Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Material

Rutin present in *Alibertia edulis* extract acts on human platelet aggregation through inhibition of cyclooxygenase/thromboxane

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Figure S1. Visual presentation of the *Alibertia edulis* plant, showing its leaves and fruit. The tree is 5 meters in height, and its fruit has an ovoid shape with a diameter of approximately 25 mm.

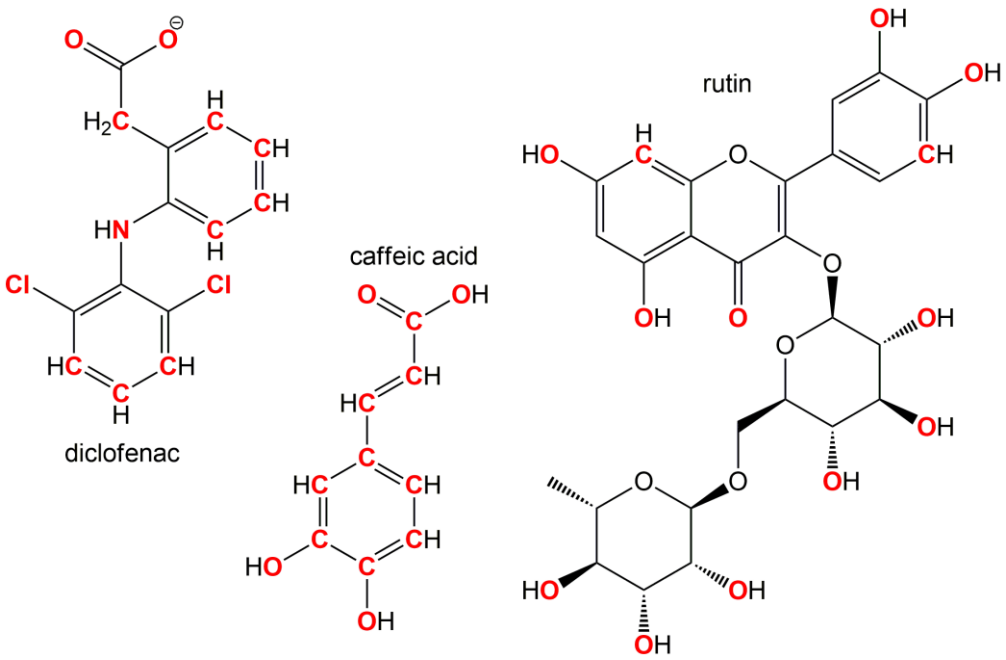

Figure S2. Atoms of the ligand highlighted in red were used as reaction coordinate (ξ) in Adaptive Biasing Force method to calculate the free-energy profiles of the protein-ligands dissociations. ξ was the distance between these atoms and alpha carbons of the following residues Met113, Val116, Arg120, Ile345, Val349, Leu352, Ser353, Tyr355, Leu359, Ile523, Gly526, Ala527, Ser530, Leu531 and Leu535.

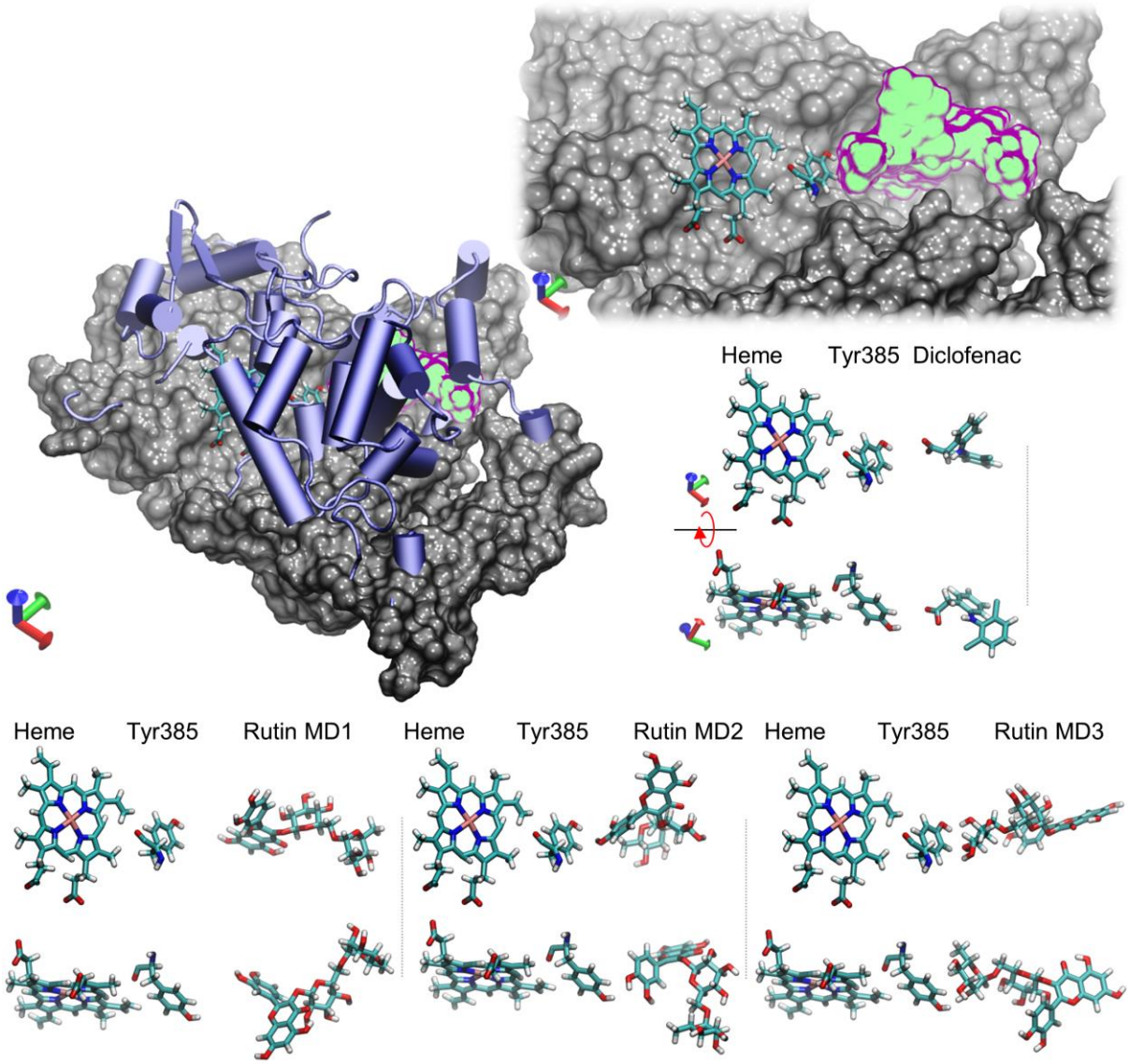

Figure S3. Details of the structures used for COX-1 and ligand conformations extracted from docking calculus and used to build the initial box for molecular dynamics simulations.

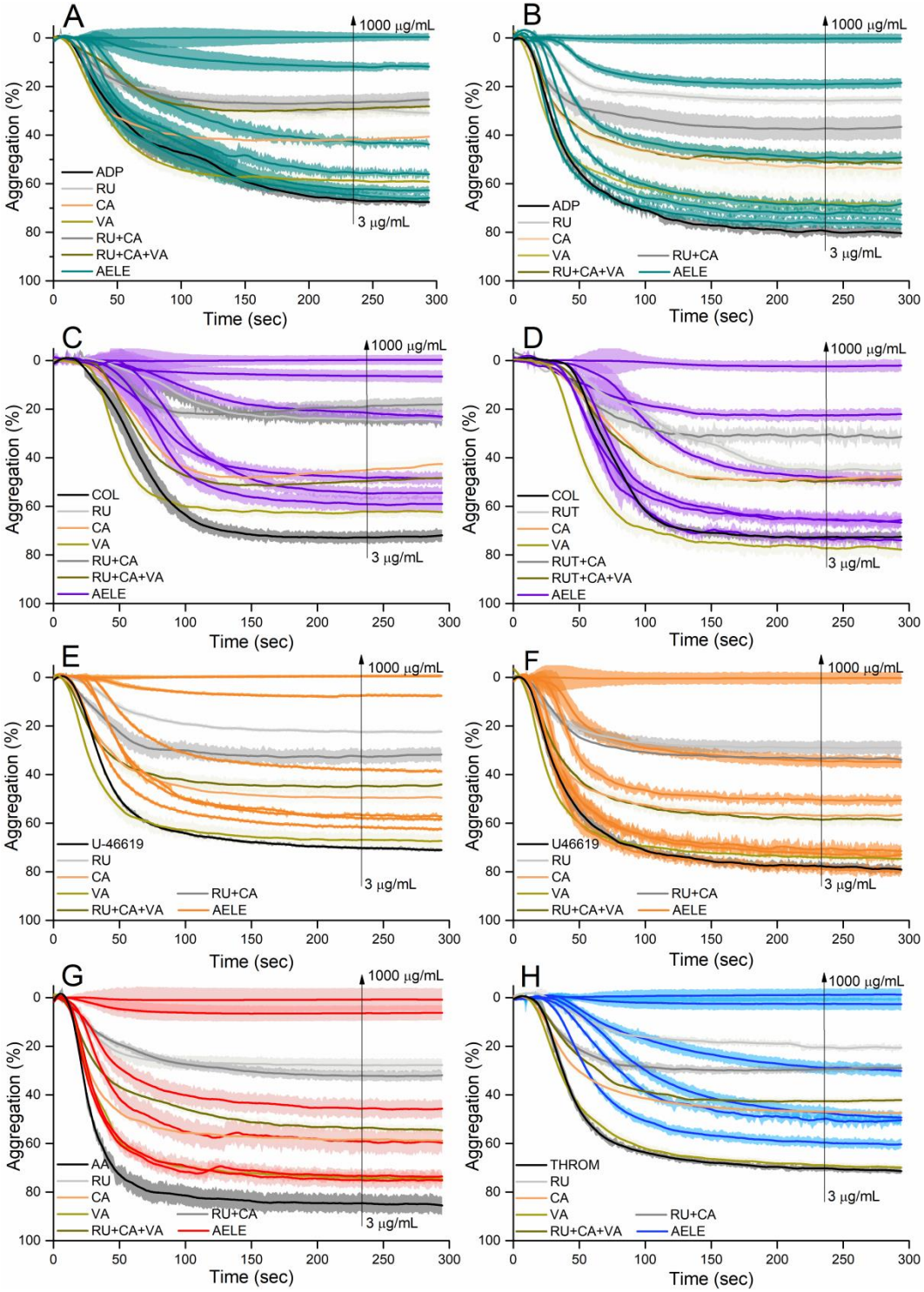

Figure S4. Illustrative curves of platelet aggregation with different agonists in washed platelet (left panels) and platelet-rich plasma (right panels) after incubation with AELE and rutin. The platelets were incubated with the AELE (3 – 1000 µg/mL) or RU - rutin (30 µM), CA - caffeic acid (30 µM), VA - vanillic acid (30 µM), RU+CA and RU+CA+VA (mixtures at same concentration - 30 µM), followed by activation with ADP (10 or 30 µM) - (Panels A for WP and B for PRP), collagen (2 µg/mL) (Panels C for WP and D for PRP), U-46619 (2 or 4 µM) (Panels E for WP and F for PRP), AA (500 µM) (Panel G for PRP) and thrombin (0.1 U/mL) (Panel H for WP). Data represent the mean values ± SEM.

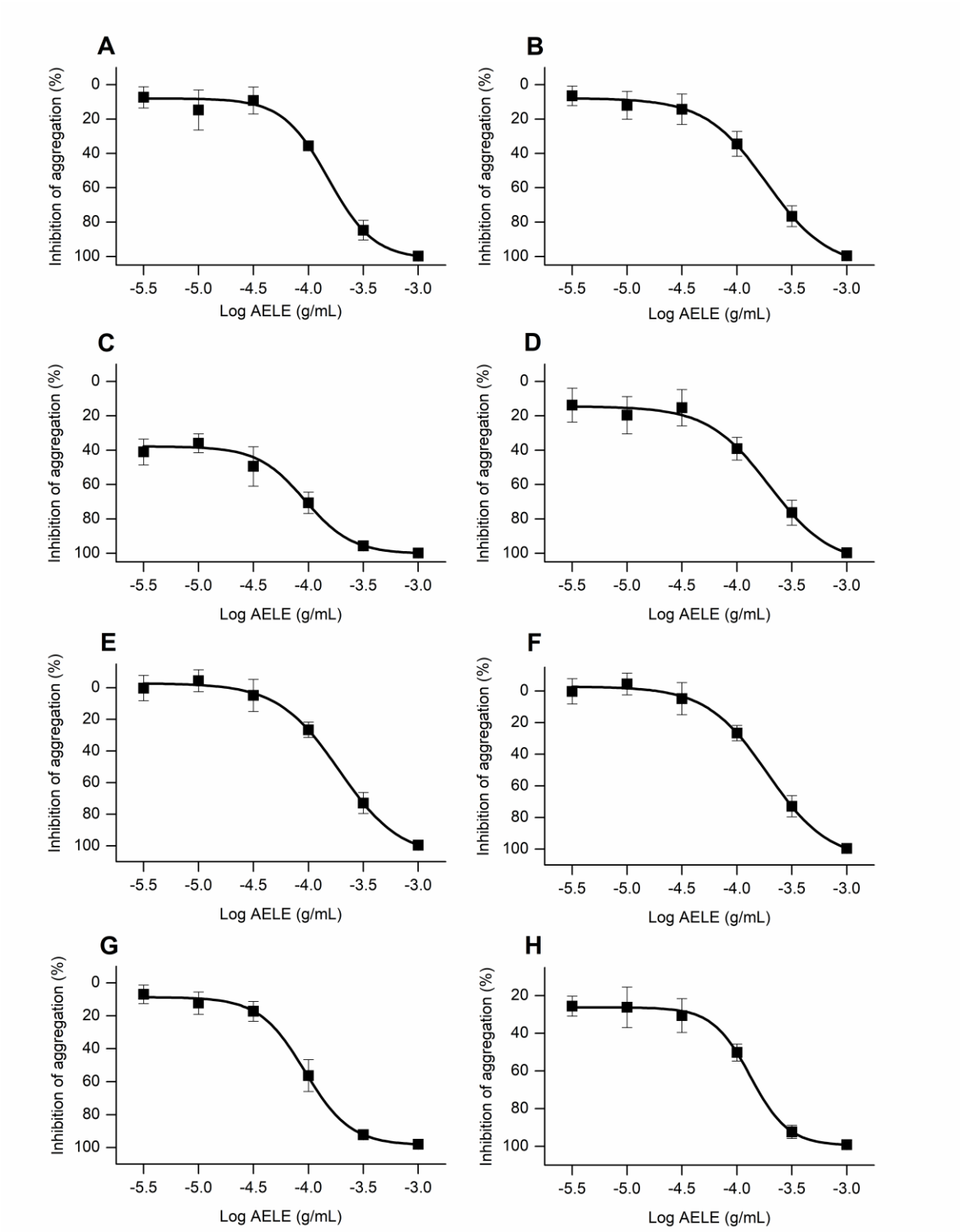

Figure S5. Concentration inhibitor curves (as % of final aggregation after 5 minutes) for AELE (3 – 1000 µg/mL) or rutin (30 µM) against aggregation induced by ADP (Panels A for WP and B for PRP), collagen (Panels C for WP and D for PRP), U-46619 (Panels E for WP and F for PRP), AA (Panel G for PRP) and thrombin (Panel H for WP). Data represent the mean values ± SEM.

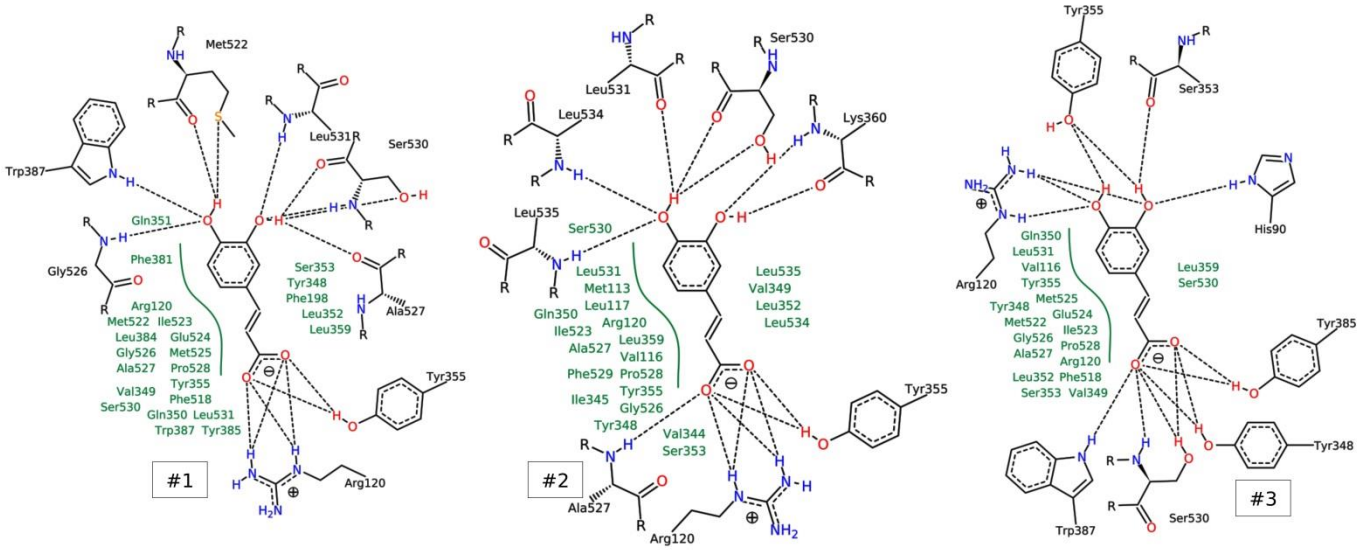

Figure S6. Specific interactions of cyclooxygenase type 1, COX-1, amino acids with caffeic acid at three different conformations. Dashed lines represent hydrogen bonds interactions. Solid green lines represent the hydrophobic contacts made by residues highlighted also in green.

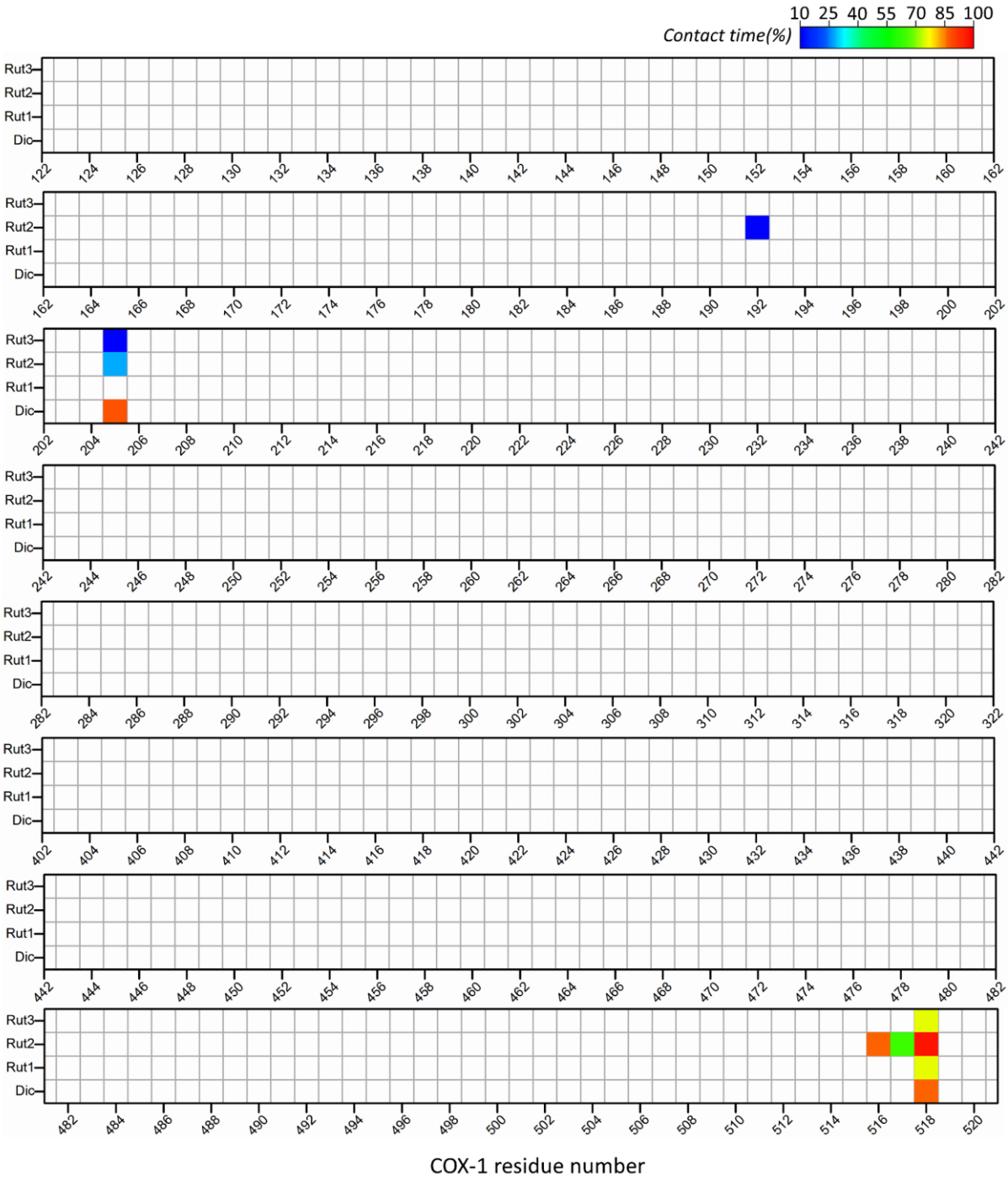

Figure S7. Complementary details about residues contacted in a distance up to 3 Å from any atom of the diclofenac and rutin ligands into the catalytic pocket. Gln192, Ser516 and Ile517 are contacted only by rutin, and Phe205 and Phe518 are contacted by both compounds.

Table S1. Docking energy parameters for each ligand computed in the catalytic site of the cyclooxygenase type 1.

Ligand/Energies (kcal/mol)	Total	Intramolecular	Van der Waals	Coulomb	Score
Diclofenac_Run1 #1	33.251	-34.634	-0.841	-33.793	-8.814
Diclofenac_Run2 #2	33.255	-34.234	-0.505	-33.729	-8.802
Diclofenac_Run3 #3	35.866	-29.940	-8.907	-21.033	-8.945
Rutin_Run1 – RuMD1	113.511	-32.836	-26.531	-6.305	-11.254
Rutin_Run2 – RuMD2	119.240	-19.708	9.791	-29.499	-11.982
Rutin_Run3 – RuMD3	125.681	-14.031	-3.424	-10.607	-11.637
Caffeic acid_Run1 #1	-9.069	-26.854	-15.771	-11.083	-7.826
Caffeic acid_Run2 #2	-8.536	-26.287	-13.644	-12.643	-7.675
Caffeic acid_Run3 #3	-6.076	-23.717	-17.557	-6.160	-7.753