Supporting information

Discovery of Highly Potent Tyrosinase Inhibitor, T1, with Significant Anti-Melanogenesis Ability by zebrafish *in vivo* Assay and Computational Molecular Modeling

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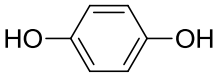
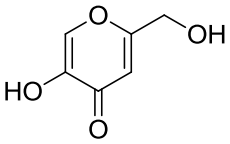
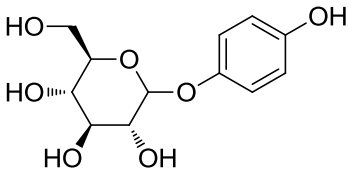
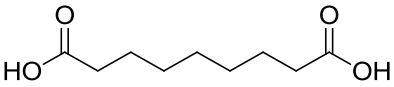
Table S1. The IC_{50} values of the compounds in cosmetics and known natural products retrieved from literatures.

Compounds in Cosmetics	**2D Structure**	**IC_{50} (μM)**	**Ref**
Hydroquinone		25.88	**1**
Kojic acid		40.69	**2**
Arbutin		368.93	**2**
Azelaic acid		>368.93	**3**
Natural products	**Scientific name**	**IC_{50} (μM)**	**Ref**
DBG	*Lespedeza cyrtobotrya*	20±3.5	**4**
Oxyresveratrol	*Mori cortex*	53.4	**5**
1,2,3,6-Tetra-*O*-galloyl-β-D-glucose	*Chinese Galls*	30	**6**
1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose	*Chinese Galls*	15	**6**
2,3,4,6-Tetra-*O*-galloyl-D-glucose	*Chinese Galls*	54	**6**
Digallic acid	*Chinese Galls*	>100	**6**
Methyl gallate	*Chinese Galls*	>100	**6**
Gallic acid	*Chinese Galls*	>100	**6**
Compund1	*Lespedeza cyrtobotrya*	5.2±3.9	**7**
Compund2	*Lespedeza cyrtobotrya*	1321.7±4.1	**7**
Compund3	*Lespedeza cyrtobotrya*	5±1.5	**7**
Compund4	*Lespedeza cyrtobotrya*	20±3.5	**7**
N-feruloylserotonin	*Carthamus tinctorius L.*	23	**8**
N-(p-coumaroyl)serotonin	*Carthamus tinctorius L.*	74	**8**
Acacetin	*Carthamus tinctorius L.*	779	**8**
Calycosin	*Astragalus membranaceus*	38.4±0.9	**9**
Cinnamic acid	*Cinnamomum cassia*	693.2	**10**
Macelignan	*Myristica fragrans*	30	**11**
Melanocins A	*Eupenicillium shearii*	0.009	**12**
Melanocins B	*Eupenicillium shearii*	>1000	**12**
Melanocins C	*Eupenicillium shearii*	>1000	**12**
Gnetin C	*Gnetum gnemon*	7	**13**
Resveratrol	*Gnetum gnemon*	7.2	**13**

Compund1	*Amberboa ramosa*	1.36±0.1	**14**
Compund2	*Amberboa ramosa*	11.68±0.4	**14**
Sophoraflavanone G	*Sophora flavescens*	6.6	**15**
Kuraridin	*Sophora flavescens*	0.6	**15**
Kurarinone	*Sophora flavescens*	6.2	**15**

Table S2. Evaluation of T1 toxicity in ICR mice via oral administration.

Sex	Dose	Mean body Weights During The Study Period(g)		
	(mg/kg)	SD1	SD8	SD15
Male	0	30.47 ± 0.66	34.68 ± 1.55	37.13 ± 0.88
	1500	30.52 ± 0.98	34.67 ± 3.67	36.93 ± 2.69
	3000	30.37 ± 0.69	35.48 ± 1.01	37.23 ± 1.09
	6000	29.85 ± 0.93	35.90 ± 1.87	37.53 ± 1.78

Sex	Dose	Body Weights Gains[a](g)	
	(mg/kg)	SD8	SD15
Male	0	4.22 ± 1.35	6.67 ± 0.52
	1500	4.15 ± 2.89	6.42 ± 2.01
	3000	5.12 ± 0.89	6.87 ± 1.00
	6000	6.05 ± 1.35	7.68 ± 1.20

[a]: Body weight (BW) gain was calculated from SD1

Figure S1. Multiple sequences alignment of human and mushroom tyrosinases. The sequence alignment was carried out by using Discovery Studio 4.0 alignment protocol. The amino acid sequence of mushroom tyrosinase (*Agaricus bisporus)* was retrieved from Protein Data Bank (PDB ID: 2Y9X, chain A) and the protein sequence of human tyrosinase (huTYR) was obtained from NCBI (NCBI Reference Sequence No: NP_000363.1). The homology of sequence and secondary structure information were rendered by ESPript program[16] (ESPript - http://espript.ibcp.fr). The numbering of residues and secondary structure elements (helices with squiggles, beta strands with arrows, turns and with TT letters) are presented according the sequence to of the *Agaricus bisporus* tyrosinase. The identical residues are highlighted by red sequence block, and the conserved residues are in red letters. The residues within 5Å near the dicopper ions in the active site are labeled with filled blue circle. The magenta stars denote the six highly conserved histidine residues. The white, cyan, and blue colors of the relative accessibility (acc) of each residue are buried, intermediate and accessible, respectively.

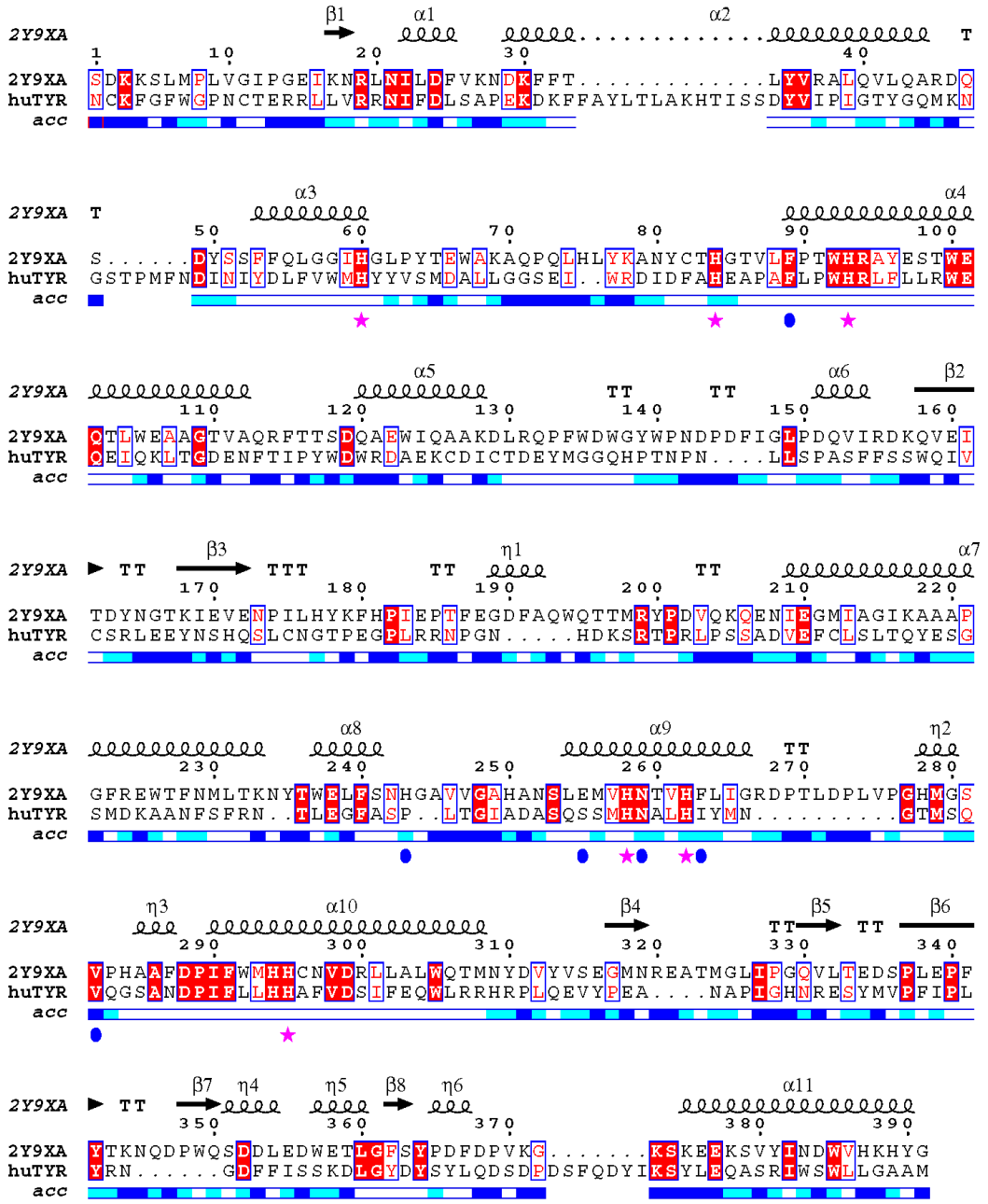


Figure S2. Dixon plot analysis of inhibition of mushroom tyrosinase. This analysis is used to determine the inhibition constant (*Ki*) of the T1 molecule.

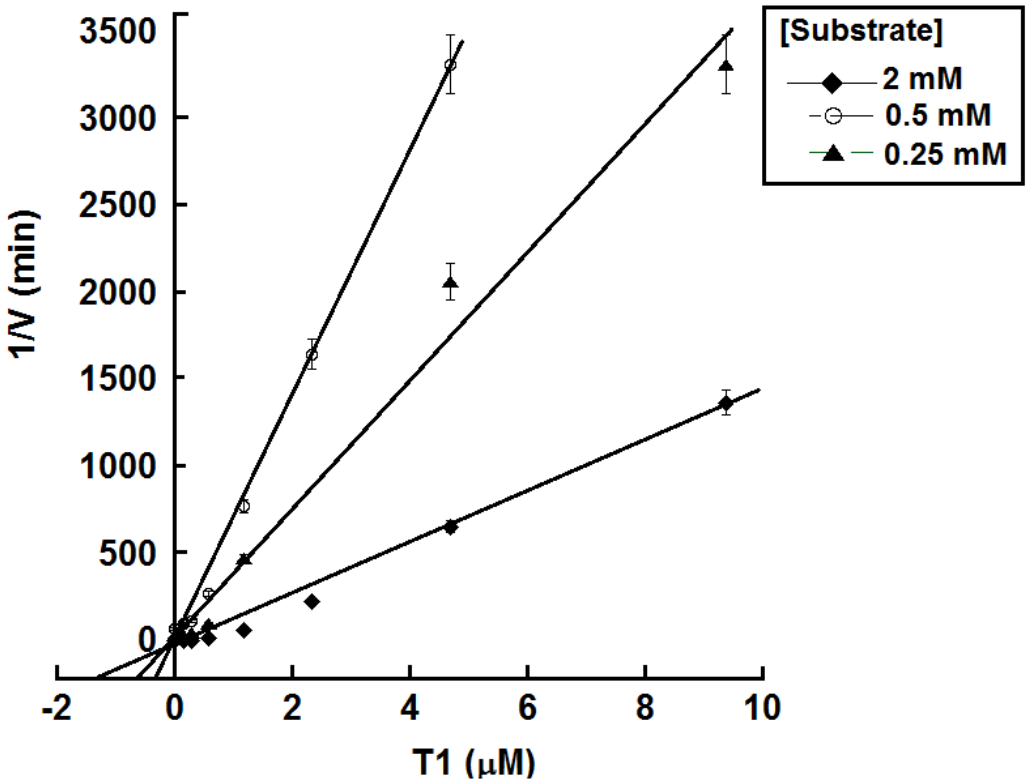


Figure S3. Molecular docking analyses of T3 and T5 toward mushroom tyrosinase. The green dash lines donate the hydrogen bond interactions, the yellow ones represent the hydrophobic interactions and the red spheres represent two cooper ions.

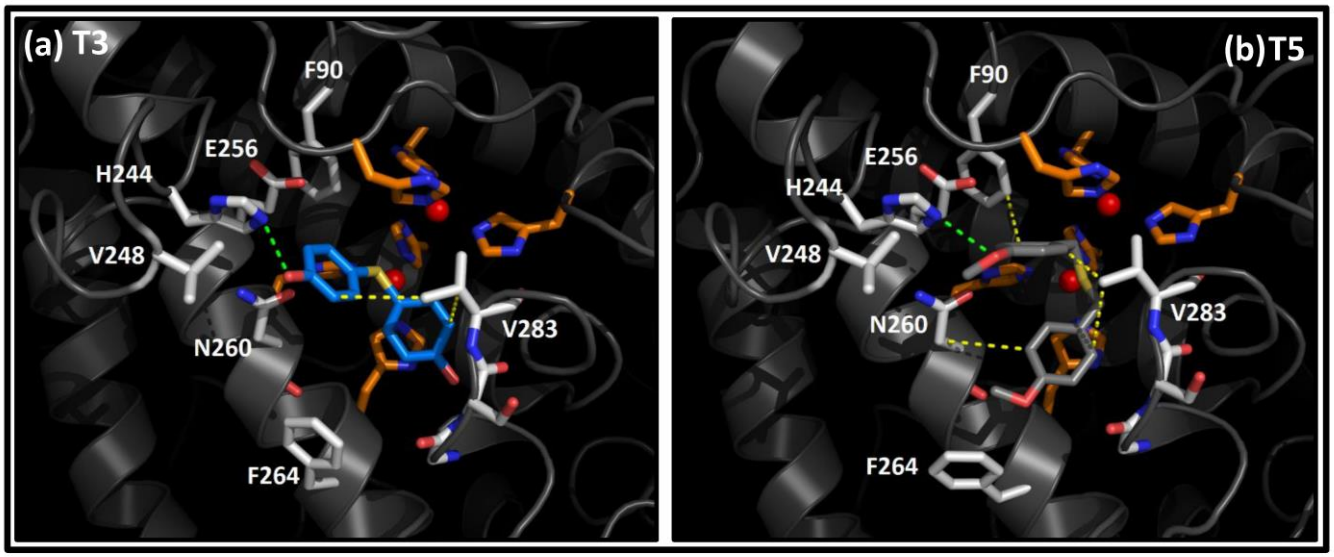
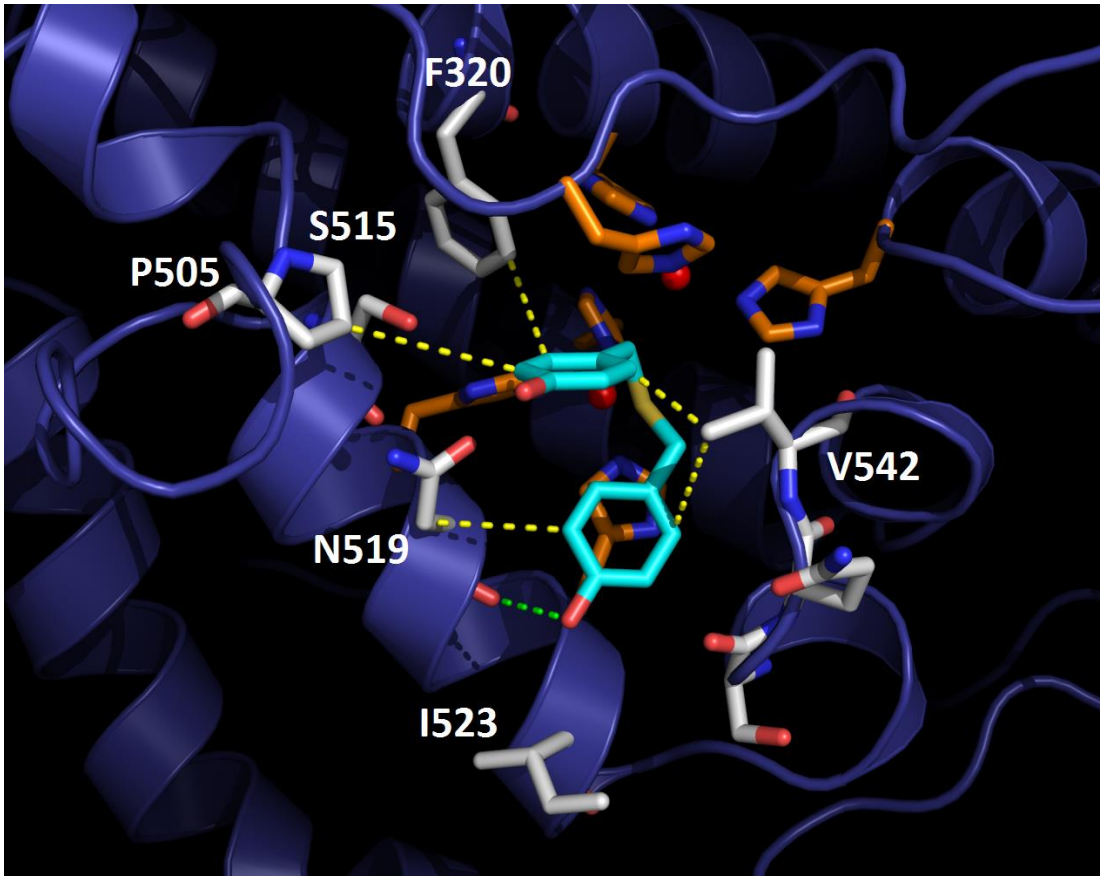


Figure S4. Molecular docking analysis of T1 toward human tyrosinase. The green dash lines donate the hydrogen bond interactions, the yellow ones represent the hydrophobic interactions and the red spheres represent two cooper ions.



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