

1 Supplementary material

2 **Synthesis and Biological Activity of Homohypotaurine Ob-**
3 **tained by the Enzyme-Based Conversion of Homocysteine sul-**
4 **finic acid Using Recombinant *Escherichia coli* Glutamate De-**
5 **carboxylase**

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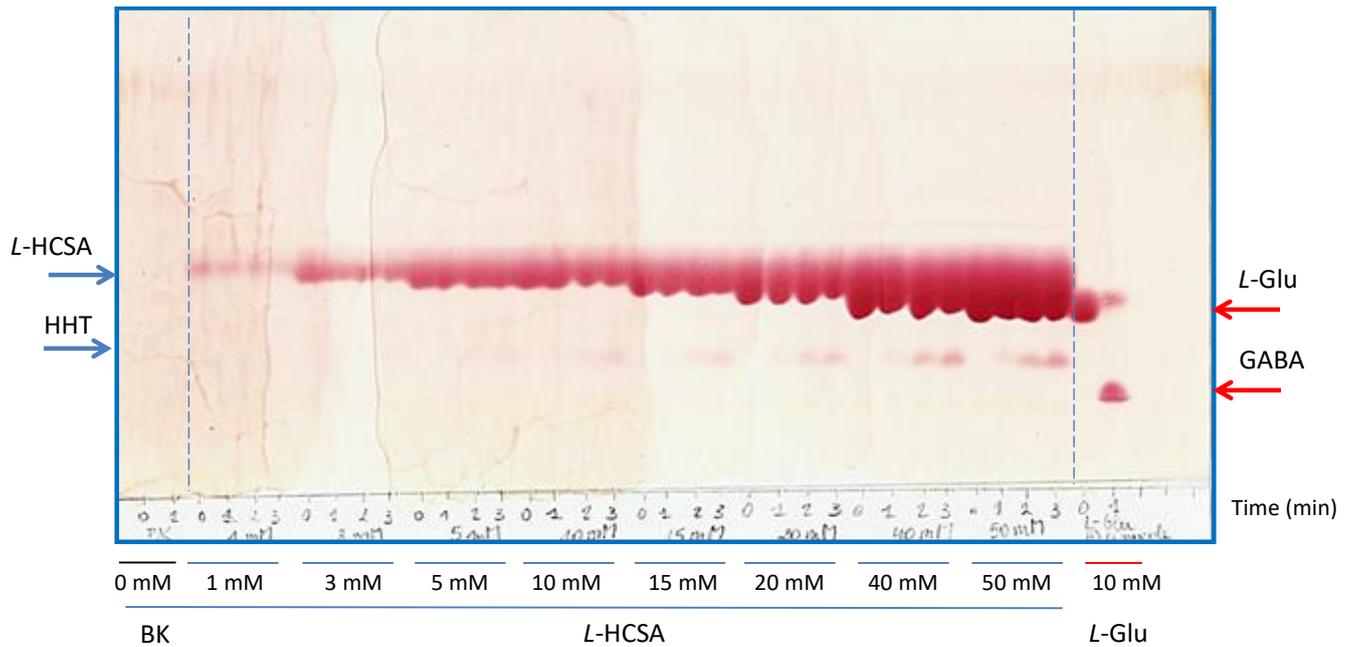
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25 **Keywords:** green chemistry; PLP; sulfinic compounds; HUVEC; H9c2; hydrogen peroxide.
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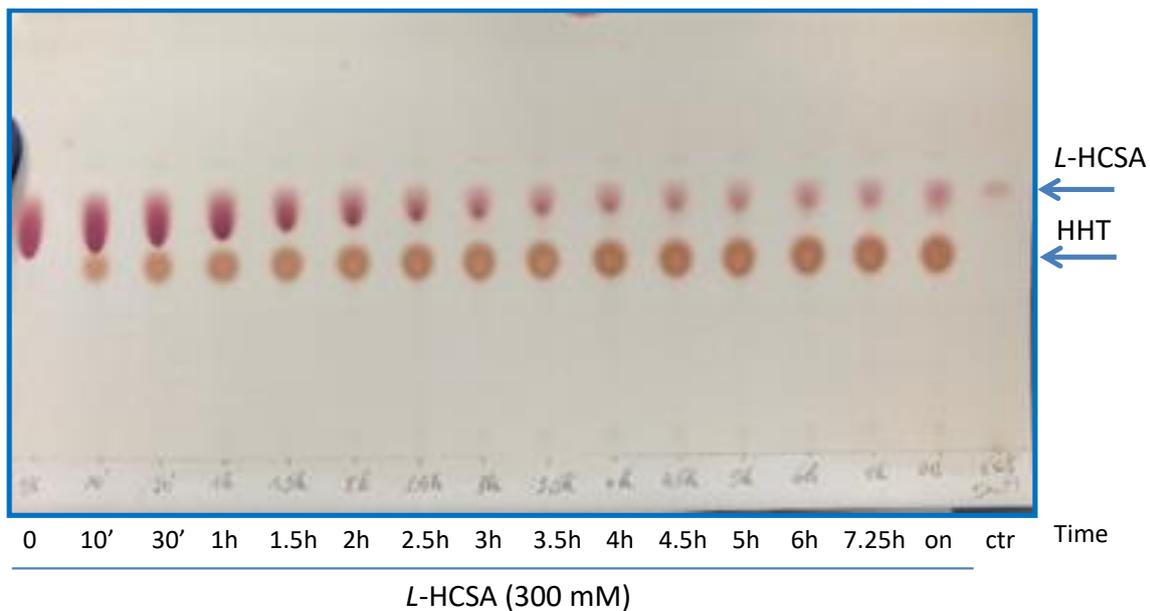


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29 **Figure S1.** Time course of *L-HCSA* bioconversion into *HHT* by *EcGadB*. The reactions were carried out using a range of concentrations
 30 of *L-HCSA* from 1 to 50 mM in 0.2 M pyridine/HCl buffer, pH 4.6, containing 1 mM PLP and 0.1 mM DTT. *EcGadB* was used at a
 31 concentration of 0.06 mg/mL and each reaction was in a final volume of 110 μ l. At each indicated time (including time 0,
 32 corresponding to no enzyme added), the reaction was halted by transferring 5 μ l of the reaction mix into 10 μ l of 50 mM NaOH
 33 and vortexed vigorously. 10 μ l (for 0-1-3-5 mM condition) and 5 μ l (for 10-15-20-40-50 mM condition) were spotted on thin layer
 34 silica sheet (on aluminium). Taking into account the dilution to halt the reactions, the minimum loading (time 0 at 1 mM *L-HCSA*)
 35 corresponded to 3.3 nmoles of *L-HCSA* whereas the maximum loading (time 0 at 50 mM *L-HCSA*) was 83.3 nmoles. On the
 36 leftmost part of the sheet, the blank (BK) with buffer only (0) and with the enzyme added (1) is shown as control, while on the
 37 rightmost part of the TLC 10 mM *L-Glu* (16 nmoles deposited) was reacted in the same conditions as above but only for 1 min, to
 38 yield GABA. The TLC was run in a vapor-saturated chamber, containing the solvent system isopropanol/ammonia 6.4 M/water in
 39 v/v/v ratio 7:0.32:2.68. The relative mobility of the compounds of interest are shown with arrows; the compounds (substrates and
 40 products) were detected by ninhydrin (0.2% in acetone) staining followed by drying with hot air to allow the development of the
 41 colored product.

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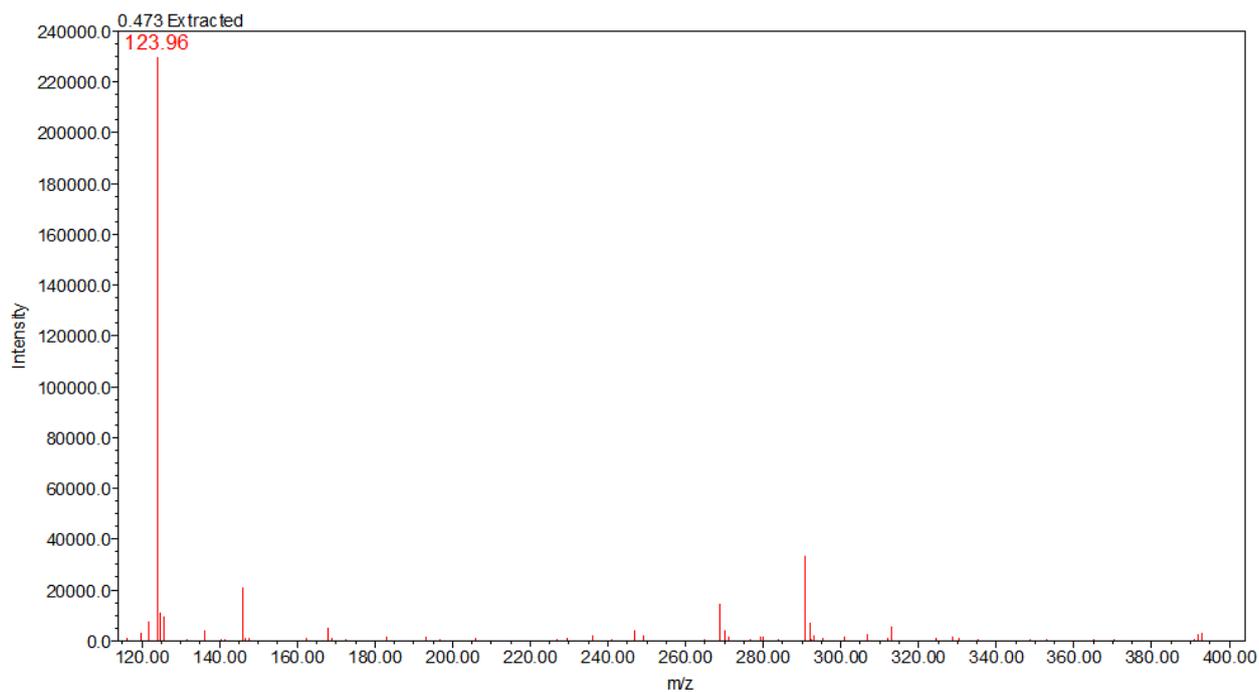
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45 **Figure S2.** Analytical TLC of the time course of the preparative scale bioconversion of L-HCSA into HHT by *EcGadB*. The reaction was carried
 46 out starting from 300 mM L-HCSA which reacted for a total of 22 hours at 37°C in 0.2 M pyridine/HCl buffer, pH 4.6, containing 1
 47 mM PLP and 1 mM DTT, and controlling the pH via the addition, when necessary, of 3 N HCl (in total, 160 µl). *EcGadB* was used at
 48 a concentration of 0.25 mg/ml in a final reaction volume of 2.5 ml. At each indicated time, the reaction was stopped by transferring 2
 49 µl into 60 µl of 50 mM NaOH; 5 µl were spotted on thin layer sheet (silica on aluminium). The loading of L-HCSA at time 0
 50 corresponded to approx. 40 nmoles, that of the control 5 mM L-HCSA corresponded to approx. 0.8 nmole. TLC conditions and
 51 staining were as in the legend to Figure S1.

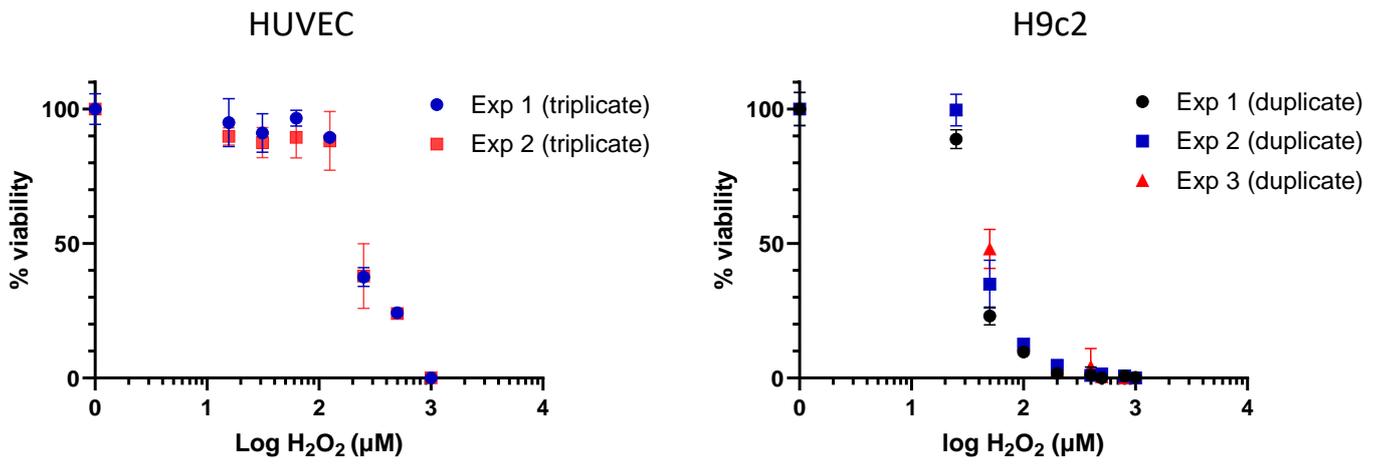
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54 **Figure S3.** *MS identification of purified HHT.* Chromatography was performed on a UPLC system as described in Materials and
55 Methods (subsection 4.2). Mass spectrometric detection was set in the positive electrospray ionization mode positive ESI [M-H]⁺,
56 using nitrogen as nebulizer gas. Analyses were performed in Total Ion Current (TIC) mode in a mass range 105–500 m/z. Capillary
57 voltage was 0.8 kV, cone voltage 15 V, ion source temperature 120 °C and probe temperature 600 °C. The mass spectrum of the
58 purified HHT confirmed its mass, i.e. calculated mass 123.96 Da vs expected monoisotopic mass 123.03 Da.

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63 **Figure S4.** Determination of IC_{50} of H_2O_2 on HUVEC and H9c2 cells. Two independent experiments were performed in triplicate for
64 HUVEC cells (left graph); three independent experiments were performed in duplicate for H9c2(2-1) cells. The tested H_2O_2
65 concentrations were from 1 to 1000 μM . The IC_{50} calculated by non linear regression (curve fit of $\log[\text{inhibitor}]$ vs. normalized
66 response - Variable slope), using GraphPad Prism were: $234.00 \pm 2.30 \mu M$ for HUVEC cells and $44.75 \pm 4.80 \mu M$ for H9c2 cells.