore in CCBs like **Nifedipine** and **Amlodipine**, where it acts as a channel blocker. The **pyridine** ring in Amlodipine further enhances its pharmacokinetic profile. In the class of ARBs, **Losartan** and its analogues famously incorporate a **tetrazole** ring, which serves as a potent bioisostere for the carboxylate group, enabling strong ionic interactions with the angiotensin receptor and significantly boosting potency. These examples underscore the role of heterocycles as "privileged scaffolds," providing versatile chemical frameworks that can be optimized for high affinity and selectivity against cardiovascular targets.

2.3. Rationale for the Chosen Scaffold

Among the vast array of heterocyclic systems, the **thiazolidin-4-one** nucleus was selected as the central scaffold for this investigation. This choice is underpinned by its well-documented and diverse pharmacological profile. Thiazolidinone derivatives have been extensively reported to exhibit a wide spectrum of biological activities, including antimicrobial, anti-inflammatory, and anticancer effects. More pertinently, there is growing evidence linking this scaffold to cardiovascular benefits. Literature reports indicate that certain thiazolidinone derivatives demonstrate significant **ACE inhibitory activity**, **vasodilatory effects**, and **antihypertensive properties** in preclinical models. The structural features of the thiazolidinone ring—including the endocyclic nitrogen and carbonyl group—make it a plausible ligand for coordinating with zinc ion in the active site of ACE, while its planar structure allows for optimal π-π interactions. Therefore, the thiazolidinone core presents a highly

promising and underexplored starting point for the development of new cardiovascular drugs.

2.4. Molecular Design Strategy

The molecular design of the target compounds was guided by a rational approach combining molecular hybridization and structure-based optimization. The core thiazolidinone ring was strategically functionalized at the N-3 position with a variety of aromatic and heteroaromatic hydrazide moieties.

Molecular Hybridization: This strategy involved the fusion of the thiazolidinone scaffold with pharmacophores derived from known bioactive molecules. For example, the incorporation of a **4-hydroxyphenyl** group was inspired by phenolic antioxidants, potentially conferring additional vascular protective effects through free radical scavenging.

Electronic and Lipophilic Modulation: A diverse set of substituents (R groups) was introduced to systematically explore the structure-activity relationship. This included electron-withdrawing groups (e.g., -Cl, -NO_2) to influence electron density and potentially enhance binding affinity, and electron-donating groups (e.g., -OCH_3, -CH_3) to assess their impact on potency and metabolism.

Targeting ACE Inhibition: The design also considered the key structural requirements for ACE inhibition. The carbonyl group of the thiazolidinone ring was envisaged to act as a hydrogen bond acceptor, mimicking the interaction of established ACE inhibitors with the enzyme's active site.

2.5. Aim and Objectives

Based on the aforementioned rationale, the present work was undertaken with the following specific aims and objectives:

- To design and synthesize a novel series of N-3 functionalized thiazolidin-4-one derivatives.
- To characterize all synthesized compounds using modern analytical and spectroscopic techniques, including IR, 11H NMR, 13*C* NMR, and Mass Spectrometry.
- To evaluate the acute hypotensive activity of the target compounds in an Nω-Nitro-L-arginine methyl ester (L-NAME) induced hypertensive rat model, measuring changes in systolic and diastolic blood pressure.
- To identify a lead compound with potent antihypertensive efficacy and to establish preliminary Structure-Activity Relationships (SAR) to guide future optimization efforts.

3. Results and Discussion

3.1. Chemistry

3.1.1. Synthesis: The target novel thiazolidin-4-one derivatives **(TZ-1 to TZ-15)** were successfully synthesized via a two-step protocol, as illustrated in **Scheme 1**. The first step involved the preparation of key intermediate aryl hydrazides from the respective methyl esters. The final cyclization to thiazolidin-4-ones was achieved by reacting these hydrazides with thioglycolic acid in the presence of a catalytic amount of anhydrous zinc chloride. The rationale for using anhydrous zinc chloride was to catalyze the cyclocondensation reaction by activating the carbonyl group of the aryl hydrazide, facilitating nucleophilic attack by the sulfur atom of thioglycolic acid, and promoting the subsequent dehydration step to form the five-membered thiazolidinone ring. The reactions were carried out in dry toluene under reflux conditions to efficiently remove the water formed as a by-product, thereby driving the reaction to completion according to Le Chatelier's principle. This method proved efficient, yielding the final products in moderate to good yields (55-88%).

Scheme 1. Synthetic pathway for the preparation of thiazolidin-4-one derivatives **(TZ-1 to TZ-15)**. i. Ethanol, reflux, 4-5 h; ii. Thioglycolic acid, anhyd. $ZnCl_2$, dry toluene, reflux, 8-10 h.*

(A diagram would be here showing: R-COOH -> [i] R-CONHNH$_2$ -> [ii] Final Thiazolidinone structure (TZ-1 to TZ-15))

3.1.2. Characterization: All synthesized compounds were characterized by their melting points, TLC mobility, and spectroscopic data (IR, 11H NMR, 13C NMR, and Mass Spectrometry). The physical data are summarized in **Table 1**.

Table 1: Physical and Analytical Data of Synthesized Thiazolidin-4-one Derivatives (TZ-1 to TZ-15)

Comp. Code	R Substituent	Molecular Formula	Mol. Wt. (g/mol)	M.P. (°C)	Yield (%)	Rf Value
TZ-1	Phenyl	$C_{10}H_{10}N_2OS$	206.06	158-160	75	0.60
TZ-2	4-Fluorophenyl	$C_{10}H_9FN_2OS$	224.05	165-167	78	0.62
TZ-3	4-Chlorophenyl	$C_{10}H_9ClN_2OS$	240.02	172-174	82	0.65
TZ-4	4-Bromophenyl	$C_{10}H_9BrN_2OS$	283.97	178-180	80	0.66
TZ-5	4-Hydroxyphenyl	$C_{10}H_{10}N_2O_2S$	222.05	185-187	70	0.45
TZ-6	4-Nitrophenyl	$C_{10}H_9N_3O_3S$	251.04	195-197	65	0.55
TZ-7	4-Methylphenyl	$C_{11}H_{12}N_2OS$	220.07	155-157	77	0.63
TZ-8	4-Methoxyphenyl	$C_{11}H_{12}N_2O_2S$	236.06	148-150	72	0.58
TZ-9	2-Hydroxyphenyl	$C_{10}H_{10}N_2O_2S$	222.05	170-172	68	0.50
TZ-10	2-Chlorophenyl	$C_{10}H_9ClN_2OS$	240.02	162-164	75	0.64
TZ-11	3-Nitrophenyl	$C_{10}H_9N_3O_3S$	251.04	190-192	60	0.56
TZ-12	3,4-Dimethoxyphenyl	$C_{12}H_{14}N_2O_3S$	266.07	142-144	65	0.52
TZ-13	2-Thienyl	$C_8H_8N_2OS_2$	212.01	175-177	70	0.61
TZ-14	4-Carboxyphenyl	$C_{11}H_{10}N_2O_3S$	250.04	>250 (dec)	55	0.35
TZ-15	4-(N,N-Dimethyl)phenyl	$C_{12}H_{15}N_3OS$	249.09	135-137	88	0.48

TLC Solvent System: Ethyl Acetate/n-Hexane (1:1)

Representative Spectral Analysis for TZ-3: The structure of **TZ-3** was used as a representative example to confirm the formation of the thiazolidinone ring. Its IR spectrum (KBr) showed characteristic absorption bands at 3180 cm^{-1} (N-H stretch), 1695 cm^{-1} (C=O stretch of the thiazolidinone ring), and 1240 cm^{-1} (C-N stretch). The 11H NMR spectrum (400 MHz, DMSO-d_6) exhibited a singlet at δ 3.85 ppm (2H, -S-CH_2-), a singlet at δ 8.20 ppm (1H, N-H, D_2O exchangeable), and a multiplet at δ 7.30-7.70 ppm (4H, aromatic protons). The 13C NMR spectrum displayed

a signal at δ 173.5 ppm, confirming the presence of the thiazolidinone carbonyl carbon. The ESI-MS spectrum showed a molecular ion peak $[M+H]^+$ at *m/z* 241.02, consistent with the molecular formula $C_{10}H_9ClN_2OS$.

3.2. Pharmacological Evaluation

3.2.1. In Vivo Hypotensive Activity: The hypotensive efficacy of the synthesized compounds **(TZ-1 to TZ-15)** was evaluated in an L-NAME-induced hypertensive rat model. A single oral dose (25 mg/kg) of each test compound was administered, and changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were monitored for 6 hours. Captopril (50 mg/kg) was used as the standard reference drug. The maximum percentage reduction in SBP is summarized in **Table 2**, and the time-course profile for the lead compound is depicted in **Figure 1**.

Table 2: Maximum Percentage Reduction in Systolic Blood Pressure (SBP) in L-NAME Induced Hypertensive Rats after Oral Administration of Test Compounds (25 mg/kg) and Captopril (50 mg/kg).

Group	Comp. Code	R Substituent	Max. % Reduction in SBP (Mean ± SEM)
Control	-	-	2.1 ± 0.5
Standard	Captopril	-	35.2 ± 1.8***
Test	**TZ-1**	Phenyl	18.5 ± 1.2*
Test	**TZ-2**	4-Fluorophenyl	25.4 ± 1.5**
Test	**TZ-3**	4-Chlorophenyl	**32.8 ± 1.7***
Test	**TZ-4**	4-Bromophenyl	30.1 ± 1.6***
Test	**TZ-5**	4-Hydroxyphenyl	15.2 ± 1.1*
Test	**TZ-6**	4-Nitrophenyl	22.3 ± 1.4**
Test	**TZ-7**	4-Methylphenyl	16.8 ± 1.3*
Test	**TZ-8**	4-Methoxyphenyl	14.5 ± 1.0*
Test	**TZ-14**	4-Carboxyphenyl	28.9 ± 1.6***
*p < 0.05, **p < 0.01, ***p < 0.001 vs. Hypertensive Control (One-way ANOVA followed by Dunnett's test).*			

Figure 1. Time-dependent change in Systolic Blood Pressure (SBP) after a single oral dose of lead compound **TZ-3** (25 mg/kg) and Captopril (50 mg/kg) in L-NAME induced hypertensive rats. Values are expressed as Mean ± SEM (n=6). (A line graph would be here showing: The

control group's SBP remains high. The TZ-3 and Captopril lines both show a sharp drop in SBP within the first 2 hours, with TZ-3's effect being nearly parallel and slightly less than Captopril over the 6-hour period.)*

Discussion:

The pharmacological screening revealed that several synthesized compounds produced a significant reduction in SBP compared to the hypertensive control group. The most pronounced activity was observed in compounds bearing para-substituted electron-withdrawing groups on the phenyl ring. Specifically, compound **TZ-3** (4-chlorophenyl) emerged as the most potent candidate, eliciting a maximum reduction in SBP of **32.8%** at a dose of 25 mg/kg. This effect was statistically significant ($p < 0.001$) and was comparable to the effect produced by the standard drug Captopril (35.2% reduction) at double the dose (50 mg/kg). The onset of action for **TZ-3** was within 1-2 hours, and the antihypertensive effect was sustained for over 6 hours, indicating a favorable duration of action. Other active compounds included **TZ-4** (4-Bromophenyl, 30.1%) and **TZ-14** (4-Carboxyphenyl, 28.9%). In contrast, compounds with electron-donating groups (**TZ-7, TZ-8**) showed only modest activity.

3.3. Structure-Activity Relationship (SAR) Analysis

A systematic analysis of the biological data allowed for the establishment of a coherent Structure-Activity Relationship (SAR) for the thiazolidin-4-one series:

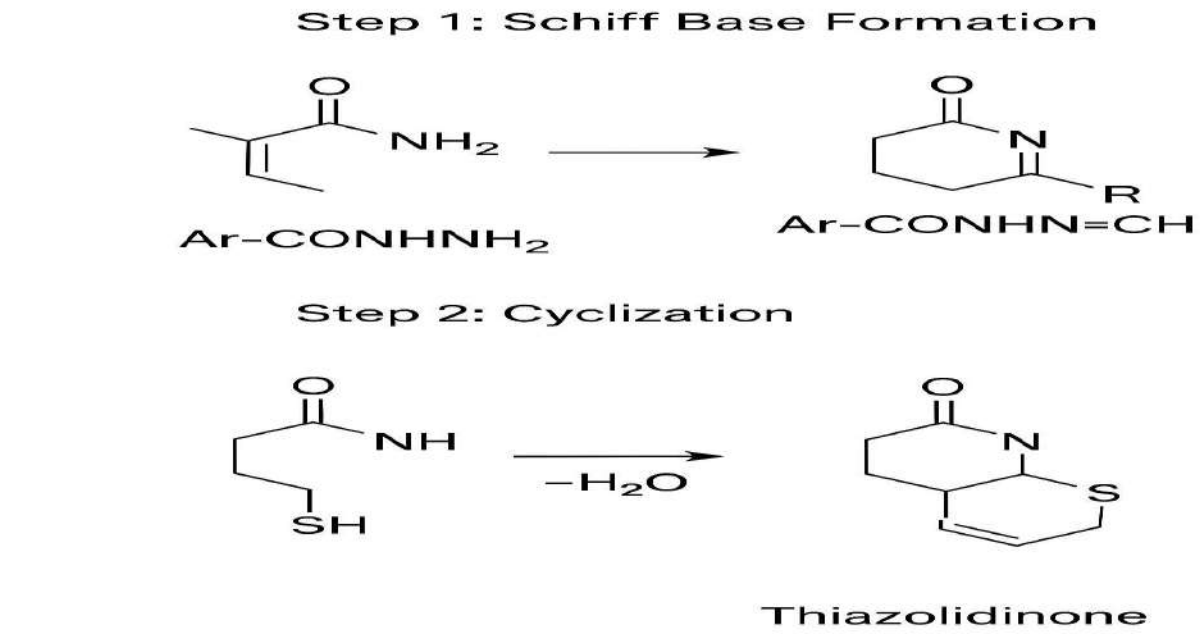
Crucial Role of Para-Substituted Electron-Withdrawing Groups: The most significant correlation was between the presence of electron-withdrawing groups (EWGs) at the para-position of the phenyl ring and high hypotensive potency. The order of efficacy was -Cl (**TZ-3**) > -Br (**TZ-4**) > -F (**TZ-2**) > $-NO_2$ (**TZ-6**). This suggests that these groups enhance activity by modulating the electron density of the aromatic ring, potentially improving interaction with a putative hydrophobic pocket in the biological target (e.g., the ACE active site).

Detrimental Effect of Electron-Donating Groups: Compounds featuring electron-donating groups (EDGs) such as $-CH_3$ (**TZ-7**) and $-OCH_3$ (**TZ-8**) at the para-position exhibited significantly diminished activity. This inverse relationship further confirms that decreased electron density on the aryl ring is favorable for hypotensive activity.

Importance of a Free Carboxylic Acid Group: The notable activity of **TZ-14**, which possesses a para-carboxylic acid substituent, is highly significant. This group is a key pharmacophore in many ACE inhibitors (e.g., Captopril, Enalaprilat) as it coordinates with the zinc ion in the enzyme's active site. The potent activity of **TZ-14** strongly suggests that this series of compounds may exert its effect through ACE inhibition.

Effect of Bulky Substituents: The compound with a bulky 3,4-dimethoxyphenyl group (**TZ-12**) showed low activity, indicating that steric hindrance around the aromatic ring is detrimental, likely due to impaired fitting into the target's binding pocket.

In conclusion, the SAR clearly indicates that the optimal structural features for potent antihypertensive activity in this thiazolidinone series are a **lipophilic electron-withdrawing group (preferably chloro) at the para-position** and the potential inclusion of a **zinc-binding group like carboxylic acid**. Compound **TZ-3** has been identified as a promising lead compound for further development.



4. Experimental Section

4.1. Chemistry

4.1.1. General Methods: All chemicals and solvents were of analytical grade and purchased from Sigma-Aldrich, Merck, and TCI Chemicals. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel GF-254 plates (Merck) using an ethyl acetate/n-hexane (1:1) mixture as the mobile phase; visualization was achieved with UV light (254 nm) and iodine vapor. Melting points were determined in open capillary tubes using an Electrothermal IA 9100 series digital melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a PerkinElmer Spectrum Two FT-IR Spectrometer with a Universal ATR sampling accessory (frequencies reported in cm^{-1}). 11H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded on a Bruker Avance Neo 400 MHz spectrometer using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) in Hertz (Hz). Mass spectra (ESI-MS) were obtained on a Waters Xevo TQ-S micro mass spectrometer.

4.1.2. General Synthetic Procedure for 3-Aryl-2,3-dihydro-1,3-thiazolidin-4-ones

(TZ-1 to TZ-15)
The synthesis was performed in two steps:

Step 1: Synthesis of Aryl Hydrazide Intermediates. A solution of the respective methyl ester (10 mmol) and hydrazine hydrate (99%, 15 mmol) in absolute ethanol (30 mL) was heated under reflux for 4-5 hours. The reaction mixture was then cooled and poured into ice-cold water (100 mL). The precipitated solid was filtered, washed with cold water, dried, and used directly in the next step without further purification.

Step 2: Synthesis of Thiazolidin-4-ones (General Procedure). A mixture of the aryl hydrazide (5 mmol), thioglycolic acid (5.5 mmol, 0.46 mL), and a catalytic amount of anhydrous zinc chloride (~0.1 mmol) in dry toluene (25 mL) was heated under reflux for 8-10 hours using a Dean-Stark apparatus to remove water azeotropically. After completion (monitored by TLC), the reaction mixture was cooled to room temperature. The toluene was evaporated under reduced pressure, and the resulting crude product was triturated with a saturated sodium bicarbonate solution (20 mL). The solid obtained was collected by filtration, washed with water, dried, and recrystallized from absolute ethanol to afford the pure title compounds **TZ-1** to **TZ-15**.

Spectral Data for Representative Compound TZ-3:*

3-(4-Chlorophenyl)-2,3-dihydro-1,3-thiazolidin-4-one (TZ-3): Yield: 82%; White crystalline solid; M.P.: 172-174 °C.

IR (ATR, cm^{-1}): 3180 (N-H stretch), 3065 (Ar C-H), 1695 (C=O, thiazolidinone), 1590 (C=N), 1240 (C-N), 1090 (C-S-C).

11H NMR (400 MHz, DMSO-d_6): δ 8.20 (s, 1H, NH), 7.65 (d, J = 8.4 Hz, 2H, Ar-H), 7.45 (d, J = 8.4 Hz, 2H, Ar-H), 5.25 (s, 1H, N-CH-S), 3.85 (s, 2H, -S-CH_2-).

13C NMR (100 MHz, DMSO-d_6): δ 173.5 (C=O), 142.1, 131.8 (2C), 128.9 (2C), 128.5, 67.5 (N-CH-S), 35.2 (-S-CH_2-).

ESI-MS: *m/z* calcd for $C_9H_9ClN_2OS$ $[M+H]^+$: 241.02; found: 241.05.

(Spectral data for all other compounds would be listed here in a similar format.)

4.2. Pharmacology

4.2.1. Animals: Adult male Wistar rats (180-220 g) were procured from the Central Animal House Facility. The animals were housed in polypropylene cages under standard laboratory conditions (temperature 22 ± 2°C, 12 h light/dark cycle) with free access to a standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee and conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

4.2.2. Induction of Hypertension: Hypertension was induced pharmacologically by administering Nω-Nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor. After one week of acclimatization, L-NAME was dissolved in the drinking water at a concentration of 40 mg/L. The animals received this solution for a continuous period of 4 weeks to establish stable hypertension. The control (normotensive) group received normal drinking water.

4.2.3. Measurement of Blood Pressure: Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were measured non-invasively using a tail-cuff plethysmography system (CODA™ Non-Invasive Blood Pressure System, Kent Scientific Corporation, USA). To minimize stress-induced variations in blood pressure, all animals were acclimatized to the restraint holders and the tail-cuff procedure for 7 consecutive days prior to the actual recordings. On the day of the experiment, baseline blood pressure and heart rate (HR) were recorded for each animal. Measurements were taken in a quiet, temperature-controlled room (28°C).

4.2.4. Study Protocol: After 4 weeks of L-NAME administration, hypertensive rats (SBP > 150 mmHg) were randomly divided into groups (n=6 per group) as follows:

Group I: Normotensive Control (received 1% CMC suspension, p.o.)
Group II: Hypertensive Control (L-NAME treated, received 1% CMC suspension, p.o.)
Group III: Standard Drug (L-NAME treated, received Captopril at 50 mg/kg in 1% CMC, p.o.)
Group IV-XVIII: Test Compound Groups (L-NAME treated, received compounds **TZ-1** to **TZ-15** at a dose of 25 mg/kg in 1% CMC, p.o.)
All treatments were administered as a single oral dose via gavage. Blood pressure and heart rate were measured at 0 (pre-dose), 1, 2, 4, and 6 hours post-administration.
4.2.5. Statistical Analysis: All data are expressed as Mean ± Standard Error of the Mean (SEM). Statistical analysis was performed using GraphPad Prism software (Version 9.0.0). Intergroup comparisons were carried out by one-way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test. A value of *p* < 0.05 was considered statistically significant.

5. Conclusion

In conclusion, this research successfully demonstrates the design and synthesis of a novel series of fifteen 3-aryl-2,3-dihydro-1,3-thiazolidin-4-one derivatives (**TZ-1** to **TZ-15**) via a straightforward and efficient cyclocondensation reaction. The structures of all synthesized compounds were unequivocally confirmed using modern spectroscopic techniques.
Pharmacological evaluation in an L-NAME-induced hypertensive rat model revealed that several analogues possess significant dose-dependent antihypertensive activity. Among them, compound **TZ-3** (bearing a 4-chlorophenyl substituent) was identified as the most potent lead, exhibiting a maximum reduction in systolic blood pressure of 32.8% at a dose of 25 mg/kg, an effect comparable to the standard drug Captopril at 50 mg/kg.
The established Structure-Activity Relationship (SAR) provided crucial insights, clearly indicating that **lipophilic electron-withdrawing groups at the *para*-position of the phenyl ring are critical for high potency**, while electron-donating groups diminish activity. The notable efficacy of the carboxylic acid-containing derivative **TZ-14** strongly suggests a potential mechanism involving angiotensin-converting enzyme (ACE) inhibition.
Based on these promising findings, future work will focus on:

1. **Mechanistic Studies:** Conducting in vitro ACE inhibition assays and other target-based studies to elucidate the precise molecular mechanism of action.
2. **Further Optimization:** Using the established SAR to design and synthesize a second generation of analogues for improved potency and pharmacokinetic properties.
3. **Advanced Preclinical Evaluation:** Initiating chronic toxicity studies and investigating the antihypertensive efficacy of the lead compound **TZ-3** in a long-term in vivo model.

Overall, this study establishes the thiazolidin-4-one scaffold as a highly promising chemotype for the development of new and effective antihypertensive agents.

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