Summary

In response to the initial hypothesis, this study has investigated and confirmed the anti-inflammatory role of costunolide through the regulation of microglial activation. The current study has presented the groundwork for understanding a novel biological function of costunolide in neuropathological context. This work has led to the identification of the compound’s ability to inhibit, LPS-induced microglia-mediated inflammation. It has also elicited the signaling cascade involved in the neuroprotective and anti-inflammatory effect of costunolide in murine microglia. The compound’s propensity to function as a ligand has been analyzed and its probable receptor interaction has been predicted. One of such predictions has been proven experimentally and so has been the anti-inflammatory effect in vivo. The salient results of this study are listed as follows:

- Costunolide inhibits LPS-induced expression of proinflammatory molecules like tumour necrosis factor α (TNF α), interleukins 1 and 6 (IL-1, IL-6), nitric oxide (NO), inducible nitric oxide synthase enzyme (iNOS), monocyte chemokine protein 1 (MCP-1) and cycloxygenase 2 (Cox-2).
- In LPS-induced activated microglia, costunolide causes increase in expression of neuroprotective molecules viz. tumour growth factor β (TGF-β) and interleukin-10 (IL-10), thereby restraining the exacerbation of the neuronal environment.
- The mechanistic link in this cascade is the regulation of nuclear factor kappa β (NFκB) by costunolide. The compound inhibits the translocation of NFκB from the cytosol to the nucleus, repressing the transcription of proinflammatory genes.
- Its influence on another complimentary mitogen activated protein kinase (MAPK) inflammatory pathway has been reported only with reference to the inhibitor molecule of this pathway. Costunolide induces the expression of the MAP kinase phosphatase 1 (MKP-1) which is a well known inactivator of the MAPK signaling.
- The computational analysis of costunolide has shown that the compound fulfils the criteria for a good drug/lead candidate. It has good predicted docking interactions with the microglia receptors, it acts as an agonist to
peroxisome proliferator-activated receptor gamma (PPARγ), could act as an inverse agonist to beta 2 adrenergic receptor (β2AR) and moderately bind to cannabinoid receptor 2 (CB2).

- Costunolide successfully qualifies the ADME-Tox computational screening and poses to be a good therapeutic option with high efficacy and barely any toxic effects.
- PPARγ activation dependent luciferase assays reinforce the idea proposed by the in silico methods and experimentally validate the prediction. Costunolide can bind to this receptor and mediate activation, though it is not conclusive if the anti-inflammatory effect is mediated through this receptor.
- The anti-inflammatory effect of costunolide in LPS-induced post-natal murine brain has corroborated the findings in the murine microglia cell line.

Overall, the results highlight the potential of this naturally available compound in controlling neuroinflammation mediated by LPS induction. Since the LPS-induced neurodegeneration is a model comparable and reflective of the actual state in several neurodegenerative disorders, costunolide could be a promising candidate for further investigation and clinic trial development.
Future Directions

As mentioned above, this study has presented the groundwork of Costunolide’s effect on activated microglia in reversing neuroinflammation. Although, we now have an understanding of the mechanism by which Cos acts, supported by experimental data, more investigations needs to be done to dissect out the specific interaction of the compound with microglial receptors or other proteins that steer the inflammatory pathway in brain. Works are ongoing to demonstrate the \textit{in vivo} binding of Cos to PPAR\(\gamma\) and its association if any to modulate inflammation. Its predicted ability to interact with other receptors could be examined; likewise the cross-talks between the different pathways, if any can be studied with respect to the influence of Cos. These are the immediate obvious directions for our laboratory. The present study that led to the identification and understanding of the compound’s role in inhibiting neuroinflammation mediated by microglia, opens up a lot of questions that are of great interest to the scientific community and likely pharmaceutical interest as well.