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RESEARCH ARTICLE

# From Channels to Circulation: Mechanistic Insights into the Calcium-Regulatory Actions of A-52

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**Abstract**

Hypertension is closely associated with disturbances in calcium ion ($Ca^{2+}$) homeostasis in vascular and cardiac tissues. In this study, the antihypertensive activity of compound A-52 was explored using combined *in silico* and *in vivo* approaches. Molecular docking analysis was conducted to assess the interaction of A-52 with selected $Ca^{2+}$-related targets, including L-type and R-type calcium channels, SERCA ($Ca^{2+}$-ATPase), RyR2 (ryanodine receptor), the $Na^{+}/Ca^{2+}$ exchanger (NCX), and renin. The docking results revealed moderate binding affinities (–5.2 to –6.1 kcal/mol), which are commonly observed in preliminary docking studies, with interactions mediated by hydrogen bonding, π–π stacking, and hydrophobic contacts with key amino acid residues. These *in silico* findings, together with vascular reactivity data, suggest a possible involvement of calcium-dependent mechanisms, although no direct electrophysiological or $Ca^{2+}$ flux measurements were performed. The *in vivo* antihypertensive effect of A-52 was evaluated in an adrenaline-induced acute hypertension model in Wistar rats. Intravenous administration of A-52 at a dose of 25 mg/kg resulted in a significant reduction of systolic and diastolic blood pressure within two hours, without evidence of severe hypotension. In contrast, a higher dose (50 mg/kg) induced a transient and unstable blood pressure response, indicating dose sensitivity under the applied conditions. Comparative evaluation with structurally related compounds (A-51 and A-54) showed that A-52 produced a more consistent antihypertensive response in this acute model. Overall, the results provide preliminary evidence that A-52 may influence pathways associated with $Ca^{2+}$ handling and blood pressure regulation. Further studies involving chronic models, larger sample sizes, pharmacokinetic profiling, toxicity assessment, and direct functional assays are required to clarify its mechanism of action and therapeutic relevance.

**Keywords:** A-52, Calcium signaling, Antihypertensive activity, L-type $Ca^{2+}$ channel, SERCA, RyR2, NCX, Renin–angiotensin pathway, *In silico* docking, *In vivo* study

## Introduction

Hypertension remains one of the most prevalent cardiovascular disorders worldwide and a leading cause of morbidity and mortality associated with stroke, myocardial infarction, and renal dysfunction [1,2]. Despite the availability of multiple classes of antihypertensive drugs—including calcium channel blockers, β-blockers, ACE inhibitors, and diuretics—a considerable proportion of patients fail to achieve optimal blood pressure control or experience dose-limiting adverse effects. This clinical challenge underscores the need for new pharmacological strategies that provide effective blood pressure reduction while

minimizing side effects, particularly in complex or stress-induced hypertensive conditions [3,4].

Calcium ions ($Ca^{2+}$) play a central role in the regulation of vascular smooth muscle contraction, cardiac excitability, and endothelial function. Intracellular $Ca^{2+}$ homeostasis is maintained through the coordinated activity of multiple transport systems, including L-type and R-type voltage-gated calcium channels, sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA), ryanodine receptor (RyR2), and the $Na^{+}/Ca^{2+}$ exchanger (NCX). Dysregulation of these pathways leads to excessive $Ca^{2+}$ influx, impaired relaxation, and sustained vasoconstriction, thereby contributing to the pathogenesis and progression of hypertension [5,6]. In parallel, activation of the renin–angiotensin system (RAS) amplifies vascular resistance by enhancing $Ca^{2+}$-dependent signaling in both endothelial and smooth muscle cells, further complicating blood pressure control [7,8].

Although several established antihypertensive drug classes already influence $Ca^{2+}$-dependent mechanisms either directly or indirectly, most clinically used calcium channel blockers predominantly target L-type $Ca^{2+}$ channels. Such a relatively narrow mechanism of action is often associated with reflex tachycardia, peripheral edema, or limited efficacy in certain patient populations. Consequently, there is growing interest in compounds capable of modulating multiple $Ca^{2+}$-associated processes in a more balanced manner, without inducing excessive $Ca^{2+}$ suppression or hemodynamic instability.

Recent advances in *in silico* modeling and molecular docking techniques have facilitated the identification of compounds with potential affinity toward multiple $Ca^{2+}$-regulating proteins. When combined with *in vivo* pharmacological validation, these approaches offer an efficient framework for exploratory screening of bioactive molecules with complex mechanisms of action. Within this context, compound A-52, a newly synthesized molecule of plant-derived origin, was selected for investigation based on its chemical features and preliminary pharmacological observations. Rather than positioning A-52 as a classical calcium channel blocker, the present study explores its potential to influence $Ca^{2+}$-related pathways through interactions with several key transport systems [9,10].

Accordingly, the present work employed an integrated *in silico* and *in vivo* approach to characterize the antihypertensive activity of A-52. Molecular docking analysis was used to examine the interaction of A-52 with $Ca^{2+}$ L-type and R-type channels, SERCA, RyR2, NCX, and renin in order to generate hypotheses regarding its multi-target interaction profile. Subsequently, the *in vivo* antihypertensive effects of A-52 were evaluated in an adrenaline-induced acute hypertension model in Wistar rats, with particular attention to dose-dependent hemodynamic responses and duration of action [11,12]. By integrating computational predictions with experimental observations, this study aimed to provide preliminary insights into the mechanisms underlying the antihypertensive effects of A-52 and to identify an appropriate dose range for further preclinical investigation.

## Materials and methods

### Animal ethics

All preoperative and experimental procedures were carefully reviewed and approved by the Institutional Committee for Animal Use and Care. The animals were housed in a vivarium under standardized conditions, including a relative humidity of 55% - 65%, a controlled ambient temperature of 22 ± 2 °C, and unrestricted access to water and standard laboratory chow. All aspects of animal care and handling were conducted in full compliance with the European Directive 2010/63/EU, which governs the protection of animals used for scientific research. Ethical clearance for this study was granted by the Animal Ethics Committee of the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan (Protocol No. 133/1a/h, dated 4 August 2016).

### Chemical and pharmacological characterization of compound A-52

Compound A-52 is chemically identified as 3,4,5-trimethoxy-4-hydroxybenzoic acid methyl ester, with the molecular formula $C_{11}H_{14}O_5$ and a molecular weight of 226.23 g/mol. Its structure contains one phenolic hydroxyl group (–OH), two methoxy groups ($–OCH_3$), and one methyl ester group ($–COOCH_3$) conjugated with an aromatic ring. These functional groups endow

the molecule with strong electron-donating and antioxidant properties (**Figure 1**).

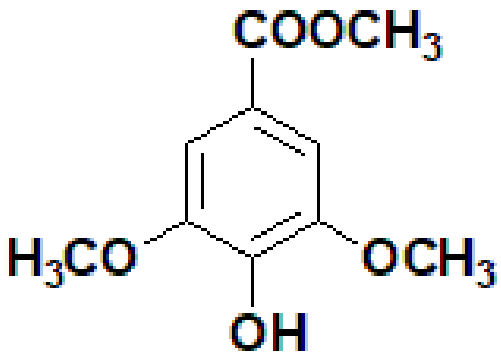



**Figure 1** Chemical structure of A-52.

A-52 belongs to the class of phenolic esters and exhibits moderate lipophilicity, which facilitates its interaction with biological membranes and protein active sites, particularly calcium transport channels. The methoxy substituents enhance the molecule's chemical stability, while the phenolic hydroxyl group contributes to free radical scavenging and reduction of oxidative stress.

**Data collection and software**

The study was conducted using publicly available datasets and open-source computational tools as described [13,14]. Three-dimensional crystal structures of key calcium and ion transport proteins were obtained from the Protein Data Bank (PDB): $Ca^{2+}$-ATPase (PDB ID: 6JJU, 1HNY), $Ca^{2+}$ R-type channel (PDB ID: To be specified), $Ca^{2+}$ L-type channel (PDB ID: 6JP5), $Na^{+}/Ca^{2+}$ exchanger (NCX) (PDB ID: 8SGI), Ryanodine receptor (PDB ID: 5C33), Angiotensin–renin complex (PDB ID: 2REN), and Sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) (PDB ID: 2ZOX). Pharmacological and physicochemical data for the selected ligands were retrieved from the PubChem database. Protein visualization and preliminary structural analyses were performed using PyMOL (version 1.2). Molecular docking was carried out with AutoDock 4.2 and AutoDock Tools (ADT), while visualization and binding analysis were performed using Discovery Studio Visualizer 4.5.

**Measurement of blood pressure in rats using the tail-cuff method**

Systolic blood pressure was measured using a non-invasive tail-cuff plethysmography system (Model *Sistola AcqKnowledge*, Neurobotics, Russia). All experiments were conducted under controlled ambient conditions (22 - 24 °C) to minimize stress-induced variability. Prior to measurements, rats were acclimatized to the restraining device for three consecutive days to reduce handling stress [15,16]. All substances were administered intravenously via the tail vein. During measurement, each rat was placed in an individual restrainer, and a pressure-sensitive cuff was attached to the tail. The cuff pressure was gradually increased until blood flow stopped, then slowly released. The systolic pressure was automatically detected when the blood pulse returned [17,18].

**Statistical analysis**

All values were expressed as mean ± standard error mean (SEM) calculated using the Origin 9.0 (OriginLab, USA). Differences between groups were assessed using the t-test (Two Populations) system. Differences were regarded as significant if *p-value* < 0.05 was adopted.

## Results and discussion

**Binding properties of compound A-52 with $Ca^{2+}$ L-Type Ion channels**

Before initiating *in vitro* and *in vivo* experiments, the biological activity of the test compounds was

evaluated *in silico* through molecular docking analyses. During this study, the binding affinity of compound A-52 toward $Ca^{2+}$ L-type ($Ca_v$) ion channels was examined, as these channels play a crucial role in the regulation of vascular tone and the pathogenesis of hypertension [19,20]. The activity of $Ca^{2+}$ L-type channels can be modulated by various compounds. Notably, 1,4-dihydropyridines (DHPs), benzothiazepines (BTZs), and phenylalkylamines (PAAs) are well-established blockers of these channels and are widely used in the treatment of hypertension and cardiovascular disorders. Previous studies have demonstrated that nifedipine, diltiazem, and verapamil inhibit $Ca^{2+}$ L-type channels through distinct mechanisms. Diltiazem and verapamil enter the central cavity of the channel and directly obstruct $Ca^{2+}$ flux, whereas nifedipine and Bay K 8644 occupy the fenestration region located at the interface of domains III and IV. However, Bay K 8644 exhibits lower stability in its active conformation compared with other ligands. Based on these structural insights, we investigated the binding energetics of compound A-52 with the $Ca^{2+}$ L-type ion channel.

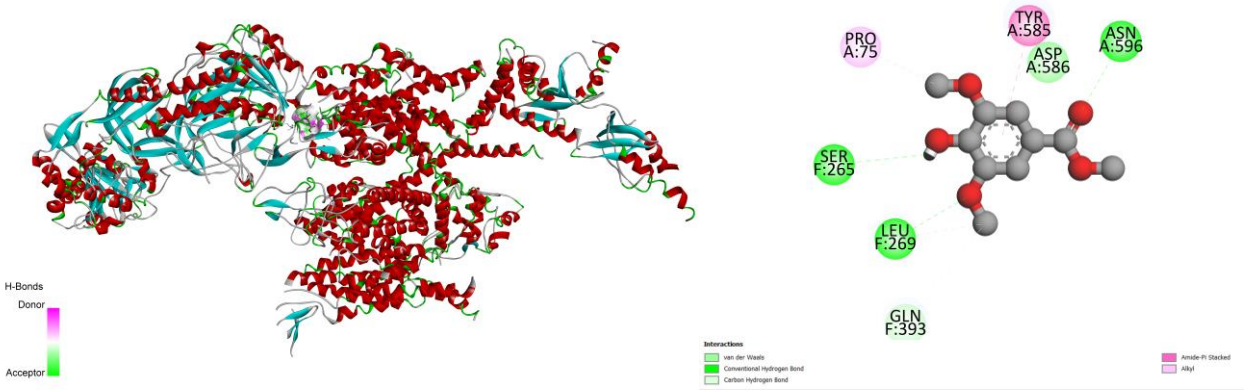



**Figure 2** Interaction of compound A-52 with $Ca^{2+}$ L-type calcium channels. (A) Overall structure of the protein and (B) detailed view of ligand interactions with amino acid residues.

Within the framework of this study, the interaction of compound A-52 with the $Ca^{2+}$ L-type channel was analyzed using *in silico* approaches. The coordinates of the protein's active site were identified using Discovery Studio, while the three-dimensional (3D) structure of the ligand was generated and optimized in Avogadro. Molecular docking was performed using AutoDock Vina, and the binding affinity of each ligand was calculated 23,24. The binding energy of compound A-52 was found to be –6.0 kcal/mol, indicating a stable interaction with the $Ca^{2+}$ L-type ion channel. The ligand established the following molecular interactions with amino-acid residues of the channel: SER F:265, LEU F:269, ASN A:596 – conventional hydrogen bonds; ASP A:586 – van der Waals interactions; GLN F:393 – carbon–hydrogen bonds; TYR A:585 – amide–π stacked interaction; PRO A:75 – alkyl interactions. The obtained results identified key amino acids that play a significant role in the blocking mechanism of $Ca^{2+}$ L-type channels. The analysis revealed that effective inhibition requires stable interactions between ligands and residues such as SER F:265, GLN F:393, LEU F:269, TYR A:1035, ASP A:586, and LEU F:269. The interactions of these residues with the ligand contribute to a deeper understanding of channel modulation and the molecular mechanism of blockade (**Figure 2**).

**Interaction of compound A-52 with $Ca^{2+}$ R-Type Ion channels**

Voltage-dependent $Ca^{2+}$ R-type ($Ca_v$) ion channels are known to be modulated by a variety of bioactive compounds, some of which are already employed in clinical practice for the treatment of hypertension and other cardiovascular disorders. Recent advances in cryo-electron microscopy (cryo-EM) have enabled the structural elucidation of $Ca_v1.1$ channels in complex with both agonists and antagonists, providing detailed insight into ligand-induced conformational changes. Within the present study, the interaction of compound A-52 with $Ca^{2+}$ R-type ion channels was analyzed using an *in silico* docking approach. The coordinates of the

channel's active site were identified in Discovery Studio, while the three-dimensional (3D) structures of the ligands were generated and optimized using Avogadro software. Molecular docking simulations were carried out in AutoDock Vina, and the binding affinities of the ligands were calculated. At the subsequent stage of analysis, the binding energetics of A-52 with the $Ca^{2+}$ R-type channels were examined in detail. The binding energy of compound A-52 with the $Ca^{2+}$ R-type ion channel was found to be –5.6 kcal/mol, indicating a stable interaction (**Figure 3**).

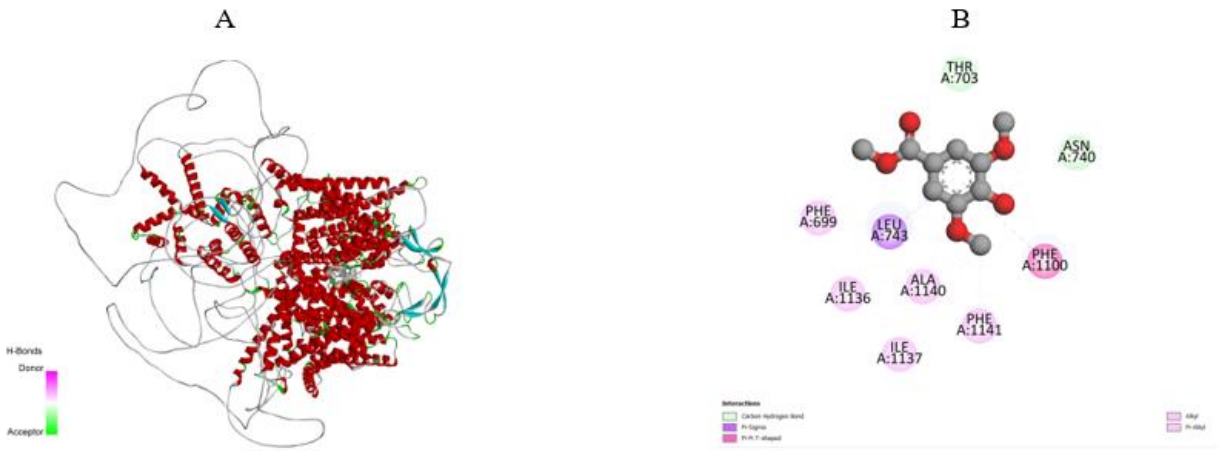



**Figure 3** Interaction of compound A-52 with $Ca^{2+}$ R-type calcium channels. (A) Overall structure of the protein and (B) detailed view of ligand interactions with amino acid residues.

The compound interacted with several amino acid residues as follows: ILE A:1136, ILE A:1137, ALA A:1140, and PHE A:1141 — π-alkyl and alkyl interactions; LEU A:743 — conventional hydrogen bond; PHE A:699 — π-sigma interaction. A subsequent docking run yielded a binding energy of –5.4 kcal/mol, further supporting the compound's strong affinity for the R-type channel. In this conformation, A-52 formed direct molecular interactions with multiple amino acid residues: THR A:703, ASN A:740 — carbon-hydrogen bonds; PHE A:699, ILE A:1136, ILE A:1137, PHE A:1141, ALA A:1140, and PHE A:1100 — π-stacking interactions. The obtained results allowed identification of the key amino acid residues involved in the binding process of A-52 to $Ca^{2+}$ R-type ion channels. Furthermore, the diverse interaction patterns observed between the ligand and channel residues provide a structural basis for understanding how A-52 may influence channel function and modulate $Ca^{2+}$ flux. These findings establish an important foundation for subsequent pharmacological investigations aimed at evaluating the therapeutic potential of A-52 as a modulator of $Ca^{2+}$-dependent cardiovascular processes.

**Binding energetics of compound A-52 with intracellular $Ca^{2+}$ transporters**

Following the analysis of binding to $Ca^{2+}$ L-type and R-type channels, we evaluated interactions with a key intracellular $Ca^{2+}$ transport mechanism—sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA)—which is central to maintaining cytosolic $Ca^{2+}$ homeostasis and thereby supports normal function of cardiac and smooth muscle tissues. Accordingly, the binding energetics of compound A-52 to SERCA and its residue-level contacts were examined in detail.

**A-52 binding to SERCA**

Docking to SERCA yielded a binding energy of –5.8 kcal/mol, indicating the formation of a reasonably stable protein–ligand complex. The interaction network comprised: Conventional hydrogen bonds: LEU A:41, ASN A:39, and PRO A:160, anchoring the ligand within the SERCA binding region. Hydrophobic contacts: π–alkyl/alkyl interactions with ILE A:232 and ALA A:142, consistent with stabilization in a lipophilic microenvironment. Carbon–hydrogen bond: A C–H·O/N type contact with GLU A:125, further supporting the pose. Donor–donor proximity: A potential donor–donor H-bond geometry involving SER A:210 was

noted; while geometrically suboptimal, such proximity can still contribute weakly to pose stabilization when supported by the surrounding hydrophobic contacts (**Figure 4**).

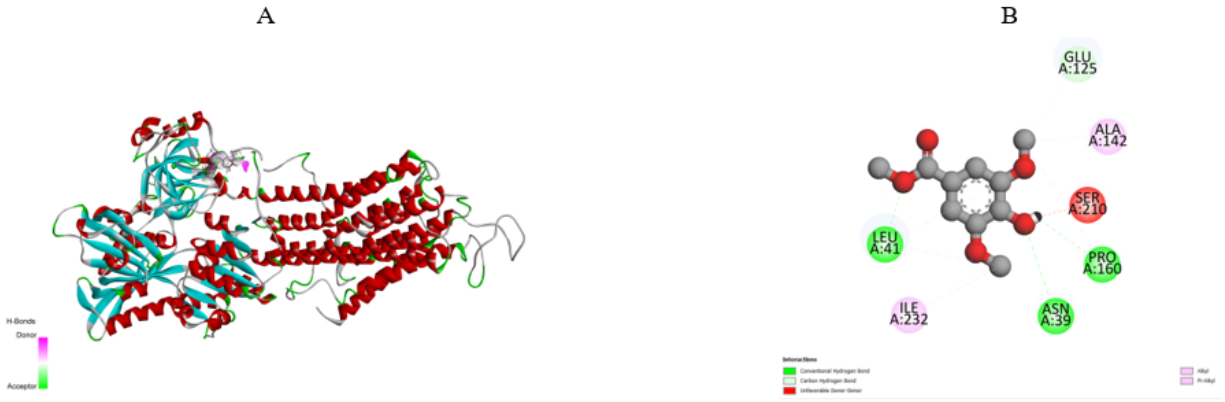



**Figure 4** Interaction of compound A-52 with SERCA $Ca^{2+}$ channels. (A) Overall structure of the protein and (B) detailed view of ligand interactions with amino acid residues.

**Binding energetics of compound A-52 with the RyR2 receptor**

In addition to the sarcoplasmic reticulum $Ca^{2+}$-ATPase (SERCA), ryanodine receptors (RyR2) play a critical role in regulating intracellular $Ca^{2+}$ homeostasis. Dysregulation of RyR2 function, particularly under conditions such as hypertension and cardiovascular diseases, can profoundly alter $Ca^{2+}$ dynamics and exacerbate pathological states. Therefore, the interaction between compound A-52 and the RyR2 receptor was investigated, and their binding energetics were evaluated.

**A-52 binding to RyR2**

Molecular docking analyses revealed that compound A-52 interacts with the cardiac ryanodine receptor isoform 2 (RyR2), a key protein that mediates $Ca^{2+}$ release from the sarcoplasmic reticulum in cardiomyocytes. The docking results demonstrated a binding energy of –5.2 kcal/mol, indicating a favorable affinity and thermodynamically stable complex formation between A-52 and RyR2. Such an energy value is pharmacologically meaningful, suggesting that A-52 could act as a potential active ligand capable of modulating RyR2 channel activity. Residue-level interaction analysis revealed that A-52 formed the following molecular contacts within the RyR2 binding pocket: GLU A:711 — conventional hydrogen bond; GLU B:711 — unfavorable acceptor–acceptor interaction (likely electrostatic repulsion between oxygen atoms); TYR A:703 — π–alkyl hydrophobic interaction (**Figure 5**).




**Figure 5** Interaction of compound A-52 with the RyR2 receptor. (A) Overall structure of the protein and (B) detailed view of ligand interactions with amino acid residues.

These results support the hypothesis that A-52 binds within the regulatory domain of RyR2 through a combination of hydrogen bonding and hydrophobic interactions. The observed binding mode provides structural insight into how A-52 might influence intracellular $Ca^{2+}$ release and contribute to cardioprotective effects through modulation of RyR2-mediated $Ca^{2+}$ signaling.

**Effect of ligands on $Ca^{2+}$-ATPase protein channels**

Maintenance of intracellular calcium ion balance depends primarily on the activity of two major protein channels: SERCA (sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase) and RyR2 (ryanodine receptor). Among them, SERCA plays a crucial role in regulating cardiac muscle contractility and arterial blood pressure by actively transporting $Ca^{2+}$ from the cytosol into the sarcoplasmic reticulum. Functional impairment of these calcium transporters is closely associated with the development of hypertension and other cardiovascular disorders. Accordingly, at this stage of the study, particular attention was given to analyzing the binding energetics between $Ca^{2+}$-ATPase and selected ligands.

**Binding of compound A-52 to $Ca^{2+}$-ATPase**

Molecular docking analysis revealed that the interaction between compound A-52 and $Ca^{2+}$-ATPase resulted in a binding energy of –6.1 kcal/mol, indicating a strong and thermodynamically favorable association between the ligand and the protein. Residue-level interaction profiling demonstrated that A-52 was stabilized within the active pocket of $Ca^{2+}$-ATPase through the following key contacts: LYS A:514, ARG A:559 — conventional hydrogen bonds, anchoring the ligand to the binding pocket; LYS A:514 — additional carbon–hydrogen interaction, reinforcing local stability; PHE A:487 — π–π stacked and π–alkyl hydrophobic interactions; MET A:494 — π–sulfur and π–alkyl contacts contributing to the overall hydrophobic stabilization of the complex. These molecular interactions collectively suggest that compound A-52 forms a robust and energetically favorable complex with $Ca^{2+}$-ATPase. The resulting stabilization of key residues within the catalytic domain implies potential modulation of SERCA-mediated $Ca^{2+}$ transport, offering mechanistic insight into the compound's possible antihypertensive and cardioprotective properties (**Figure 6**).

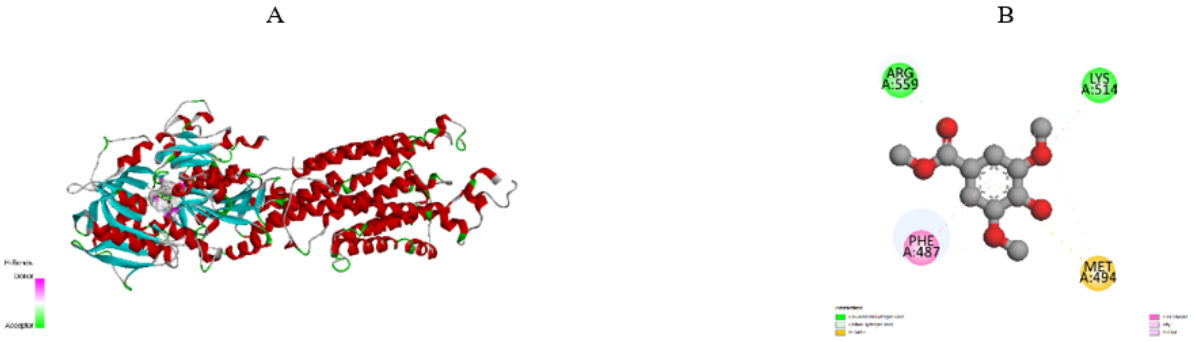



**Figure 6** Interaction of compound A-52 with $Ca^{2+}$-ATPase. (A) Overall structure of the protein and (B) detailed view of ligand interactions with amino acid residues.

**Interaction of compound A-52 with the $Na^{+}/Ca^{2+}$ exchanger (NCX)**

Calcium homeostasis and ion transport systems play a central role in the pathogenesis of hypertension. The intracellular concentration of $Ca^{2+}$ ions directly regulates essential physiological processes such as myocardial contraction, vascular tone, and cardiac output. In this context, three major ion-transporting proteins—SERCA ($Ca^{2+}$-ATPase), RyR2 (ryanodine receptor), and the $Na^{+}/Ca^{2+}$ exchanger (NCX)—function cooperatively to maintain calcium equilibrium. SERCA facilitates relaxation by pumping $Ca^{2+}$ ions back into the sarcoplasmic reticulum, whereas RyR2 releases $Ca^{2+}$ into the cytoplasm to trigger contraction. The NCX protein, in turn, plays a vital role in removing excess intracellular calcium, especially during the diastolic

phase of the cardiac cycle. The coordinated activity of these three proteins ensures stable cardiac rhythm, stroke volume, and blood pressure regulation.

**Binding of compound A-52 to NCX**

Docking simulations revealed that compound A-52 binds effectively to the $Na^+/Ca^{2+}$ exchanger, forming a stable complex with a binding energy of –6.1 kcal/mol. This value indicates a strong and thermodynamically favorable interaction between the ligand and the NCX protein. Residue-level interaction analysis showed the following binding contacts: ASP A:829, GLY A:833 — conventional and carbon–hydrogen bonds; VAL A:99, HIS A:165, LEU A:222, PHE A:169, ILE A:832, ILE A:163 — π–alkyl and alkyl hydrophobic interactions; VAL A:168 — π–sigma interaction (**Figure 7**).




**Figure 7** Interaction of compound A-52 with the $Na^+/Ca^{2+}$ exchanger. (A) Overall structure of the protein and (B) detailed view of ligand interactions with amino acid residues.

These interactions suggest that A-52 binds within the hydrophobic transmembrane region of the NCX protein, where it may influence the $Ca^{2+}/Na^+$ exchange process. Overall, the –6.1 kcal/mol binding energy and multiple stabilizing contacts indicate that A-52 could modulate NCX activity, potentially contributing to the compound's overall antihypertensive and cardioprotective profile by regulating intracellular calcium efflux.

**Interaction analysis of ligands with the Renin–protein complex**

In this study, the renin–angiotensin signaling pathway was examined due to its critical role in the regulation of arterial blood pressure and the development of hypertension. Accordingly, the molecular interactions between ligands A-51, A-52, and A-54 and the renin protein were analyzed in detail using *in silico* docking approaches.

**Binding of A-52 to the renin protein**

Docking analysis revealed that compound A-52 binds effectively to the renin protein, with a binding energy of –5.3 kcal/mol, indicating a stable and energetically favorable interaction. Residue-level interaction mapping identified several key contacts responsible for stabilizing the ligand within the renin active site: SER A:84, THR A:85, ASP A:38 — conventional and carbon–hydrogen bonds; PHE A:119, VAL A:36, VAL A:127 — π–alkyl and alkyl hydrophobic interactions; TYR A:83 — π–π stacked aromatic interaction (**Figure 8**).

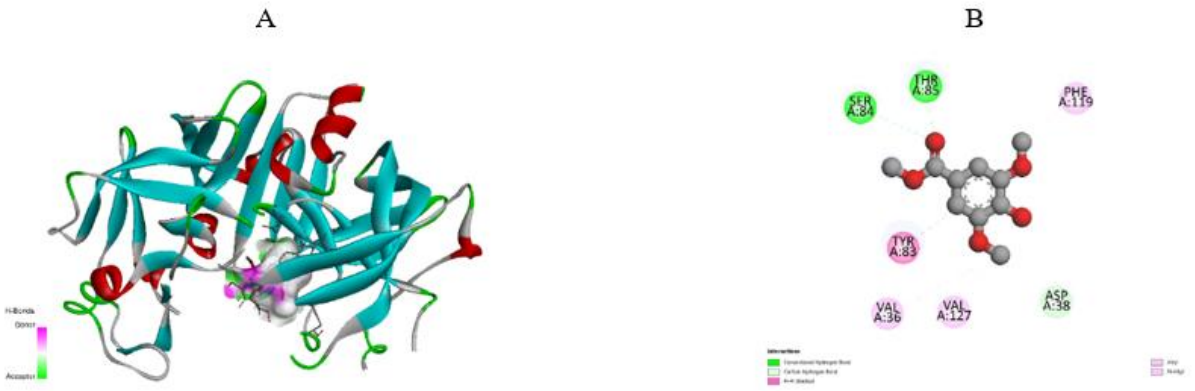



**Figure 8** Interaction of compound A-53 with the renin–angiotensin complex. (A) Overall structure of the protein and (B) detailed view of ligand interactions with amino acid residues.

These interactions demonstrate that A-52 occupies the renin catalytic cleft through a combination of hydrogen bonding and hydrophobic contacts, consistent with typical renin inhibitor binding modes. The binding energy (–5.3 kcal/mol) supports the notion that A-52 may moderately stabilize the renin–protein complex, potentially interfering with the enzymatic conversion of angiotensinogen to angiotensin I. Such a mechanism may underlie the compound's potential antihypertensive activity via modulation of the renin–angiotensin system.

***In vivo* evaluation and comparative analysis of the antihypertensive activity of compound A-52**

Following the *in silico* and *in vitro* findings, the *in vivo* antihypertensive activity of compound A-52 was evaluated to confirm its pharmacological potential in a biological system. Experiments were conducted in an adrenaline-induced hypertension model in rats, assessing both systolic (SBP) and diastolic blood pressure (DBP) responses over time. The study aimed to characterize the dose–response relationship, determine the optimal therapeutic range, and compare the pharmacodynamic profiles of A-52 with related analogs (A-51 and A-54). The resulting data allowed identification of the most effective, stable, and safe candidate compound for further pre-clinical development [21,22].

**Experimental design**

Healthy adult male Wistar rats (n = 3 per group) were used. All animals underwent routine physiological screening prior to experimentation. Blood pressure was measured by a non-invasive tail-cuff plethysmography system under controlled ambient conditions (22 - 24 °C). Two doses of A-52 (25 and 50 mg/kg) were administered intravenously via the tail vein, and dynamic changes in blood pressure were recorded at 1, 2, and 3 h post-administration [23,24].

**Effect of the 25 mg/kg dose**

At baseline, hypertensive rats exhibited elevated blood pressure values typical for this model (SBP = 130.0 ± 12.8 mmHg; DBP = 98.5 ± 10.5 mmHg). Within the first hour after A-52 administration, a pronounced reduction in blood pressure was observed (SBP = 118.8 ± 12.3 mmHg; DBP = 85.8 ± 7.8 mmHg), reflecting the compound's rapid onset of action. By the second hour, A-52 reached its maximal antihypertensive effect (SBP = 108.0 ± 10.5 mmHg; DBP = 75.0 ± 7.0 mmHg). The sustained decrease suggests vasodilatory and/or $Ca^{2+}$-channel-blocking properties, potentially linked to endothelium-dependent mechanisms. At the third hour, blood pressure slightly rebounded yet remained below baseline (SBP = 110.0 ± 6.8 mmHg; DBP = 78.0 ± 4.9 mmHg), indicating a moderate but persistent antihypertensive effect. Overall, the 25 mg/kg dose provided an optimal, stable reduction in arterial pressure without evidence of hypotension or rebound effects, and was therefore selected as the target dose for studies (**Figure 9**).

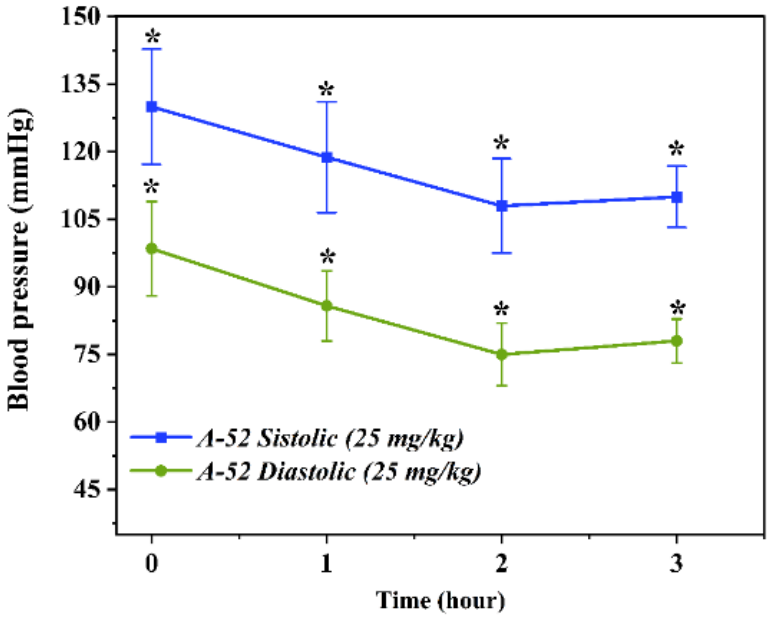



**Figure 9** Effect of A-52 (25 mg/kg) on systolic and diastolic blood pressure. The graph illustrates time-dependent changes in blood pressure (hours). Each point represents the mean ± SEM (n = 3). **p-value* < 0.05 indicates a statistically significant difference compared to the control group.

**Effect of the 50 mg/kg dose**

In the high-dose group (50 mg/kg), the initial mean blood pressure values were slightly lower (SBP = 108.8 ± 10.7 mmHg; DBP = 74.5 ± 7.3 mmHg), suggesting variable hypertension induction across animals. One hour after injection, a sharp fall in blood pressure was recorded (SBP = 79.0 ± 7.6 mmHg; DBP = 56.5 ± 5.4 mmHg), approaching the hypotensive threshold. Such an abrupt decline may reflect excessive vasodilation or a transient reduction in cardiac output, potentially posing pharmacological risk (**Figure 10**).

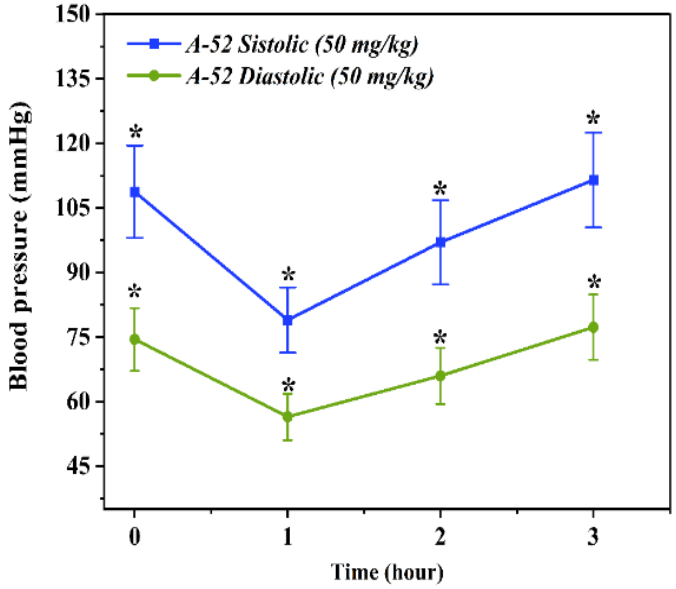



**Figure 10** Effect of A-52 (50 mg/kg) on systolic and diastolic blood pressure. The graph shows time-dependent changes in blood pressure (hours). Each point represents the mean ± SEM (n = 3). **p-value* < 0.05 indicates a statistically significant difference compared to the control group.

During the second hour, partial recovery occurred (SBP = 97.0 ± 9.8 mmHg; DBP = 66.0 ± 6.5 mmHg), and by the third hour, pressures nearly returned to baseline (SBP = 111.5 ± 11.0 mmHg; DBP = 77.3 ± 7.6 mmHg).

These data suggest that while the higher dose elicits a strong initial response, the effect is transient and unstable, indicating activation of physiological compensatory mechanisms. Consequently, the 50 mg/kg dose was excluded from subsequent trials due to potential safety concerns. The observed non-linear dose–response relationship suggests that A-52 exhibits a narrow therapeutic window, where overdosing may lead to diminished or adverse effects rather than enhanced efficacy.

**Comparative evaluation in the adrenaline-induced hypertension model**

To further validate the antihypertensive potential of A-52, experiments were performed using four experimental groups: Control group – received adrenaline only (no treatment). A-52 (25 mg/kg) – received A-52 after adrenaline administration. A-51 (25 mg/kg) – comparison ligand. A-54 (25 mg/kg) – comparison ligand. Each group consisted of 3–4 rats, and SBP/DBP values were monitored over three hours following adrenaline injection (**Figure 11**).

**Baseline (Pre-treatment)**

Before administration, blood pressure values were within normal physiological limits (control SBP = 106.3 ± 10.5 mmHg; DBP = 76.8 ± 7.4 mmHg). A-52-treated animals showed comparable baseline readings (SBP = 95.5 ± 9.4 mmHg; DBP = 75.5 ± 7.4 mmHg).

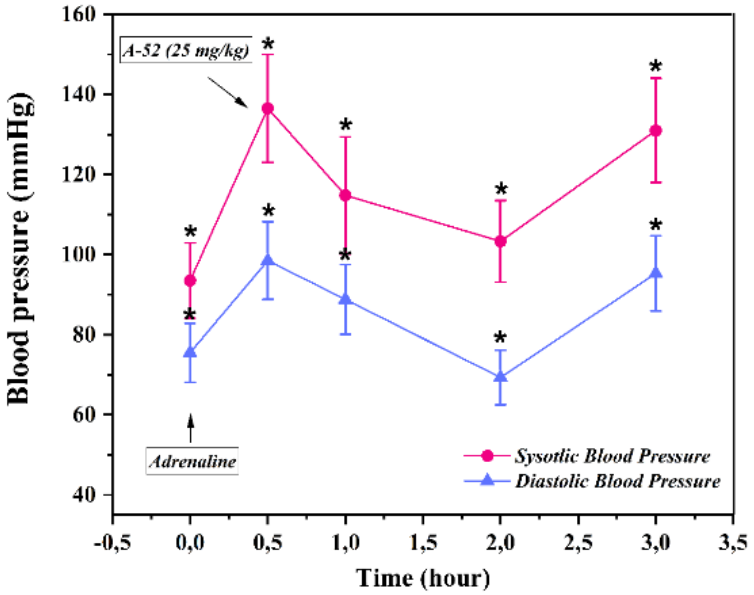



**Figure 11** Effect of A-52 (25 mg/kg) on systolic and diastolic blood pressure in adrenaline-induced hypertension. The graph illustrates time-dependent changes in blood pressure (hours). Each point represents the mean ± SEM (n = 3). **p-value* < 0.05 indicates a statistically significant difference compared to the control group.

**30 minutes after adrenaline injection**

A marked hypertensive response confirmed successful model induction. In the control group, SBP and DBP rose to 160.5 ± 16.3 mmHg and 119.5 ± 11.8 mmHg, respectively—an increase exceeding 50 % above normal baseline values.

**1 hour - onset of antihypertensive response**

At this point, the control group still exhibited elevated pressures (SBP = 147.8 ± 11.6 mmHg; DBP = 118.8 ± 9.8 mmHg). Conversely, A-52 significantly reduced both parameters (SBP = 114.8 ± 14.6 mmHg; DBP = 88.8 ± 8.7 mmHg), demonstrating clear antihypertensive efficacy. In comparison, A-54 showed

weaker effects (SBP = 135.0 ± 10.8 mmHg; DBP = 110.0 ± 11.2 mmHg).

**2 hours - peak antihypertensive effect**

A-52 produced its maximum response at this time, reducing SBP and DBP to 103.3 ± 10.2 mmHg and 69.3 ± 6.8 mmHg, respectively—values approaching the normal physiological range. This stage represents the peak antihypertensive efficacy and reflects sustained pharmacodynamic activity likely mediated by calcium-channel modulation and endothelium-dependent vasorelaxation.

**3 hours - sustained activity and recovery phase**

At the third hour, partial rebound was observed. The control group remained hypertensive (SBP = 129.0 ± 11.8 mmHg; DBP = 98.0 ± 9.2 mmHg), while the A-52 group showed moderate elevation (SBP = 131.0 ± 13.0 mmHg; DBP = 95.3 ± 9.4 mmHg). This likely reflects metabolic degradation or short systemic half-life of the compound. Nevertheless, blood pressure did not return to hypertensive levels, suggesting residual pharmacological activity [25,26].

**Discussion**

The present study explored the antihypertensive effects of compound A-52 using a combined *in silico* and *in vivo* experimental framework. The molecular docking analysis indicated that A-52 is capable of interacting with several proteins involved in $Ca^{2+}$ handling, including $Ca^{2+}$ L-type and R-type channels, SERCA, RyR2, and the $Na^{+}/Ca^{2+}$ exchanger (NCX), with moderate binding affinities in the range of −5.2 to −6.1 kcal/mol. Such affinity values are typical for exploratory docking studies and suggest potential interaction propensity rather than definitive functional modulation.

Docking simulations demonstrated that A-52 can occupy regions proximal to known ligand-binding sites of the L-type $Ca^{2+}$ channel, forming hydrogen bonding, π–π stacking, and hydrophobic interactions with amino acid residues located near the channel pore. While these residues overlap spatially with regions targeted by classical calcium channel blockers, this observation alone does not confirm channel inhibition. Instead, the docking results support the hypothesis that A-52 may influence $Ca^{2+}$-dependent signaling pathways, which requires validation using direct functional assays such as electrophysiology or $Ca^{2+}$ flux measurements.

Similar moderate docking affinities were observed for SERCA, RyR2, and NCX. Although interaction with SERCA has been interpreted as potentially favoring $Ca^{2+}$ reuptake into the sarcoplasmic reticulum, such an effect remains speculative in the absence of enzymatic activity measurements. Likewise, predicted interactions with RyR2 and NCX should be regarded as indicative of possible binding rather than evidence of altered $Ca^{2+}$ release or efflux. Therefore, the multi-target interaction profile of A-52 inferred from docking analysis is best interpreted as hypothesis-generating, reflecting a capacity to engage multiple $Ca^{2+}$-related proteins rather than balanced or coordinated modulation.

The *in vivo* experiments provided preliminary functional evidence that A-52 can reduce arterial blood pressure in an acute adrenaline-induced hypertension model. Administration of A-52 at 25 mg/kg produced a significant reduction in systolic and diastolic blood pressure over a two-hour period without inducing marked hypotension. However, considerable variability was observed, likely reflecting the small sample size, stress sensitivity of the tail-cuff method, and the acute nature of the model. At a higher dose (50 mg/kg), A-52 induced a transient and pronounced decrease in blood pressure followed by recovery, suggesting dose sensitivity under the tested conditions. Rather than defining a strict therapeutic window, these findings indicate that the hemodynamic response to A-52 is dose-dependent, and additional dose-ranging studies are required to characterize safety margins.

Comparative evaluation with structurally related analogs (A-51 and A-54) suggested that A-52 produced a more consistent antihypertensive response in this acute model. Nevertheless, the limited number of compounds and the absence of quantitative pharmacokinetic or receptor-binding data restrict firm conclusions regarding structure–activity relationships. The SAR observations presented here should therefore be considered qualitative and preliminary, serving as a basis for future rational optimization rather than definitive structure-based conclusions.

Several limitations of the present study should be acknowledged. These include the small sample size (n = 3 per group), reliance on a single acute hypertension model, absence of chronic or disease-relevant models

(e.g., spontaneously hypertensive rats), use of non-invasive blood pressure measurement only, and the intrinsic constraints of docking-based interpretation. Additionally, no biochemical, electrophysiological, or toxicity-related endpoints were assessed, limiting insight into systemic effects and safety.

## Conclusions

The present study combined *in silico* predictions with *in vivo* observations to explore the antihypertensive effects of compound A-52. The docking analysis suggested that A-52 is capable of interacting with several $Ca^{2+}$-related molecular targets, indicating a potential for multi-target engagement; however, these findings should be regarded as hypothesis-generating due to the predictive and static nature of docking approaches. In an acute adrenaline-induced hypertension model, A-52 produced a dose-dependent reduction in arterial blood pressure, with the 25 mg/kg dose showing a more consistent antihypertensive response compared to higher dosing under the tested conditions.

When evaluated alongside structurally related analogs, A-52 demonstrated comparatively greater efficacy in this acute experimental setting. Nevertheless, the limited sample size, use of a single acute model, and absence of direct functional, pharmacokinetic, and toxicity assessments restrict definitive conclusions regarding mechanism, safety, and long-term therapeutic relevance. Overall, these findings provide preliminary evidence supporting further investigation of A-52 as a bioactive scaffold for studying calcium-dependent pathways in blood pressure regulation, rather than establishing it as a confirmed lead compound.

## Declaration of Generative AI in Scientific Writing

Only minimal assistance was used from QuillBot for paraphrasing selected sentences. All scientific content, interpretation, and conclusions were developed independently by the authors.

## CRediT Author Statement

**Rohatoy Sayidaliyeva:** Conceptualization, Methodology, Data curation, Writing – Original Draft. **Anvar Zaynabiddinov:** Formal analysis, Validation, Visualization. **Izzatullo Abdullaev** and **Ziyoyiddinov Ziyodullokh:** Investigation, Laboratory experiments, Data collection. **Ulugbek Gayibov:** Supervision.

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