Novel Nrf2/ARE Activator, *trans*-Coniferylaldehyde, Induces a HO-1-Mediated Defense Mechanism through a Dual p38α/MAPKAPK-2 and PK-N3 Signaling Pathway

Huang-Hui Chen[a, b], Tai-Chi Wang[c], Yen-Chen Lee[a], Pei-Ting Shen[a], Jang-Yang Chang [d, e], Teng-Kuang Yeh[a], Chih-Hsiang Huang[a], Hsih-Huei Chang[a], Shu-Ying Cheng[a], Chin-Yu Lin[a], Chuan Shih[a], Chiung-Tong Chen[a], Wei-Min Liu[b,f], Ching-Hui Chen[b,f], Ching-Chuan Kuo[a, e,g *]

[a] Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan, Taiwan

[b] Department of Obstetrics and Gynecology, Taipei Medical University Hospital, Taipei, Taiwan

[c] Department of Pharmacy, Tajen University, Pingtung, Taiwan

[d] National Institute of Cancer Research, National Health Research Institutes, Tainan, Taiwan

[e] Institute of Clinical Pharmacy and Pharmaceutical Sciences, National Cheng Kung University Medical College, Tainan, Taiwan

[f] Department of Obstetrics and Gynecology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

[g] Graduate Program for Aging, China Medical University, Taichung, Taiwan

Corresponding author:

Ching-Chuan Kuo, Ph.D., Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, No. 35, Keyan Road, Zhunan 35053, Taiwan. Phone: +886-37-246166 ext. 35758; Fax: +886-37-586456; E-mail: cckuo@nhri.org.tw

Table of Contents Graphic

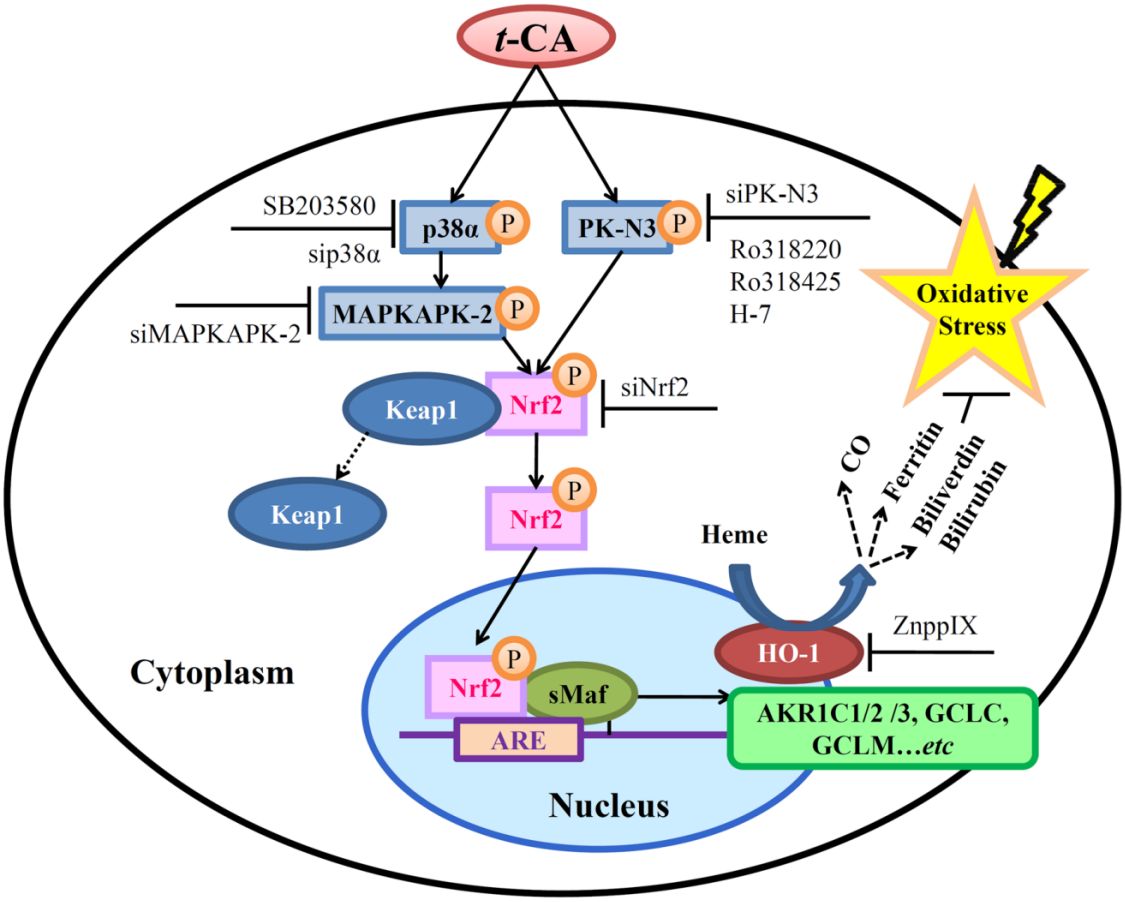

Table S1. Primers used for real-time RT-PCR

Primers	Sequences
GR	forward: 5’ CACGGAGGAGCTGGAGAAC, reverse: 5’ CGACAAAGTCTTTTTAACCTCCTT;
TR	forward: 5’ CAGACGGGGAGGCTTTTC, reverse: 5’ CCGAGAGCGTTCCTTTCA;
Prx-1	forward: 5’ CACTGACAAACATGGGGAAGT, rverse: 5’ TTTGCTCTTTTGGACATCAGG;
GCLM	forward: 5’ GACAAAACACAGTTGGAACAGC, reverse: 5’ CAGTCAAATCTGGTGGCATC;
GCLC	forward: 5’ ATGCCATGGGATTTGGAAT, reverse: 5’ AGATATACTGCAGGCTTGGAATG;
HO-1	forward: 5’ TTCAGAAGGGCCAGGTGA, reverse: 5’ CCTCCAGGGCCACATAGAT;
NQO-1	forward: 5’ ATGTATGACAAAGGACCCTTCC, reverse: 5’ TCCCTTGCAGAGAGTACATGG;
AKR1C1	forward: 5’ CATGCCTGTCCTGGGATTT, reverse: 5’ AGAATCAATATGGCGGAAGC;
AKR1C2	forward: 5’ GCAGCGCATCAGACAGAAC, reverse: 5’ CAAGGGTCAAATATCGCACA;
AKR1C3	forward: 5’ CATTGGGGTGTCAAACTTCA, reverse: 5’ CCGGTTGAAATACGGATGAC;
ABCC1	forward: 5’ TGTGGGAAAACACATCTTTGA, reverse: 5’ CTGTGCGTGACCAAGATCC;
ABCC2	forward, 5’AGCATGCTTCCCATGATGA, reverse, 5’ TCTAGCCGCTCTGTGGAAAC;
ABCC3	forward, 5’ TGCTCTCCTTCATCAATCCA, reverse, 5’ TGGGGTTGGAGATAAACCTG;
ABCC4	forward, 5’ AAGCGCCTGGGATCTACAA, reverse, 5’ CCCCTGGAGAGAAGATGACA;
ABCC5	forward, 5’ CATCCACGCCTACAATAAAGG, reverse, 5’ GCATCGCACACGTAAACAAA;
GAPDH	forward: 5’ AGCCACATCGCTCAGACAC, reverse: 5’ GCCCAATACGACCAAATCC

Table S2. Target sequences of siRNA

Target name	NCBI accession number	Target sequences
AKT-1	NM006164	sense: AUACCGGCAAAGAAGCGAUGCUGCA; antisense: UGCAGCAUCGCUUCUUUGCCGGUAU
p38α MAPK	NM001315	sense: GCUGUUGACUGGAAGAACAUUGUUU; antisense: AAACAAUGUUCUUCCAGUCAACAGC
p38β MAPK	NM002751	sense: UCAACGCUCUCAUCAUAUGGCUCGG; antisense: CCGAGCCAUAUGAUGAGAGCGUUGA
MSK-1	NM004755	sense: UCCUUUGUUGCUCCUUCCAUCCUAU; antisense: AUAGGAUGGAAGGAGCAACAAAGGA
MAPKAPK-2	NM004759	sense: CCACUCCUUGUUAUACACCGUACUA; antisense: UAGUACGGUGUAUAACAAGGAGUGG
PKCδ	NM006254	sense: UGUCGAUGCAUUUCUUGUGGAUGGC; antisense: GCCAUCCACAAGAAAUGCAUCGACA
PKCε	NM005400	sense: UCACAUACACUCUUCCUUCUGGCUC; antisense: GAGCCAGAAGGAAGAGUGUAUGUGA
PKCθ	NM006257	sense: UAUCCACCUCAUCCAACGGAGACUC; antisense: GAGUCUCCGUUGGAUGAGGUGGAUA
PKCη	NM006255	sense: UUACACUGAAGUCCUUGUCGCAUUA; antisense: UAAUGCGACAAGGACUUCAGUGUAA
PKCι	NM002740	sense: AAAUCUGGCAUGUUCUUCAGGAAGU; antisense: ACUUCCUGAAGAACAUGCCAGAUUU
PKCζ	NM002744	sense: AUCGAUAACUGGCUUAAGGUCCUCC; antisense: GGAGGACCUUAAGCCAGUUAUCGAU
PK-N1	NM213560	sense: AAUCCUCUCAAUGACAGGGUUGCGG; antisense: CCGCAACCCUGUCAUUGAGAGGAUU
PK-N2	NM006256	sense: UUGGCAGCCCAUAAGACGAACUUCC; antisense: GGAAGUUCGUCUUAUGGGCUGCCAA
PK-N3	NM013355	sense: UUGGGCAGGAAAUUACUGGGAGUGG; antisense: CCACUCCCAGUAAUUUCCUGCCCAA

Table S3. Effect of *t*-CA on intracellular GSH and GSSG amount in HSC-3 cells

t-CA (μM)	GSH (μM/μg)	GSSG (μM/μg)	GSH/GSSG ratio
0	10.43 ± 0.67	1.2 ± 0.06	8.69
6.25	10.07 ± 0.65	1.22 ± 0.04	8.25
12.5	13.14 ± 2.33	1.32 ± 0.04	9.95
25	14.74 ± 1.18	1.38 ± 0.04	10.68
50	18.21 ± 0.25	1.43 ± 0.02	12.73

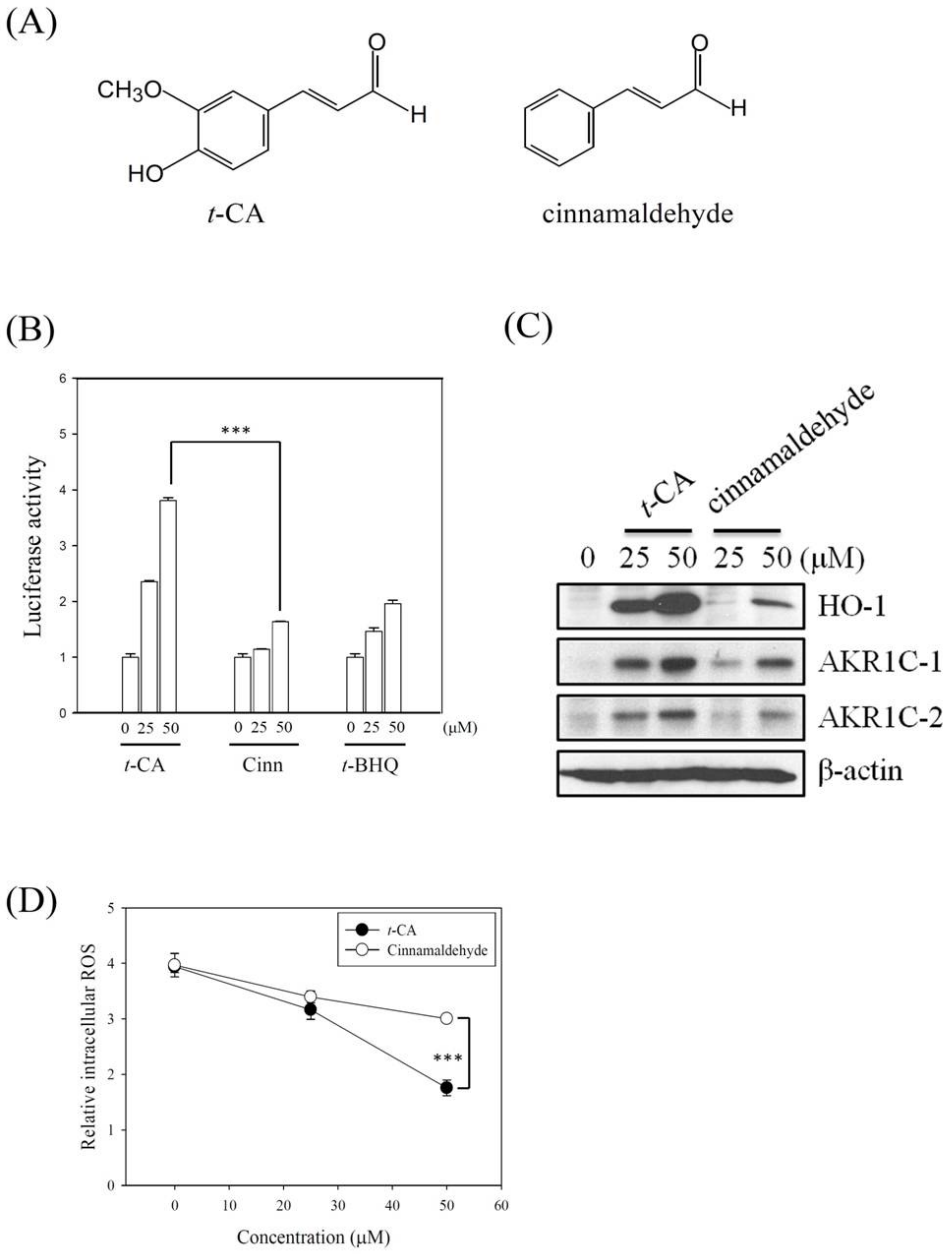

Figure S1. Comparison of the effects of *t*-CA and cinnamaldehyde on the Nrf2/ARE-dependent gene induction and cytoprotective activity. (A) Chemical structure of *t*-CA and cinnamaldehyde. **(B)** HSC3-ARE9 cells were treated with 25 and 50 μM of *t*-BHQ, *t*-CA and cinnamaldehyde (Cinn) for 24 h and luciferase activity was analyzed as described in Materials and Methods. **(C)** HSC-3 cells were treated with 25 and 50 μM of *t*-CA and cinnamaldehyde for 24 h and then the protein levels of HO-1, AKR1C1, and AKR1C2 were detected using immunobloting. β-actin was used as an internal control. **(D)** HSC-3 cells were pre-incubated with indicated concentrations of *t*-CA and cinnamaldehyde for 24 h and then exposed to 200 μM *t*-BHP for an additional 4 h. The levels of intracellular ROS were measured using DCFH-DA fluorescence. Significantly different: $*p < 0.05$ and $***p < 0.005$.