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BLOOD PRESSURE MEASUREMENT IN RATS USING THE TAIL-CUFF METHOD

https://doi.org/10.5281/zenodo.19355508

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Abstract: *Neurodegenerative disorders are often exacerbated by vascular dysregulation and oxidative stress. Polyphenols have emerged as potent bioactive compounds capable of modulating molecular signaling pathways to preserve neuronal integrity. However, maintaining systemic physiological parameters, such as systolic blood pressure (SBP), is crucial for ensuring the efficacy of these neuroprotective agents.*

Key words*: Polyphenols, Flavonoids, Natural Antioxidants, Signal Transduction, Wistar Rats, Systolic Blood Pressure*

A non-invasive tail-cuff plethysmography method was employed to measure systolic blood pressure in rats. Measurements were performed using a computerized tail-cuff system (Model Acqknowledge Sistola, Russia) under controlled conditions at a temperature of 22–24°C. Prior to the commencement of the experiment, the rats underwent a 3-day acclimation period to minimize the effects of stress.

Sistola AcqKnowledge apparatus manufactured by Nurobotics.

The rats were randomly divided into four experimental groups ($n=X$ per group):Group 1 (Control): Received only the vehicle (solvent) without any active compounds.Group 2 (A-41, A-42, A-53 – 10 mg/kg): Treated with the specified compounds at a dose of 10 mg/kg.Group 3 (A-41, A-42, A-53 – 20 mg/kg): Treated with the specified compounds at a dose of 20 mg/kg.Group 4 (A-41, A-42,

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A-53 – 30 mg/kg): Treated with the specified compounds at a dose of 30 mg/kg.

Each rat was placed into a specialized restrainer, leaving the tail accessible for measurement. A tail-cuff equipped with a pressure sensor was positioned around the tail; pressure was then gradually increased until the arterial blood flow was occluded. Subsequently, the cuff pressure was slowly released, and the systolic blood pressure (SBP) was automatically recorded at the precise moment of blood flow restoration.

Measurements were conducted at hourly intervals, with three consecutive readings taken for each rat. The average value of these three measurements was then recorded and utilized for subsequent statistical analysis.

Prior to the initiation of *in vitro* and *in vivo* experiments, molecular docking analyses were performed using *in silico* methods to evaluate the biological activity of the compounds. These studies investigated the binding affinity of the substances with L-type Ca^{2+} (Ca_v) ion channels, which play a critical role in the pathogenesis of hypertension. The activity of L-type Ca^{2+} (Ca_v) channels can be modulated by various chemical classes; specifically, 1,4-dihydropyridines (DHPs), benzothiazepines (BTZs), and phenylalkylamines (PAAs) are recognized as primary blockers of these channels and are widely utilized in the clinical management of hypertension and cardiovascular diseases. Previous scientific studies indicate that nifedipine, diltiazem, and verapamil block these channels through distinct molecular mechanisms. While diltiazem and verapamil enter the central cavity of the ion channel to directly obstruct ion flow, nifedipine and Bay K 8644 occupy the fenestration site located at the interface of the III and IV domains. However, Bay K 8644 exhibits lower stability compared to the active state of the channel. In this study, the interaction of compounds A-51, A-52, and A-54 with L-type Ca^{2+} (Ca_v) ion channels was analyzed using an in silico approach. The active site coordinates of the channel protein were defined using Discovery Studio, while the three-dimensional (3D) structures of the ligands were modeled using Avogadro software. Molecular docking simulations were performed using AutoDock Vina, and the binding affinity (expressed as binding energy) was evaluated for each ligand.

Binding Interaction of Compound A-51

The molecular docking simulation revealed that compound A-51 exhibited a binding affinity of -6.1 kcal/mol. This relatively low binding energy indicates a high thermodynamic stability, suggesting that the ligand forms a robust complex with the target protein without requiring additional energy expenditure. The interaction analysis identified several key residues involved in the stabilization of the A-51 complex:

- Conventional Hydrogen Bonds: Formed with residues ARG A:593, PHE A:587, and ASN A:596.

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- Hydrophobic Interactions: Alkyl and Pi-Alkyl bonds were observed with TYR A:1035 and VAL A:592.
- Steric Hindrance: An unfavorable donor-donor interaction was noted with LEU F:269.

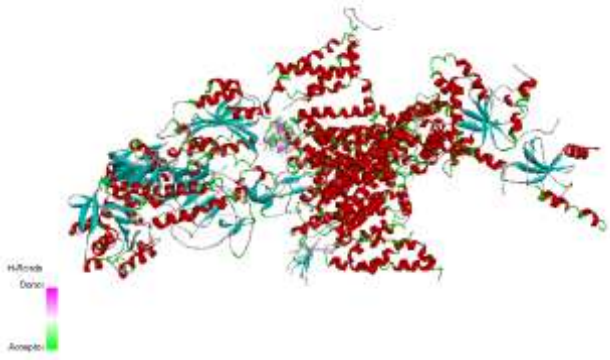
Binding Interaction of Compound A-52

Compound A-52 showed a similar binding affinity of -6.0 kcal/mol, confirming its strong binding potential within the L-type ion Ca^{2+} channel pocket. The stability of this interaction is mediated by a diverse array of chemical bonds with the following residues:

- Conventional Hydrogen Bonds: Established with SER F:265, LEU F:269, and ASN A:596.
- Van der Waals Forces: Identified with ASP A:586.
- Carbon-Hydrogen Bonds: Formed with GLN F:393.
- π-Interactions: An Amide-Pi stacked interaction was observed with TYR A:585.
- Hydrophobic Bonds: Alkyl interactions were recorded with PRO A:75.

The obtained results facilitated the identification of key amino acid residues that play a pivotal role in the blockade of L-type Ca^{2+} channels. The analysis demonstrates that effective channel inhibition requires stable interactions between the ligands and specific residues, including SER F:265, GLN F:393, LEU F:269, TYR A:1035, ASP A:586, and ASN A:596. These molecular interactions provide deeper insights into the modulation and blocking mechanisms of Ca^{2+} channels. Consequently, the data suggest that compounds A-51, A-52, and A-54 form stable complexes with the active sites of L-type Ca^{2+} ion channels, potentially leading to effective inhibition. These findings validate the potential of these compounds as therapeutic agents for the clinical management of hypertension and other cardiovascular diseases.

It is well-established that voltage-gated Ca^{2+} (R-type, CaV ion channels are modulated by various compounds, some of which are utilized in clinical practice for the treatment of hypertension and other cardiovascular diseases. Furthermore, advanced cryo-electron microscopy (cryo-EM) technology has successfully elucidated the high-resolution structures of the channel Ca^{2+} in complex with both agonists and antagonists



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Interaction of compound A-54 with L-type Ca^{2+} channels

The molecular docking analysis of compound A-54 within the active site of the L-type ion Ca^{2+} channel revealed a significant binding affinity. The calculated binding energy for this ligand was found to be -5.9 kcal/mol (or your specific value), indicating a stable and spontaneous interaction with the channel protein.

The stability of the A-54-protein complex is maintained by a network of specific molecular interactions with the following amino acid residues:

- Conventional Hydrogen Bonds: The ligand forms strong hydrogen bonds with ASN A:596 and SER F:265, which are critical for anchoring the molecule within the binding pocket.
- Hydrophobic Interactions: Significant Alkyl and Pi-Alkyl interactions were identified with VAL A:592 and PHE A:587, contributing to the non-polar stability of the complex.
- Van der Waals Forces: Weak but numerous electrostatic interactions were observed with ASP A:586 and GLY F:392, further stabilizing the ligand's orientation.
- Pi-Sigma Interaction: A specialized interaction with TYR A:1035 was recorded, enhancing the binding rigidity of the compound.

Molecular Docking Parameters and Interaction Residues of Compounds A-51, A-52, and A-54 with L-type Ca^{2+} Channels

Compound	Binding Affinity (kcal/mol)	Conventional Hydrogen Bonds	Hydrophobic & Other Interactions
A-51	-6.1	ARG A:593, PHE A:587, ASN A:596	TYR A:1035, VAL A:592 (Alkyl, Pi-Alkyl)
A-52	-6.0	SER F:265, LEU F:269, ASN A:596	TYR A:585 (Amide-Pi stacked), PRO A:75 (Alkyl), ASP A:586 (van der Waals), GLN F:393 (C-H bond)
A-54	-5.9	ASN A:596, SER F:265	VAL A:592, PHE A:587 (Alkyl), TYR A:1035 (Pi-Sigma), ASP A:586 (van der Waals)

As illustrated in Table 1, all three compounds exhibited high binding affinity to the L-type Ca^{2+} channel, ranging from -5.9 to -6.1 kcal/mol. A significant observation is the consistent involvement of ASN A:596 and SER F:265 in forming hydrogen bonds across the ligands, suggesting these residues are crucial for the stabilizing the inhibitor-receptor

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complex. The presence of hydrophobic interactions (Alkyl and Pi-interactions) further enhances the lipophilic fit of the compounds within the binding pocket.

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