Exploring the structure-activity relationship of various sEH inhibitors in promoting brown adipogenesis

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Introduction

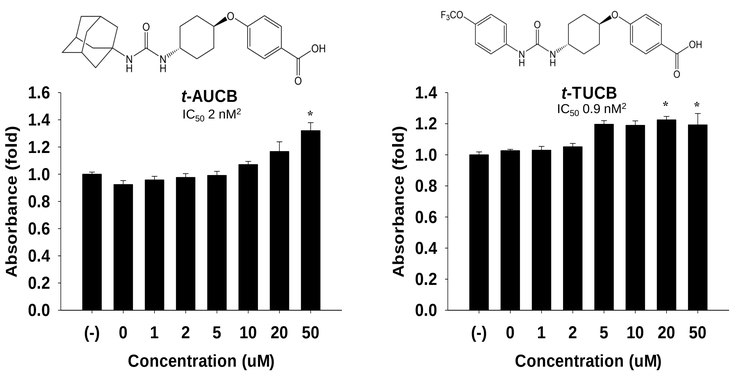
Brown adipose tissue has emerged as a novel target for obesity prevention and treatment due to its responsibility for heat production. Pharmaceutical agents that can promote brown adipogenesis, i.e. formation of new brown adipocytes, will be beneficial for this purpose. Soluble epoxide hydrolase (sEH) is a cytosolic enzyme that degrades epoxy fatty acids (EpFAs) (lipid signaling molecules) into inactive diols.[1] Potent sEH inhibitors (sEHIs) are beneficial for many chronic diseases as they stabilize endogenous EpFAs by blocking their degradation.[1] Our preliminary results have shown that *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB) and *trans*-4-{4-[3-(4-trifluoromethoxyphenyl)-ureido] cyclohexyloxy} benzoic acid (*t*-TUCB), both potent sEH inhibitors, dose-dependently promote brown adipogenesis.

The objective of this study is to investigate the structure and activity relationship (SAR) of various sEHIs with variations to those of *t*-AUCB and *t*-TUCB to identify the structure properties that make a potent drug to promote brown adipogenesis. Oil Red O (ORO) stained lipids accumulation was used as a marker to evaluate brown adipogenesis.

Methods

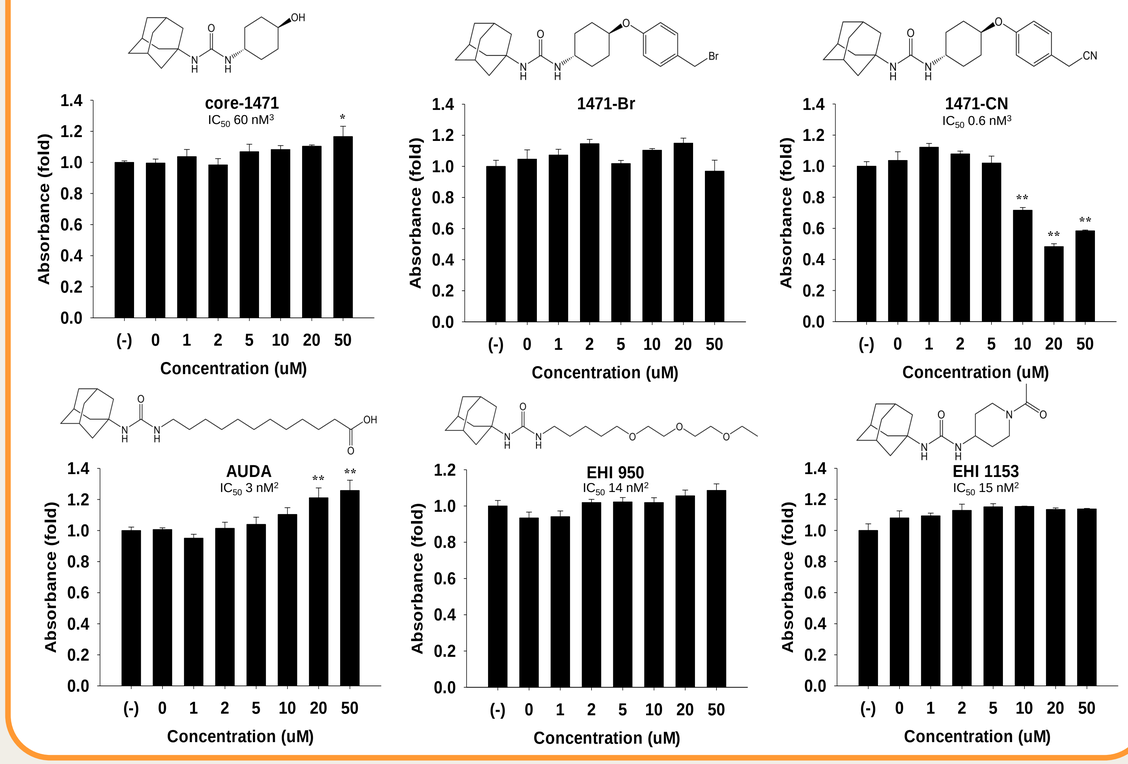
- **Murine brown adipogenesis**: murine brown pre-adipocytes were induced to differentiate in the presence of various doses of sEHIs during the differentiation process
- **ORO staining and absorbance**: the cells were fixed after differentiation and were stained with ORO dye; ORO absorbance was measured at 500 nm wavelength
- **Statistical analysis**: ANOVA with RM was used to detect the significance of various sEHIs compared to the controls

Effects of *t*-AUCB and *t*-TUCB

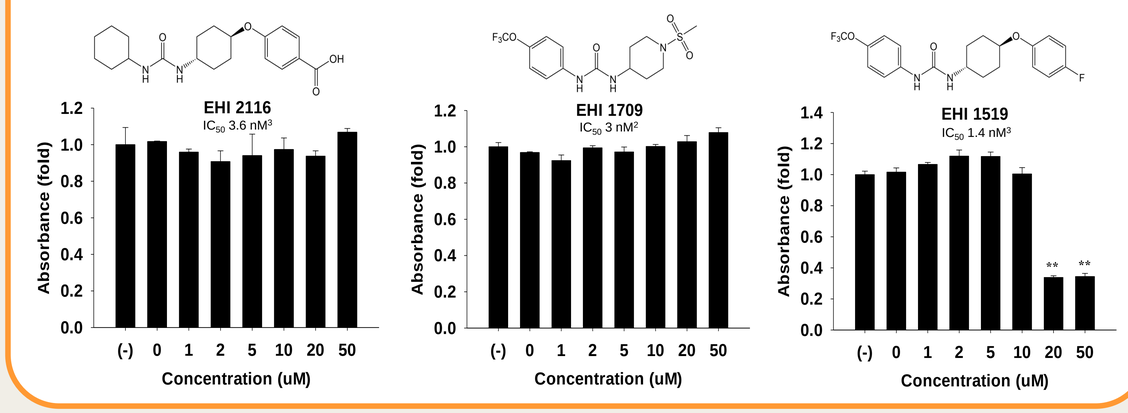


***** represents significant difference with p<0.05
****** represents significant difference with p<0.01

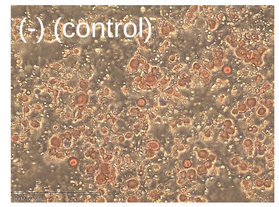
Effects of *t*-AUCB-derived sEHIs

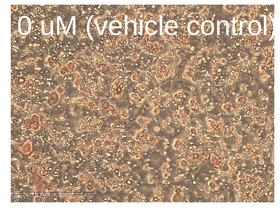


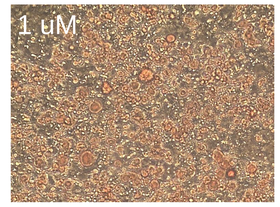
Effects of *t*-TUCB-derived sEHIs

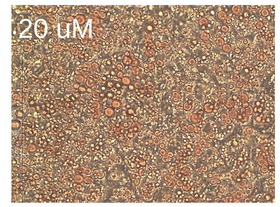


Example of ORO-stained brown adipocytes micrographs (AUDA)









Conclusion

- Among the *t*-AUCB-related sEHIs tested, core-1471 and AUDA significantly promoted brown adipogenesis, suggesting the importance of the adamantane in the structure for promoting lipids accumulation; however, higher doses of 1471-CN showed cell toxicity.
- Among the *t*-TUCB-related sEHIs tested, none of them significantly promoted brown adipogenesis; however, higher doses of EHI 1519 showed cell toxicity.
- Activities of promoting brown adipogenesis do not appear to be associated with IC_{50} values of sEHs.
- Further analysis of brown marker gene expression will be needed to validate ORO lipids accumulation results.

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References:

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[2] Tsai, H. J., Hwang, S. H., Morisseau, C., Yang, J., Jones, P. D., Kasagami, T., Hammock, B. D. (2010). Pharmacokinetic screening of soluble epoxide hydrolase inhibitors in dogs. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, *40*(3), 222–238. doi:10.1016/j.ejps.2010.03.018

[3] Unpublished results.