

SUITABILITY OF RECOMBINANT *ZOPHOBAS MORIO* LUCIFERASE-LIKE ENZYME FOR TOXICITY BIOASSAYS OF PHARMACEUTICAL COMPOUNDS

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The accumulation of toxic compounds of pharmaceutical origin can display severe effects on the environment and living organisms. The luciferase-like enzyme previously cloned from the Malpighian tubules of the non-luminous mealworm *Zophobas morio* produces weak luminescence in presence of ATP and firefly D-luciferin, a xenobiotic for this organism. Therefore, we started to investigate whether this enzyme could be involved in carboxylic xenobiotic metabolism, and its possible application as a luminescent biosensor for the carboxylic xenobiotics. To do this, we compared the effect of different carboxylic compounds at the concentration of 1 mM on the cellular growth of *E.coli* bacteria expressing the luciferase-like enzyme and their luminescence. We also tested the effect of these compounds on the *in vitro* luminescence activity of the enzyme. Among several compounds tested, we found that the anti-inflammatory drug, diclofenac, displays a strong inhibitory effect on the luminescence activity of this enzyme. This compound had no effect on the cellular growth and cell viability, which were followed spectrophotometrically at 600 nm and by the tetrazolium cell viability assay (TTC), respectively. However, both the *in vivo* and *in vitro* luminescent activities in the presence of 1 mM diclofenac showed ~ 98% inhibition of luminescence activity, indicating that the decrease of luminescence was essentially due to enzymatic inhibition. A dose/effect curve showed that diclofenac, displayed a P₅₀ of 15 µM. Analysis of the inhibition kinetics of diclofenac for D-luciferin and ATP, showed mixed type inhibition regarding both substrates, indicating that this compound binds to another site of the enzyme. These results are promising, showing the potential usefulness of this luciferase-like enzyme for development of a novel luminescent biosensor for diclofenac and similar pharmaceutical compounds.

Key words: Luminescence, Diclofenac, Luciferase-like enzyme, Biosensor.

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