*Supplementary Material*

# Long-Lasting Anti-Inflammatory and Antinociceptive Effects of Acute Ammonium Glycyrrhizinate Administration: Pharmacological, Biochemical, and Docking Studies

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## FLAP

We performed molecular docking experiment on the 5-lipoxygenase–activating protein (FLAP), known for activating the conversion of arachidonic acid into 5-(*S*)-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HpETE) and leukotriene $A_4$ ($LTA_4$) [1]. The crystal structure of the human FLAP bound to a known inhibitor (PDB: 2Q7M) [1] was used during computational studies by software Glide [2–4]. The grid center was set on the ligand (X = 65.86, Y = 83.68, and Z = 31.92) and the inner box was extended for 24 Å in the three dimensions. The molecular docking (Figure S1) of both MK-591 and AG was carried out in the XP mode saving 10,000 poses and the best 800 poses were minimized. Afterwards, a postdocking optimization was carried out, setting the rejection cut-off at 0.8 kcal/mol, and a maximum of 30 poses per ligand.

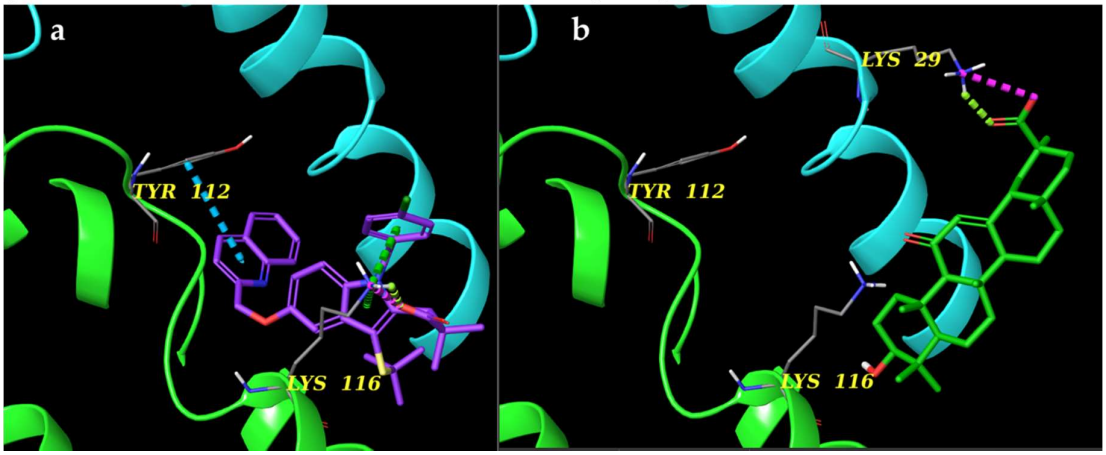


**Figure 1.** (**a**) 3D models of the inhibitor MK-591 (colored by atom types: C purple, O red, S yellow, N blue) and (**b**) AG (colored by atom types: C green, O red, polar H white) in the binding site of 5-lipoxygenase–activating protein (FLAP). Protein chains are A and B are reported in green and cyan (chain C not shown). Light green dotted lines represent H-bonds, dark green dotted lines are π-cation interactions, pink dotted lines represent salt bridges, and cyan dotted lines are π-π stacking. Important residues are labelled in yellow.

Comparing the binding mode of AG with respect to the co-crystallized inhibitor, MK-591 forms several interactions with Lys116 and a π-π interaction with Tyr112 (Figure S1a) essential for the inhibitory activity. On the other hand, AG was not able to interact with these key amino acids; the only bonds formed are with Lys29, far from the binding cavity (Figure S1b). The results suggested a non-optimal binding between AG and FLAP.

## References


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