Supporting Information

Title:

A Synthetic 7,8-Dihydroxyflavone Derivative Promotes Neurogenesis and Exhibits Potent Antidepressant Effect

Authors:

Xia Liu, Chi-Bun Chan, Sung-Wuk Jang, Sompol Pradoldej, Junjian Huang, Kunyan He, Lien H. Phun, Stefan France, Ge Xiao, Yonghui Jia, Hongbo R. Luo and Keqiang Ye

Content:

Supplemental Table 1. CBC analysis of TrkB agonists-treated mice

Parameter	Control	7,8-DHF	4'-DMA-7,8-DHF
WBC (K/ul)	7.67±0.803	8.69±0.746	6.15±0.191
NE (K/ul))	1.11±0.078	2.99±0.906	1.36±0.559
LY (K/ul)	6.16±0.687	5.12±0.206	4.34±0.373
MO K/ul	0.23±0.069	0.29±0.063	0.20±0.033
EO (K/ul)	0.13±0.033	0.21±0.107	0.17±0.023
BA (K/ul)	0.05±0.019	0.08±0.012	0.07±0.03
RBC (K/ul)	7.87±0.886	8.43±0.393	8.47±0.28
Hb (K/ul)	10.67±1.453	11.00±0.265	10.87±0.384
HCT (K/ul)	39.07±4.431	40.13±2.019	40.90±1.724
MCV (K/ul)	49.60±0.451	47.60±1.258	48.30±1.353
MCH (K/ul)	13.47±0.384	13.10±0.321	12.83±0.12
MCHC (K/ul)	27.17±0.867	27.47±0.857	26.57±0.524
RDW (K/ul)	18.83±0.639	19.53±0.754	18.73±0.273
PLT (K/ul)	858.67±73.676	1113.00±22.234	1041.33±153.563 *
MPV (K/ul)	5.43±0.088	5.37±0.273	4.63±0.437

* Although PLT is significantly different between control and drug treated group, they are in the normal range.

Supplemental Figure Legends

Supplemental Figure 1. Structure-activity relationship study

(A & B) Chemical structures of flavone derivatives. (C) Phospho-Akt ELISA assay. The lysates from cells, treated with various flavones or related compounds for 15 min, were analyzed with p-Akt ELISA. 7,8-dihydroxy groups are essential for the agonistic effect of 7,8-DHF.

Supplemental Figure 2. 4'-DMA-7,8-DHF is more potent than 7,8-DHF in triggering TrkB activation in primary neurons.

Different concentrations of both compounds were employed to incubate with primary neurons for 15 min. The cell lysates were analyzed by immunoblotting with anti-p-TrkB 816 antibody.

Supplemental Figure 3. TrkB is activated by 7,8-DHF via oral administration in a dose dependent manner.

Different doses of 7,8-DHF solutions were administrated to C57BL/6 mice via oral gavage. The mice were sacrificed 2 h after drug treatment, and the hippocampal region and spinal cord were homogenated and analyzed by immunoblotting with anti-p-TrkB.

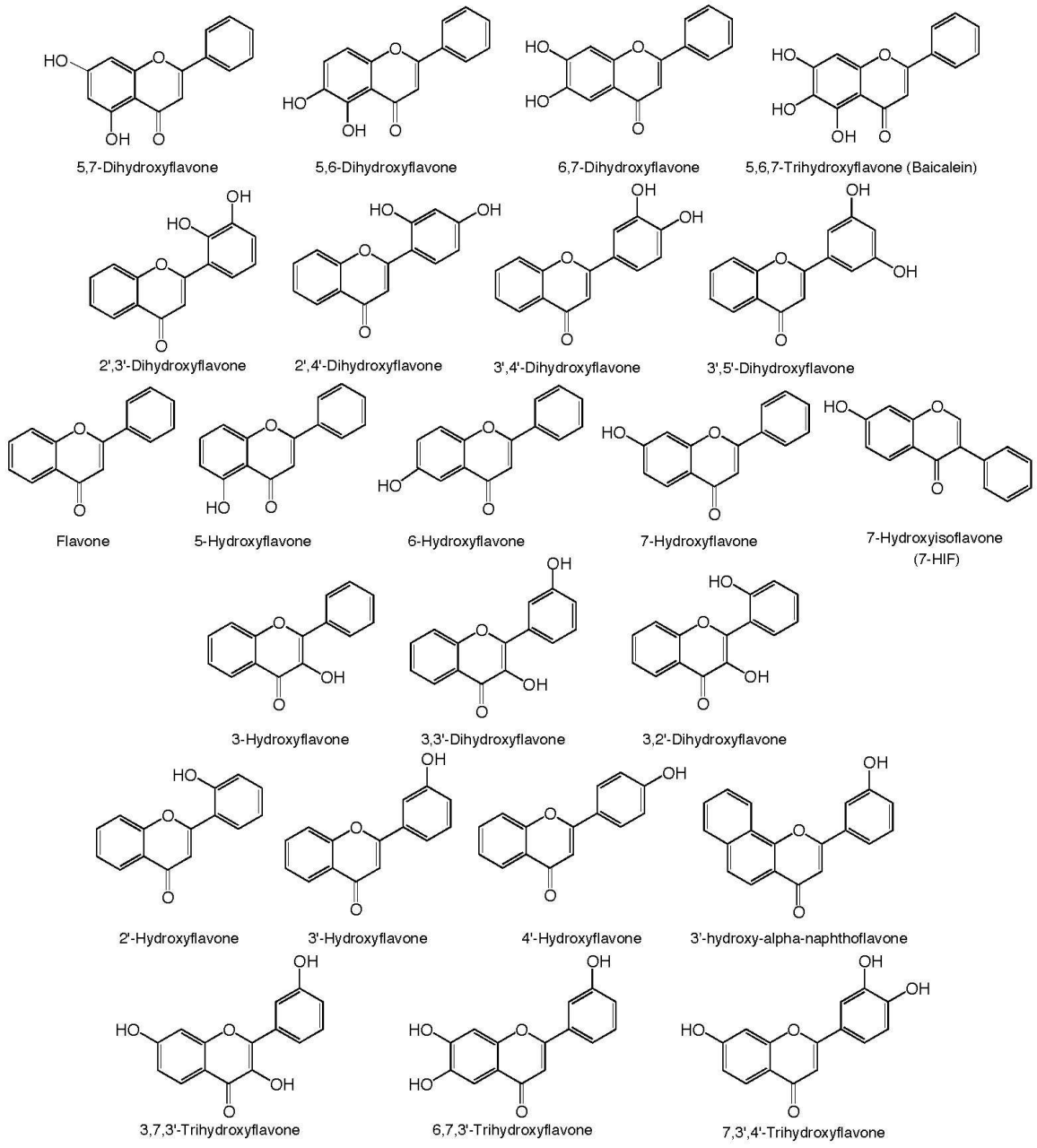
Supplemental Figure 4. Histology examination of drug-treated mice and control saline-treated mice.

The organs from drug-treated mice exhibited no demonstrable pathological changes compared with the vehicle-treated control mice.

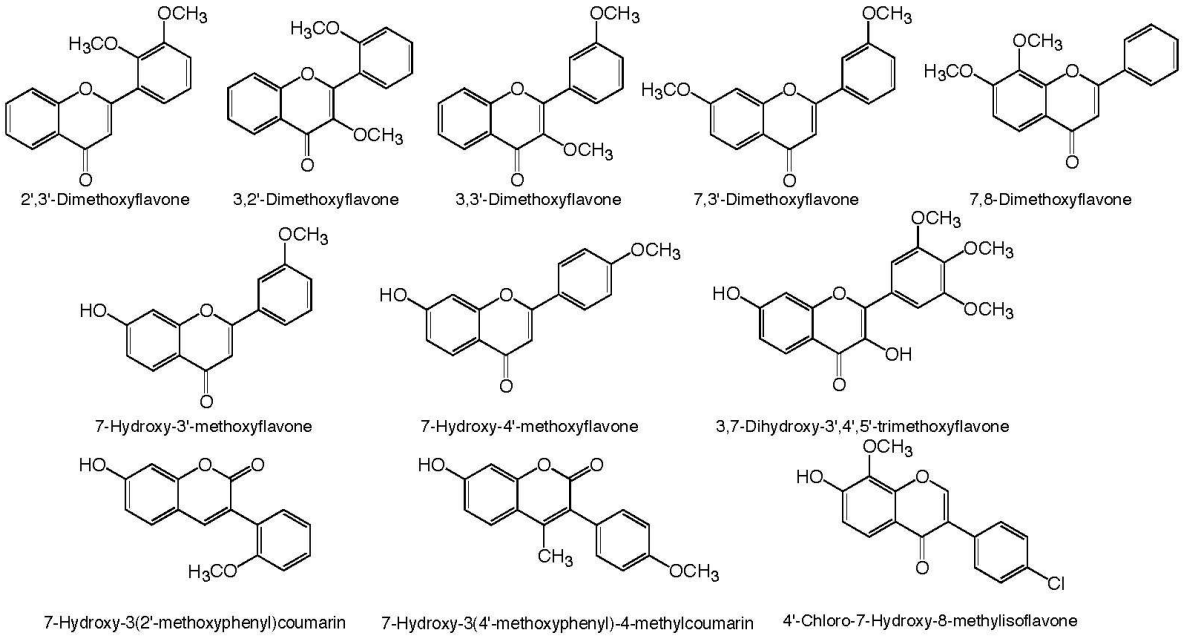
Supplemental Figure 5. Cell growth and cytotoxicity assays

(A & B) 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Different concentrations of the compounds and control vehicle incubate with HEK293 cells in 6-well plates. The cell proliferation was analyzed by an MTT assay. Neither 4'-dimethylamino-7,8-dihydroxyflavone nor 7,8-dihydroxyflavone blocked HEK293 cell proliferation. (C & D) Lactate dehydrogenase (LDH) assay. Different concentrations of the compounds and control vehicle incubate with HEK293 cells in 6-well plates. The cell proliferation was monitored by an LDH assay. Neither 4'-dimethylamino-7,8-dihydroxyflavone nor 7,8-dihydroxyflavone demonstrated toxicity on HEK293 cells. (E & F) LDH assay. Different concentrations of the compounds and control vehicle were incubated with primary cortical neurons (DIV 7) in 6-well plates. The cellular toxicity was monitored by an LDH assay. Neither 4'-dimethylamino-7,8-dihydroxyflavone nor 7,8-dihydroxyflavone demonstrated toxicity on primary neurons at concentration up to 5 μM.

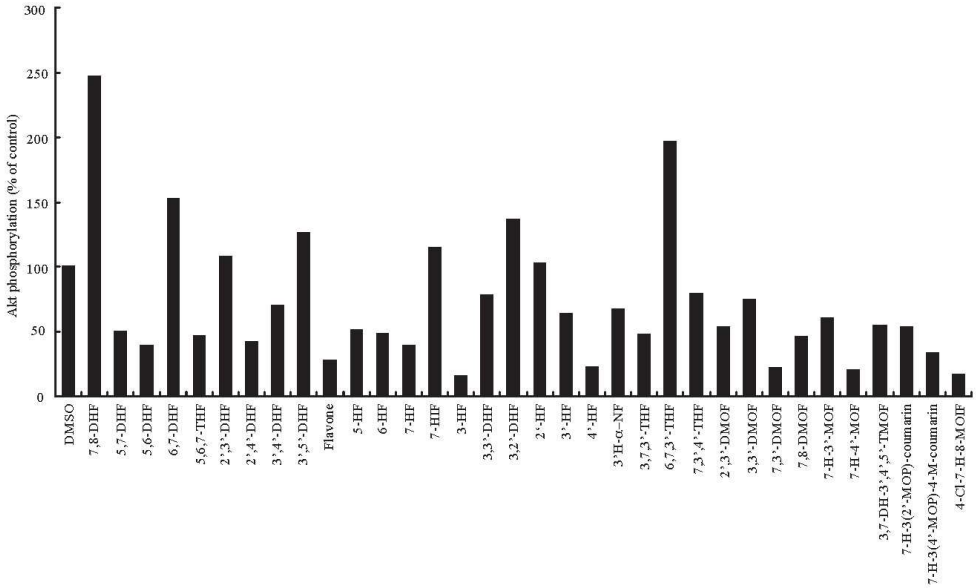
Supplemental Figure 1a



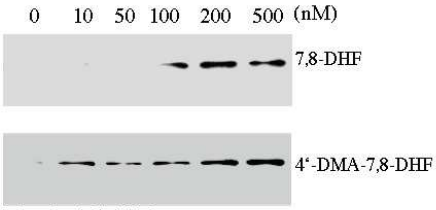
Supplemental Figure 1b



Supplemental Figure 1c



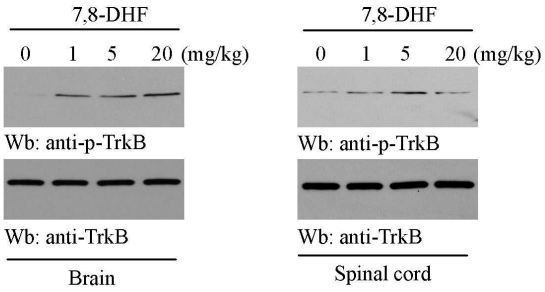
Supplemental Figure 2



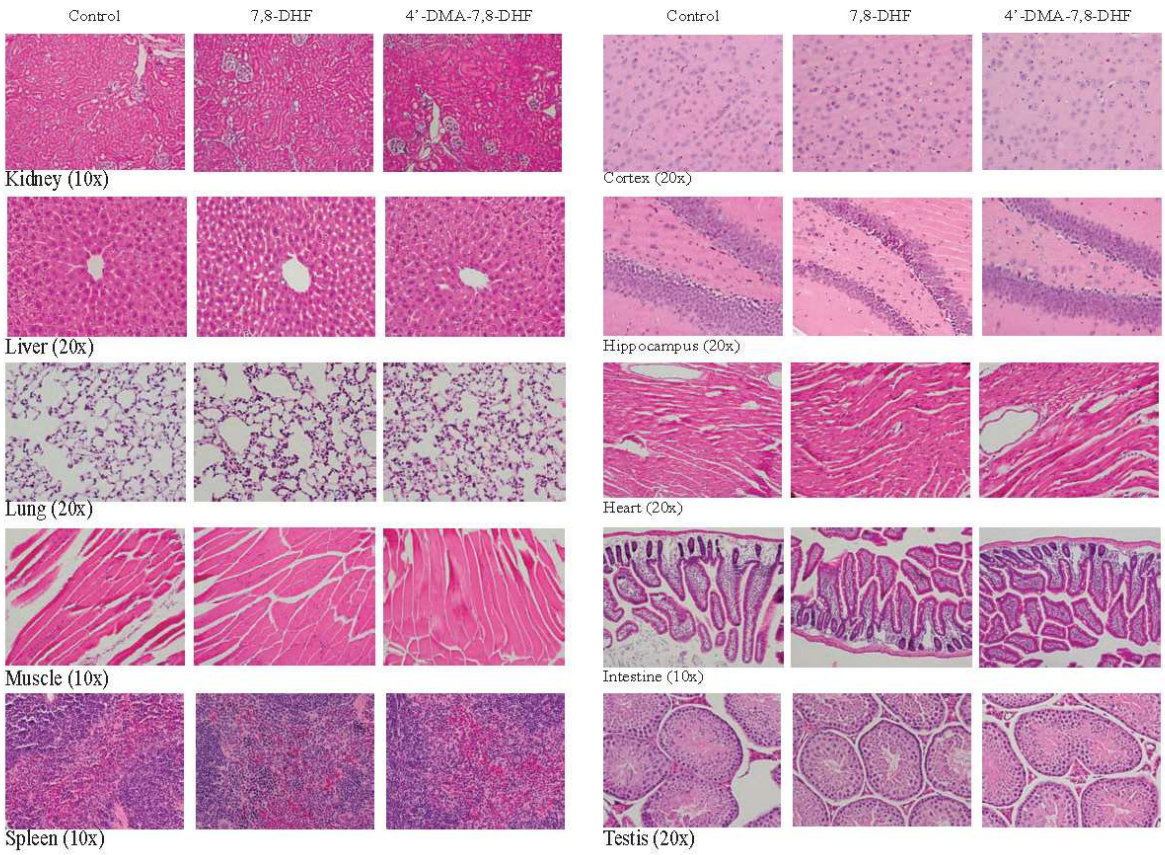
IB: anti-p-TrkB 816

Primary neurons

Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5