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Research Article

# *In Silico* Docking and *In Vivo* Studies of the Natural Compound, 5-(Hydroxymethyl) Furfural

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## Abstract

5-(Hydroxymethyl) furfural, a bioactive natural product showed anti-inflammatory activity against ear edema induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). The maximal anti-inflammatory activity (inhibition of 32.5%) was obtained at dose of 0.3 mg/ear of 5-HMF. Also, this compound and indomethacin markedly reduced myeloperoxidase activity, with inhibitions of 43.0% for 5-HMF (0.3 mg/ear) and 78.7% for indomethacin (1 mg/ear), respectively. Microscopic analysis of the sections of mice ears treated with TPA, showed an intense dermal edema, infiltration of inflammatory cells and epidermal thickness as compared with the control group, treated with the vehicle. These events were markedly reduced in the ear´s tissue of the animals treated with 0.3 mg of 5-HMF and 1 mg indomethacin.

5-HMF has been examined *in silico* as a ligand against Cyclooxygenase 1 (COX-1) and 2 (COX-2), utilizing Auto Dock Vita as docking tool. The docking energies were found to be -5 Kcal/ mol for COX-1 and -4.9 Kcal/mol for COX-2. The results of docking investigation suggest a good binding interaction with COX, although, there is no selective for any COX, since the calculated selectivity was 0.84.

In conclusion, 5-HMF showed *in vivo* anti-inflammatory effects, suggesting that the compound might represent a potential therapeutic option for the treatment of inflammation processes. The reduction in MPO activity and the *in silico* results could explain, at least in part, the underlying mechanism involved in the protective effects of 5-HMF on the acute inflammation process.

***Keywords:*** *5-(Hydroxymethyl) Furfural; Inflammation; COX; Docking Study*

## Abbreviations

5-HMF: 5-(Hydroxymethyl) Furfural; COX-1: Cyclooxygenase-1 Protein; COX-2: Cyclooxygenase-2 Protein; TPA: 12-O-Tetradecanoylphorbol-13-Acetate; SCD: Sickle Cell Disease; MPO: Myeloperoxidase

## Introduction

Natural products have been an important drug source of leads for the development of new drugs. Owing to the diverse biological activities and medicinal potentials, different civilizations have accumulated knowledge of their use. In this sense, traditional Chinese medicine is famous for its extensive use of natural products such as Jieyu-wan and Xiaoyao-san, which have been used for centuries to manage stress, depression, and other mood disorders and contain curcumin (diferuloylmethane) as active ingredient. This bright yellow plant pigment, the principal curcuminoid in Indian saffron or turmeric (*Curcuma longa*), is also used in Ayurvedic medicine to treat various inflammatory conditions, such as arthritis and ulcers [1]. But, other significant drugs developed from natural sources could be described, so studies on natural anti-inflammatory compounds can make an important contribution to treat inflammation that it is known to be one of the important cause responsible for many diseases.

5-Hydroxymethylfurfural (Figure 1) is a five carbon-ring aromatic aldehyde that exists naturally in coffee, honey, dried fruits, fruit juices and flavouring agents. It is also a product of the Maillard reaction and it is mainly generated by acid-catalysed thermal dehydration of fructose and identified as a flavoring substance in a wide variety of heat-processed products. Despite the previous concern on the dangers of 5-HMF [2], it has been reported that it has benefic biological effects such as anti-oxidant, anti-hypoxia and hepatoprotective activities [3-5].

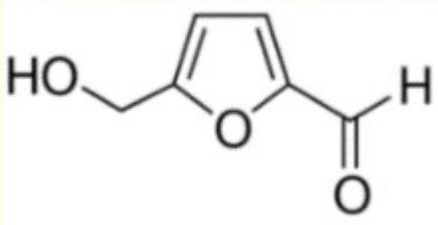


***Figure 1:*** *5-(Hydroxymethyl) furfural.*

Also 5-HMF showed beneficial properties in Sickle cell disease (SCD), a haemoglobinopathy caused by abnormal haemoglobin that polymerizes under hypoxic conditions, causing rigidity and distortion of red blood cells. In this sense, preclinical studies had demonstrated favorable effects [6,7]. But also, it was developed an orally bioavailable drug for chronic therapy for SCD named Aes-103, whose active ingredient is 5-HMF. This drug, in phase 1 clinical trial did not show significant side effects and this drug is currently being tested in Phase 2 clinical trials [8,9].

Related to inflammatory process, it was shown that crude Maillard reaction products or their fractions have antioxidant and anti-inflammatory activities on Caco cells and 5-HMF was identified as one of components of those fractions [10].

On the basis of these considerations and taking into account that no scientific evaluation of this compound related to the inflammatory process has been carried out to date on *in vivo* model, the present study aims to examine the effects of 5-HMF on topical inflammatory model. Also, attempts have been made to further investigate some of the possible mechanisms that underlie the pharmacological activity of the compound by *in vivo* and *in silico* strategies.

## Materials and Method

### Drugs

Indomethacin, 12-O-tetradecanoylphorbol-13-acetate (TPA), hexadecyltrimethylammonium, O-dianisidine dihydrochloride were purchased from Sigma Chemical Co., St. Louis, MO.

### Animals

Female Swiss mice (25 - 30g) were obtained from Animal House of the Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. The experiments were carried out taking into account international guiding principles and local regulations concerning the care and use of laboratory animals for biomedical research (Institute of Laboratory Animal Resources, 1996). The experiments were approved by the local Ethics Committee (Exp-FyB: 0738658/2011). The animals had free access to a standard commercial diet and water ad libitum and were kept in rooms maintained at 22 ∘ C ± 1 ∘ C with a 12h light/dark cycle.

### Ear edema test

Groups of 6 mice each were used. The right ear of each mouse received a topical application of 2.5 μg of TPA in 0.125 μg/μl acetone solution (10 μl to each side of the ear). HMF was dissolved in EtOH 80% and was topically applied immediately after TPA (0.03, 0.1 and 0.3 mg/ear) [11]. The left ear, used as control, received the vehicle (EtOH 80%). Indomethacin (1.0 mg/ear/20 μl) was used as the anti-inflammatory reference drug. After 4h, animals were sacrificed and disks of 6 mm diameter were removed from each ear and their weights determined. The swelling was measured as the difference in weight between the punches from right and left ears, and the percentage inhibition of edema was calculated in comparison to inflamed ear from control animals.

### Myeloperoxidase (MPO) activity

MPO activity, as an indicator of polymorphonuclear leukocyte accumulation was determined by Paiva., *et al* [12]. A punch of each ear was homogenized in a solution containing 0.5% hexadecyltrimethylammonium bromide dissolved in 50 mM potassium phosphate buffer (pH 6), before sonication in an ice bath for 10s. The homogenates were freeze-thawed three times, repeating the sonication. Then, they were centrifuged for 20 min at 20,000 rpm at 4ºC. The level of MPO activity was measured spectrophotometrically. 0.1 ml of the material to be measured was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was then measured for 5 minutes using a Metrolab spectrophotometer (Metrolab 325 BD). Myeloperoxidase activity was expressed in optic density (DO)*100/mg of tissue.

### Histological study

Samples of ears were fixed in 10% buffered formalin and embedded in paraffin. Sections of 5 µm thickness were prepared and stained with hematoxylin and eosin and examined under a light microscope.

### Statistical analysis

The pharmacological results are expressed as means ± standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by the Dunnet test. P values lower than 0.05 were considered to be statistically significant (Graph Pad Prism 5, version 5.03 for windows).

### Virtual study

The molecular structures of 5-HMF was generated and optimized using ACDLabs (http://www.acdlabs.com/download//). The experimental coordinates cyclooxygenase-1 protein (COX-1, PDB: 3N8X) and cyclooxygenase-2 protein (COX-2, PDB: 1CX2) structures were taken from PDB (www.rcsb.org/pdb/). Ligands were removed from the binding sites of COX-1 and COX-2 and the enzymes cleaned from any unwanted interaction. The ligands were docked in the active domain of COX-1 and COX-2 using program AutoDockVina [13]. For COX-1: -32.155, -43.520 and 47.185 were selected for center of x, y and z, respectively and size 20 for the three axis, with nine of binding modes and eight exhaustiveness of search. For COX-2: -24.263, 21.528 and 16.496 were selected for center of x, y and z, respectively and size 20 for the three axis, with nine of binding modes and eight exhaustiveness of search. Lig Plot program was used for automatic generation of 2D ligand-protein interaction (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). The selectivity was calculated by formula

$\delta$: Kb (COX-2) / Kb (COX-1) = $e^{(\Delta G\ b\ (COX\text{-}1)-\ \Delta G\ b\ (COX\text{-}2))/RT}$

$\Delta Gb$: -RT log (Kb)

Kb (COX-1) = $e^{-\Delta G\ b\ (COX\text{-}1)/RT}$

Kb (COX-2) = $e^{-\Delta G\ b\ (COX\text{-}2)/RT}$

ΔGb represents binding energy (kcal/mol); Kb, binding constant; R, gas constant and T, temperature

## Results and Discussion

The TPA model of ear inflammation is useful for screening prospective topical anti-inflammatory compounds that act at variety of levels. An early hallmark of local inflammation in the TPA model is thickening due to edema and swelling within the dermis. 5-HMF´s topical anti-inflammatory activity was tested in the ear edema in mice and the results are shown in figure 2. The maximal anti-inflammatory activity (inhibition of 32.5%) was obtained at dose of 0.3 mg/ear of 5-HMF, meanwhile, the anti-inflammatory reference drug, indomethacin (1 mg) exhibited anti-inflammatory activity with an inhibition of 78.1% (Figure 2A).

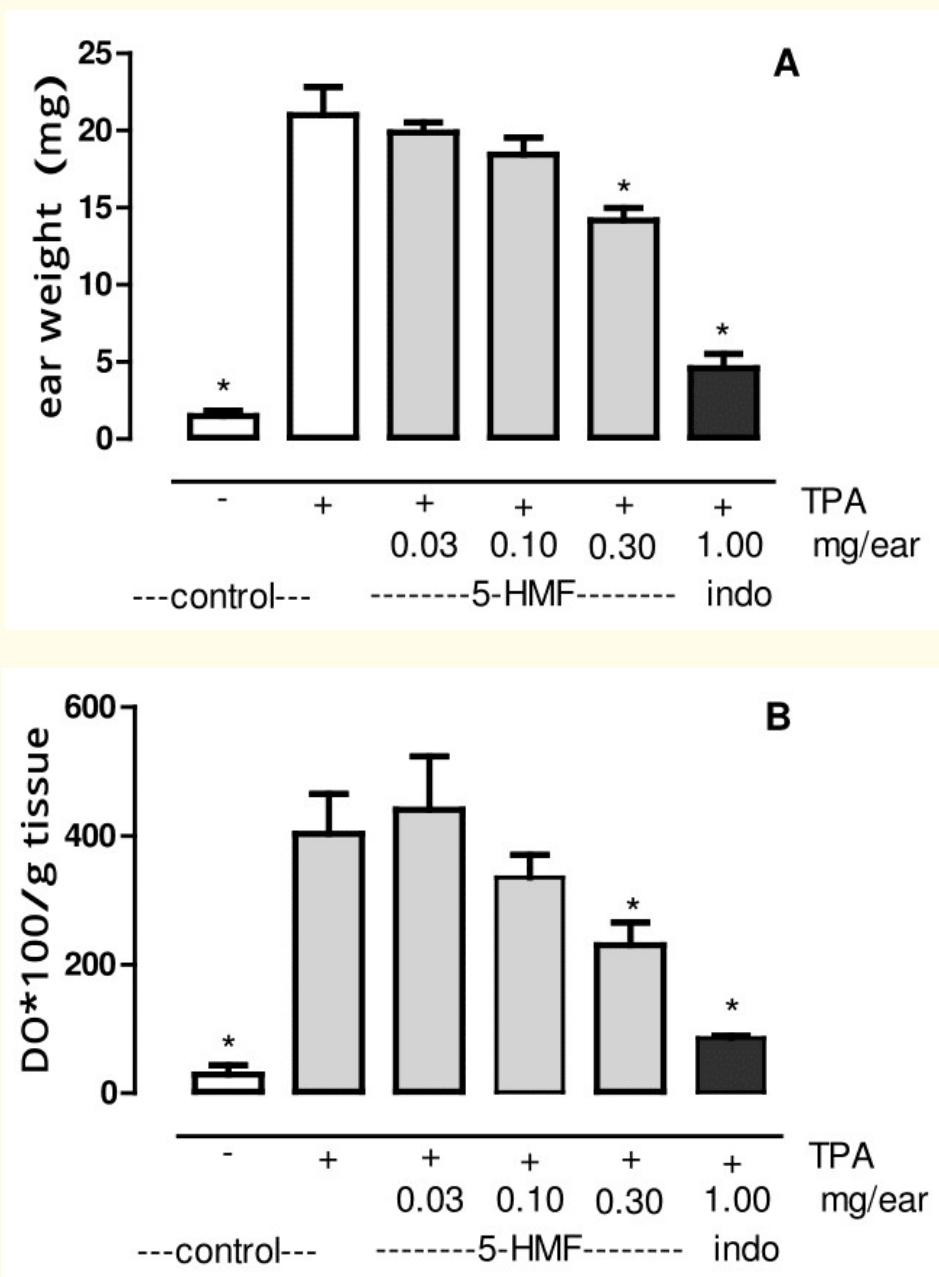


***Figure 2:*** *Effect on ear weight (A) and activity of MPO (B) on TPA induced mouse ear edema. Results were obtained by topical administration of 0.03, 0.1 and 0.3 of 5-HMF and 1 mg of indomethacin. Each value represents the mean ± ESM of the results from 6 mice. Statistical differences from inflamed control group were determined by ANOVA followed by Dunnet´s test *p < 0.05.*

In inflamed tissues, MPO activity is significantly increased, indicating that neutrophils accumulation is a critical event in the pathogenesis of inflammation. MPO is a member of the haem peroxidase-cyclooxygenase superfamily found primarily in azurophilic granules of neutrophils and it is used as a marker for tissue neutrophil content [14]. Since, the inflammation caused by TPA is related to an increase polymorphonuclear leukocyte migration to the dermis, the MPO activity was studied. 5-HMF and indomethacin markedly reduced this activity, with inhibitions of 43.0% for 5-HMF (0.3 mg/ear) and 78.7% for indomethacin, respectively (Figure 2B). 5-HMF isolated from marine red alga, *Laurencia undulate*, showed similar effect on MPO activity in isolated cells [15].

Microscopic analysis of the sections of mice ears 4h after TPA application, showed intense dermal edema, infiltration of inflammatory cells and epidermal thickness as compared with the control ears, treated with the vehicle. These events were markedly reduced in the ear´s tissue of the animals treated with 0.3 mg of 5-HMF and indomethacin (Figure 3).

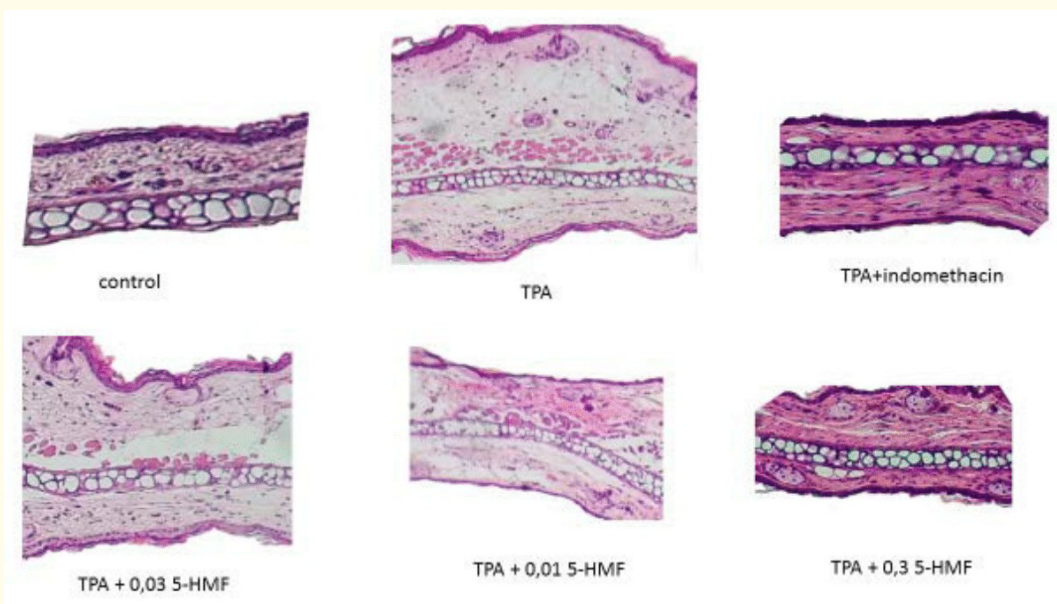


***Figure 3:*** *Effect of 5-HMF on histological alterations induced by TPA: Representative photomicrograph of transversal cut of mice ear (HE20x) 4 h after TPA application*

Topical application of TPA has been shown to induce the expression of COX and its mRNA transcript by activating the intracellular signalling [16], so the potential activity of 5-HMF on COX-1 and COX-2 was investigated by *in silico* model.

Molecular docking is a computational procedure that attempts to predict noncovalent binding of macromolecules (receptor) and a small molecule (ligand) efficiently. The goal is to predict the bound conformations and the binding affinity [13,17,18].

Molecular Docking revealed interaction between 5-HMF with both active site of COX-2 and COX-1 enzymes successfully (Figure 4).

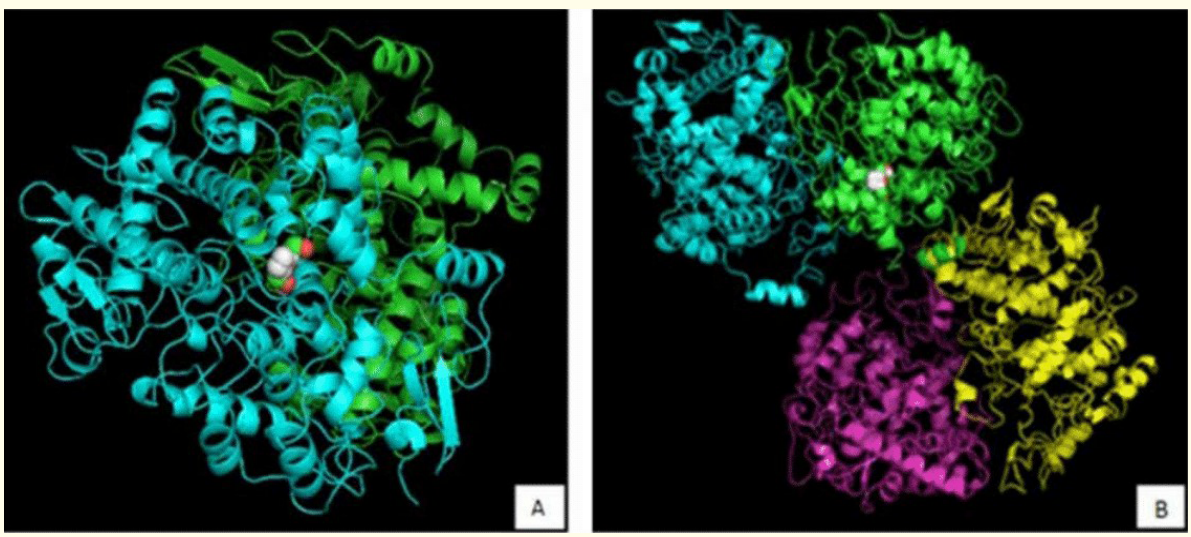


***Figure 4:*** *COX-1 (A) and COX-2 (B) ribbon representations and 5-HMF as spheres.*

The docked conformations of 5-HMF were ranked into clusters based on the binding energy and the most favorable binding conformation with the lowest free energies was selected as the binding pose, -5 Kcal/ mol for COX-1 and -4.9 Kcal/mol for COX-2. The most adequate Root-mean-square-deviation (RMSD) was also represented for both enzymes (Table 1).

| COX-1 | | | | COX-2 | | | |
|---|---|---|---|---|---|---|---|
| **Mode** | **Affinity** | **RMSD rmsd/ld** | **RMSD rmsd/ud** | **Mode** | **Affinity** | **RMSD rmsd/ld** | **RMSD rmsd/ud** |
| 1 | -5.0 | 0.000 | 0.000 | 1 | -4.9 | 0.000 | 0.000 |
| 2 | -4.8 | 0.520 | 3.743 | 2 | -4.9 | 9.190 | 10.624 |
| 3 | -4.8 | 2.328 | 3.481 | 3 | -4.9 | 9.487 | 10.632 |
| 4 | -4.7 | 2.474 | 4.634 | 4 | -4.8 | 0.208 | 3.674 |
| 5 | -4.7 | 8.210 | 8.986 | 5 | -4.8 | 10.799 | 12.582 |
| 6 | -4.6 | 2.487 | 5.034 | 6 | -4.8 | 11.942 | 12.879 |
| 7 | -4.5 | 2.392 | 3.105 | 7 | -4.7 | 10.089 | 11.265 |
| 8 | -4.4 | 2.286 | 3.031 | 8 | -4.6 | 16.154 | 16.952 |
| 9 | -4.4 | 3.061 | 4.874 | 9 | -4.6 | 10.398 | 11.157 |

***Table 1:*** *Affinity and Root mean square deviation calculated by AutodockVina.*

Furthermore, hydrogen bonding interactions between 5-HMF and COX-1 and COX-2 were analyzed using Lig Plot. 5-HMF showed common hydrogen bond interactions with Ile 523(B), Met 522(B), Ala 527(B), Arg 120(B) and Tyr 355(B) of COX-1 (Figure 5).

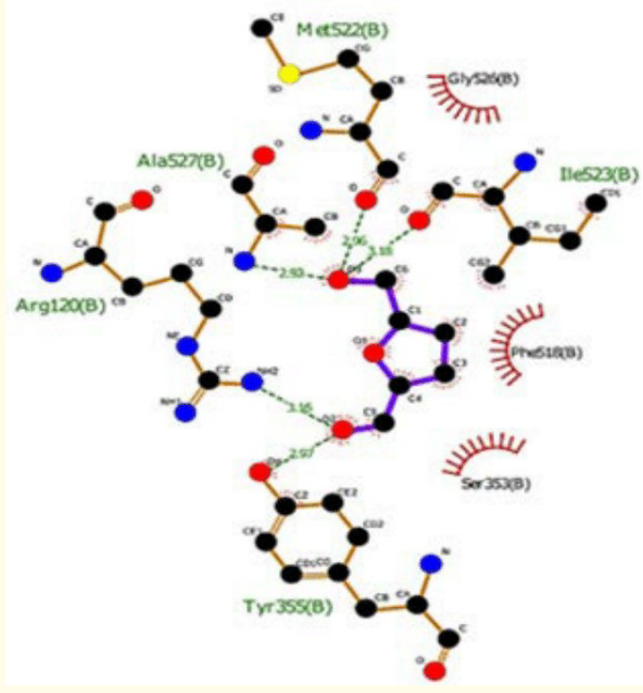


***Figure 5:*** *Interaction COX1:5-HMF: Hydrogen bond and its length (A°) in green colour. Violet structure represents 5-HMF.*

Also 5-HMF showed common hydrogen bond interactions with Glu 524(A) and three hydrogen bonds with Arg 120 (A) of COX-2 (Figure 6).

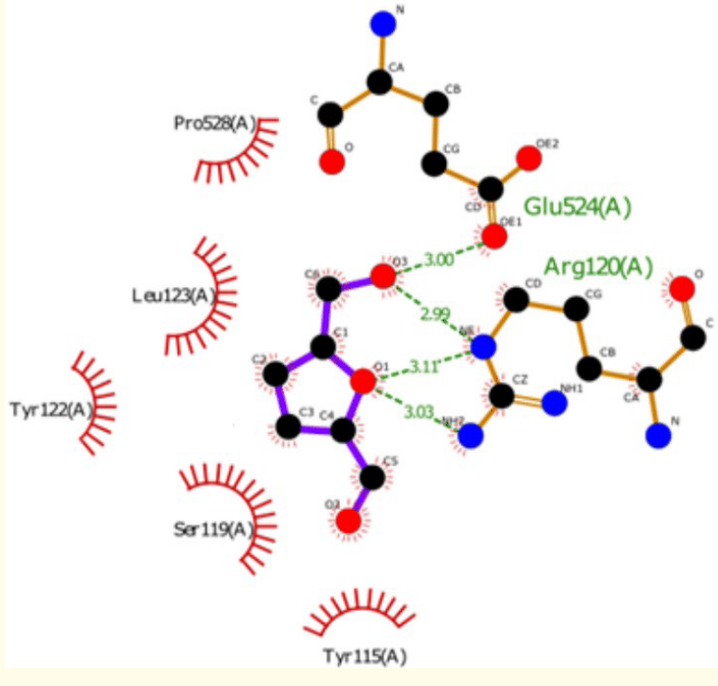


***Figure 6:*** *Interaction COX2:5-HMF: Hydrogen bond and its length (A°) in green colour. Violet structure represents 5-HMF.*

The results of docking investigation suggested a good binding interaction with COX, although, there is no selective for any COX, since 0.84 was the calculated selectivity. This results could explain, at least in part, the significant biological activity of 5-HMF on *in vivo* test and its potential mechanism of action.

Furthermore, taking into account that number of hydrogen bond acceptor and donors were in the range of 4 - 5 for 5-HMF, its molecular weight is 126.11, its log P-0.778, thus the compound does not violated the Lipinski's rule of five parameters, so it could be possible that 5-HMF could have properties that would make it a likely orally active drug in humans [19].

Despite the benefits of 5-HMF on human SCD and other potential uses, probable threats must be taken into account, since it could be activated to 5-sulfoxymethylfurfural (SMF) by sulfotransferases and SMF showed a possible carcinogenic potential [20]. But the number of studies conducted and the species used are limited, so the contradictory findings on the possible carcinogenicity of 5-HMF must be studied in depth but it should not be discarded [2].

Although part of the mechanisms responsible for beneficial effect induced by 5-HMF were studied, future research needs to fully evaluate the clinical potential of the compound to treat inflammatory process.

## Conclusion

In conclusion, this work reveals, for the first time, that 5-HMF has *in vivo* anti-inflammatory effects, suggesting that the compound might represent a potential therapeutic option for the treatment of inflammatory processes. The reduction in MPO activity and the *in silico* results could explain, at least in part, the underlying mechanism involved in the protective effects of 5-HMF on the acute inflammatory process.

## Acknowledgements

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## Conflict of Interest

No conflict of interest exists.

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