Bo-Yeong Yu and coworkers demonstrate the anti-melanogenesis effects of dimethyl itaconate in B16F10 cells as well as the SAR analysis of itaconic acid. In addition, the link between Nrf2 and melanogenesis is discussed. The manuscript is highly intriguing, however it has to address various difficulties.

In introduction:

1. Need more information regarding the connection between oxidative stress, Nrf2, and melanogenesis.
2. The author should comment on some aspect of the biological function of itaconic acid and its derivatives.
3. The author must explain how itaconic acid is derived from natural sources.
4. Dimethyl itaconic acid has been shown to inhibit melanogenesis in B16F10 cells, as reported in molecules. (Molecules 2022, 27, 4183. https://doi.org/10.3390/molecules27134183). So what is the originality of this study?
5. What is the study's hypothesis and how do the authors refute it? Should be described in the introduction's final paragraph.

Materials and methods

6. The authors asserted that monomethyl itaconate (MMI) is available from Sigma. However, as far as I am aware, MMI is unavailable in sigma. So please provide the catalog numbers for all substances in each vendor's inventory.
7. The determination of melanin production using melanoma cell lines is dependent on numerous variables, including culture medium, pH, culture conditions, culture duration, the number of cells passes, and the growth rate of the cells. Therefore, authors should describe the validation technique for cell proliferation and melanin formation. Also offer the equation for measuring intracellular melanin.
8. Provide the equation of cell viability measurement.

Results

9. The authors claim that reduction of Nrf2 in B16F10 cells reverses the anti-melanogenesis activity of DMI. In this section, authors should address a particular topic in order to better comprehend the anti-melanogenesis action of DMI via the Nrf2 pathway.
    - Does DMI enhance Nrf2 expression in B16F10 cells? To overcome this issue, the author should confirm that DMI upregulates Nrf2 expression in B16F10 cells using a western blot experiment.
    - In addition, authors should verify that shNrf2 inhibits Nrf2 expression in B16F10 cells.
    - In accordance with my suggestion, the author should conduct an immunoblot experiment with shNrf2 to examine Tyrosinase and MITF expression in B16F10 cells.

Discussion

10. Recent discoveries should be incorporated into the discussion of the SAR activity of itaconate.
11. Additional debate is required about the role of PAH in melanogenesis, with reference to other drugs that likewise block PAH.
12. The authors examine the SAR of itaconic acid for Nrf2 activator. However, there is no relative experiment to support this assertion in the present study. To support this assertion,

the author must do the activation of Nrf2 experiment using various itaconic acid derivatives.

13. In addition, some information regarding how itaconic acid and its derivatives suppress oxidative stress by upregulating Nrf2 is needed to discuss.
14. The author must include a discussion of the relationship between oxidative stress, Nrf2 signaling, and melanogenesis, along with an appropriate citation. And offer information on drugs that inhibit oxidative stress and melanogenesis via Nrf2 signaling.

Minor issue

1. In the materials and methods section, the authors present information on 193T cells. In the present investigation, however, there are no data utilizing this cell. Please append.