7.1 Major findings

Considering the complex nature of inflammation and its ramified implications under the control of immune/redox/metabolic processes, conventional anti-inflammatory agents seem to miss the big picture while addressing individual targets in isolation. Therefore, we thought it worth evaluating certain pleiotropic compounds in our laboratory against the backdrop of multiple pathways that govern inflammatory conditions.

1. **Anti-oxidant, anti-inflammatory and MC stabilization *in-vitro***: DHPO, DHFO, DMFO and DMPI were efficient antioxidants *in-vitro*. DHPO, DHFO, DMFO and DMPI inhibited both ROS and NO in LPS-stimulated macrophages, DHPO and DMFO being potent ROS and NO inhibitor respectively. DMFO effectively inhibited MC degranulation induced by both C48/80 and A23187, with a better activity against A23187-induced MC degranulation.

2. **Acute inflammation**: DMFO, DMPI and DHFO dose-dependently reduced carrageenan-induced rat paw oedema in the third hour suggesting NSAID-like COX inhibition, DMFO being the most efficacious. Test compounds reduced inflammation in air pouch. DMFO reduced leucocyte infiltration into pouch with efficacy comparable to diclofenac.

3. **Ulcerogenicity**: All the test compounds reduced free acidity, while DMPI reduced total/titrable acidity. As the test compounds are non-ulcerogenic and anti-inflammatory, an additional mechanism, different from non-specific blockade of COX isozymes, might be operating.

4. **Chronic inflammation**:
   a. **Colitis**: The test compounds significantly reduced MPO and IL6 in TNBS-induced colitis with an efficacy comparable to sulfasalazine, thereby
indicating their therapeutic potential in a T\textsubscript{H}1, T\textsubscript{H}17-mediated chronic inflammation. Only DMFO and DMPI reduced colon weight/length ratio.

b. **Allergy:** In the antigen-induced MC degranulation model, only DMFO significantly reduced Evan’s blue dye extravasation, with an efficacy comparable to ketotifen. The ability of DMFO to stabilize MCs in both antigen and non-antigen-induced models (cell-based assay), suggests a multimodal activity independent of IgE.

c. **Arthritis:** DMFO and DHPO significantly reduced arthritic score. Only DMFO reduced contralateral paw oedema in Freund’s adjuvant-induced arthritis model. DMFO and DHFO improved pain threshold in the ipsilateral paw, while DHFO and DHPO were effective against inflammatory pain in the contralateral paw. Additionally, DMFO reduced serum TNF\textalpha to undetectable levels. The test compounds were non-ulcerogenic on chronic dosing.

5. **Immune modulation via T\textsubscript{H}:** DHFO, DMFO and DMPI dose-dependently increased anti-inflammatory T\textsubscript{H}2 polarization (DMPI>>DHFO>DMFO). DHPO, an established AMPK activator, dose-dependently decreased pro-inflammatory T\textsubscript{H}1 (DHPO>DHFO>DMFO>DMPI) and T\textsubscript{H}17 polarization (DHPO>DMFO>DMPI>DHFO).

6. **Redox Modulation via NF\kappa B, Nrf2:** While DHFO and DMFO were better than curcumin in inhibiting the downstream marker of NF\kappa B activation. DMPI and DHPO inhibited COX2 expression moderately. Moreover, DHPO and DMFO enhanced HO1 expression, downstream marker of Nrf2 activation. This could underlie the observed non-ulcerogenicity of these compounds. COX2 inhibition and HO1 upregulation together provide crucial evidence for non-ulcerogenic anti-inflammatory activity.

7. **Metabolic regulation via AMPK:** In induced fit docking studies on AMPK\alpha, DMPI binding exhibited least overall energy comparable to curcumin.
AMPKα phosphorylation matched with AMPKα docking score (XP GScore). Additionally, all test compounds increased glucose uptake in L6 myotubes in accordance with the AMPK activation predicted by XP GScore. The highest glucose uptake by DMPI is consistent with its lowest XP GScore in AMPKα docking and highest AMPKα phosphorylation.

Though most effective in both cell-based and in-vivo models of inflammation, DMFO was uniformly moderate in mechanistic studies, further demonstrating the value of moderation in modulating inflammatory pathways. The observed non-ulcerogenic anti-inflammatory activity of test compounds, particularly DMFO, could be attributed at least partly to a combination of T_H cell modulatory activity regulated by AMPK activation through NF-κB inhibition and Nrf2 activation. This novel class of synthetic small molecules probably represents a “first-in-class” category of safe, non-ulcerogenic, anti-inflammatory drug candidates operating in parallel, through multiple pathways.

### 7.2 List of Publications/Patent


7.3 List of Conference/Symposium Presentations


7.4 Other publications/book chapter


