Design, Synthesis, and in Vitro Evaluation of 4-(4-Hydroxyphenyl)piperazine-Based Compounds Targeting Tyrosinase

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Table S1: Smiles strings for compounds 2-35

| entry | SMILE |
|-------|-------|
| 2     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3 |
| 3     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccc(cc3)c4ccccc4 |
| 4     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3F |
| 5     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccc(F)cc3 |
| 6     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccc(F)cc3 |
| 7     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3Cl |
| 8     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3Cl |
| 9     | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3Br |
| 10    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3Br |
| 11    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3Br |
| 12    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3Br |
| 13    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3Br |
| 14    | Cc1cccc1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 15    | Cc1cccc1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 16    | Cc1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 17    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3C(F)(F)F |
| 18    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3C(F)(F)F |
| 19    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3C(F)(F)F |
| 20    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3C(F)(F)F |
| 21    | Co1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 22    | Co1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 23    | Co1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 24    | Co1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 25    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3[N+](=O)[O-] |
| 26    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3[N+](=O)[O-] |
| 27    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3[N+](=O)[O-] |
| 28    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3[N+](=O)[O-] |
| 29    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3[N+](=O)[O-] |
| 30    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3[N+](=O)[O-] |
| 31    | Oc1ccc(cc1)N2CCN(CC2)C(=O)c3ccccc3[N+](=O)[O-] |
| 32    | Nc1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 33    | Nc1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 34    | Nc1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
| 35    | Nc1ccc1c1C(=O)N2CCN(CC2)c3ccc(O)cc3 |
**Mushroom tyrosinase inhibition assay**

Aliquots (0.05mL) of test compound at various concentrations (0.5–140μM) were firstly mixed with 0.5 mL of L-tyrosine or L-DOPA solution (1.25mM), 0.9 mL of phosphate buffer (0.05M, pH 6.8). Then, the reaction mixture was preincubated at 25 °C for 10 min. Subsequently, 0.05 mL of an aqueous solution of mushroom tyrosinase (333 U/mL) was added. The linear increase in absorbance was measured after 60 or 1 min of incubation time in the reaction mixture containing L-tyrosine or L-DOPA, respectively. The inhibitory activity was expressed as inhibition percentage respect to control sample. The concentrations leading to 50 % activity loss (IC50) were also calculated by interpolation of the dose-response curves. Kojic acid [5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one], a fungal secondary metabolite used as skin whitening agent, was employed as a positive standard (10–50 μM). A spectrophotometer (Agilent Cary 60 UV-Vis Spectrophotometer) was used for absorbance measurements.

![Figure S69](image)

**Figure S69**: Inhibition effects of compounds 7, 10, 11, 17 and 21 (40 μM) on monophenolase activity of AbTYR compared to Kojic Acid

**Kinetic analysis of the tyrosinase inhibition**

The inhibition kinetics on the tyrosinase were studied using Lineweaver-Burk double reciprocal plots. The reaction mixture consisted of four different concentrations of the substrate L-DOPA (0.6–5 mM), and mushroom tyrosinase in phosphate buffer (0.05 M, pH 6.8). Three different concentrations were used for the analysis. Preincubation and measurement time were the same as discussed in mushroom tyrosinase inhibition assay. The Michaelis-Menten constant (Km) and maximal velocity (Vmax) of tyrosinase were determined by Lineweaver-Burk plots.
Figure S70: 2D interaction diagrams of compounds 7 (A), 10 (B), 11 (C), 17 (D), 21 (E) in the catalytic cavity of AbTYR
Figure S7: Effect of compounds 7, 10, 11, 17 and 21 on B16F10 cell viability. Cells were treated with different concentrations of compounds (4-100 μM) and their viability was evaluated by MTT assay. Data represent mean ± SD of triplicate experiments.