# Investigation of 4-amino-TEMPO spin label interactions with some dihydroxycoumarins

[1] Krzysztof ŻAMOJĆ, [1] Wiesław WICZK, [2] Maciej WITWICKI, [1] Magdalena ZDROWOWICZ, [3] Aleksandra HAĆ, [1] Paweł B. RUDNICKI-VELASQUEZ, [1] Dariusz WYRZYKOWSKI, [1] Lech CHMURZYŃSKI

*[1]University of Gdansk, Faculty of Chemistry, W. Stwosza 63, 80-308 Gdansk, Poland.*
*[2]University of Wrocław, Faculty of Chemistry, F. Joliot-Curie 14, 50-383 Wrocław, Poland.*
*[3]University of Gdansk, Faculty of Biology, W. Stwosza 59, 80-308 Gdansk, Poland.*

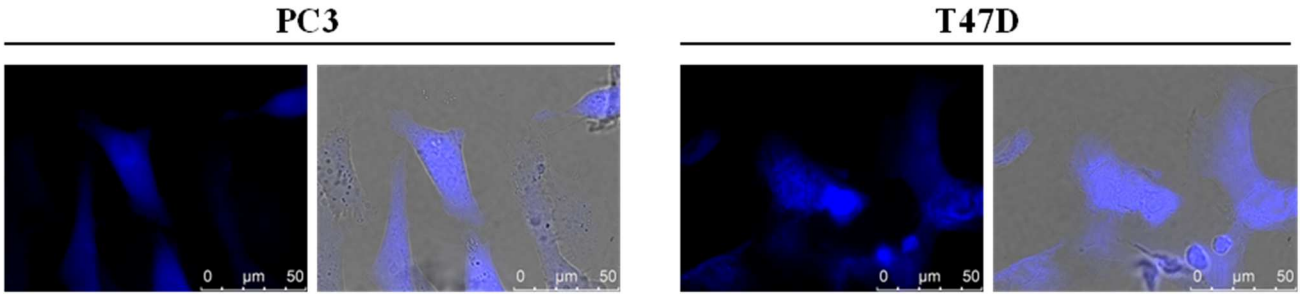
We report herein dihydroxycoumarins as fluorescent probes suitable for the detection and determination of 4-amino-TEMPO in aqueous solution. Among different coumarins studied, only dihydroxy-substituted derivatives show high sensitivity, specificity and selectivity for the nitroxide radical.

In this assay, dihydroxy-substituted coumarins under the action of 4-amino-TEMPO undergo very fast and significant increase in the fluorescence intensity. In the presence of 100 μM nitroxide, 6,7-dihydroxycoumarin (esculetin) exhibits the strongest fluorescence enhancement (up to 40 times) with estimated limits of the detection and quantitation equal to 0.43 μM and 1.04 μM, respectively. A linear relationship has been observed between the fluorescence enhancement of the chosen dihydroxycoumarins and 4-amino-TEMPO concentration up to 50 μM. The mechanism of the interaction between 6,7-dihydroxycoumarin and 4-amino-TEMPO has been examined with the use of a series of complementary techniques, such as steady-state and time-resolved fluorescence spectroscopy, UV-Vis spectroscopy, electron paramagnetic resonance spectroscopy (EPR) and the potentiometric titration. It has been proven that the only route of the reaction in the system studied is a proton transfer from the molecule of esculetin to the amino group of the nitroxide radical.

Furthermore, in the current project, it has been shown that 6,7-dihydroxycoumarin incorporates easily into the cancer cells (probably contrary to normal cells). It has been confirmed that esculetin exhibits anticancer properties[1]. Additionally, the cytotoxicity of that derivative was evaluated on the prostate (PC3) and breast (T47D) cancer cell lines as well as non-cancerous cells (normal fibroblasts) by the MTT assay.

[1] S. Emami & S. Dadashpour, Current developments of coumarin-based anti-cancer agents in medicinal chemistry. Eur. J. Med. Chem., 2015, 102, 611–630.

**Figure:** Representative images of 6,7-dihydroxycoumarin fluorescence in prostate (PC3) and breast (T47D) cancer cells. Phase contrast and fluorescence images were aquired with fluorescence microscope with a 100x oil objective.

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