# NRF2 activator attenuates the progression of nonalcoholic steato-hepatitis (NASH) in diet-induced NASH mice

The Environmental Response V
17th JBS Biofrontier Symposium
Date: September 12-14
Tohoku University Seiryo Auditorium, Sendai, Japan

**Yuko Katayama, Shigemitsu Matsumoto, Tsuyoshi Maekawa, Yukio Yamada, Masanori Watanabe**

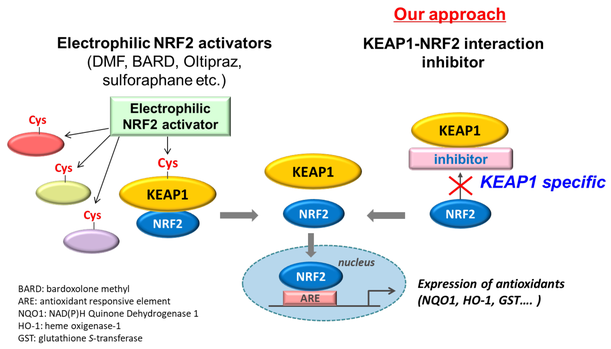
**SCOHIA PHARMA, Inc., Kanagawa, Japan**

SCOHIA

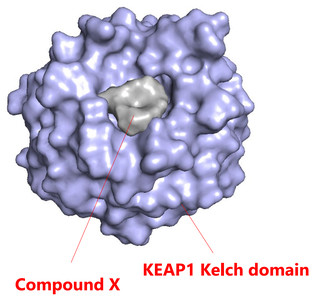
## Background

Nonalcoholic steatohepatitis (NASH) is a complex disease characterized by liver steatosis with inflammation (hepatitis) and fibrosis and can progress into cirrhosis or liver cancer. Lipid accumulation, oxidative stress and inflammation are important pathophysiological mechanisms in NASH. NRF2 activators could suppress these pathways and could be NASH therapeutic agents. There are some potent NRF2 activators, such as oltipraz, sulforaphane and bardoxolone methyl that are electrophilic compounds binding to cysteine residues of KEAP1, an endogenous inhibitory protein of NRF2. To identify NRF2 activator without off-target effect, we are trying to discover non-electrophilic KEAP1-NRF2 interaction inhibitor.

### Two types of NRF2 activators




### Docking model of compound X with the KEAP1 Kelch domain (2FLU*)




1. **We discovered potent protein protein interaction inhibitor (Compound X)**
2. **Analogues of Compound X binding to Kelch domain was confirmed by X-ray cocrystal structure with KEAP1-Kelch domain.**
3. **Compound X binding to Kelch domain is assumed.**

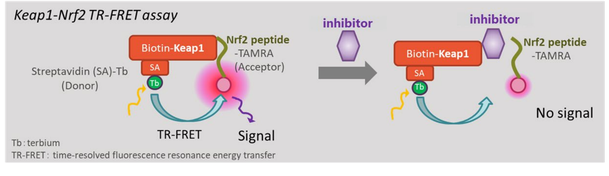
◆ Electrophilic Nrf2 activators (Irreversible binder type) bind Cys151 on BTB domain of Keap1.

◆ BTB and IVR domain is not shown on this image.

*2FLU = code No. of Crystal Structure of the Kelch-Neh2 Complex (Protein Data Bank)

## Methods

***In vitro 1*** : The inhibitory activity of NRF2-KEAP1 binding of the compounds was evaluated using Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) technology.




***In vitro 2*** : The effects of compounds on antioxidant response element (ARE)-mediated transcriptional activity was evaluated using HEK293T cells stably transfected with nano-luc ARE reporter plasmid. The cells were incubated with compounds for 6 h.

***In vivo*** :

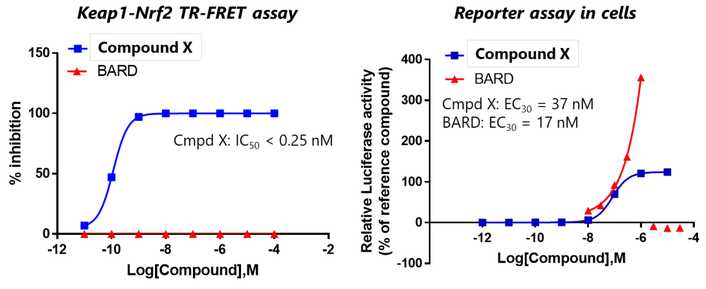
- Animal: 8w Male C57BL/6 mice (n=8)
- Diet: CDAHFD (choline-deficient, amino acid-defined, high-fat dietA06071302, Research diet)
- CDAHFD diet duration: 6 weeks
- Treatment period: 6 weeks
- Readout:
  Liver histology (NAS)
  Liver collagen content
  plasma ALT/AST


- Group: (q.d.)
  1. CDAHFD-Vehicle (p.o.)
  2. CDAHFD-Compound X (0.03 mg/kg, p.o.)
  3. CDAHFD-Compound X (0.1 mg/kg, p.o.)
  4. CDAHFD-Compound X (0.3 mg/kg, p.o.)
  5. CDAHFD-pioglitazone (50 mg/kg, p.o.)
  6. Normal chow-Vehicle (p.o.)

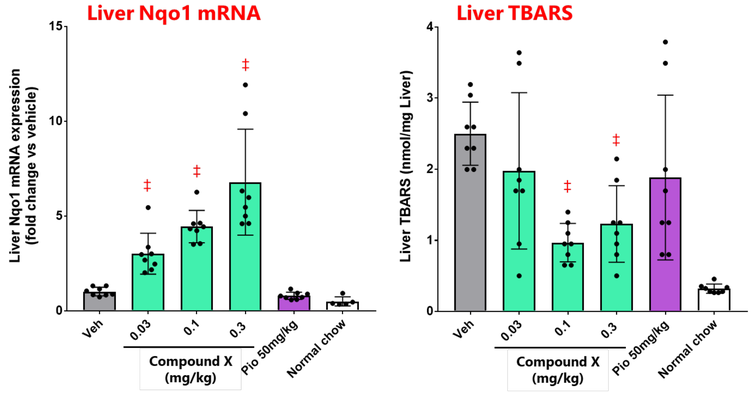
## Results

### In vitro profile:

**Compound X inhibits KEAP1-NRF2 interaction**
**Compound X increased ARE- reporter activity**

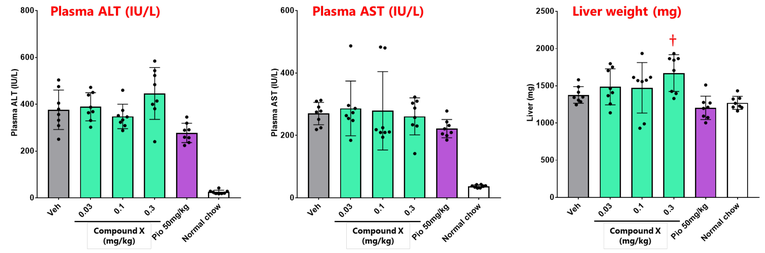



### Compound X increased PD marker, Nqo1 mRNA and suppressed oxidative stress marker, TBARS in the liver in CDAHFD-induced NASH mice

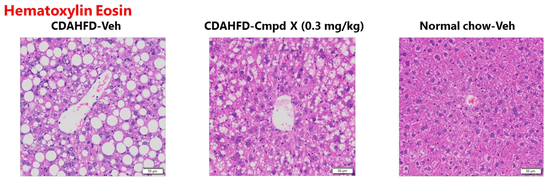



N=8, mean±SD, ‡p<0.025vs vehicle by Shirley Williams' test

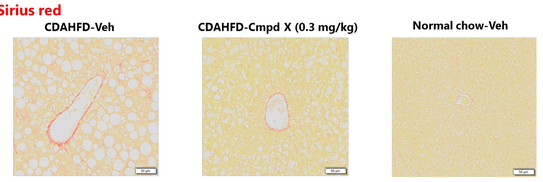
### Compound X prevented the progression of NASH in CDAHFD-induced NASH mice

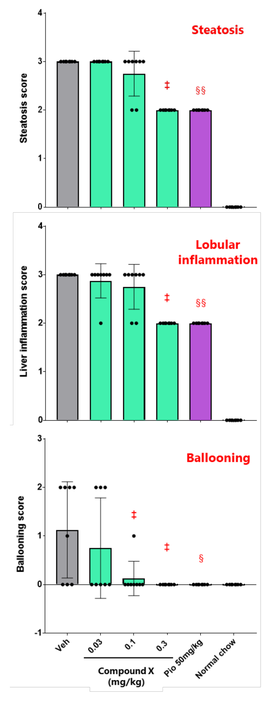


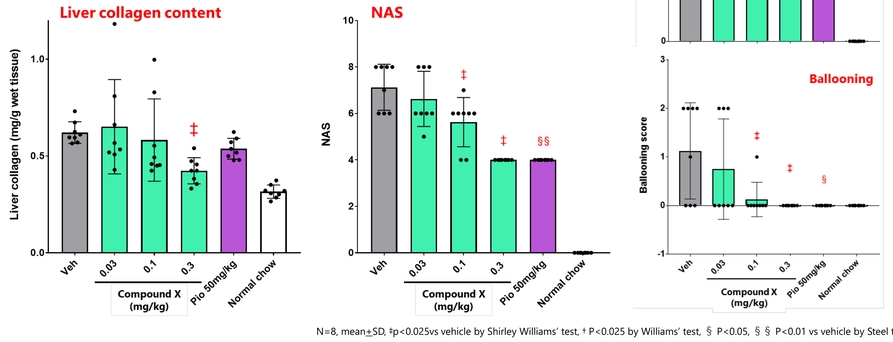

### Hematoxylin Eosin




### Sirius red



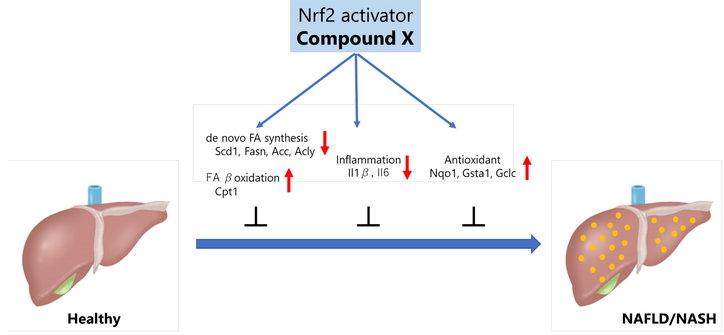




N=8, mean±SD, ‡p<0.025vs vehicle by Shirley Williams' test, † P<0.025 by Williams' test, § P<0.05, § § P<0.01 vs vehicle by Steel test

## Conclusion

We discovered a potent KEAP1-NRF2 interaction inhibitor, compound X that strongly activated NRF2 in vivo, and prevented NASH progression in diet-induced mouse NASH model. Compound X is expected as a NASH treatment.

### Potential mechanism of NRF2 activator as anti-NASH drug




**SCOHIA PHARMA, Inc.**
Contact information
https://www.scohia.com/eng/sys/contact_research_or_pipeline/