Supporting Information

Resorcinol-based hemiindigoid derivatives as human tyrosinase inhibitors and melanogenesis suppressors in human melanoma and melanocyte cells

Brayan Roulier,¹,# Inbal Rush,²,# Leticia M. Lazinski,¹,³ Basile Pérès,¹ Hamza Olleik,⁴ Guy Royal,³ Ayelet Fishman,² Marc Maresca⁴ and Romain Haudecoeur*,¹

1. Univ. Grenoble Alpes, CNRS 5063, DPM, 38000 Grenoble, France.
2. Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa, 3200003, Israel.
4. Aix Marseille Univ., CNRS, Centrale Marseille, iSm2, 13397 Marseille, France.

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*¹B.R. and I.R. have contributed equally to this work.*
**Part 1.** $^1$H and $^{13}$C NMR spectra of evaluated compounds.

**Figure S1.** Systematic numbering of reported scaffolds.
HO
\[\text{OH} \quad 5\]

\(12.0 \quad 11.5 \quad 11.0 \quad 10.5 \quad 10.0 \quad 9.5 \quad 9.0 \quad 8.5 \quad 8.0 \quad 7.5 \quad 7.0 \quad 6.5 \quad 6.0 \quad 5.5 \quad 5.0 \quad 4.5 \quad 4.0 \quad 3.5 \quad 3.0 \quad 2.5 \quad 2.0 \quad 1.5 \quad 1.0 \quad 0.5 \quad 0.0\)

\(210 \quad 200 \quad 190 \quad 180 \quad 170 \quad 160 \quad 150 \quad 140 \quad 130 \quad 120 \quad 110 \quad 100 \quad 90 \quad 80 \quad 70 \quad 60 \quad 50 \quad 40 \quad 30 \quad 20 \quad 10 \quad 0\)

S6
Part 2. Representative HPLC traces.

Compound 10

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Part 3. Inhibition curves for the screening against MNT-1 lysates.
Inhibitor Concentration (µM)

% Activity
Part 4. Validation of hsTYR.

Following purification on a Ni affinity column, hsTYR presence was validated by SDS-PAGE and western blotting (Figures S2 and S3, respectively). Samples containing hsTYR were then dialyzed in 25 mM Tris-HCl, pH 7.5 and 50 mM NaCl buffer prior to performing activity assays. The broad hsTYR band in the Figures at 60–75 kDa (red arrow in Figure S2 and black smear in Figure S3) confirm the presence of glycosylated forms of the enzyme. The glycosylations of hsTYR were validated via their elimination either by the use a glycosylation enzymatic inhibitor (PNGase) or by mutating the putative glycosylation sites. Both methods have led to a decrease in the molecular weight and in activity (data not shown), as was reported previously by Dolinska & Sergeev.¹

Figure S2. SDS-PAGE visualization of protein purification fractions of hsTYR. Protein samples were loaded into 12% acrylamide gel and stained with Coomassie Blue after Ni column purification. (M) protein marker in kDa; (Media) media collected after 5 days of expression, prior to concentration; (Load) media after concentration in binding buffer; (FT) flow through fractions collected at sample

loading; (W) wash fractions; (E) elution fractions at 500 mM imidazole step. Samples were loaded in their original eluted concentration in 30 µL volume. The red arrow indicates the hsTYR.

**Figure S3. Western blot visualization of protein purification fractions of hsTYR.** Protein samples were loaded into 12% acrylamide gel after Ni column purification, then transferred to a membrane overnight and incubated with anti His-tag antibody (R&D systems, MAB050H, 1:4,000). (M) protein marker in kDa; (Media) media collected after 5 days of expression, prior to concentration; (Load) media after concentration in binding buffer; (FT) flow through fractions collected at sample loading; (W) wash fractions; (E) elution fractions at 500 mM imidazole step. Samples were loaded in their original eluted concentration in 30 µL volume.
**Part 5. hsTYR kinetics.**

Figure S4 describes the kinetic performance of hsTYR without inhibitors. Constants obtained are: $K_m = 0.32 \pm 0.01$ mM, $V_{max} = 5.35 \pm 0.10 \mu$mol.min$^{-1}$.mg$^{-1}$, $k_{cat} = 5.95$ s$^{-1}$. Inhibition by KA is described in Figure S5. Inhibition by 10 and 23 are described in Figure S6.

**Figure S4.** Michaelis-Menten and Lineweaver-Burk plots of hsTYR with L-DOPA. L-DOPA concentrations: 0.08–1.7 mM. All measurements were performed in triplicates.
Figure S5. Michaelis-Menten and Lineweaver-Burk plots of hsTYR with L-DOPA as substrate and kojic acid as inhibitor in mixed (full) inhibition model. L-DOPA concentrations: 85–1700 µM. Kojic acid concentrations (µM): (a ●) 0, (b ▽) 100, (c ♦) 300, (d ◊) 500 and (e ■) 700. All measurements were performed in triplicates.
Figure S6. Plots for inhibition potency determination of compounds 10 (left panel) and 23 (right panel). Michaelis-Menten (A) and Lineweaver-Burk (B) plots of hsTYR with L-DOPA as substrate and 10 (left panel) and 23 (right panel) as inhibitors. L-DOPA concentrations: 65–1,375 µM. Inhibitor concentrations: 0 µM (a ●), 0.5 µM (b ▽), 2 µM (c ●) and 8 µM (d □). All measurements were performed in triplicates.
Part 6. Inhibition curves for the cytotoxicity assay.
Part 7. Inhibition curves for the MNT-1 whole cells assay.

![Graphs showing inhibition curves for different inhibitors. The x-axis represents inhibitor concentration in µM, and the y-axis represents % melanin content. The graphs show the inhibition of melanin content at various concentrations of inhibitors.]

Kojic acid
Part 8. Therapeutic interest of hsTYR inhibitors.

Figure S7. Main therapeutic and dermocosmetic applications of hsTYR inhibitors.