Voltammetric determination of binding constant of newly synthesized potential COX-2, 5-LOX dual inhibitors with human serum albumin

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Many research papers showed strong inflammatory correlation with some cancer types [1]. Transferring of dual COX-2 and 5-LOX inhibitors into the oncology field has been intensively studied in last 15 years so the aim of this work was to examine the redox behaviour, as well as interaction with human serum albumin (HSA), of newly synthesized dual COX-2 and 5-LOX inhibitors: 1-(4-aminophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (4FNH2), 1-(4-aminophenyl)--3-(3-fluorophenyl)prop-2-en-1-one (3FNH2) and (1-(4-aminophenyl)-3-(2-fluorophenyl)prop-2-en-1-one (2FNH2).
Voltammetric techniques, cyclic voltammetry (CV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) with GCE were used. When analysing the CVs, all compounds were found to have an oxidation peak (I_a) around + 0.75 V and a reduction peak (I_c) at potentials around −1.0 V. No significant influence of the substituents on the electrochemical behaviour of the compounds was observed. For all three compounds, a preliminary study of the interaction with HSA was performed at a concentration of each compound of 5×10^{-5} M and different HSA concentrations (5×10^{-8} M - 10^{-5} M) using DPV. The decrease in current intensity was similar for all three compounds after interaction with HSA, while the most intense change in the position of the oxidation peak after interaction was observed for compound 4FNH2 (ΔE_p = 122 mV). The results of relative decrease in peak current and shifting of peak potential upon the interaction with HSA for all compounds are shown in Table 1.

Table 1 Normalized peak currents and DPV peak potentials shift after the interaction of compounds with HSA

Compound	Normalized peak current, %	ΔE_p / mV
4FNH2	46.42	122
3FNH2	45.97	85
2FNH2	48.56	58

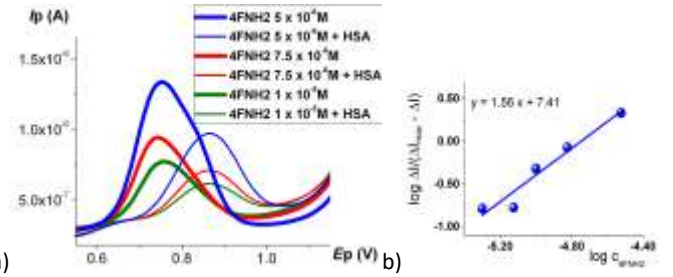

Figure 1. a) DPV voltammograms of different concentration of 4FNH2 before and after interaction with HSA b) Dependence of log (ΔI /(ΔI_{max} - ΔI)) vs. log c_{4FNH2} used for 4FNH2–*HSA binding constant determination(c_{HSA} = 2×10⁻⁶ M)*

Since compound 4FNH2 showed the most intense interaction with HSA, the detailed investigation of its electrochemical behaviour is presented. The peak current of 4FNH2 increased linearly with the square root of ν ($I_{p,Ia}$ / μA = 5.36×10^{-6} $\nu^{1/2}$ / $V^{1/2}$ $s^{-1/2}$ - 2.04×10^{-7}); r = 0.993) indicating that the oxidation process was diffusion controlled. This is confirmed by the log $I_{p,Ia}$ *vs.* log ν linear dependence [2], with slope values of 0.67, which was close to theoretical value of 0.5 for diffusion-limited process. In addition to diffusion of electroactive material, some adsorption also took place, as indicated by the small intercept in the $I_{p,Ia} = f(\nu^{1/2})$ regression equation and small variance of the slope of log $I_{p,Ia} = f$ (log ν) plot from theoretical value of 0.5.
The DPV was used to thoroughly investigate the interaction 4FNH2 with HSA and to determine the binding constant (Fig. 1). The concentration of 4FNH2 was increased (5×10^{-6} - 5×10^{-5} M) while the HSA concentration was kept constant (2×10^{-6} M). The differential peak current intensity was measured before and after the interaction of 4FNH2 with HSA and by using log (ΔI/(ΔI_{max} - ΔI)) *vs.* log c_{4FNH2} dependence [3], the binding constant, K was calculated, K = 5.62×10^{4} M^{-1}.

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