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Interaction of quercetin and its derivatives with Ca^{2+}-ATPase from sarcoplasmic reticulum: Kinetic and molecular modeling studies

Petronela Rezbarikova[1], Jana Viskupicova[1], Magdalena Majekova[1] and Lubica Horakova[1]

[1] *Centre of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia*

Abstract. Sarcoplasmic reticulum Ca^{2+}-ATPases (SERCAs) regulate cellular calcium homeostasis and are targeted for age-related diseases. Among 14 SERCA mRNA splice variants, SERCA1a is specific to adult fast-twitch skeletal muscle. Quercetin derivatives (monochloropivaloylquercetin (CPQ), IC_{50} = 195.7 μM; 2-chloro-1,4-naphthoquinonequercetin (CHNQ), IC_{50} = 60.3 μM) were studied for their impact on SERCA1a using molecular modeling and enzyme kinetics. While there were some similarities in kinetic parameters and molecular modeling, the compounds exhibited diverse actions on SERCA1a. Quercetin reduced activity by 48% at 250 μM by binding to the cytosolic ATP-binding pocket with increased ATP affinity. CPQ bound near the Ca^{2+}-binding site, possibly altering the transmembrane domain. CHNQ significantly reduced activity by 94% at 250 μM without binding to substrate sites. It was proposed that CHNQ induced global protein structure changes, inhibiting Ca^{2+}-ATPase activity.

Key words: Ca^{2+}-ATPase — Quercetin — Monochloropivaloylquercetin — 2-chloro-1,4-naphthoquinonequercetin — Kinetics — Molecular docking

Abbreviations: CHNQ, 2-chloro-1,4-naphthoquinonequercetin; CPQ, monochloropivaloylquercetin; SERCA1, sarcoplasmic reticulum Ca^{2+}-ATPase isoform 1.

Highlights

- Quercetin and its derivatives affected Ca^{2+}-ATPase activity by different mechanisms
- Quercetin interfered with the ATP pocket of SERCA1
- Monochloropivaloylquercetin decreased activity by altering Ca^{2+}-binding pathways
- 2-chloro-1,4-naphthoquinonequercetin caused extensive impairment of SERCA1 conformation

Introduction

Sarcoplasmic reticulum Ca^{2+}-ATPase isoform 1 (SERCA1) is a P-type ATPase that is predominantly expressed in skeletal muscle. This enzyme actively transports Ca^{2+} from the cytosol into the sarcoplasmic reticulum (SR), thus maintaining Ca^{2+} homeostasis and creating an intracellular Ca^{2+} store. Once its activity inhibition occurs, basal intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is elevated (Wray and Burdyga 2010; Aguayo-Ortiz and Espinoza-Fonseca 2020).

Proteins are flexible molecules that undergo conformational changes as part of their interactions with other proteins or drug molecules (Haspel et al. 2010). The basic model of the transportation cycle of P-ATPases was proposed by Albers (1967) and Post et al. (1972), and to

Correspondence to: Petronela Rezbarikova, Centre of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia
E-mail: petronela.zizkova@savba.sk

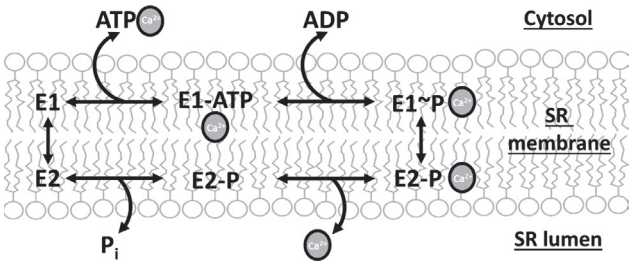

Figure 1. Schematic representation of the reaction cycle of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1a). SERCA1a exists in two main forms, E1 and E2, and several intermediate states. E1 conformation is defined by binding of two Ca^{2+} ions from the cytoplasmic side of the membrane, whereas E2 is characterized by dephosphorylation after releasing calcium ions into the lumen of SR.

this day structural biology has covered almost the entire sequence with high-resolution snapshots (Toyoshima et al. 2000, 2003; Zhang and Zhang 2019). SERCA1 undergoes several conformational transitions, which are important for the active transport of two ions of calcium from the cell cytoplasm into SR. The transport cycle comprises at least six different states with two major E1 and E2 conformations (Fig. 1) (Wang et al. 2021). Their phosphorylated counterparts, E1P and E2P, link ATP hydrolysis to Ca^{2+} transport *via* different affinities and specificities for ATP and Ca^{2+} (Mahaney et al. 1995).

The transport cycle starts at the E1 state where ATP-binding sites and Ca^{2+}-binding sites are To measure the kinetic parameters of SERCA1a in the presence of quercetin and its derivatives. accessible and ready for substrate binding (Rui et al. 2018). This state is characterized by a high affinity toward Ca^{2+}. On the contrary, in the E2 state, the affinity toward Ca^{2+}-binding sites is low, which enables the release of Ca^{2+} into the lumen of SR (Wray and Burdyga 2010; Inesi and Tadini-Buoninsegni 2014).

SERCA1 expresses biphasic kinetics when the reaction rate is calculated as a function of ATP (Coll and Murphy 1991). The membranous Ca^{2+}-ATPase is first activated at low micromolar concentrations of ATP (usually 1–100 μM), followed by the modulation of the E2→E1 transition (Jensen et al. 2006) at higher millimolar concentrations (Kosk-Kosicka et al. 1983).

Flavonoids are dietary compounds that exert protective effects in several disease conditions, including cancer (Abotaleb et al. 2018), cardiovascular diseases (Vazhappilly et al. 2019), and neurodegenerative disorders (Devi et al. 2021). Flavonoids can interact with the ATP-binding sites of different proteins, including mitochondrial ATPase, calcium plasma membrane ATPase, protein kinase A, protein kinase C, and topoisomerase (Williams et al. 2004; Ontiveros et al. 2019). Flavonoids were reported to inhibit SERCA1 and induce apoptotic cell death *via* the intrinsic (mitochondrial) pathway, which is associated with $[Ca^{2+}]_i$ overload (Ogunbayo and Michelangeli 2014).

Quercetin (3,3',4',5,7-pentahydroxyflavone) belongs to the most studied flavonoids. A molecule of quercetin contains five hydroxyl groups whose presence determines the compound's reactivity, biological activity, and the possible number of derivatives (Materska 2008); however, bioavailability in humans is very low (~ 2%) and its absorption varies from 3% to 17%, which means that its medical use is limited (Iside et al. 2020; Alizadeh and Ebrahimzadeh 2022).

Therefore, we tried to improve this drawback *via* the structural modification of the quercetin molecule (Veverka et al. 2013). The synthesized novel structures are depicted in Figure 2.

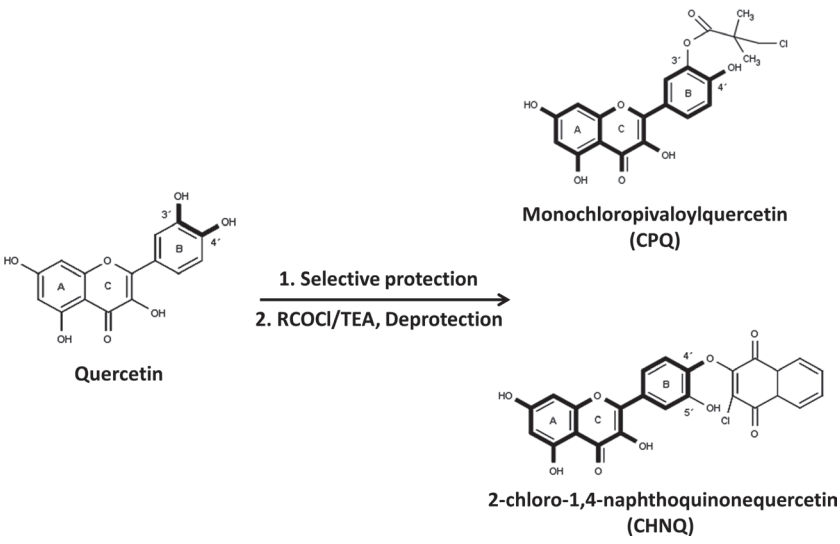

Figure 2. Novel quercetin derivatives monochloropivaloylquercetin (CPQ) and 2-chloro-1,4-naphthoquinonequercetin (CHNQ) were synthesized *via* selective protection procedures of quercetin and acylation with respective acyl chlorides according to Veverka et al. (2013). Highlighted positions on the quercetin B-ring depict the preferential binding of substituents.

Previously published studies have indicated that these novel quercetin analogs possess higher bioavailability and solubility as well as altered antioxidant/prooxidant properties compared to the parent molecule quercetin (Kuniaková et al. 2015; Milackova et al. 2015; Enayat et al. 2016; Soltesova-Prnova et al. 2016; Zizkova et al. 2017).

The aims of this study are as follows:

1. To measure the kinetic parameters of SERCA1a in the presence of quercetin and its derivatives.
2. To utilize molecular modeling techniques to gain insights into the preferential binding sites of these compounds within the enzyme molecule and assess their impact on ATP and Ca^{2+} binding sites.
3. To enhance our understanding of the distortion of the SERCA1a molecule, which could aid in the design of new drug-like compounds with a desirable effect on the enzyme for the management of diseases.

Material and Methods

Chemicals

All chemicals were at least reagent grade and were purchased from Merck/MilliporeSigma (Denmark) unless otherwise indicated.

Isolation of SR

SR vesicles were isolated from the spinal region of the fast-twitch skeletal muscle and from the hindlimbs of a female New Zealand rabbit (about 2.5 kg) according to Warren et al. (1974a, 1974b) and modified by Andriamainty et al. (1997).

Ca^{2+}-ATPase activity measurement

SERCA1a activity was measured spectrophotometrically by a NADH-coupled enzyme assay, as outlined by Warren et al. (1974a, 1974b). The final amount of SR vesicles was 12.5 μg protein/cuvette. The assay mixture containing Hepes (40 mM, pH 7.2), KCl (0.1 M), $MgSO_4$ (5.1 mM), ATP (2.1 mM), phosphoenolpyruvate (0.52 mM), EGTA (1 mM), NADH (0.15 mM), pyruvate kinase (7.5 IU), and lactate dehydrogenase (18 IU) was incubated at 37°C for 2 min with their respective flavonoids (quercetin, monochloropivaloylquercetin (CPQ), or 2-chloro-1,4-naphthoquinonequercetin (CHNQ)). A concentration range of 10–150 μM was used for activity measurement, and a single concentration of 50 μM for kinetic study. The reaction was started by the addition of 1 mM $CaCl_2$, while the reaction rate was determined by measuring the decrease in NADH absorbance at 340 nm on a SPECORD 40 at 37°C (Analytik Jena, Denmark). Corresponding graphs were created using the GraphPad Prism 8 program.

Kinetic parameters of Ca^{2+}-ATPase activity

a) Analysis of the ATP dependence of Ca^{2+}-ATPase activity: ATP dependence of Ca^{2+}-ATPase activity was measured by a NADH-coupled enzyme assay, as described above. The pre-incubated samples contained SR vesicles and an assay mixture without ATP. The individual concentrations of ATP in the range from 0.1 μM to 2.1 mM were incubated separately at 37°C in Hepes (40 mM, pH 7.2). The respective concentration of ATP was added to the assay mixture with SR, and the reaction was started by the addition of 1 mM $CaCl_2$. The dependence of Ca^{2+}-ATPase activity on ATP could be modeled assuming a bi-Michaelis-Menten process where ATP independently reacted with high affinity (Hi) and low affinity (Lo) sites. Parameters for V_{max} and K_m were calculated based on non-linear two-site specific binding with excluded outlier values using GraphPad Prism 8 software: high-affinity binding site (E1) = V_{maxHi}*[Mg.ATP]/K_{mHi} + [Mg.ATP], low-affinity binding site (E2) = V_{maxLo}*[Mg.ATP]/K_{mLo} + [Mg.ATP], Total activity: Y = E1 + E2; where V_{max} is the activity at saturating concentrations of the substrate and K_m is the Michaelis constant. The indices Hi and Lo mean high- and low-affinity binding sites, respectively. Calculated biphasic dose-response curve is the intersection of two points, one at low ATP concentrations yielding V_{maxHi} and K_{mHi} in the catalytic state (E1), and the second at high ATP concentrations with corresponding V_{maxLo} and K_{mLo} in the modulatory state (E2). Error bars were calculated as the standard deviation (SD) of three independent measurements.
b) Analysis of the Ca^{2+} dependence of Ca^{2+}-ATPase activity: The Ca^{2+} dependence of SERCA1a activity was measured in a wide range of calcium concentrations (0.024–13.5 μM) and analyzed as mentioned in the above section. The SR sample was pre-incubated with an assay mix containing 2.1 mM ATP. The reaction was started by addition of individual Ca^{2+} concentrations. Concentrations of $Ca^{2+}{}_{free}$ ($[Ca^{2+}]_{free}$) were calculated by the Maxchelator program and using the binding affinities described by Gould et al. (1986).The kinetic parameters V_{max}, K_m, and cooperativity coefficient (Hill coefficient) were calculated based on non-linear least squares fitting of the activity data to the specific binding with Hill slope: Activity = V_{max}*$[Ca^{2+}]^h/K_m{}^h + [Ca^{2+}]^h$; where V_{max} is the activity of Ca^{2+}-ATPase at saturating concentrations of the substrate, K_m is the $[Ca^{2+}]_{free}$ corresponding to one-half of V_{max}, and h is the Hill coefficient, an indicator of the steepness of the curve. All computations were done with GraphPad Prism 8 software. Error bars were calculated as SD of three independent measurements.

In silico study

In order to model the SERCA1a interactions with quercetin, CPQ, and CHNQ, we used the crystal structures from protein

data bank (PDB) with PDB ID 4xou and 3w5c for the E1 and E2 states, respectively. Starting geometries of the compounds were obtained by a Monte Carlo equilibrium conformer search – (MMFF94) through the program SPARTAN'08 (Wavefunction Inc., USA). The structures of protein were treated in order to correct the bonds and hydrogens using the protocol Clean in the software Yasara (www.yasara.org). Then a global docking search was performed using the AMBER14 force field. The AutodockLGA method was chosen, with maximum runs = 25, rmsdmin = 5.0 for identifying complexes in different clusters. Complexes with the best score (given by the value of binding energy) were fully optimized using the Energy Minimization protocol, which consists of the combination of gradient optimization, molecular dynamics, and simulated annealing, and was subsequently taken as the representative of the predicted geometry.

Statistical evaluation

Statistics were performed with one-way ANOVA using the Dunnett test. Values are mean ± SD of at least three independent experiments, where each sample was measured by two or more duplicates (details given under the specific figure). Statistical significance was set at * $p < 0.0331$, ** $p < 0.0021$, *** $p < 0.0002$, **** $p < 0.0001$.

Results

SERCA1 possesses two substrate-binding sites in the cytosolic region and the transmembrane region in order to bind ATP and two Ca^{2+} ions, respectively. To understand the mechanisms of SERCA1 inhibition by quercetin derivatives, Ca^{2+}-ATPase activity was studied in relation to increasing concentrations of the individual substrates ATP (Fig. 3A) and free Ca^{2+} (Fig. 3B). The selected concentration of 50 μM evoked changes in both the cytosolic and the transmembrane domain of SERCA1, as indicated by our previous experiments (Žižková et al. 2014).

Quercetin

Quercetin exhibited a concentration-dependent decrease in SERCA1a activity within the range of 0–50 μM (Fig. 4A). However, determining the exact IC_{50} value was challenging due to an unusual concentration response, which is likely attributed to the complex and multimodal mechanism of action of quercetin. We will delve into this topic in subsequent discussions. To further investigate the enzyme's behavior, we conducted preincubation experiments with the enzyme in the absence of Ca^{2+} and varying concentrations of ATP. The relationship between enzyme activity and ATP concentration, spanning from 0.1 μM to 2.1 mM, was fitted using the bi-Michaelis-Menten equation. This equation was deemed more appropriate for our enzyme model than the standard Michaelis-Menten equation, as it better accommodates the characteristics of our experimental system (Taylor and Hattan 1979).

The biphasic model of SERCA1a assumes that ATP interacts independently with both high-affinity catalytic sites and low-affinity modulatory sites (Ogunbayo et al. 2008). By employing this model, we gain a more comprehensive

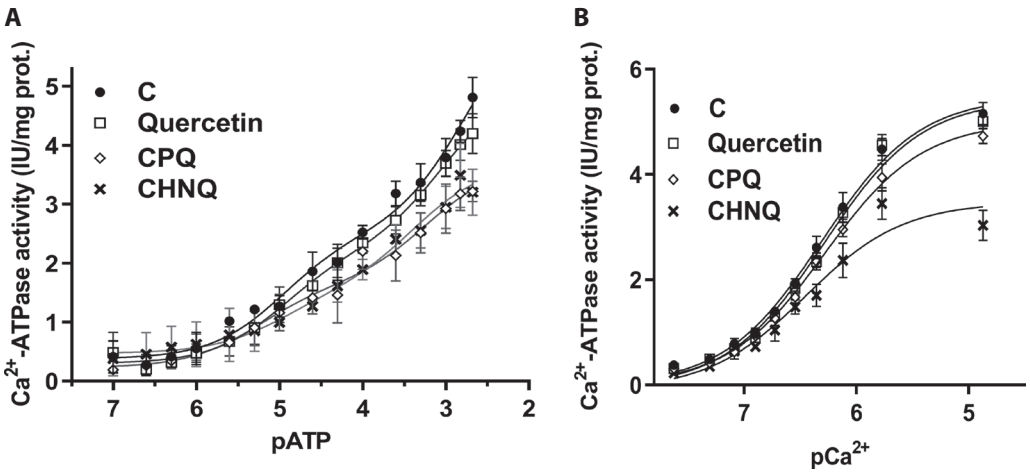

Figure 3. Changes in the activity of SERCA1a as a function of ATP (**A**) and Ca^{2+} (**B**) in the presence of quercetin and its derivatives CPQ and CHNQ (all 50 μM). SERCA1a activity was determined as described in the section "Materials and Methods" and through the dependence of increasing ATP and Ca^{2+} concentrations. Values are mean ± SD of six independent measurements. Kinetic parameters were calculated with the bi-Michaelis-Menten equation for ATP and with the Hill equation for Ca^{2+} using GraphPad Prism 8. pCa^{2+}, natural logarithm of Ca^{2+} concentration; pATP, natural logarithm of ATP concentration.

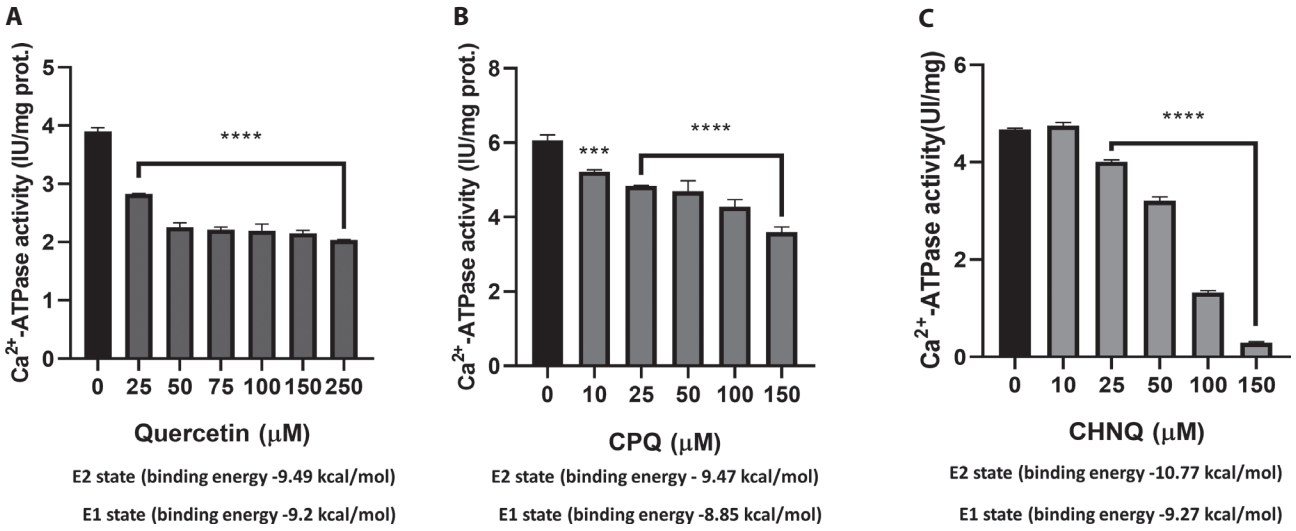

Figure 4. Effect of quercetin (**A**) and its derivatives CPQ (**B**) and CHNQ (**C**) on the activity of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1a). SERCA1a activity was measured by a NADH-coupled assay. Values are mean ± SD of three independent experiments, where each sample was measured in two parallels. *** $p < 0.0002$, **** $p < 0.0001$ are significant differences between control (0 μM) and samples pretreated with quercetin and its derivatives.

understanding of the intricate dynamics of SERCA1a and its interactions with ATP.

We considered the SERCA1a E2 conformation a ground state (free from all ligands), and our results are presented/described according to this layout. Quercetin exhibited notable effects on the enzymatic activity of the protein. Specifically, at the modulatory E2 step, quercetin led to a decrease in the kinetic parameter $V_{max(ATP)}$ from a control value of 3.32 ± 0.17 IU/mg to 2.95 ± 0.19 IU/mg. Additionally, we observed an increase in $K_{m(ATP)}$ from 0.43 ± 0.1 μM to 0.57 ± 0.17 μM (Table 1).

Through molecular docking analysis, we discovered that quercetin formed hydrogen bonds with four amino acids (Gln 244, Asp245, Ser338, and Gln692) within the cytosolic domain of the enzyme, near the stalk region (Fig. 5).

In Table 1, it can be observed that quercetin did not have an impact on the maximal rate ($V_{max(ATP)}$) in the E1 state. However, during the catalytic step, we observed an approximately 2-fold decrease in the affinity of SERCA1a for ATP, as evidenced by the increase in $K_{m(ATP)}$ values (control = 2.07 ± 0.48 μM, quercetin = 4.33 ± 0.95 μM). Notably, molecular modeling analysis revealed that quercetin interacted with six specific amino acids (Arg489, Arg560, Cys561, Asp627, Arg678, and Glu680) in the E1 conformation, which are located near the ATP-binding pocket (Fig. 5). According to Table 2, quercetin did not alter the affinity of SERCA1a for Ca^{2+}.

Novel quercetin derivatives: CPQ and CHNQ

The pivaloyl derivative of quercetin – CPQ demonstrated a concentration-dependent reduction in SERCA1a activity, with an IC_{50} value of 195.7 μM (Fig. 4B). In the presence of CPQ at the modulatory E2 state, we observed a decrease

Table 1. SERCA1 affinity for ATP in the presence of quercetin and its derivatives

	Modulatory (E2) mode		Catalytic (E1) mode		r^2
	V_{max} (IU/mg)	K_m (mM)	V_{max} (IU/mg)	K_m (μM)	
C	3.32 ± 0.17 (1)	0.43 ± 0.1 (1)	1.75 ± 0.13 (1)	2.07 ± 0.48 (1)	0.97
Quercetin	2.95 ± 0.19 (0.88)	0.57 ± 0.17 (1.3)	1.85 ± 0.14 (1.06)	4.33 ± 0.95 (2.1)**	0.96
CPQ	2.56 ± 0.13 (0.77)**	0.32 ± 0.04 (0.74)	1.33 ± 0.10 (0.76)**	1.85 ± 0.61 (0.89)	0.93
CHNQ	2.15 ± 0.15 (0.65)****	0.15 ± 0.11 (0.34)*	0.85 ± 0.14 (0.48)****	0.21 ± 0.13 (0.1)*	0.91

SERCA1 activity was determined as mentioned above and dependent on increasing ATP concentrations. Values are mean ± SD of six independent measurements. Numbers in brackets represent relative values. * $p < 0.0331$, ** $p < 0.0021$, **** $p < 0.0001$ are significant differences between control (C) and samples pretreated with quercetin and its derivatives. V_{max}, activity at saturating concentrations of the substrate; K_m, Michaelis constant.

in the reaction rate of the enzyme, $V_{max(ATP)}$, from 3.32 ± 0.17 IU/mg (control) to 2.56 ± 0.13 IU/mg (CPQ). Additionally, the kinetic parameter $K_{m(ATP)}$ was altered from 0.43 ± 0.1 μM to 0.32 ± 0.04 μM (Table 1).

Despite similarities in the binding sites of the compounds under study in the E2 conformation, there were notable differences in their effects on the kinetic parameters and the amino acids involved. The compounds exhibited diverse impacts on these parameters and interacted with distinct amino acids within the enzyme structure.

In Figure 5, the preferred binding position of CPQ in the E2 state is depicted, showing hydrogen bonds with the amino acids Glu113, Asn114, Ile116, Gln244, and Glu732. During the E1 mode, the reaction rate was decreased from 1.75 ± 0.13 IU/mg to 1.33 ± 0.1 IU/mg, and the affinity for ATP was reduced from 2.07 ± 0.48 μM to 1.85 ± 0.61 μM (Table 1). CPQ was predominantly localized in the actuator domain at the E1 mode and likely interacted with four amino acids: Glu125, Lys141, Ser210, and Gly227 (Fig. 5). The main interactions between SERCA1a and CPQ involved hydrogen bonds. The binding energies calculated for both E2 (−9.47 kcal/mol) and E1 (−8.85 kcal/mol) states suggest that the E2 state was more favorable for CPQ binding. The calculated kinetic parameters for Ca^{2+} showed reduced $V_{max(Ca2+)}$ (control = 5.35 ± 0.08 IU/mg, CPQ = 4.91 ± 0.07 IU/mg) and changed $K_{m(Ca2+)}$ (control = 0.46 ± 0.02 μM, CPQ = 0.49 ± 0.02 μM), as indicated in Table 2.

On the other hand, CHNQ exerted significant changes in the SERCA1a affinity towards ATP (Fig. 3A), as well as towards Ca^{2+} (Fig. 3B). We also observed a concentration-dependent inhibitory effect on SERCA1a activity, with an IC_{50} value of 60.3 μM (Fig. 4C). It caused a decrease in all ATP-dependent kinetic parameters related to SERCA1a. In the modulatory E2 state, $V_{max(ATP)}$ decreased to 2.15 ± 0.15 IU/mg (control = 3.32 ± 0.17 IU/mg) and $K_{m(ATP)}$ decreased to 0.15 ± 0.11 mM (control = 0.43 ± 0.1 μM) (Table 1). The extent of inhibition induced by CHNQ indicated a pronounced impairment of SERCA1a function. CHNQ docked into Ca^{2+}-ATPase revealed that the residues Gln244, Asp245, Lys246, and Lys713 were involved in hydrogen bonding with CHNQ in the E2 state (Fig. 5). In the catalytic E1 state, CHNQ reduced $V_{max(ATP)}$ to 0.85 ± 0.14 IU/mg (control = 1.75 ± 0.13 IU/mg) and $K_{m(ATP)}$ to 0.21 ± 0.13 μM (control = 2.07 ± 0.48 μM) (Table 1). In the E1 state, CHNQ exclusively bound to the SERCA1a actuator domain *via* Glu44, Glu45, and Glu121 (Fig. 5). The difference in binding energies between the E2 (−10.77 kcal/mol) and E1 (−9.27 kcal/mol) states was the most prominent among all calculated derivatives, indicating a strong preference for the E2 state. Furthermore, CHNQ-mediated changes included a decrease in $V_{max(Ca2+)}$ from 5.35 ± 0.08 IU/mg to 3.12 ± 0.11 IU/mg and a decrease in $K_{m(Ca2+)}$ from 0.46 ± 0.02 μM to 0.32 ± 0.3 μM, indicating an uncompetitive type of inhibition (Table 2).

Discussion

In our study, we evaluated quercetin and its new derivatives: CPQ and CHNQ, previously found to be inhibitors of SERCA1 (Blaškovič et al. 2013; Žižková et al. 2014), which indicates their affinities toward this enzyme. In addition to the experimental kinetic study, molecular modeling analysis of SERCA1a interaction with quercetin and its derivatives was determined.

Quercetin

It is known that flavonoids, including quercetin, chrysin, kaempferol, or rutin, can directly affect proteins involved in cell signaling cascades by binding into their ATP-binding sites (Rafał et al. 2018; Chambers et al. 2020). This action may result in a variety of cellular effects, including modulation of neuronal function, cancer cell proliferation, and inflammation responses (Passamonti et al. 2009). Several authors found a decrease in SERCA1a activity induced by quercetin (Shoshan 1981; Anderson et al. 1984; Ogunbayo and Michelangeli 2014) and the inhibitory effects of quercetin on Ca^{2+}-ATPases from red platelets and plasma membrane (Fischer et al. 1987; Ontiveros et al. 2019).

Table 2. SERCA1 affinity for Ca^{2+} in the presence of quercetin and its derivatives

	V_{max} (IU/mg)	K_m (μM)	h	r^2
C	5.35 ± 0.08 (1)	0.46 ± 0.02 (1)	1.10 ± 0.04 (1)	0.98
Quercetin	5.22 ± 0.07 (0.98)	0.48 ± 0.02 (1.04)	1.15 ± 0.04 (1.04)	0.97
CPQ	4.91 ± 0.07 (0.92)**	0.49 ± 0.02 (1.07)	1.01 ± 0.03 (0.92)	0.96
CHNQ	3.12 ± 0.11 (0.58)****	0.32 ± 0.3 (0.70)***	1.18 ± 0.11 (1.10)	0.90

SERCA1 activity was determined as mentioned above and dependent on increasing Ca^{2+} concentrations. Values are mean ± SD of six independent measurements. Numbers in brackets represent relative values. ** $p < 0.0021$, *** $p < 0.0002$, **** $p < 0.0001$ are significant differences between control (C) and samples pretreated with quercetin and its derivatives. h; Hill coefficient. For more abbreviations, see Table 1.

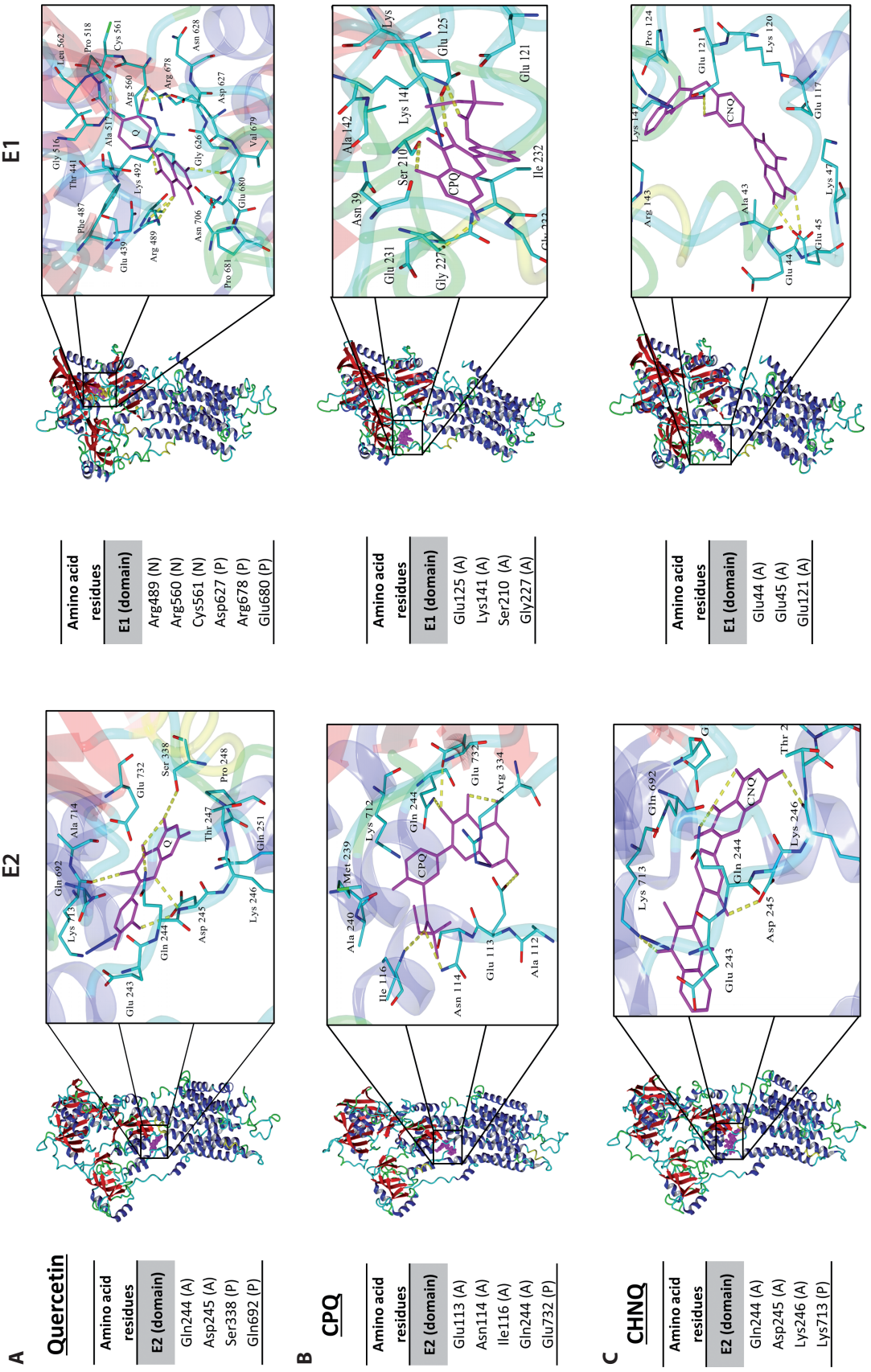

Figure 5. The structural arrangement of the SERCA1a in E2 (PDB code 3w5c) and in the E1 state (PDB code 4xou) with bound quercetin (**A**) and its derivatives CPQ (**B**) and CHNQ (**C**). Yellow dashed lines represent H-bonds between quercetin and its derivatives, and amino acids. A, actuator domain; N, nucleotide domain; P, phosphorylation domain. (See online version for color figure.)

SERCA1 activity is also modulated by the oxidation/reduction of critical amino acids and the modification of Cys349 by peroxynitrite, which induces the loss of the enzyme function (Viner et al. 1999). The presence of quercetin (Žižková et al. 2014) led to a notable reduction in free thiol content, which was correlated with a decrease in SERCA1 activity. Experiments conducted by Fraqueza et al. (2012) showed that quercetin protected cysteines against oxidation by decavanadate but did not reverse the inhibition of SERCA (Fraqueza et al. 2012). On contrary to these observations, our study with peroxynitrite revealed that quercetin did not protect SH-groups against oxidation and even deepened effect of peroxynitrite (Žižková et al. 2014). The role of quercetin seems to depend on the nature of the oxidizing agent used.

The calcium transport cycle of SERCA1 is a very dynamic process, during which Ca^{2+}-ATPase domains are relocated and repositioned and any ligand molecule bond could affect different amino acid residues at the same time (Jensen et al. 2006).

Many residues from the SERCA1 cytosolic region, including nucleotide, phosphorylation, and actuator domains, have been identified as participating in nucleotide binding in various structural stages, as determined by mutagenesis and crystallography (Autry et al. 2012; Páez-Pérez et al. 2016; de la Cruz-Torres et al. 2020). While the SERCA1 ATP-binding site flips between the catalytic (Ca^{2+}E1-ATP) and modulatory (E2-ATP) mode (Jensen et al. 2006; Clausen et al. 2011; Aguayo-Ortiz and Espinoza-Fonseca 2020; Kabashima et al. 2020), ATP serves as an interdomain-interaction mediator at various stages of the transport cycle. The majority of amino acid residues involved in ATP binding are almost identical for both the E1 and E2 conformation (Autry et al. 2012; Clausen et al. 2014; Páez-Pérez et al. 2016; de la Cruz-Torres et al. 2020).

Molecular modeling revealed that quercetin interacted with Gln244, which was located in the stalk region in the E2 mode. This amino acid, connected with the modulatory effect of ATP, was reported to be responsible for the decrease in SERCA1 activity if deleted (Holdensen and Andersen 2009). Therefore, the interaction of quercetin with Gln244 could be responsible for the SERCA1 activity drop in the E2 state as found in this study.

Moreover, the docking of the quercetin molecule in the E1 conformational state revealed interactions with amino acids involved in the ATP binding. The interaction of Phe487 and Lys515 with adenine moiety is required for the catalytic binding of ATP in SERCA1, whereas Arg489 and Arg560 interact with the polyphosphate tail. Modulatory binding was described as the binding of adenine between "pinchers" formed by Arg489 and Arg678 (Autry et al. 2012), although a few other possibilities of ATP-binding have been outlined. Jensen et al. (2006) published a list of the 25 amino acid residues involved in ATP binding in different functional states of SERCA. Molecular docking revealed a direct interaction of quercetin with amino acids responsible for ATP binding, i.e., Arg489, Arg560, Cys561, Asp627, and Arg678, whereas Arg489 and Arg560 belong to the group of the most critical amino acids needed for ATP-binding (McIntosh et al. 2003). As quercetin prefers to bind near catalytic and modulatory binding sites of ATP according to our calculations, its overall effect may be a sum of several partial actions, blocking both the catalytic and modulatory functions of ATP.

In the previous studies, we observed conformational changes in both SERCA1 domains, the cytosolic and transmembrane, respectively (Žižková et al. 2014). The commonly used fluorescent label FITC specifically labels Lys515 near the ATP binding site, and while it prevents the high-affinity binding of ATP, it permits enzyme phosphorylation (Coll and Murphy 1991).

We can thus conclude that decreased FITC fluorescence (Žižková et al. 2014) and changes in the kinetic parameters together with molecular modeling affirm that quercetin affected SERCA1a activity through binding into both the catalytic and modulatory ATP-binding pockets.

Quercetin derivatives exerted indirect effects on SERCA

CPQ

CPQ possesses pivalate incorporated through an ester linkage at position C-3 (Blaškovič et al. 2013) and may directly modulate SERCA1a function through halogen bonding (Parisini et al. 2011; Žižková et al. 2014). SERCA1a activity decrease was confirmed by NADH-coupled assay. As mentioned above, a decrease in SERCA1a activity is tightly connected with the oxidation or nitration of critical amino acids. CPQ decreased cysteine SH-groups in the concentration range tested, which was used for activity measurement, and was able to react with protein-thiolates through the chloropivaloyl group (Eaton 2006). Kinetic parameters revealed that CPQ evoked an uncompetitive inhibition in the modulatory E2 step as well as in the catalytic E1 step of the transport cycle. Furthermore, molecular docking of CPQ in the E2 indicated binding to the Glu113, Asn114, and Gln244 in the stalk region, which is an important region for the translocation of conformational changes from the cytosolic domain to the transmembrane domain during enzyme phosphorylation. Clarke et al. (1989) reported that mutation of Asn114 resulted in a 50% decrease in protein activity due to incompetent handling of the Ca^{2+} after ATP utilization.

CPQ seems to interact mainly with the A domain, which is necessary for Ca^{2+} translocation, E1P-E2P transition, and their coupling (Katoh et al. 2021; Kobayashi et al. 2021). Conformational changes in the cytosolic domain near the ATP-binding pocket were confirmed by decreased FITC flu-

orescence (Žižková et al. 2014). Lowered fluorescence could indicate that CPQ altered amino acids near the ATP-binding pocket rather than through direct interaction, as showed by molecular docking. Molecular modeling also revealed that CPQ occupied the actuator domain, which explained the decrease in SERCA1a activity: blocking actuator domain rotation could interrupt the domain movement important for the SERCA1a cycle. In the transmembrane domain, CPQ bound near Glu231, thus distorting or decreasing the accessibility of Ca^{2+}-binding sites, as calculated (simulated) by Yasara. Also in the transmembrane domain are second ligand-binding sites for Ca^{2+}.

N-cyclohexyl-N'-(dimethylamino)-carbodiimide (NCD-4) is a probe used for detecting changes in the transmembrane environment of calcium-binding sites by labeling residues in a segment between Glu231 and Glu309 (Munkonge et al. 1989). NCD-4 modified SERCA1a activity *via* the inhibition of high-affinity Ca^{2+}-binding sites (Velasco-Guillén et al. 1998): although SERCA1 was able to hydrolyze ATP, the translocation of Ca^{2+} was blocked because the enzyme did not undergo the conformational changes coupled with Ca^{2+}-binding (Pick and Weiss 1985). Moreover, we detected a decrease in NCD-4 fluorescence in the previous study (Žižková et al. 2014). Since ATP utilization by Ca^{2+}-ATPase is Ca^{2+}-dependent (Kobayashi et al. 2021) and CPQ could alter Ca^{2+}-binding sites or their neighborhood, we assumed that this mechanism contributed to the reduction of SERCA1a function.

CHNQ

1,4-naphthoquinones are common metabolites of plants and animals. The biological properties and effects of their derivatives are quite diverse and include antimicrobial, antifungal, antiviral, antiprotozoal, cytotoxic, antitumor (Aminin and Polonik 2020), neuroprotective (Menchinskaya et al. 2021), and other properties. CHNQ exhibited strong inhibitory action toward SERCA1 and acted as an uncompetitive inhibitor. Its inhibitory action on SERCA1a could be linked through a connection to enzyme-free cysteine-thiol moieties.

CHNQ did not compete with either ATP or Ca^{2+} for binding sites; nevertheless, it changed their affinities toward enzymes and deformed the active site, thus altering SERCA1 catalytic properties (Ouertani et al. 2019). *In silico* methods involving molecular docking are indispensable tools for revealing potential molecular targets for CHNQ in relation to SERCA1a. Analogically to CPQ, CHNQ preferred binding into the actuator domain, which contributed to impaired SERCA1a activity because the domain rotations required for Ca^{2+} transfer were blocked. We have previously reported changes in the NCD-4 and tryptophane fluorescence in the transmembrane region near the Ca^{2+}-binding sites (Žižková et al. 2014). Even though molecular modeling excluded the binding of CHNQ into this domain, CHNQ could affect it through the SERCA1 stalk region, which transfers domain movement from the cytosolic to the transmembrane region. Previously detected CHNQ-induced conformational changes in the cytosolic and transmembrane domain with corresponding fluorescent probes (Žižková et al. 2014) provided evidence supporting these findings.

Finally, the molecular modeling of CHNQ revealed interactions with amino acids at the actuator domain. This domain is important for the rotation and movement that causes the E1→E2 transition. We presumed that CPQ and CHNQ could block this motion and lock SERCA1 in the E1 state, which was supported by the decrease in FITC fluorescence (Žižková et al. 2014). However, according to the analysis of the binding sites and the E2-preferred state of SERCA, we suggest that the most probable mechanism of action for both CPQ and CHNQ is the interactions with the residues Glu113 and Glu243 for CPQ and Asp245 for CHNQ.

Conclusions

Enzyme kinetics and molecular modeling studies helped us profile the interactions of quercetin and its derivatives with SERCA1. Different compounds showed varying degrees of inhibition of SERCA1a, suggesting distinct modes of binding to SERCA1a.

Quercetin decreased SERCA1a activity by binding near the ATP-binding sites, as validated by changes in the kinetic parameters, molecular modeling, and previously reported decreased thiol-content and FITC fluorescence (Žižková et al. 2014). Considering the existence of neighboring modulatory binding sites for ATP besides the catalytic one, we assume that the overall effect of quercetin could also be multimodal yet always connected with the binding of ATP, as supported by the increase of K_m for ATP (E1 state) in the presence of quercetin.

The inhibitions of SERCA1a by CPQ and CHNQ are probably associated with binding to Glu113 (CPQ) and Glu243 together with Asp245 (CHNQ) in the E2 state, as those residues are involved in the initial interactions before Ca^{2+}-binding (Musgaard et al. 2012).

In conclusion, these findings suggest that quercetin and its derivatives can modulate SERCA1a activity through different modes of inhibition, highlighting their potential as agents for the management of diseases associated with SERCA1. Inhibitors of natural origin can be useful in elucidating the underlying molecular mechanisms influencing SERCA1a functionality and related pathophysiological conditions. However, further research is needed to fully understand and exploit the therapeutic potential of these compounds in the treatment of specific diseases.

Author's contribution. P.R. and J.V. performed the *in vitro* experiments; M.M. provided *in silico* results with descriptions; P.R. wrote the manuscripts. The manuscript was edited by M.M., J.V., and L.H. All authors read and approved the final manuscript.

Conflict of interest. The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abotaleb M, Samuel SM, Varghese E, Varghese S, Kubatka P, Liskova A, Büsselberg D (2018): Flavonoids in cancer and apoptosis. Cancers **11,** 28 https://doi.org/10.3390/cancers11010028

Aguayo-Ortiz R, Espinoza-Fonseca ML (2020): Linking biochemical and structural states of SERCA: Achievements, challenges, and new opportunities. Int. J. Mol. Sci. **21,** 1-32 https://doi.org/10.3390/ijms21114146

Albers RW (1967): Biochemical aspects of active transport. Annu. Rev. Biochem. **36,** 727-756 https://doi.org/10.1146/annurev.bi.36.070167.003455

Alizadeh SR, Ebrahimzadeh MA (2022): Quercetin derivatives: Drug design, development, and biological activities, a review. Eur. J. Med. Chem. **229,** 114068 https://doi.org/10.1016/j.ejmech.2021.114068

Aminin D, Polonik S (2020): **1,**4-Naphthoquinones: Some biological properties and application. Chem. Pharm. Bull. **68,** 46-57 https://doi.org/10.1248/cpb.c19-00911

Anderson KW, Coll RJ, Murphy AJ (1984): Inhibition of skeletal muscle sarcoplasmic reticulum CaATPase activity by calmidazolium. J. Biol. Chem. **259,** 11487-11490 https://doi.org/10.1016/S0021-9258(18)90887-2

Andriamainty F, Filípek J, Devínsky F, Balgavý P (1997): Effect of N,N-dimethylalkylamine N-oxides on the activity of purified sarcoplasmic reticulum (Ca-Mg)ATPase. Pharmazie **52,** 240-242

Autry JM, Rubin JE, Svensson B, Li J, Thomas DD (2012): Nucleotide activation of the Ca-ATPase. J. Biol. Chem. **287,** 39070-39082 https://doi.org/10.1074/jbc.M112.404434

Blaskovic D, Zizkova P, Drzik F, Viskupicova J, Veverka M, Horakova L (2013): Modulation of rabbit muscle sarcoplasmic reticulum Ca2+-ATPase activity by novel quercetin derivatives. Interdiscip. Toxicol. **6,** 3-8 https://doi.org/10.2478/intox-2013-0001

Chambers CS, Viktorová J, Řehořová K, Biedermann D, Turková L, Macek T, et al. (2020): Defying multidrug resistance! Modulation of Related Transporters by Flavonoids and Flavonolignans. J. Agric. Food Chem. **68,** 1763-1779 https://doi.org/10.1021/acs.jafc.9b00694

Clarke DM, Maruyama K, Loo TW, Leberer E, Inesi G, MacLennan DH (1989): Functional consequences of glutamate, aspartate, glutamine, and asparagine mutations in the stalk sector of the Ca2+-ATPase of sarcoplasmic reticulum. J. Biol. Chem. **264,** 11246-11251 https://doi.org/10.1016/S0021-9258(18)60455-7

Clausen JD, Holdensen AN, Andersen J (2014): Critical roles of interdomain interactions for modulatory ATP binding to sarcoplasmic reticulum Ca2+-ATPase. J. Biol. Chem. **289,** 29123-34 https://doi.org/10.1074/jbc.M114.571687

Clausen JD, McIntosh DB, Woolley DG, Andersen JP (2011): Modulatory ATP binding affinity in intermediate states of E2P dephosphorylation of sarcoplasmic reticulum Ca2+-ATPase. J. Biol. Chem. **286,** 11792-11802 https://doi.org/10.1074/jbc.M110.206094

Coll RJ, Murphy AJ (1991): Kinetic evidence for two nucleotide binding sites on the CaATPase of sarcoplasmic reticulum. Biochemistry **30,** 1456-1461 https://doi.org/10.1021/bi00220a002

De la Cruz-Torres V, Cataño Y, Olivo-Rodríguez M, Sampedro JG (2020): ANS interacts with the Ca2+-ATPase nucleotide binding site. J. Fluoresc. **30,** 483-496 https://doi.org/10.1007/s10895-020-02518-x

Devi S, Kumar V, Singh SK, Dubey AK, Kim JJ (2021): Flavonoids: Potential candidates for the treatment of neurodegenerative disorders. Biomedicines **9,** 99 https://doi.org/10.3390/biomedicines9020099

Eaton P (2006): Protein thiol oxidation in health and disease: techniques for measuring disulfides and related modifications in complex protein mixtures. Free Radic. Biol. Med. **40,** 1889-1899 https://doi.org/10.1016/j.freeradbiomed.2005.12.037

Enayat S, Şeyma Ceyhan M, Taşkoparan B, Stefek M, Banerjee S (2016): CHNQ, a novel 2-Chloro-1,4-naphthoquinone derivative of quercetin, induces oxidative stress and autophagy both in vitro and in vivo. Arch. Biochem. Biophys. **596,** 84-98 https://doi.org/10.1016/j.abb.2016.03.004

Fischer TH, Campbell KP, White GC (1987): An investigation of functional similarities between the sarcoplasmic reticulum and platelet calcium-dependent adenosinetriphosphatases with the inhibitors quercetin and calmidazolium. Biochemistry **26,** 8024-8030 https://doi.org/10.1021/bi00398a070

Fraqueza G, Ohlin CA, Casey WH, Aureliano M (2012): Sarcoplasmic reticulum calcium ATPase interactions with decaniobate, decavanadate, vanadate, tungstate and molybdate. J. Inorg. Biochem. **107,** 82-89 https://doi.org/10.1016/j.jinorgbio.2011.10.010

Gould GW, East JM, Froud RJ, McWhirter JM, Stefanova HI, Lee AG (1986): A kinetic model for the Ca2+ + Mg2+-activated ATPase of sarcoplasmic reticulum. Biochem. J. **237,** 217-227 https://doi.org/10.1042/bj2370217

Haspel N, Moll M, Baker ML, Chiu W, Kavraki LE (2010): Tracing conformational changes in proteins. BMC Struct. Biol. **10,** S1 https://doi.org/10.1186/1472-6807-10-S1-S1

Holdensen A, Andersen JP (2009): The length of the A-M3 linker is a crucial determinant of the rate of the Ca2+ transport cycle of sarcoplasmic reticulum Ca2+-ATPase. J. Biol. Chem. **284,** 12258-12265

https://doi.org/10.1074/jbc.M900977200
Inesi G, Tadini-Buoninsegni F (2014): Ca2+/H+ exchange, lumenal Ca2+ release and Ca2+/ATP coupling ratios in the sarcoplasmic reticulum ATPase. J. Cell Commun. Signal. **8,** 5-11 https://doi.org/10.1007/s12079-013-0213-7
Iside C, Scafuro M, Nebbioso A, Altucci L (2020): SIRT1 activation by natural phytochemicals: An overview. Front. Pharmacol. **11,** 1-14 https://doi.org/10.3389/fphar.2020.01225
Jensen AML, Sørensen TLM, Olesen C, Møller JV, Nissen P (2006): Modulatory and catalytic modes of ATP binding by the calcium pump. EMBO J. **25,** 2305-2314 https://doi.org/10.1038/sj.emboj.7601135
Kabashima Y, Ogawa H, Nakajima R, Toyoshima C (2020): What ATP binding does to the Ca2+ pump and how nonproductive phosphoryl transfer is prevented in the absence of Ca2+. Proc. Natl. Acad. Sci. USA **117,** 18448-18458 https://doi.org/10.1073/pnas.2006027117
Katoh TA, Daiho T, Yamasaki K, Danko S, Fujimura S, Suzuki H (2021): Angle change of the A-domain in a single SERCA1a molecule detected by defocused orientation imaging. Sci. Rep. **11,** 1-12 https://doi.org/10.1038/s41598-021-92986-3
Kobayashi C, Matsunaga Y, Jung J, Sugita Y (2021): Structural and energetic analysis of metastable intermediate states in the E1P-E2P transition of Ca2+-ATPase. Proc. Natl. Acad. Sci. USA **118,** e2105507118 https://doi.org/10.1073/pnas.2105507118
Kosk-Kosicka D, Kurzmack M, Inesi G (1983): Kinetic characterization of detergent-solubilized sarcoplasmic reticulum adenosinetriphosphatase. Biochemistry **22,** 2559-2567 https://doi.org/10.1021/bi00279a037
Kuniakova M, Mrvova N, Knezl V, Rackova L (2015): Effect of novel quercetin pivaloyl ester on functions of adult rat microglia. Biologia **70,** 690-702 https://doi.org/10.1515/biolog-2015-0082
Mahaney JE, Thomas DD, Froehlich JP (1995): Conformational transitions of the sarcoplasmic reticulum Ca-ATPase studied by time-resolved EPR and quenched-flow kinetics. Biochemistry **34,** 4864-4879 https://doi.org/10.1021/bi00014a044
Materska M (2008): Quercetin and its derivatives: chemical structure and bioactivity – a review. Pol. J. Food Nutr. Sci. **58,** 407-413
McIntosh DB, Clausen JD, Woolley DG, MacLennan DH, Vilsen B, Andersen JP (2003): ATP Binding Residues of Sarcoplasmic Reticulum Ca2+-ATPase. Ann. N. Y. Acad. Sci. **986,** 101-105 https://doi.org/10.1111/j.1749-6632.2003.tb07145.x
Menchinskaya E, Chingizova E, Pislyagin E, Likhatskaya G, Sabutski Y, Pelageev D, Polonik S, Aminin D (2021): Neuroprotective effect of **1,**4-naphthoquinones in an in vitro model of paraquat and 6-OHDA-induced neurotoxicity. Int. J. Mol. Sci. **22,** 9933 https://doi.org/10.3390/ijms22189933
Milackova I, Rackova L, Majekova M, Mrvova N, Stefek M (2015): Protection or cytotoxicity mediated by a novel quinonoid-polyphenol compound? Gen. Physiol. Biophys. **34,** 51-64 https://doi.org/10.4149/gpb_2014028
Munkonge F, East JM, Lee AG (1989): Positions of the sites labeled by N-cyclohexyl-N'-(4-dimethylamino-1-naphthyl)carbodiimide on the (Ca2+ + Mg2+)-ATPase. Biochim. Biophys. Acta **979,** 113-120 https://doi.org/10.1016/0005-2736(89)90530-0
Musgaard M, Thøgersen L, Schiøtt B, Tajkhorshid E (2012): Tracing cytoplasmic Ca(2+) ion and water access points in the Ca2+-ATPase. Biophys. J. **102,** 268-277 https://doi.org/10.1016/j.bpj.2011.12.009
Ogunbayo OA, Harris RM, Waring RH, Kirk CJ, Michelangeli F (2008): Inhibition of the sarcoplasmic/endoplasmic reticulum Ca2+-ATPase by flavonoids: A quantitative structure-activity relationship study. IUBMB Life **60,** 853-858 https://doi.org/10.1002/iub.132
Ogunbayo OA, Michelangeli F (2014): Related flavonoids cause cooperative inhibition of the sarcoplasmic reticulum Ca2+ ATPase by multimode mechanisms. FEBS J. **281,** 766-777 https://doi.org/10.1111/febs.12621
Ontiveros M, Rinaldi D, Marder M, Espelt MV, Mangialavori I, Vigil M, et al. (2019): Natural flavonoids inhibit the plasma membrane Ca2+-ATPase. Biochem. Pharmacol. **166,** 1-11 https://doi.org/10.1016/j.bcp.2019.05.004
Ouertani A, Neifar M, Ouertani R, Saleheddine Masmoudi A, Cherif A (2019): Effectiveness of enzyme inhibitors in biomedicine and pharmacotherapy. Adv. Tissue Eng. Regen. Med. **5,** 85-90 https://doi.org/10.15406/atroa.2019.05.00104
Páez-Pérez ED, De La Cruz-Torres V, Sampedro JG (2016): nucleotide binding in an engineered recombinant Ca2+-ATPase N-domain. Biochemistry **55,** 6751-6765 https://doi.org/10.1021/acs.biochem.6b00194
Parisini E, Metrangolo P, Pilati T, Resnati G, Terraneo G (2011): Halogen bonding in halocarbon-protein complexes: a structural survey. Chem. Soc. Rev. **40,** 2267-2278 https://doi.org/10.1039/c0cs00177e
Passamonti S, Terdoslavich M, Franca R, Vanzo A, Tramer F, Braidot E, Petrussa E, Vianello A (2009): Bioavailability of flavonoids: a review of their membrane transport and the function of bilitranslocase in animal and plant organisms. Curr. Drug Metab. **10,** 369-394 https://doi.org/10.2174/138920009788498950
Pick U, Weiss M (1985): Spectral and catalytical properties of the sarcoplasmic reticulum Ca-ATPase labeled with N-cyclohexyl-N'-(4-dimethylamino-1-naphthyl)-carbodiimide. Eur. J. Biochem. **152,** 83-89 https://doi.org/10.1111/j.1432-1033.1985.tb09166.x
Post RL, Hegyvary C, Kume S (1972): Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. J. Biol. Chem. **247,** 6530-6540 https://doi.org/10.1016/S0021-9258(19)44725-X
Rafał IG, Króliczewski BJ, Górniak I, Bartoszewski R, Króliczewski ÁJ (2018): Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem. Rev. **18,** 241-272 https://doi.org/10.1007/s11101-018-9591-z
Rui H, Das A, Nakamoto R, Roux B (2018) Proton countertransport and coupled gating in the sarcoplasmic reticulum calcium pump. J. Mol. Biol. **430,** 5050-5065 https://doi.org/10.1016/j.jmb.2018.10.014

Shoshan V, MacLennan DH (1981): Quercetin interaction with the (Ca2+ + Mg2+)-ATPase of sarcoplasmic reticulum. J. Biol. Chem. **256,** 887-892
https://doi.org/10.1016/S0021-9258(19)70062-3

Soltesova-Prnova M, Milackova I, Stefek M (2016): 3'-O-(3-Chloropivaloyl)quercetin, α-glucosidase inhibitor with multi-targeted therapeutic potential in relation to diabetic complications. Chem. Pap. **70,** 1439-1444
https://doi.org/10.1515/chempap-2016-0078

Taylor JS, Hattan D (1979): Biphasic kinetics of ATP hydrolysis by calcium-dependent ATPase of the sarcoplasmic reticulum of skeletal muscle. Evidence for a nucleoside triphosphate effector site. J. Biol. Chem. **254,** 4402-4407
https://doi.org/10.1016/S0021-9258(17)30022-4

Toyoshima C, Nakasako M, Nomura H, Ogawa H (2000): Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 A resolution. Nature **405,** 647-655
https://doi.org/10.1038/35015017

Toyoshima C, Nomura H, Sugita Y (2003): Structural basis of ion pumping by Ca2+-ATPase of sarcoplasmic reticulum. FEBS Lett. **555,** 106-110
https://doi.org/10.1016/S0014-5793(03)01086-X

Vazhappilly CG, Ansari SA, Al-Jaleeli R, Al-Azawi AM, Ramadan WS, Menon V, Hodeify R, Siddiqui SS, Merheb M, Matar R, Radhakrishnan R (2019): Role of flavonoids in thrombotic, cardiovascular, and inflammatory diseases. Inflammopharmacology **27,** 863-869
https://doi.org/10.1007/s10787-019-00612-6

Velasco-Guillén I, Corbalán-García S, Gómez-Fernández JC, Teruel JA (1998): Location of N-cyclohexyl-N'-(4-dimethylamino-alpha-naphthyl)carbodiimide- binding site in sarcoplasmic reticulum Ca2+-transporting ATPase. FEBS J. **253,** 339-344
https://doi.org/10.1046/j.1432-1327.1998.2530339.x

Veverka M, Gallovic J, Svajdlenka E, Veverkova E, Pronayova N, Milackova I, Stefek M (2013): Novel quercetin derivatives: synthesis and screening for anti-oxidant activity and aldose reductase inhibition. Chem. Pap. **67,** 76-83
https://doi.org/10.2478/s11696-012-0240-5

Viner RI, Williams TD, Schöneich C (1999): Peroxynitrite modification of protein thiols: oxidation, nitrosylation, and S-glutathiolation of functionally important cysteine residue(s) in the sarcoplasmic reticulum Ca-ATPase. Biochemistry **38,** 12408-12415
https://doi.org/10.1021/bi9909445

Wang S, Gopinath T, Larsen EK, Weber DK, Walker C, Uddigiri VR, Mote KR, Sahoo SK, Periasamy M, Veglia G (2021): Structural basis for sarcolipin's regulation of muscle thermogenesis by the sarcoplasmic reticulum Ca2+-ATPase. Sci. Adv. **7,** 1-11
https://doi.org/10.1126/sciadv.abi7154

Warren GB, Toon PA, Birdsall NJ, Lee AG, Metcalfe JC (1974a): Reconstitution of a calcium pump using defined membrane components. Proc. Natl. Acad. Sci. USA **71,** 622-626
https://doi.org/10.1073/pnas.71.3.622

Warren GB, Toon PA, Birdsall NJ, Lee AG, Metcalfe JC (1974b): Reversible lipid titrations of the activity of pure adenosine triphosphatase-lipid complexes. Biochemistry **13,** 5501-5507
https://doi.org/10.1021/bi00724a008

Williams RJ, Spencer JPE, Rice-Evans C (2004): Flavonoids: antioxidants or signalling molecules? Free Radic. Biol. Med. **36,** 838-849
https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Wray S, Burdyga T (2010): Sarcoplasmic reticulum function in smooth muscle. Physiol. Rev. **90,** 113-1478
https://doi.org/10.1152/physrev.00018.2008

Zhang XC, Zhang H (2019): P-type ATPases use a domain-association mechanism to couple ATP hydrolysis to conformational change. Biophys. Rep. **5,** 167-175
https://doi.org/10.1007/s41048-019-0087-1

Zizkova P, Blaskovic D, Majekova M, Svorc L, Rackova L, Ratkovska L, Veverka M, Horáková L (2014): Novel quercetin derivatives in treatment of peroxynitrite-oxidized SERCA1. Mol. Cell. Biochem. **386,** 1-14
https://doi.org/10.1007/s11010-013-1839-8

Zizkova P, Stefek M, Rackova L, Prnova M, Horakova L (2017): Novel quercetin derivatives: From redox properties to promising treatment of oxidative stress related diseases. Chem. Biol. Interact. **265,** 36-46
https://doi.org/10.1016/j.cbi.2017.01.019

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