

November 21, 2023

Dear. Reviewer #2.

We appreciate your review of our manuscript entitled “STAT3 inhibitory activities of lignans isolated from the stems of *Lindera obtusiloba* Blume (scipharm-2704450)”.

We have revised the manuscript based on the reviewer comments and all corrections are marked in red.

Sincerely yours,

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Reviewer #2.

Comments and Suggestions for Authors

Lee and coworkers described the isolation of compounds 1-7 from *L. obtusiloba* and evaluated their IL-6-induced STAT3 inhibitory. In view of the reviewer, the manuscript in its present state leaves certain questions, in particular pertaining to the novelty of this work, unanswered and would benefit significantly from an additional revision.

The following points of concern could be addressed:

1. Most compounds 1-7 were reported previously, and the stems of *Lindera obtusiloba* Blume are known to have inhibitory effects on various inflammatory responses such as neuroprotective activity and anti-allergic activity. Can authors highlight the novelty of this work in the manuscript?

→ Thank you for comment. Thank you for your feedback. However, there are no reports on whether the stem extract of *Lindera obtusoloba* Blume and compounds derived from it act on IL-6-induced STAT3 activation, one of the important cytokines in inflammatory response. We think there is something novel about this.

2. Lines 244-247, the chiral centers 7,7' of compound **1** are not consistent with reference 11. Didn't find compounds 3 and 4, 6 in reference 21 and 23, respectively.

→ Thank you for comment. The compound structure at chiral center 7, 7' was modified, and asarinin episesamin are the same compound. Compound was reported in the indicated reference 4 and compounds 4 and 6 were reported in reference 24. Rewritten to match the reference.

3. Line 250-264 Section 3.2, the extract and fractions are a mixture and may contain unknown components in the mixture. It doesn't make any sense to evaluate the inhibition of IL-6-induced pSTAT3 luciferase activity of the mixture.

→ Thank you for comment. In separating compounds, isolation was conducted focusing on fractions that showed activity. As a result, active compounds were isolated in fractions 5 and 7.

4. Line 283, Authors demonstrated that compounds **1** and **5** could be promising IL-6/STAT3 inhibitors. What's the target of these compounds? IL-6, IL-6Rs, JAK or STAT3?

→ Thank you for comment. Through this study, the effect of inhibiting JAK and STAT3 phosphorylation induced by IL-6 was confirmed. However, we plan to

conduct additional research in the future to determine whether it directly inhibits IL-6 or IL-6r or inhibits receptor-ligand interaction.

5. Figure 3, Please label A, B, and C for each figure. For each subfigure, which column means compound 1, which means compound 5?

→ Thank you for comment. Figure 3 was modified. Labels are indicated, and compounds 1 and 5 are expressed separately.

6. SI, for all the ¹H NMR, please show the spectrum from 0-10 ppm; for ¹³C NMR please show the spectrum from 0-160 ppm. As compounds 1-7 have been reported, please add a table to compare the NMR with previous data.

→ Thank you for comment. The spectrum presented by modifying it to the requested ppm value and added a table to compare the NMR with previous data in Supplementary data.