

EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF SOME NEW FERULLIC ACID DERIVATIVES

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Abstract

The anti-inflammatory potential of new thiazolidin-4-one derivatives of ferulic acid was evaluated using *in vitro* assays based on bovine serum albumin denaturation and human red blood cell membrane stabilization. The most intense anti-denaturation effect was showed by compounds **1e** (R = 2-NO₂), **1f** (R = 2-OH) and **1d** (R = 4-NO₂) for which the anti-denaturation activity was more intense than ferulic acid and comparable with diclofenac. Considering the ability of the tested compounds to protect the erythrocytes membrane it was observed that at 500 µg/mL, all compounds showed a protection comparable or higher than diclofenac and ferulic acid. The results support the favourable influence of the thiazolidine-4-one moiety on the anti-inflammatory effect of the ferulic acid derivatives.

Rezumat

Potențialul antiinflamator al unor noi derivați de acid ferulic cu structură de tiazolidin-4-onă a fost evaluat utilizând teste *in vitro* bazate pe inhibarea denaturării albuminei serice bovine și testul de stabilizare a membranei eritrocitare. Cea mai intensă capacitate de inhibare a denaturării proteice au demonstrat-o compușii **1e** (R = 2-NO₂), **1f** (R = 2-OH) și **1d** (R = 4-NO₂), a căror activitate de anti-denaturare a fost mai intensă decât a acidului ferulic și comparabilă cu cea a diclofenacului. În ce privește capacitatea compușilor testați de a stabiliza membrana eritocitară, aceasta a fost comparabilă sau mai intensă decât a diclofenacului și a acidului ferulic, la concentrația de 500 µg/mL. Rezultatele obținute demonstrează influența favorabilă a nucleului de tiazolidin-4-onă asupra potențialului antiinflamator al derivaților de acid ferulic.

Keywords: ferulic acid, thiazolidin-4-one, anti-inflammatory activity

Introduction

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound widely distributed in plants. It is a strong natural antioxidant, being able to protect DNA and biological lipids from oxidative stress [1, 2]. As an antioxidant, it has been recently investigated for its potential in Alzheimer's and Parkinson diseases [3], hypertension, atherosclerosis, various inflammatory diseases [4] and for its skin protective properties [2]. Also, it was found that ferulic acid provides protection from excessive glutamate-induced excitotoxicity, which suggests that it may act as an antagonist of NMDA receptors (N-methyl-D-aspartate).

On the other hand, recent studies have established a strong correlation between inflammation and oxidative stress. The human body is constantly aggressed by the endogenous or exogenous highly reactive free radicals, which may damage proteins, enzymes, lipids, DNA, causing inflammatory reactions and metabolic disturbances [5]. Also it has been shown that chronic inflammation is responsible for

the increased levels of reactive oxygen species (ROS) in cells (neutrophils, monocytes, macrophages, eosinophils) and for the decreased levels of antioxidant enzymes (catalase, superoxide dismutase, glutathione-peroxidase). ROS are also involved in metabolic conversion of arachidonic acid to pro-inflammatory intermediates and prostaglandins, conversion which is mediated by cyclooxygenase-1 (COX-1) and lipoxygenase. The reactive oxygen species activate redox-sensitive transcription factors as nuclear factor kappa B type (NK-kB) and activator protein 1 (AP-1). Disturbances in these proteins regulation are correlated with cancer, viral infections, autoimmune diseases etc. [7].

Based on these considerations the goal of this study was to evaluate *in vitro* anti-inflammatory potential of new thiazolidin-4-one derivatives of ferulic acid.

Materials and Methods

The used reagents: bovine serum albumin (BSA), 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris), diclofenac sodium, phosphate buffer (pH=7.4),

isosaline solution (NaCl 0.85%), hyposaline solution (NaCl 0.36%), acetic acid, methanol, dimethylformamide (DMFA) were purchased from Sigma Aldrich and Fluka.

Bovine serum albumin denaturation assay

The tested compounds were dissolved in methanol in order to obtain a stock solution of concentration 10 mg/mL [8]. From stock solution of each compound were measured different volumes (100 μ L, 200 μ L and 500 μ L) which were diluted with methanol to obtain a final volume of 1000 μ L. To 50 μ L of

each dilution was added 5 mL bovine serum albumin (BSA) 0.2% (in Tris buffer saline, pH = 6.8). A mixture of 50 μ L methanol and 5 mL bovine serum albumin 0.2% was used as control. The samples and the control were incubated at 37°C for 20 min and then at 72°C for 5 min. Finally the samples and the control were cooled for 10 min and the turbidity was measured at 660 nm relative to Tris buffer saline solution.

The inhibition of protein denaturation (%) was calculated using the following formula:

$$\text{Inhibition of denaturation (\%)} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

where: A_{control} = absorbance of the control and A_{sample} = absorbance of the tested compounds.

Diclofenac (0.1 mg/mL) was used as standard anti-inflammatory drug being processed in a similar manner with the samples. All determinations were performed in triplicate and the values are expressed as mean \pm standard deviation (SD) [9, 10].

Human red blood cell (HRBC) membrane stabilization assay

The blood was collected from healthy volunteers [11]. The collected blood was mixed with an equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged at 3000 rpm. The human red blood cells were washed with isosaline solution (NaCl 0.85%) and a 10% (v/v) suspension was made with isosaline.

The tested compounds were dissolved in DMFA in order to obtain a stock solution of concentration

10 mg/mL. Different volumes (100 μ L, 200 μ L and 500 μ L) from stock solution were diluted with isosaline solution 0.85% to obtain a final volume of 1000 μ L. To this solution 1 mL of phosphate buffer (pH = 7.4), 2 mL of hyposaline solution (0.36%) and 0.5 mL of human red blood cells (HRBC) suspension (10% v/v) were added. A mixture between 1 mL of phosphate buffer (pH = 7.4), 2 mL of hyposaline solution (0.36%) and 0.5 mL of human red blood cells (HRBC) (10% v/v) was used as control. The samples and the control were incubated at 37°C for 30 min and then centrifuged at 3000 rpm. The haemoglobin content in the supernatant solution was estimated by using a spectrophotometric method at 560 nm [11]. The erythrocytes' membrane stability (%) was calculated using the following formula:

$$\text{Erythrocyte membrane stability (\%)} = 100 - (A_{\text{sample}}/A_{\text{blood}})$$

where: A_{sample} = absorbance of the tested compound and A_{blood} = absorbance of the control.

Diclofenac (0.1 mg/mL) was used as standard anti-inflammatory drug being processed in a similar manner with the samples. All determinations were performed in triplicate and the values are expressed as mean \pm standard deviation (SD) [12].

Results and Discussion

The structure of the tested compounds, thiazolidin-4-one derivatives of ferulic acid, is presented in Figure 1.

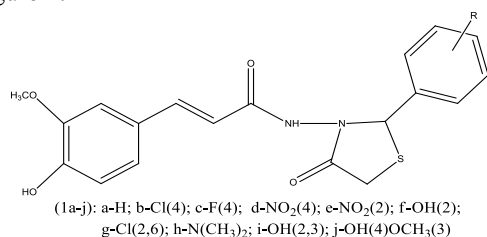


Figure 1.

Thiazolidine-4-one derivatives of ferulic acid (1a-j)

Anti-denaturation activity

Recent data support that protein denaturation are among the causes of inflammatory and rheumatic diseases [9]. By heating, bovine serum albumin (BSA) undergoes denaturation with the release of antigens responsible for several hypersensitivity reactions, which lead to various diseases such as glomerulonephritis, lupus erythematosus etc. [9]. Thus, the agents that can prevent the protein denaturation could be useful for inflammatory diseases. It is considered that any compound presenting a percentage inhibition of protein denaturation higher than 20% could be studied as potential anti-inflammatory agent [10]. Anti-inflammatory drugs as indomethacin, diclofenac, ibufenac, acetylsalicylic acid, flufenamic acid, besides the inhibition of cyclooxygenase showed also a considerable ability to prevent the BSA denaturation [13].

The anti-denaturation activity of the tested compounds (1a-j) at different concentrations, are presented in Figure 2. It was observed that the inhibition of BSA

denaturation is increasing with the concentration, the better anti-denaturation activity being observed at 500 µg/mL. At this concentration the most active compounds were **1e** (R = 2-NO₂, I% = 98.38 ± 0.012%), **1f** (R = 2-OH, I% = 99.00 ± 0.017%) and **1d** (R = 4-NO₂, I% = 96.32 ± 0.009%) for which the anti-denaturation activity was comparable to diclofenac (I% = 97.88 ± 0.001).

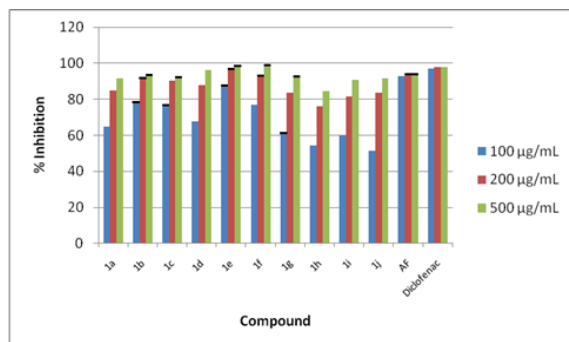


Figure 2.

The BSA denaturation inhibition (%) of thiazolidin-4-one derivatives (**1a-j**) at different concentrations

Membrane stabilizing activity

The human red blood cell (HRBC) membrane stabilization assay is, often used for assessing the anti-inflammatory effect [11]. Human erythrocytes membrane is similar to lysosomal membrane and its stability limits the inflammatory response by inhibiting the release of cellular constituents that activate neutrophils, bactericidal enzymes and proteases causing inflammation of tissue and cell damage [9, 12, 13].

The membrane stabilizing activity of the tested compounds (**1a-j**) at different concentrations, are presented in Figure 3.

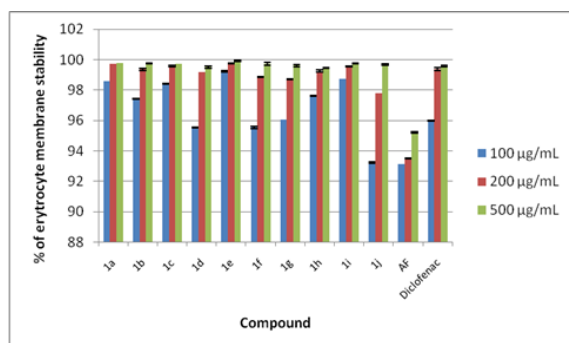


Figure 3.

The erythrocyte membrane stability (%) of thiazolidin-4-one derivatives (**1a-j**) at different concentrations

For all tested compounds it was observed that protection of erythrocyte membrane was higher than ferulic acid and comparable with diclofenac for all tested concentrations: 100 µg/mL, 200 µg/mL and 500 µg/mL. Except **1d** (R = 4-NO₂), **1f** (R = 2-OH),

1g (R = 2,6-diCl) and **1j** (R = 4-OH, 3-OCH₃), all other compounds showed a membrane stabilizing activity higher than diclofenac and ferulic acid at 100 µg/mL and 200 µg/mL. At 500 µg/mL all the tested compounds showed a membrane protection comparable or higher than diclofenac (99.56 ± 0.045) and ferulic acid (95.20 ± 0.057) respectively.

Conclusions

New thiazolidin-4-one derivatives of ferulic acid were investigated for their *in vitro* anti-inflammatory activity using BSA denaturation and HRBC membrane stabilization assays. The results support that the albumin anti-denaturation and erythrocyte membrane stabilizing activity of the tested compounds are depending of concentration, the best results being obtained at 500 µg/mL. Compared with diclofenac, used as standard anti-inflammatory drug, the most active compounds were **1e** (R = 2-NO₂), **1f** (R = 2-OH) and **1d** (R = 4-NO₂). These compounds showed an anti-denaturation and membrane stabilizing activity comparable and higher than diclofenac at 500 µg/mL.

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