

In Silico Modeling and Quantification of Synergistic Effects of Multi-Combination Compounds:
Case Study of the Attenuation of Joint Pain Using a Combination of Phytonutrients

V.A. Shiva Ayyadurai^{1*}, Prabhakar Deonikar¹

¹Systems Biology Group, CytoSolve Research Division, CytoSolve, Inc., Cambridge, MA, USA

Supplementary Information

Supplementary Material 1
CytoSolve® Operating Guide Protocol Summary

1.1. Introduction to CytoSolve® System

CytoSolve is a well-established computational systems biology framework of technology and processes that provides the capability to derive molecular mechanisms of action, to create quantitative and predictive models of those mechanisms, and to employ resultant models to simulate complex biomolecular phenomena [1–5]. In neurovascular studies, the CytoSolve framework elicited and derived a multi-layered engineering molecular systems architecture by integrating the anatomy of the neurovascular unit, molecular mechanisms, and disease to demonstrate the commonality of multiple neurovascular diseases as communication dysfunctions in common molecular signaling sub-systems and compounds [4].

In oncology, CytoSolve's capability has been employed for the in silico modeling of pancreatic cancer to identify and optimize a multi-combination therapeutic that was subsequently allowed for clinical trials by the United States Food and Drug Administration [6] and has been used to identify the molecular systems architecture of interactome in acute myeloid leukemia (AML) microenvironment [7]; moreover, it has been independently recognized by leading cancer researchers as a platform for developing multi-combination therapies [3]. In cardiovascular

research, CytoSolve has been used to accurately model the release of nitric oxide (NO) production in endothelial cells subjected to shear stress [5].

In the area of plant biology, CytoSolve enabled a quantitative molecular systems understanding of C1 metabolism—a critical system of molecular pathways inherent to all plants, fungi, and bacteria—to understand the systemic effects oxidative stress and genetic modification on C1 metabolism in soy [8–11]. Recently, CytoSolve was used to discover and model the mechanisms of immunomodulatory effect of bioactive compound in green tea on organ transplant tolerance [12] and elucidated the effect of bioactive compounds from fruit, berry, and vegetable (FBV) juice power on low-grade chronic inflammation [13].

1.2. CytoSolve® System Capabilities

The method used in this study provides a scalable computational framework for modeling large-scale biological systems by a dynamic integration of an ensemble of multiple molecular pathway models [1]. This method enables the development of large-scale models of complex biological systems that span multiple temporal and spatial scales as well as across diverse domains. Rather than attempting to monolithically model systems of biochemical reactions, a distributed engineering systems approach—a relatively novel concept in systems biology—is employed that breaks a large scale biological system into an ensemble of smaller molecular pathway models that are computationally coupled. This approach makes the modeling of large-scale biological systems both tractable and scalable.

1.3. Key Elements of CytoSolve® System Protocol

There are six (6) steps that comprise the protocol to use the CytoSolve® system. Fig. A1 illustrates the steps of the protocol.

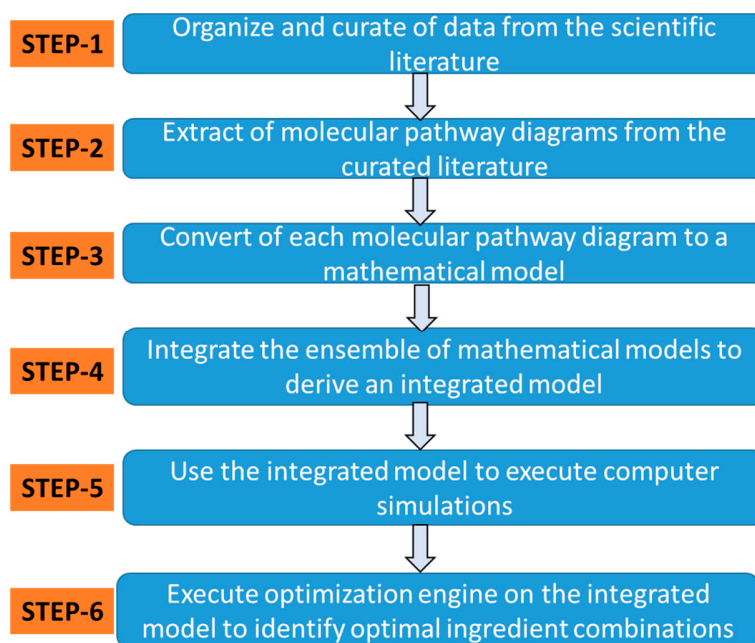


Figure S1: CytoSolve® Protocol Overview. The six steps involved in the CytoSolve® protocol. Steps 1 and 2 relate to performing a systematic literature review to identify molecular pathways and the biochemical parameters required for computational modeling. Steps 3 to 5 relate to the construction of individual models, the integration of individual models, and executing simulations using the integrated modes. Step 6 provides the option to employ a CytoSolve® optimization engine to discover the optimal combination of inputs (ingredients/compounds) for a specific range of values of outputs (biomarkers associated with particular biomolecular functions).

The six (6) steps are listed below:

- 1) Organize and curate data from the scientific literature (Section 1.4.);
- 2) Extract molecular pathway diagrams from the curated literature (Section 1.5.);
- 3) Convert each molecular pathway diagram to a mathematical model (Section 1.6.);
- 4) Integrate individual mathematical models to derive an integrated model (Section 1.7.);
- 5) Use the integrated model to execute computer simulations to analyze the effect of ingredients on interest, individually as well as in combination (Section 1.8.);

- 6) Execute optimization engine on the integrated model to identify optimal ingredient combination (Section 1.9.).

1.4. CytoSolve® Protocol for the Organization and Curation of Data from the Scientific Literature

This protocol step is the first step of the overall CytoSolve® Protocol indicated in Fig.A1. In this step, the scientific literature is searched to identify journal papers that contain research on the *area of interest*, i.e., inflammation, apoptosis, joint pain, cardiovascular health, etc. For a particular area of interest and *ingredients of interest*, molecular pathways for the area of interest and the effect of ingredients of interest on those molecular pathways are identified and organized.

Four (4) specific steps are executed as per the CytoSolve® Protocol to organize and curate the journal papers, as itemized below:

- i. Create a list of Medical Subject Headings (MeSH) *keywords* to optimize recall and the precision of peer-reviewed articles. The keywords are typically a list of words in combination with Boolean operators i.e. “AND”, “OR”, etc.
- ii. Search and retrieve the relevant peer-reviewed articles published during a specific *time period* from PubMed, Medline, and Google Scholar. These sets of articles are stored as an “Initial Set” repository. The time period is a date range, i.e., “January 1989 to April 2022,” etc.
- iii. Screen the titles and abstracts of articles in the Initial Set repository to identify most relevant articles based on our inclusion criteria. These sets of articles are stored as the “Final Set” repository.
- iv. Perform a full-length review of peer-reviewed articles from the Final Set repository.

Abstracts and unpublished literature were not sought as they have not been peer reviewed adequately to authenticate their results.

Articles obtained from the systematic literature review are categorized into three categories: 1) articles related to molecular pathways implicated in the area of interest, 2) articles related to interactions of ingredients of interest with molecular targets in the area of interest pathways, and 3) articles related to pharmacokinetic properties of the ingredients of interest.

1.5. CytoSolve® Protocol for Extraction of Data from the Scientific Literature

Journal articles in Group 1 are reviewed to gather data relevant to molecular pathways involved in the area of interest. The steps to extract and represent molecular pathways diagrammatically are itemized below:

1. Identify and extract:
 - a. Chemical species involved in the molecular pathways;
 - b. Types of cells;
 - c. Cellular components (e.g., cytosol, mitochondria, nucleus, etc.) where the chemical species are present in each cell type;
2. Identify and diagrammatically represent biochemical interactions;
3. Interconnect biochemical reactions to create molecular pathway diagram in each cell type.

Journal articles in Group 2 are reviewed to gather data relevant to pharmacokinetics of ingredients of interest.

Journal articles in Group 3 are reviewed to extract the following information:

1. Reaction rate constants of biochemical reactions involved in molecular pathways;
2. Molecular targets of ingredients of interest in the molecular pathways of interest.

The kinetic parameters used in this study are derived using the principles of Michaelis–Menten kinetics that are based on the steady-state approximation of biochemical reactions [14,15]. This information is provided in Sections B1, B2, B3, and B4 of Appendix B.

1.6. CytoSolve® Protocol for the Setup of Individual In Silico Mathematical Models

The steps to convert molecular pathway diagrams to mathematical models are itemized below:

1. Convert biochemical reactions involved in each of the molecular pathway into ordinary differential equations (mathematical expressions that describe the rate of change);
2. Represent each molecular pathway as a system of ordinary differential equations;
3. Encode the system of differential equations in a computer software source-code format known as Systems Biology Markup Language (SBML)[16] to construct a mathematical model for a particular molecular pathway;
4. Store each model as a separate SBML file.

1.7. CytoSolve® Protocol for the Integration of Individual In Silico Mathematical Models

In order to create an integrative quantitative model of the area of interest, it is necessary to mathematically couple the solutions across the ensemble of individual molecular pathway models. Such mathematical coupling is performed using the CytoSolve [2,12,17] computational engine, which is described in detail in Ayyadurai and Dewey, 2011[1], and Ayyadurai et al., 2022. The computational architecture of CytoSolve enables the integration of a plurality of molecular pathway models [1,17].

The steps to integrate the ensemble of individual mathematical models are listed below:

1. Upload individual SBML files, constructed in Section 1.6., to the CytoSolve engine;
2. Update the initial conditions for the molecular species in all mathematical models in the graphical user interface;
3. Update the simulation period specified in the graphical user interface;
4. Review and confirm molecular species and reaction duplicates across all individual models in the graphical user interface;
5. Commence the integration of individual models.

For a computational system biology analysis wherein the simulation of biochemical reactions is being executed and the governing equations are well known, as is in this case, the error bounds are set prior to executing simulations [18,19]. Herein, the error bounds are set to 10^{-6} prior to the execution of simulations. This means that the solutions to the governing equations used in the simulation of specific biochemical reactions must be within these error bounds. Therefore, in silico—computational—results from such simulations will not have error bars, which customarily appear in results reported from in vitro and in vivo experimental studies [20].

1.8. CytoSolve® Protocol for the Simulation of an Integrated In Silico Mathematical Model

The following steps are performed to execute the computer simulations:

1. Input biochemical reactions for interaction between ingredients of interest and molecular pathways involved in the area of interest;
2. Input the kinetic rate constants for each of biochemical reaction;
3. Input the initial concentrations for each of the molecular species in the biochemical reaction;
4. Input the time period for the simulation of integrative models and dose levels of the ingredients of interest;
5. Execute the integrative model under control conditions;
6. Execute the integrative model in the presence of the ingredients of interest individually;
7. Execute the integrative model in the presence of the combination of ingredients.

The steps for analyzing simulation output data are itemized below:

1. Export the raw data to Microsoft Excel;
2. Extract the steady state levels of biomarkers;
3. Plot steady state levels of biomarkers as a function of simulation time in the presence and absence of ingredients of interest, individually as well as in combination.

1.9. CytoSolve® Protocol to Execute the Optimization Engine on the Integrated Model to Identify Optimal Ingredient Combinations

The following steps were performed to execute the optimization engine:

1. Simulate the integrative model in the presence of the ingredients of interest;

2. Select “Optimize Results” on the CytoSolve® graphical user interface to initiate the optimization engine;
3. In the optimization engine, choose “Objective” for the ingredients of interest and the biomarkers affected by the ingredients of interest. The objective is set to “optimize” for the ingredients of interest, “maximize” for the biomarkers that are related to positive outcome, and “minimize” for the biomarkers that are related to negative outcomes;
4. Execute the optimization engine and record the results.

The steps for analyzing simulation output data are itemized below:

1. Export the raw data to Microsoft Excel;
2. Extract the values of ingredients of interest that have either minimized or maximized the biomarker.

1.10. CytoSolve® System Constraints

Although the framework developed in this study provides a detailed mechanistic understanding of joint pain that matches well with published clinical data, some model components’ parameters were derived from experiments using different cell types, as well as different experimental conditions such as variations in culture conditions, which adds to the uncertainty of the model’s predictions [21]. Such issues of parameter estimation, however, are not unique to this study. They are common to a number of cellular mathematical models [21] and do warrant further experimental investigation and validation.

Supplementary Material 2

Details of mathematical models used to assess the effects of apigenin and hesperidin on joint pain are given below. Four mathematical models were constructed to represent four different signaling transduction pathways involved in joint pain. For each models, the section below lists the initial conditions for the parameters, biochemical reactions, the corresponding rate equations, and the rate constants.

Section 2.1. contains the information used to model the COX-2 synthesis pathway involved in joint pain in cartilage that is initiated by IL-1 β and leads to the formation of COX-2.

Section 2.2. contains the information used to model the arachidonic acid metabolism pathway involved in joint pain in the cartilage that leads to the formation of PGE2.

Section 2.3. contains the information used to model the PGE2 signaling pathway involved in joint pain in cartilage that leads to the activation of TRPV1 and the expression of CGRP.

Section 2.4. contains the information used to model the oxidative stress pathway involved in endothelial cells that leads to the formation of ROS.

Additional literature was identified to obtain biochemical reactions, kinetic parameters, and initial conditions to simulate in silico models and is available in the bibliography of Supplementary Information.

2.1. In Silico Modeling of COX-2 Synthesis Pathway

Table S1. Initial Concentrations

Species	Value (nM)	Reference
IL-1 β	0.00323	Imamura et al., 2014 [22]
IL1 β R	150.0	Kablar & Kvrgi, 2012 [23]
myD88	50.0	Kablar & Kvrgi, 2012 [23]
IRAK	50.0	Kablar & Kvrgi, 2012 [23]
TRAF6	50.0	Kablar & Kvrgi, 2012 [23]
IKK	100.0	Kablar & Kvrgi, 2012 [23]
IKB(a)-NF κ B	100.0	Kablar & Kvrgi, 2012 [23]

Glossary:

IL-1 β —Interleukin 1 beta

IL1 β R—Interleukin 1 beta receptor

MyD88—myeloid differentiation primary-response protein 88

IRAK—Interleukin-1 receptor-associated kinase 1

TRAF6—TNF Receptor Associated Factor 6

NF κ B—Nuclear factor kappa-light-chain-enhancer of activated B cells

IKK—I κ B kinase

Table S2. Biochemical Reactions and their Rate Equations

Description	Rate Equation
$[IL-1\beta] + [IL1\beta R] \leftrightarrow [IL-1\beta_IL-1\beta R]$	$[IL-1\beta] * [IL1\beta R] * a14 - [IL-1\beta_IL-1\beta R] * d18$
$[IL-1\beta_IL-1\beta R] + [myD88] \leftrightarrow IL-1\beta_IL-1\beta R_myD88 * d15$	$[IL-1\beta_IL-1\beta R] * myD88 * a15 - [IL-1\beta_IL-1\beta R_myD88] * d15$
$[IL-1\beta_IL-1\beta R_myD88] + [IRAK] \leftrightarrow [IL-1\beta_IL-1\beta R_myD88_IRAK]$	$[IL-1\beta_IL-1\beta R_myD88] * [IRAK] * a16 - [IL-1\beta_IL-1\beta R_myD88_IRAK] * d16$
$[IL-1\beta_IL-1\beta R_myD88_IRAK] + [TRAF6] \leftrightarrow [IL-1\beta_IL-1\beta R_myD88_IRAK_TRAF6]$	$[IL-1\beta_IL-1\beta R_myD88_IRAK] * [TRAF6] * a17 - [IL-1\beta_IL-1\beta R_myD88_IRAK_TRAF6] * d17$
$[IKK] * [IL-1\beta_IL-1\beta R_myD88_IRAK_TRAF6] \leftrightarrow [IL-1\beta_IL1R_myD88_IRAK_TRAF6_IKK]$	$[IKK * IL-1\beta_IL-1\beta R_myD88_IRAK_TRAF6] * a18 - [IL-1\beta_IL1R_myD88_IRAK_TRAF6_IKK] * d18$
$[IL-1\beta_IL1R_myD88_IRAK_TRAF6_IKK] \rightarrow [IKK(a)] + [IL-1\beta_IL-1\beta R_myD88_IRAK_TRAF6]$	$[IL-1\beta_IL1R_myD88_IRAK_TRAF6_IKK] * (1 + ROS^*) * k19$
$\Phi \rightarrow [COX-2\ mRNA_N]$	$[NF\kappa B [N]] * k_{syn_COX2_mRNA}$

$[\text{COX-2 mRNA}_C] \rightarrow \Phi$	$[\text{COX-2 mRNA}_C] * \text{kdegCOX2mRNA}$
$[\text{COX-2 mRNA}_N] \rightarrow [\text{COX-2 mRNA}_C]$	$[\text{COX-2 mRNA}_N] * \text{ktrans}$
$\Phi \leftrightarrow [\text{COX-2}]$	$[\text{COX-2 mRNA}_C] * \text{ksyn_COX2_protein} - [\text{COX-2}] * \text{kdegCOX2protein}$
$[\text{IKK (a)}] + [\text{IKB(a)}] \leftrightarrow [\text{IKK-IKB(a)}]$	$a1 * [\text{IKK (a)}] * [\text{IKB(a)}] - [\text{IKK-IKB(a)}] * d1$
$[\text{NFkB}] + [\text{IKK-IKB(a)}] \leftrightarrow [\text{IKK-IKB(a)-NFkB}]$	$a4 * [\text{NFkB}] * [\text{IKK-IKB(a)}] - [\text{IKK-IKB(a)-NFkB}] * d4$
$[\text{IKB(a)}] \rightarrow \Phi$	$\text{deg1} * [\text{IKB(a)}]$
$[\text{IKK (a)}] \rightarrow \Phi$	$k02 * [\text{IKK (a)}]$
$[\text{IKK-IKB(a)}] \rightarrow [\text{IKK (a)}]$	$r1 * [\text{IKK-IKB(a)}]$
$[\text{NFkB [N]}] \rightarrow [\text{NFkB}]$	$[\text{NFkB [N]}] * k01$
$[\text{NFkB}] + [\text{IKB(a)}] \leftrightarrow [\text{IKB(a)-NFkB}]$	$[\text{NFkB}] * [\text{IKB(a)}] * a4 - [\text{IKB(a)-NFkB}] * d4$
$\Phi \rightarrow [\text{IKB(a)}]$	$[\text{tr1}] * [\text{IKB(a)mRNA}]$
$\Phi \rightarrow [\text{IKB(a)mRNA}]$	$[\text{tr2a} + \text{tr2}] * [\text{NFkB [N]}] * [\text{NFkB [N]}]$
$[\text{IKB(a)mRNA}] \rightarrow \Phi$	$[\text{tr3}] * [\text{IKB(a) mRNA}]$

Table S3: Chemical Kinetic Parameters

Parameter	Value	Unit	Reference
a1	$2.25 \cdot 10^{-5}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a2	$6.0 \cdot 10^{-6}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a3	$9.0 \cdot 10^{-6}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a4	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a5	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a6	$5.00 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a7	$1.85 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a8	$4.8 \cdot 10^{-5}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
a9	$7.0 \cdot 10^{-5}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
d1	0.00125	S^{-1}	Hoffmann et al., 2002 [24]
d2	0.00175	S^{-1}	Hoffmann et al., 2002 [24]
d3	0.00175	S^{-1}	Hoffmann et al., 2002 [24]
d4	$5.0 \cdot 10^{-4}$	S^{-1}	Hoffmann et al., 2002 [24]
r1	0.00407	S^{-1}	Hoffmann et al., 2002 [24]
r4	0.0204	S^{-1}	Hoffmann et al., 2002 [24]
r6	0.011	S^{-1}	Hoffmann et al., 2002 [24]
deg1	$1.00 \cdot 10^{-4}$	S^{-1}	Hoffmann et al., 2002 [24]
tr2a	0.0015	nM S^{-1}	Hoffmann et al., 2002 [24]
tr1	0.004	S^{-1}	Hoffmann et al., 2002 [24]

tr2	$1.7 \cdot 10^{-5}$	$\text{nM}^{-1} \text{S}^{-1}$	Hoffmann et al., 2002 [24]
tr3	$2.8 \cdot 10^{-4}$	S^{-1}	Hoffmann et al., 2002 [24]
tp1	$3.0 \cdot 10^{-4}$	S^{-1}	Hoffmann et al., 2002 [24]
tp2	$2.0 \cdot 10^{-4}$	S^{-1}	Hoffmann et al., 2002 [24]
k02	$1.2 \cdot 10^{-4}$	S^{-1}	Hoffmann et al., 2002 [24]
k22	0.07	S^{-1}	Estimated
d11	$8.0 \cdot 10^{-5}$	S^{-1}	Kablar & Kvrgi, 2012 [23]
a12	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Kablar & Kvrgi, 2012 [23]
d12	$5.0 \cdot 10^{-4}$	S^{-1}	Kablar & Kvrgi, 2012 [23]
a14	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Kablar & Kvrgi, 2012 [23]
a15	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Kablar & Kvrgi, 2012 [23]
a16	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Kablar & Kvrgi, 2012 [23]
a17	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Kablar & Kvrgi, 2012 [23]
a18	$5.0 \cdot 10^{-4}$	$\text{nM}^{-1} \text{S}^{-1}$	Kablar & Kvrgi, 2012 [23]
d14	$5.0 \cdot 10^{-4}$	S^{-1}	Kablar & Kvrgi, 2012 [23]
d15	$5.0 \cdot 10^{-4}$	S^{-1}	Kablar & Kvrgi, 2012 [23]
d16	$5.0 \cdot 10^{-4}$	S^{-1}	Kablar & Kvrgi, 2012 [23]
d17	$5.0 \cdot 10^{-4}$	S^{-1}	Kablar & Kvrgi, 2012 [23]
d18	$5.0 \cdot 10^{-4}$	S^{-1}	Kablar & Kvrgi, 2012 [23]
k19	$1.2 \cdot 10^{-7}$	$\text{nM}^{-1} \text{S}^{-1}$	Kablar & Kvrgi, 2012 [23]
k13	0.0138	S^{-1}	Kablar & Kvrgi, 2012 [23]
ksyn COX2 mRNA	$1.352 \cdot 10^{-6}$	S^{-1}	Boerboom et al., 2004 [25]
ksyn COX2 mRNA	0.00002657	S^{-1}	Learn et al., 2000 [26]
ksyn COX2 protein	0.00168	S^{-1}	Boerboom et al., 2004 [25]
k18	16.666	S^{-1}	Yang et al., 2007 [27]
ki3	30000.0	nM	Yang et al., 2007 [27]
ks	500000.0	nM	Yang et al., 2007 [27]
k20	50.0	S^{-1}	Yang et al., 2007 [27]
ki1	300.0	nM	Yang et al., 2007 [27]
kdegCOX2protein	$1.6 \cdot 10^{-5}$	S^{-1}	Yang et al., 2007 [27]
kdegCOX2mRNA	$9.625 \cdot 10^{-5}$	nM	Yang et al., 2007 [27]
ki hespt NFkB	5000	nM	Yang et al., 2007 [27]
ki hespd Nfkb	25000	nM	Ghorbani et al., 2012 [28]
ki Api IKK	10000	nM	Shukla et al., 2015 [29]

2.2. In Silico Modeling of Arachidonic Acid Metabolism Pathway

Table S4. Initial Concentrations

Species	Value (nM)	Reference
5-LOX	5000	Yang et al., 2007 [27]
15-LOX	1500	Yang et al., 2007 [27]
PLA2	1500	Yang et al., 2007 [27]
PGHPx	800	Yang et al., 2007 [27]
12-LOX	500	Yang et al., 2007 [27]
TXAS	500	Yang et al., 2007 [27]
PGES	500	Yang et al., 2007 [27]
COX-2	200	Yang et al., 2007 [27]
Arachidonic acid	34716.0	King et al., 2008 [30]

Glossary:

5-LOX—5-lipoxygenase

15-LOX—15-lipoxygenase

PLA2—Phospholipase A2

PGHPx—Phospholipid-hydroperoxide glutathione peroxidase

12-LOX—12-lipoxygenase

TXAS—Thromboxane-A synthase

PGES—Prostaglandin synthetase

COX-2—Cyclooxygenase 2

PGE2—Prostaglandin E2

Table S5: Biochemical Reactions and their Rate Equations

Reaction	Rate Equation
$\Phi \leftrightarrow [\text{Arachidonic acid}]$	$\frac{\text{lin} \cdot K15 \cdot [\text{PLA2}]}{(\text{lin} + k15 \cdot (1 + \frac{[\text{Arachidonic acid}]}{Ks} + \frac{[12\text{-HPETE}]}{K19} + \frac{[15\text{-HPETE}]}{K120} + \frac{[\text{LTA4}]}{K121} + \frac{[5\text{-HETE}]}{K122})) \cdot -[\text{Arachidonic acid}] \cdot kd17}$
$[12\text{-LOX}] + [\text{Arachidonic acid}] \rightarrow [12\text{-HPETE}]$	$\frac{K17 \cdot [12\text{-LOX}] \cdot [\text{Arachidonic acid}]}{(k17 \cdot (1 + \frac{[12\text{-HPETE}]}{ki18} + \frac{[15\text{-HETE}]}{ki16}) + [\text{Arachidonic acid}])}$
$[12\text{-LOX}] + [15\text{-HPETE}] \rightarrow \Phi$	$ki17 \cdot [12\text{-LOX}] \cdot [15\text{-HPETE}]$
$\text{PGHPx} + [12\text{-HPETE}] \rightarrow [12\text{-HETE}]$	$\frac{[\text{PGHPx}] \cdot [12\text{-HPETE}] \cdot K24}{(k24 \cdot (1 + \frac{[12\text{-HETE}]}{Ks}) + [12\text{-HPETE}])}$
$\Phi \leftrightarrow [15\text{-LOX}]$	$\frac{a24 \cdot [\text{PGE2}] \cdot [\text{PGE2}]}{([\text{PGE2}] \cdot [\text{PGE2}] + K124 \cdot K124)} - kd16 \cdot [15\text{-LOX}]$
$[15\text{-LOX}] + [\text{Arachidonic acid}] \rightarrow [15\text{-HPETE}]$	$\frac{K16 \cdot [15\text{-LOX}] \cdot [\text{Arachidonic acid}]}{(k16 \cdot (1 + \frac{[15\text{-HPETE}]}{Ks}) + [\text{Arachidonic acid}])}$
$[\text{PGHPx}] + [15\text{-HPETE}] \rightarrow [15\text{-HETE}]$	$\frac{K24 \cdot [\text{PGHPx}] \cdot [15\text{-HPETE}]}{(k24 \cdot (1 + \frac{[15\text{-HETE}]}{Ks}) + [15\text{-HPETE}])}$
$[15\text{-HPETE}] \rightarrow \Phi$	$kd2 \cdot [15\text{-HPETE}]$
$[15\text{-HETE}] \rightarrow \Phi$	$kd3 \cdot [15\text{-HETE}]$
$\Phi \leftrightarrow [5\text{-LOX}]$	$K123 \cdot [5\text{-LOX}] - (ki10 \cdot [5\text{-HPETE}] \cdot [5\text{-LOX}] + ki6 \cdot [5\text{-LOX}] \cdot [15\text{-HPETE}] + [\text{LTA4}] \cdot [5\text{-LOX}] \cdot ki9)$
$[\text{Arachidonic acid}] + [5\text{-LOX}] \rightarrow [5\text{-HPETE}]$	$\frac{K21 \cdot [\text{Arachidonic acid}] \cdot [5\text{-LOX}]}{([\text{Arachidonic acid}] + k21 \cdot (1 + \frac{[5\text{-HPETE}]}{Ks} + \frac{[12\text{-HETE}]}{ki7} + \frac{[15\text{-HETE}]}{ki8} + \frac{[\text{PGE2}]}{ki11} + \frac{[5\text{-HETE}]}{ki12}))}$
$[\text{PGHPx}] + [5\text{-HPETE}] \rightarrow [5\text{-HETE}]$	$\frac{K24 \cdot [\text{PGHPx}] \cdot [5\text{-HPETE}]}{(k24 \cdot (1 + \frac{[5\text{-HETE}]}{Ks}) + [5\text{-HPETE}])}$
$[5\text{-LOX}] + [5\text{-HPETE}] \rightarrow [\text{LTA4}]$	$\frac{K21 \cdot [5\text{-LOX}] \cdot [5\text{-HPETE}]}{((k21 \cdot (1 + \frac{[\text{LTA4}]}{Ks} + \frac{[12\text{-HETE}]}{ki7} + \frac{[15\text{-HETE}]}{ki8} + \frac{[\text{PGE2}]}{ki11} + \frac{[5\text{-HETE}]}{ki12}) + [5\text{-HPETE}]) \cdot (1 + \text{Hesperetin}/kihespLoX + \text{Apigenin}/kiApi5lox))}$
$[\text{COX-2}] + [\text{Arachidonic acid}] \rightarrow [\text{PGH2}]$	$\frac{K18 \cdot [\text{COX-2}] \cdot [\text{Arachidonic acid}]}{([\text{Arachidonic acid}] + k18 \cdot (1 + \frac{[\text{PGE2}]}{ki3} + \frac{[\text{PGH2}]}{Ks}))}$

$[PGES] + [PGH2] \rightarrow [PGE2]$	$\frac{K19 * [PGES] * [PGH2]}{(k19 * (1 + \frac{[PGE2]}{ks} + \frac{[Arachidonic\ acid]}{ki1} + \frac{[15-HETE]}{ki2}) + [PGH2])}$
$[TXAS] + [PGH2] \rightarrow [TXA2]$	$\frac{K20 * [TXAS] * [PGH2]}{(k20 * (1 + \frac{[TXA2]}{ks}) + [PGH2])}$
$[TXAS] + [15-HPETE] + [PGH2] \rightarrow \Phi$	$ki4 * [15-HPETE] * [TXAS] + ki5 * [PGH2] * [TXAS]$
$[TXA2] \rightarrow [TXB2]$	$[TXA2] * kd8$
$[TXA2] \rightarrow \Phi$	$[TXA2] * kd8$
$[TXB2] \rightarrow \Phi$	$kd9 * [TXB2]$

Table S6: Chemical Kinetic Parameters

Parameter	Value	Unit	Reference
kd17	0.00167	S ⁻¹	Yang et al., 2007 [27]
K15	60	S ⁻¹	Yang et al., 2007 [27]
ks	500000	nM	Yang et al., 2007 [27]
KI22	500000	nM	Yang et al., 2007 [27]
KI21	500000	nM	Yang et al., 2007 [27]
KI20	200000	nM	Yang et al., 2007 [27]
KI19	500000	nM	Yang et al., 2007 [27]
k15	2600000	nM	Yang et al., 2007 [27]
lin	12000	nM	Yang et al., 2007 [27]
K17	16.67	S ⁻¹	Yang et al., 2007 [27]
ki18	10000	nM	Yang et al., 2007 [27]

ki16	10000	nM	Yang et al., 2007 [27]
k17	50000	nM	Yang et al., 2007 [27]
ki17	$1.67 \cdot 10^{-4}$	$\text{nM}^{-1}\text{S}^{-1}$	Yang et al., 2007 [27]
K24	8.33	S^{-1}	Yang et al., 2007 [27]
k24	70000	nM	Yang et al., 2007 [27]
kd16	$1.67 \cdot 10^{-4}$	S^{-1}	Yang et al., 2007 [27]
a24	150	nM	Yang et al., 2007 [27]
KI24	0.023	nM	Yang et al., 2007 [27]
K16	16.67	S^{-1}	Yang et al., 2007 [27]
k16	70000	nM	Yang et al., 2007 [27]
kd2	$8.33 \cdot 10^{-4}$	S^{-1}	Yang et al., 2007 [27]
kd3	$1.67 \cdot 10^{-4}$	S^{-1}	Yang et al., 2007 [27]
KI23	$8.833 \cdot 10^{-7}$	$\text{nM}^{-1}\text{S}^{-1}$	Yang et al., 2007 [27]
ki10	$1.67 \cdot 10^{-7}$	$\text{nM}^{-1}\text{S}^{-1}$	Yang et al., 2007 [27]
ki9	$2.9167 \cdot 10^{-6}$	$\text{nM}^{-1}\text{S}^{-1}$	Yang et al., 2007 [27]
ki6	$1.67 \cdot 10^{-7}$	$\text{nM}^{-1}\text{S}^{-1}$	Yang et al., 2007 [27]
K21	83.33	S^{-1}	Yang et al., 2007 [27]
ki12	6300	nM	Yang et al., 2007 [27]
ki11	15000	nM	Yang et al., 2007 [27]
ki8	4000	nM	Yang et al., 2007 [27]

ki7	30000	nM	Yang et al., 2007 [27]
k21	5000	nM	Yang et al., 2007 [27]
K18	16.67	S ⁻¹	Yang et al., 2007 [27]
ki3	30000	nM	Yang et al., 2007 [27]
k18	50000	nM	Yang et al., 2007 [27]
K19	50	S ⁻¹	Yang et al., 2007 [27]
ki2	30000	nM	Yang et al., 2007 [27]
ki1	300	nM	Yang et al., 2007 [27]
k19	160000	nM	Yang et al., 2007 [27]
K20	26.65	S ⁻¹	Yang et al., 2007 [27]
k20	4000	nM	Yang et al., 2007 [27]
ki5	1.67*10 ⁻⁶	nM ⁻¹ S ⁻¹	Yang et al., 2007 [27]
ki4	1.0*10 ⁻⁵	nM ⁻¹ S ⁻¹	Yang et al., 2007 [27]
kd8	0.00167	S ⁻¹	Yang et al., 2007 [27]
kd9	1.67*10 ⁻⁵	S ⁻¹	Yang et al., 2007 [27]
ki_hesp_LOX	10000	nM	Alcaraz and Ferrandiz, 1987 [31]
ki_Api_5lox	8000	nM	Franke and Schilche, 2005 [32]
kiApi_15lox	2000	nM	Franke and Schilche, 2005 [32]
kiapi_12lox	14000	nM	Franke and Schilche, 2005 [32]

2.3. In Silico Modeling of PGE2 Signaling in Chondrocytes

Table S7: Initial Concentrations

Species	Value (nM)	Ref
PIP2	10000	McLaughlin et al. 2002 [33]
ATP	8300	Estimated
ADP	6000	Estimated
PKA (i)	2040	Willoughby and Cooper, 2007 [34]
Rap1	1506	https://www.genecards.org/cgi-bin/carddisp.pl?gene=RAP1A&keywords=RAp1
Adenyl Cyclase (i)	590.0	Willoughby and Cooper, 2007 [34]
PLC(i)	200	Kawaguchi and Hirano, 2013 [35]
PKC	200	Wei et al., 2011 [36]
PDE4	200	Calebiro et al., 2009 [37]
PPAse	100	Markevich et al., 2004 [38]
epac	100	Salonikidis et al., 2008 [39]
EP4	34	Willoughby and Cooper, 2007 [34]
TRPV1	0.135	https://www.genecards.org/cgi-bin/carddisp.pl?gene=TRPV1&keywords=TRPV1
CREB	66	(Biggin, 2011) [40]
PMCA	2279	Www.genecards.org 2016b
Ca2+(e)	2000000	Dolan and Diamond, 2014 [41]
Ca2+(i-post)	75	Dolan and Diamond, 2014 [41]
CaMKII (i)	200	Saucerman and Bers, 2008
Calmodulin	26	Dolan and Diamond, 2014 [41]

Glossary

PIP2—Phosphatidylinositol bisphosphate

ATP—Adenosine triphosphate

ADP—Adenosine diphosphate

PKA (i) —Protein Kinase A

Rap1—Ras-associated protein-1

Adenyl Cyclase (i) —Adenyl cyclase inactive

PLC(i) —Phospholipase C

PKC—Protein Kinase C

PDE4—Phosphodiesterase

PPAse—Phosphotase

Epac—Exchange proteins activated by cAMP

EP4—Prostanoid Receptor

TRPV1—Transient receptor potential cation channel subfamily V member 1

CREB—CREB transcription factor

PMCA—Plasma membrane Ca²⁺ ATPase

$\text{Ca}^{2+}(\text{e})$ — Ca^{2+} concentration in extracellular space

$\text{Ca}^{2+}(\text{i-post})$ — Calcium concentration in cytosol

CaMKII (i) — Inactive Calcium calmodulin-dependent kinase

Table S8: Biochemical Reactions and Rate Equations

Description	Rate Equation
$[\text{Adenyl Cyclase (i)}] * [\text{EP4 active}] \leftrightarrow [\text{Adenyl Cyclase (a)}]$	$[\text{Adenyl Cyclase (i)}] * [\text{EP4 active}] * k_{AC_a} - [\text{Adenyl Cyclase (a)}] * k_{AC_r}$
$[\text{ATP}] + [\text{Adenyl Cyclase (a)}] \rightarrow [\text{cAMP}]$	$[\text{ATP}] * [\text{Adenyl Cyclase (a)}] * K_{camp} / (K_{mcamp} + [\text{ATP}])$
$[\text{EP4}] * [\text{PGE2 ECM}] \leftrightarrow [\text{EP4 active}]$	$[\text{EP4}] * [\text{PGE2 ECM}] * k_{bEP4} / (K_{pge2EP4} + [\text{PGE2 ECM}]) - k_{dEP4} * [\text{EP4 active}]$
$[\text{PKA (i)}] + [\text{cAMP}] \leftrightarrow [\text{PKA-cAMP}]$	$[\text{PKA (i)}] * [\text{cAMP}] * k_{campPKAf} - [\text{PKA-cAMP}] * k_{camppkar}$
$[\text{cAMP}] + [\text{PKA-cAMP}] \leftrightarrow [\text{PKA-[2CAMP]}]$	$[\text{cAMP}] * [\text{PKA-cAMP}] * k_{2camppkaf} - [\text{PKA-[2CAMP]}] * k_{2camppkar}$
$[\text{PKA-[2CAMP]}] + [\text{cAMP}] \leftrightarrow [\text{PKA-[3cAMP]}]$	$[\text{PKA-[2CAMP]}] * [\text{cAMP}] * k_{3camppkaf} - [\text{PKA-[3cAMP]}] * k_{3camppkar}$
$[\text{cAMP}] + [\text{PKA-3cAMP}] \leftrightarrow [\text{PKA (a)}]$	$[\text{cAMP}] * [\text{PKA-3cAMP}] * k_{3camppkaf} - [\text{PKA (a)}] * k_{3camppkar}$
$[\text{cAMP}] + [\text{PDE4}] \rightarrow [\text{AMP}]$	$[\text{cAMP}] * [\text{PDE4}] * k_{Pde4} / ([\text{cAMP}] + k_{mpde4})$
$[\text{PKA (a)}] + [\text{PKC_a}] + [\text{TRPV1}] \leftrightarrow [\text{TRPV1_P}]$	$([\text{PKA (a)}] + [\text{PKC_a}]) * [\text{TRPV1}] * k_{TRPV1senti} / (1 + 4) - [\text{TRPV1_P}] * k_{TRPV1desenti}$
$[\text{AMP}] + [\text{ATP}] \leftrightarrow [\text{ADP}]$	$[\text{AMP}] * [\text{ATP}] * k_{ATP_ADP} / (k_{mATP_ADP} + [\text{ATP}]) - [\text{ADP}] * [\text{ADP}] * k_{ADP_ATP} / (k_{mADP_ATP} + [\text{ADP}])$
$[\text{epac_cAMP}] \rightarrow [\text{epac_A}]$	$[\text{epac_cAMP}] * k_{act_epac}$
$[\text{epac_A}] + [\text{Rap1}] \rightarrow [\text{Rap1_a}]$	$[\text{Rap1}] * [\text{epac_A}] * k_{rap1_act} / ([\text{Rap1}] + k_{rap1a})$
$[\text{PLC(i)}] + [\text{Rap1_a}] \leftrightarrow [\text{PLC_Rap1}]$	$J_{21_k1} * [\text{PLC(i)}] * [\text{Rap1_a}] / (1 + [\text{Hesperetin}] / k_{i_hes_PLC}) - J_{21_k2} * [\text{PLC_Rap1}]$
$[\text{PLC_PIP2_Rap}] \rightarrow [\text{IP3}] + [\text{DAG}] + [\text{PLC_Rap1}]$	$J_{24_k1} * [\text{PLC_PIP2_Rap}]$

$[\text{PKC_DAG}] \rightarrow [\text{PKC_a}]$	$[\text{PKC_DAG}] * k_f \text{PKC_a} - [\text{PKC_a}] * k_b \text{PKC_a}$
$[\text{PKC}] + [\text{DAG}] \leftrightarrow [\text{PKC_DAG}]$	$[\text{PKC}] * [\text{DAG}] * k_f \text{PKC_DAG} / (1 + [\text{Apigenin}] / k_i \text{api_PKC}) - [\text{PKC_DAG}] * k_b \text{PKC_DAG}$
$[\text{PLC_Rap1}] + [\text{PIP2}] \leftrightarrow [\text{PLC_PIP2_Rap}]$	$[\text{PLC_Rap1}] * [\text{PIP2}] * J25_k1 - [\text{PLC_PIP2_Rap}] * J25_k2$
$[\text{Rap1_a}] + [\text{PPAse}] \rightarrow [\text{Rap1}]$	$[\text{Rap1_a}] * [\text{PPAse}] * K_{cat_MAPKdp} / (k_m \text{MAPK_dp} + [\text{Rap1_a}])$
$[\text{cAMP}] + [\text{epac}] \leftrightarrow [\text{epac_cAMP}]$	$[\text{cAMP}] * [\text{epac}] * k_{ass_Epac_camp} * scale4 - [\text{epac_cAMP}] * k_{diss_Epac_camp}$
$[\text{Ca2}] \rightarrow [\text{Ca2+(i-post)}]$	$[\text{Ca2}] \rightarrow [\text{Ca2+(i-post)}]$
$\log([\text{Ca2+(e)}] / [\text{Ca2+(i-post)}]) * DL * [\text{TRPV1_P}]$	$\log([\text{Ca2+(e)}] / [\text{Ca2+(i-post)}]) * DL * [\text{TRPV1_P}]$
$[\text{Ca2+(i-post)}] + [\text{calmodulin}] \leftrightarrow [\text{ca2_calmodulin}]$	$[\text{Ca2+(i-post)}] + [\text{calmodulin}] \leftrightarrow [\text{ca2_calmodulin}]$
$[\text{Ca2+(i-post)}] * [\text{Ca2+(i-post)}] * [\text{calmodulin}] * k_{ca2_calmodulin} / (1 + 75 * 75) - [\text{ca2_calmodulin}] * k_{ca2_calmodulin}$	$[\text{Ca2+(i-post)}] * [\text{Ca2+(i-post)}] * [\text{calmodulin}] * k_{ca2_calmodulin} / (1 + 75 * 75) - [\text{ca2_calmodulin}] * k_{ca2_calmodulin}$
$[\text{Ca2+(i-post)}] + [\text{ca2_calmodulin}] \leftrightarrow [\text{Ca4_calmodulin}]$	$[\text{Ca2+(i-post)}] + [\text{ca2_calmodulin}] \leftrightarrow [\text{Ca4_calmodulin}]$
$[\text{Ca2+(i-post)}] * [\text{Ca2+(i-post)}] * [\text{ca2_calmodulin}] * k_{ca4_calmodulin} / (1 + 75 * 75) - [\text{Ca4_calmodulin}] * k_{ca4_calmodulin}$	$[\text{Ca2+(i-post)}] * [\text{Ca2+(i-post)}] * [\text{ca2_calmodulin}] * k_{ca4_calmodulin} / (1 + 75 * 75) - [\text{Ca4_calmodulin}] * k_{ca4_calmodulin}$
$[\text{Ca4_calmodulin}] + [\text{CaMKii(i)}] \leftrightarrow [\text{CaMKII_cacaM_b}]$	$[\text{Ca4_calmodulin}] + [\text{CaMKii(i)}] \leftrightarrow [\text{CaMKII_cacaM_b}]$
$[\text{Ca4_calmodulin}] * [\text{CaMKii(i)}] * k_{IB} - [\text{CaMKII_cacaM_b}] * k_{BI}$	$[\text{Ca4_calmodulin}] * [\text{CaMKii(i)}] * k_{IB} - [\text{CaMKII_cacaM_b}] * k_{BI}$
$[\text{CaMKII_cacaM_b}] \rightarrow [\text{CaMKII_CaCaM_P}]$	$[\text{CaMKII_cacaM_b}] \rightarrow [\text{CaMKII_CaCaM_P}]$
$k_A * [\text{CaMKII_cacaM_b}]$	$k_A * [\text{CaMKII_cacaM_b}]$
$[\text{CaMKII_CaCaM_P}] \rightarrow [\text{CaMKII_cacaM_b}]$	$[\text{CaMKII_CaCaM_P}] \rightarrow [\text{CaMKII_cacaM_b}]$
$[\text{CaMKII_CaCaM_P}] * k_{PP1}$	$[\text{CaMKII_CaCaM_P}] * k_{PP1}$
$[\text{CaMKII_P_A}] \leftrightarrow [\text{CaMKII_a_N}]$	$[\text{CaMKII_P_A}] \leftrightarrow [\text{CaMKII_a_N}]$
$[\text{CaMKII_P_A}] * k_{CaMt} - [\text{CaMKII_a_N}] * k_{CaMtr}$	$[\text{CaMKII_P_A}] * k_{CaMt} - [\text{CaMKII_a_N}] * k_{CaMtr}$
$[\text{CREB}] \rightarrow [\text{CREB_P}]$	$[\text{CREB}] \rightarrow [\text{CREB_P}]$
$[\text{CREB}] * [\text{CaMKII_a_N}] * k_{CREB_P} / ([\text{CREB}] + k_m \text{CREB_P})$	$[\text{CREB}] * [\text{CaMKII_a_N}] * k_{CREB_P} / ([\text{CREB}] + k_m \text{CREB_P})$

Table S9: Chemical Kinetic Parameters

Parameter	Value	Units	References
J20_k1	0.1	nM ⁻¹ S ⁻¹	Greget et al. 2011 [42]
J20_k2	30	S ⁻¹	Greget et al. 2011 [42]
kfPKC_ca	3.00*10 ⁻⁴	nM ⁻¹ S ⁻¹	Wei et al., 2011 [36]
kbPKC_ca	0.01	S ⁻¹	Wei et al., 2011 [36]
kfPKC_DAG	3.00*10 ⁻⁴	nM ⁻¹ S ⁻¹	Wei et al., 2011 [36]
kbPKC_DAG	0.001	S ⁻¹	Wei et al., 2011 [36]
kfPKC_a	0.00178	nM ⁻¹ S ⁻¹	Wei et al., 2011 [36]
kbPKC_a	0.01	S ⁻¹	Wei et al., 2011 [36]
kfca_PLA2	0.001	nM ⁻¹ S ⁻¹	Chang et al., 2009 [43]
kbca_PLA2	0.1	S ⁻¹	Chang et al., 2009 [43]
k9	44.6	S ⁻¹	Wu et al., 2012 [44]
k9m	5400	nM	Wu et al., 2012 [44]
kAC_a	0.5	nM ⁻¹ S ⁻¹	Calebiro et al., 2009 [37]
kAC_r	1	S ⁻¹	Calebiro et al., 2009 [37]
Kcamp	8.5	S ⁻¹	Calebiro et al., 2009 [37]
Kmcamp	315000	nM	Calebiro et al., 2009 [37]
kcampPKAf	6.0*10 ⁻⁶	nM ⁻¹ S ⁻¹	Calebiro et al., 2009 [37]
kcamppkar	3.0*10 ⁻⁴	S ⁻¹	Calebiro et al., 2009 [37]
k2camppkaf	6.0*10 ⁻⁶	nM ⁻¹ S ⁻¹	Calebiro et al., 2009 [37]
k2camppkar	3.0*10 ⁻⁴	S ⁻¹	Calebiro et al., 2009 [37]
k3camppkaf	0.00835	nM ⁻¹ S ⁻¹	Calebiro et al., 2009 [37]
k3camppkar	0.0617	S ⁻¹	Calebiro et al., 2009 [37]
kPde4	8	S ⁻¹	Calebiro et al., 2009 [37]
kmpde4	1300	nM	Calebiro et al., 2009 [37]
kTRPV1senti	0.0012	S ⁻¹	Buschow, 2014 [45]

kTRPV1desenti	0.012	S ⁻¹	Buschow, 2014 [45]
kATP_ADP	63.33	nM S ⁻¹	Zuo et al., 2008 [46]
kmATP_ADP	23000	nM	Zuo et al., 2008 [46]
kADP_ATP	30	nM S ⁻¹	Zuo et al., 2008 [46]
kmADP_ATP	43000	nM	Zuo et al., 2008 [46]
KbEP4	0.057	S ⁻¹	Marshall et al., 1997 [47]
kdEP4	0.04	S ⁻¹	Marshall et al., 1997 [47]
k33	0.6	nM ⁻¹ S ⁻¹	Calebiro et al., 2009 [37]
k_33	6.0	S ⁻¹	Calebiro et al., 2009 [37]
KAC_a	30.0	nM ⁻¹ S ⁻¹	Calebiro et al., 2009 [37]
KAC_r	60.0	S ⁻¹	Calebiro et al., 2009 [37]
kcampf	510	S ⁻¹	Calebiro et al., 2009 [37]
kmcamp	0.1	nM	Calebiro et al., 2009 [37]
kpde4	30	S ⁻¹	Calebiro et al., 2009 [37]
Kmpde4	3.00*10 ⁻⁴	nM	Calebiro et al., 2009 [37]
J21_k1	0.01	nM ⁻¹ S ⁻¹	Greget et al. 2011 [42]
J21_k2	3.00*10 ⁻⁴	S ⁻¹	Greget et al. 2011 [42]
J23_k1	0.001	nM ⁻¹ S ⁻¹	Greget et al. 2011 [42]
J24_k1	0.00178	nM ⁻¹ S ⁻¹	Greget et al. 2011 [42]
J25_k1	0.01	nM ⁻¹ S ⁻¹	Greget et al. 2011 [42]
J25_k2	0.001	S ⁻¹	Greget et al. 2011 [42]
kact_epac	0.1	S ⁻¹	Ponsioen et al., 2004 [48]
kass_Epac_camp	44.6	nM ⁻¹ S ⁻¹	Estimated from Ponsioen et al., 2004 [48]
kdiss_Epac_camp	5400	S ⁻¹	Calebiro et al., 2009 [37]
Kcat_MAPKdp	0.5	S ⁻¹	Markevich et al., 2004 [38]
kmMAPK_dp	1	nM	Markevich et al., 2004 [38]
kdeg_TRPV1	8.5	S ⁻¹	Ogawa et al., 2016 [49]

ki_Ai_PKA	315000	nM	Long et al., 2008 [50]
ki_api_PKC	$6.0 \cdot 10^{-6}$	nM	Lin et al., 1997 [51]
ki_hes_PLC	$3.0 \cdot 10^{-4}$	nM	Jin et al., 2007 [52]
ki_hesp_PKA	$6.0 \cdot 10^{-6}$	nM	Boonpawa et al., 2017 [53]
ki_hesp_TRPV1	$3.0 \cdot 10^{-4}$	nM	Martínez et al., 2011 [54]
krap1_act	0.00835	S ⁻¹	Rehmann, 2013 [55]
krap1a	0.0617	nM	Rehmann, 2013 [55]
I_4	0.0025	nM ⁻¹ S ⁻¹	Dolan and Diamond, 2014 [41]
L5	54700	nM	Dolan and Diamond, 2014 [41]
I6	4707	S ⁻¹	Dolan and Diamond, 2014 [41]
I_6	11.4	S ⁻¹	Dolan and Diamond, 2014 [41]
k21ca	0.21	S ⁻¹	Dolan and Diamond, 2014 [41]
k_21ca	0.5	S ⁻¹	Dolan and Diamond, 2014 [41]
kca2_calmodulin	$2.66 \cdot 10^{-6}$	nM ⁻² S ⁻¹	Dolan and Diamond, 2014 [41]
k_ca2_calmodulin	2.682	S ⁻¹	Dolan and Diamond, 2014 [41]
kca4_calmodulin	$1.704 \cdot 10^{-4}$	nM ⁻² S ⁻¹	Dolan and Diamond, 2014 [41]
k_ca4_calmodulin	1.551	S ⁻¹	Dolan and Diamond, 2014 [41]
kCREB_P	0.95	S ⁻¹	Enslen et al., 1994 [56]
kmCREB_P	1000	nM	Enslen et al., 1994 [56]
kdp_creb	$3.3 \cdot 10^{-4}$	S ⁻¹	Tomaiuolo et al., 2016 [57]
ksynCGRP	$1.83 \cdot 10^{-6}$	nM S ⁻¹	Neeb et al., 2011 [58]
kdegCGRP	0.001155	S ⁻¹	Ma, 2010 [59]
ksynCGRPmRNA	$3.37 \cdot 10^{-5}$	S ⁻¹	Ma, 2010 [59]
kdegCGRPmRNA	0.000240	S ⁻¹	Shimozawa et al., 2007 [60]
J37_k1	0.94	nM ⁻¹ S ⁻¹	Greget et al. 2011 [42]
J37_k2	4000	S ⁻¹	Greget et al. 2011 [42]
J38_k1	80000	S ⁻¹	Greget et al. 2011 [42]
J39_k1	0.25	nM ⁻¹ S ⁻¹	Greget et al. 2011 [42]
J39_k2	200	S ⁻¹	Greget et al. 2011 [42]
J40_k1	50	S ⁻¹	Greget et al. 2011 [42]
J41_k1	1	S ⁻¹	Greget et al. 2011 [42]
J42_k1	1.5	S ⁻¹	Greget et al. 2011 [42]

ktrans	0.005775	S ⁻¹	Dargemont and Kuhn, 1992 [61]
kCaMt	0.01155	S ⁻¹	Deisseroth and Tsien, 1998 [62]
kCaMtr	0.000257	S ⁻¹	Deisseroth and Tsien 1998 [62]
kmp	100	nM	Falcke et al., 2000 [63]
Vmp	14.5	nM S ⁻¹	Falcke et al., 2000 [63]
kpmex	900	nM	Falcke et al., 2000 [63]
Vpmex	145	nM S ⁻¹	Falcke et al., 2000 [63]
kIB	0.01	nM ⁻¹ S ⁻¹	Dupont et al., 2003 [64]
kBI	0.8	S ⁻¹	Dupont et al., 2003 [64]
kPT	1	S ⁻¹	Dupont et al., 2003 [64]
kPP1	1.72	S ⁻¹	Saucerman and Bers, 2008 [65]
SV	0.015	dimensionless	Shi et al., 2008 [66]
DL	0.0015	S ⁻¹	Shi et al., 2008 [66]
kA	0.29	S ⁻¹	Dupont et al., 2003 [64]

2.4. In Silico Modeling of Oxidative Stress Pathways in Endothelial Cells

Table S10: Initial Concentrations

Species	Value (nM)	Ref
LH	351000	Babbs and Steiner, 1990 [67]
O ₂	10000	Atunes et al., 1996 [68]
H ₂ O	5.5 × 10 ⁷	Shi et al., 2013 [69]
Fe ²⁺	100.0	Atunes et al., 1996 [68]
Fe ³⁺	6800.0	Atunes et al., 1996 [68]
SOD	700.0	Kavdia et al., 2011 [70]
H ₂ O ₂	0.38	Atunes et al., 1996 [68]
Catalase	41.03	Aydemir and Kuru, 2003 [71]
GSH	1000	Shi et al., 2013 [69]
GPr	1	Shi et al., 2013 [69]
NADPH Oxidase	3.06 × 10 ⁻⁴	Atunes et al., 1996 [68]

Glossary

LH—Lipid peroxide
 O₂—Oxygen
 H₂O—Water
 Fe²⁺—Ferrous ion
 Fe³⁺—Ferric ion
 SOD—Superoxide dismutase
 H₂O₂—Hydrogen peroxide
 GSH—Glutathione
 GPr—Glutathione peroxidase
 NADPH—Nicotinamide adenine dinucleotide phosphate
 L*—Lipid radical
 OH*—Hydroxyl radical
 LOO*—Lipid peroxy radical
 LOOH—Lipid hydroperoxide
 LO*—lipid alkoxy radical
 H⁺—Hydrogen ion
 O₂^{-•}—Superoxide radical
 OH⁻—Hydroxide ion
 GSSH—Glutathione persulfide
 LOH—Lipid hydroxide
 GPo—Glutathione peroxidase
 ROS—Reactive oxygen species
 GSSG—Oxidized glutathione
 HO₂*—Hydroperoxyl radical
 HO₂⁻—Hydroperoxyl ion

Table S11: Biochemical Reactions and Rate Equations

Description	Rate Equation
$[L^*] + [O_2] \rightarrow [LOO^*]$	$k_{LPO} * [L^*] * [O_2]$
$[LH] + [LOO^*] \rightarrow [LOOH] + [L^*]$	$[LH] * [LOO^*] * k_{LR1}$
$[LOOH] + [Fe^{3+}] \rightarrow [LOO^*] + [Fe^{2+}] + [H^+]$	$[LOOH] * [Fe^{3+}] * k_{LRFe2}$
$[LOOH] + [Fe^{2+}] \rightarrow [LO^*] + [Fe^{3+}] + [OH^-]$	$[LOOH] * [Fe^{2+}] * k_{LRFe1}$
$[H_2O_2] + [Catalase] \rightarrow [H_2O] + [O_2]$	$[H_2O_2] * [Catalase] * k_{cat}$
$[LH] + [LO^*] \rightarrow [LOH] + [L^*]$	$[LH] * [LO^*] * k_{LR2}$
$[GPO] + [GSH] \rightarrow [GSGP] + [GSSG] + [H_2O]$	$[GPO] * [GSH] * k_{GSGP}$
$[GSH] + [GSGP] \rightarrow [GPr] + [GSSG] + [H_2O]$	$[GSH] * [GSGP] * k_{GPr}$
$[H_2O_2] + [GPr] \rightarrow [H_2O] + [GPO]$	$[H_2O_2] * [H^+] * [H^+] * [GPr] * k_{GPO}$
$[H^+] + [O_2^*] + [SOD] \rightarrow [H_2O_2]$	$[H^+] * [O_2^*] * [SOD] * k_{SOD}$
$[H_2O] \leftrightarrow [H^+] + [OH^-]$	$[H_2O] * k_{dH_2O} - [H^+] * [OH^-] * K_{H_2O}$
$[LH] + [OH^*] \rightarrow [L^*] + [H_2O]$	$[LH] * [OH^*] * k_{initLR}$
$[H_2O_2] + [Fe^{2+}] \rightarrow [Fe^{3+}] + [OH^*] + [OH^-]$	$[H_2O_2] * [Fe^{2+}] * k_{Fe1}$
$[OH^*] + [Fe^{2+}] \rightarrow [Fe^{3+}] + [OH^-]$	$[OH^*] * [Fe^{2+}] * k_{Fe6}$
$[Fe^{3+}] + [H_2O_2] \rightarrow [Fe^{2+}] + [HO_2^*] + [H^+]$	$k_{Fe5} * [Fe^{3+}] * [H_2O_2]$
$[HO_2^*] + [Fe^{3+}] \rightarrow [Fe^{2+}] + [O_2] + [H^+]$	$[HO_2^*] * [Fe^{3+}] * k_{Fe3}$
$[O_2^*] + [Fe^{3+}] \rightarrow [Fe^{2+}] + [O_2]$	$[O_2^*] * [Fe^{3+}] * k_{Fe3}$
$[H_2O_2] + [OH^*] \rightarrow [H_2O_2^*] + [H_2O]$	$[H_2O_2] * [OH^*] * k_{Fe4}$
$[OH^*] + [HO_2^*] \rightarrow [H_2O] + [O_2]$	$[OH^*] * [HO_2^*] * k_{Fe7}$
$[H_2O_2^*] + [H_2O_2] \rightarrow [OH^*] + [H_2O] + [O_2]$	$[H_2O_2^*] * [H_2O_2] * k_{Fe9}$
$[H_2O_2^*] + [H_2O_2^*] \rightarrow [H_2O_2]$	$[H_2O_2^*] * [H_2O_2^*] * k_{Fe8}$

Table S12: Chemical Kinetic Parameters

Parameter	Value	Units	References
kinitLR	0.5	$\text{nM}^{-1} \text{s}^{-1}$	Antunes et al., 1996 [68]
kLPO	0.3	$\text{nM}^{-1} \text{s}^{-1}$	Antunes et al., 1996 [68]
kLR1	1.4×10^{-8}	$\text{nM}^{-1} \text{s}^{-1}$	Antunes et al., 1996 [68]
kFe1	7.6×10^{-8}	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe6	0.35	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe3	3.1×10^{-4}	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kLR2	0.0066	$\text{nM}^{-1} \text{s}^{-1}$	Atunes et al., 1996 [68]
kLRFe1	0.015	$\text{nM}^{-1} \text{s}^{-1}$	Xue et al., 2012 [73]
kLRFe2	1×10^{-6}	$\text{nM}^{-1} \text{s}^{-1}$	Xue et al., 2012 [73]
kGPo	0.021	$\text{nM}^{-1} \text{s}^{-1}$	Buettner et al., 2006 [74]
kGSGP	4×10^{-5}	$\text{nM}^{-1} \text{s}^{-1}$	Buettner et al., 2006 [74]
kGPr	0.01	$\text{nM}^{-1} \text{s}^{-1}$	Buettner et al., 2006 [74]
kSOD	1.6	$\text{nM}^{-1} \text{s}^{-1}$	Edwards et al., 2011 [75]
kcat	0.034	$\text{nM}^{-1} \text{s}^{-1}$	Edwards et al., 2011 [75]
KH2O	140.0	$\text{nM}^{-1} \text{s}^{-1}$	Edwards et al., 2011 [75]
kdH2O	2.5×10^{-5}	s^{-1}	Edwards et al., 2011 [75]
kFe2	0.0012	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe4	0.027	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe5	2.7×10^{-10}	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe7	7.0	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe8	0.017	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe9	5×10^{-10}	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kFe10	5.5	$\text{nM}^{-1} \text{s}^{-1}$	Henle et al., 1996 [72]
kT_LOO	0.001	$\text{nM}^{-1} \text{s}^{-1}$	Xue et al., 2012 [73]
kVE_LOO_deg	2×10^{-5}	$\text{nM}^{-1} \text{s}^{-1}$	Xue et al., 2012 [73]
kBC_O2	1.8×10^{-17}	$\text{nM}^{-1} \text{s}^{-1}$	Xue et al., 2012 [73]
kBCR_O2	0.2	$\text{nM}^{-1} \text{s}^{-1}$	Ozhogina and Kasakina, 1995 [76]
kBC kBCR	8×10^{-7}	$\text{nM}^{-1} \text{s}^{-1}$	Ozhogina and Kasakina, 1995 [76]
kBC_LOO	3×10^{-6}	$\text{nM}^{-1} \text{s}^{-1}$	Ozhogina and Kasakina, 1995 [76]
kBC_VITE	10	$\text{nM}^{-1} \text{s}^{-1}$	Haila, 1999 [77]
kBCO_LOO	1×10^{-4}	$\text{nM}^{-1} \text{s}^{-1}$	Haila, 1999 [77]
kAsc_OH	0.00855	$\text{nM}^{-1} \text{s}^{-1}$	Haila, 1999 [77]
kAsc_O2	0.01	$\text{nM}^{-1} \text{s}^{-1}$	Haila, 1999 [77]
kAScR_O2	0.26	$\text{nM}^{-1} \text{s}^{-1}$	Haila, 1999 [77]
kIQ_XO	280	nM	Haila, 1999 [77]
k32	720	$\text{nM} \text{s}^{-1}$	Haila, 1999 [77]
kEpi_ROS	0.0073	$\text{nM}^{-1} \text{s}^{-1}$	Haila, 1999 [77]
kdegROS	0.085	s^{-1}	Macfarlane and Miller, 1992 [78]
kNADPHOxi	1720	s^{-1}	Koshkin et al., 1997 [79]
km_NADPHoxi	30000	nM	Koshkin et al., 1997 [79]
kI_Epi_NDAPhoxi	5000	nM	Estimated from Koshkin et al., 1997 [79]

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Supplementary Material 3

3.1 Medical Subject Headings (MeSH) keywords

The specific list of Medical Subject Headings (MeSH) keywords is provided below.

Table S13: Search strings used to identify the literature.

Literature Search Keywords
1. “Apigenin”
2. “Hesperidin”
3. “Apigenin AND joint pain”
4. “Hesperidin AND joint pain”
5. “Apigenin AND COX-2”
6. “Apigenin AND PGE2”
7. “Apigenin AND CGRP”
8. “Apigenin AND TRPV1”
9. “Apigenin AND ROS”
10. “Hesperidin AND COX-2”
11. “Hesperidin AND PGE2”
12. “Hesperidin AND CGRP”
13. “Hesperidin AND TRPV1”
14. “Hesperidin AND ROS”

Articles obtained from the systematic literature review are categorized into three categories: 1) Articles related to molecular pathways implicated in joint pain, 2) articles related to interactions of apigenin and hesperidin with molecular targets in joint pain pathways, and 3) articles related to apigenin and hesperidin pharmacokinetic properties.

3.2 Initial Simulation Results

Initial simulations were conducted for a dose range of 0 to 100 mg for apigenin and 0 to 5000 mg for hesperidin to identify an efficacious dose level for each of phytonutrient on all four mechanisms of action analyzed in this study. The initial results indicated that 30 mg of apigenin and 1000 mg of hesperidin were most effective in reducing all five biomarkers (COX-2, PGE2, TRPV1, CGRP, and ROS) of joint pain. Increasing the dose levels of apigenin to more than 30 mg and hesperidin to more than 1000 did not further reduce the biomarker levels significantly. Figures S2A, S2B, S2C, S2D, and S2E represent the effect of apigenin on COX-2, PGE2, TRPV1, CGRP, and ROS, respectively. Figures S3A, S3B, S3C, S3D, and S3E represent the effect of apigenin on COX-2, PGE2, TRPV1, CGRP, and ROS, respectively. These results show that increasing the amount of apigenin beyond 30 mg and increasing the amount of hesperidin beyond 1,000 mg did not improve the reduction in all five biomarkers of joint pain.

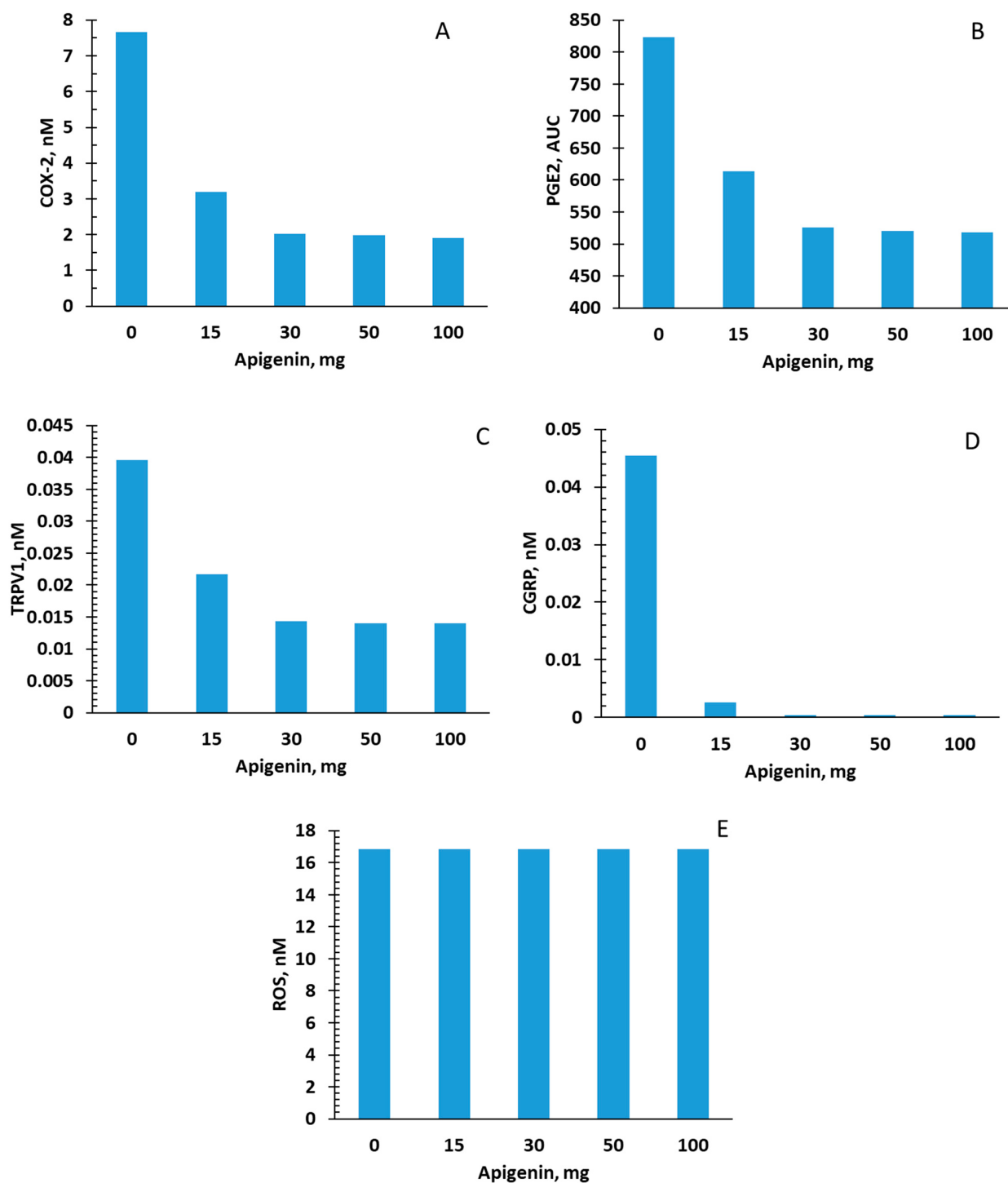


Figure S2: Dose determination for apigenin. Initial simulations were performed for apigenin dose level of 15, 30, 50, and 100 mg to determine the effective apigenin dose. Panels A, B, C, D, and E represent the results for COX-2, PGE2, TRPV1, CGRP, and ROS as a function of apigenin dose. Apigenin lowered all biomarkers except ROS.

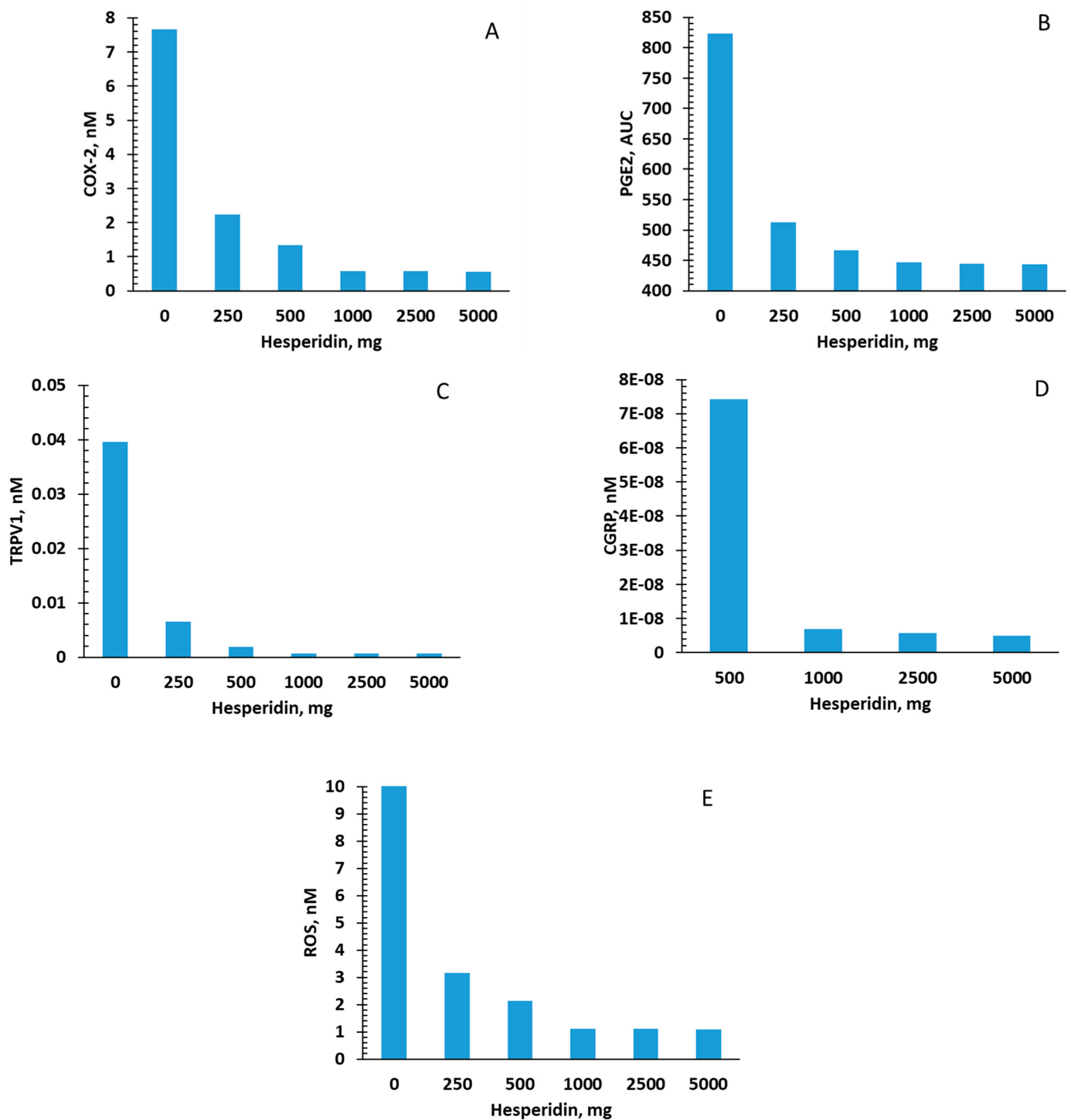


Figure S3: Hesperidin dose determination. Initial simulations were performed for hesperidin dose level of 250, 500, 2,500, and 5,000 mg to determine the effective hesperidin dose. Panels A, B, C, D, and E represent the results for COX-2, PGE2, TRPV1, CGRP, and ROS as a function of hesperidin dose. Hesperidin lowered all biomarkers.