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Colorimetric assay for the of Alcohol Dehydrogenase activity assessment.

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PURPOSE OF THE ABSTRACT

It is a matter of fact that the importance of enzymatic catalysis has significantly increased during the last decades, indeed, biocatalysts seem to be one of the most promising answers to the urgent need of environmentally friendly chemical processes. Although a plethora of new enzymes have been discovered or bioengineered, their employment at preparative scale always requires the knowledge of activity and stereoselectivity. For example, the Alcohol Dehydrogenases (ADH), which are very likely one of the most popular redox enzymes, can reduce prochiral ketones and aldehydes into alcohols, but the determination of their activity is tedious and a time-consuming practice [1]. Herein we describe a new colorimetric assay suitable to the rapid and efficient screening of ADH activity [2]. Our colorimetric probe is formed by the levulinc carboxylic acid coupled to the resorufin dye, and it should be a suitable substrate for the ADHs. The chromogenic signaling is due to the ADH triggered release of resorufin dye, that when it is free exhibits a brilliant pink color, instead, when it is functionalized has a pale-yellow color, as shown in the Figure. The chemoselective bio-reduction of the ketone group of levulinate affords the corresponding hydroxyester, which in turn ring-closes very rapidly affording the γ-lactone and the resorufin dye. The suggested ADH triggered cyclization was studied by 1H-NMR, UV-vis, and fluorescence measurements.

FIGURES

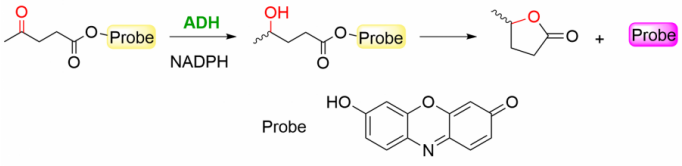

FIGURE 1
Colorimetric assay for the screening of Alcohol Dehydrogenase activity

FIGURE 2

KEYWORDS
colorimetric assay | ADH activity | chemoselective bio-reduction | resorufin dye

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