6.1 Conclusion

Current research trends do not favour focusing exclusively on individual biological targets (Lounkine et al., 2012) or a target-based therapeutic approach in general (Swinney, 2014).

This work is based on the following premises:

1. Efficacy is more important than nanomolar selectivity (Higgs, 2004, Balram, 2004).

2. Complex pathologies such as inflammation (Medina-Franco et al., 2013, Zimmermann et al., 2007) require multi-target drugs (Lounkine et al., 2012, Kotz, 2012).

3. Contemporary literature supports phenotypic drug discovery, which:
   - explores interaction of compounds with complex biological systems and molecular pathways (Lounkine et al., 2012), and
   - The pharma industry which did not favour compounds with novel mechanisms (Cuatrecasas, 2006), have now approved the revival of phenotypic drug discovery (Kotz, 2012).

4. Multi-target drugs are more efficacious and safer as demonstrated by the popularity of polypharmacology (involving combinations and/or multi-target drugs) for complex diseases (Lu et al., 2012, Bolognesi, 2013, Morphy and Harris, 2012).

5. Multi-target drugs, even if they are only partially effective, can regulate/restore the complex equilibrium of wholesome cellular networks rather than single-target drugs (Csermely et al., 2005). Such a strategy is akin to relatively harmless natural products which afford a comprehensive holistic therapeutic action by working in moderation (Unnikrishnan et al., 2014).

The current innovation deficit in drug discovery encouraged us to shift our focus towards a multi-target-based approach (Mathew et al., 2014). Our aim has been to establish a new
approach to anti-inflammatory therapy, as against fine tuning the activity profile of a
restricted chemical class addressing a single isolated drug target.
AMPK is a pleiotropic upstream nodal point regulating complex metabolic pathways in
inflammation (Zhang et al., 2009, Zhou et al., 2001, Kanellis et al., 2006). Though the test
compounds are DHPO analogs, except for the common ring structure from
dihydroxyindandione, the compounds are structurally diverse, in tune with the large structural
diversity in AMPK activators (Hardie, 2013). Structure activity relationship (SAR) is usually
applied when attempting to correlate chemical structure with activity towards a single
receptor (Ning et al., 2009, Frye, 1999). Therefore, SAR was not a priority in the current
study.
Pleiotropism (multi-target effect) implies that different aspects of the molecular structure will
produce variable outcomes on the many-sided biological responses. We presume that the
pleiotropism arising from the structural identities/peculiarities of the test compounds may be
causally related to the observed non-uniformity in biological activity in the various
experimental models of inflammation in Chapter 3 and 4. This is exemplified by the variable
molecular mechanisms of anti-inflammatory activity in Chapter 6. A similar observation is
made in Chapter 2 dealing with preliminary in-vitro and cell-based studies.
Though the biological activity is divergent across the different inflammatory models, it is
interesting to note that DMFO was more or less equally effective in all animal models of
inflammation. Additionally, DMFO was uniformly moderate in mechanistic studies in spite
of being most effective in both cell-based and in-vivo models of inflammation, further
demonstrating the value of moderation in modulating inflammatory pathways. Quite by
contrast, the remaining compounds (DMPI, DHPO,DHFO), which seemed more effective and
potent individually in different mechanistic studies, were not as effective in cell-based and in-
vivo models. Among the four test compounds, DMFO and DHFO seem to have
comparatively more similarities in their chemical structure. DMFO, being more efficacious than DHFO, thus appears to have a favourable structure, which may be, at least partly, attributed to the presence of dehydrozingerone moiety (one half of the curcumin molecule, a pleiotropic natural product).

The hypothesis that TH cell modulatory agents that activate AMPK, thereby stimulating Nrf2 and inhibiting NFκB, may work as non-ulcerogenic anti-inflammatory agents, is probably validated by the non-ulcerogenic anti-inflammatory activity of DMFO, consistently demonstrated in all cell-based and in-vivo models of inflammation. By modulating multiple molecular mechanisms of inflammation simultaneously in moderation, the success of DMFO further substantiates our earlier argument that addressing multiple targets of inflammation more holistically, in moderation, is probably a more viable strategy than addressing individual targets brutally in isolation. The test compounds may indeed be a novel “first-in-class” category of non-ulcerogenic anti-inflammatory agents targeting immune, redox and metabolic processes associated with the very complex inflammatory pathology (Figure 6.1).

**Figure 6.1** Comprehensive multimodal anti-inflammatory mechanism of test compounds

**6.2 Future Perspectives**

1. TH17 and T_{reg} being cross-regulatory (Lee et al., 2009), suppression of TH17 polarisation also suggests possible promotion of T_{reg} differentiation which requires further investigation.
2. A combination of DMPI and DHPO might form a two-pronged strategy to treat chronic inflammation because it simultaneously operates on pro-inflammatory Th1 and Th17 on the one hand (DHPO) and anti-inflammatory Th2 on the other (DMPI). This strategy is worth further investigation.

3. As the test compounds DHFO and DMFO were significantly better than diclofenac in reducing inflammatory pain in the ipsilateral paw, and DHPO (at a low dose of 10mg/kg) and DHFO were comparable in efficacy to diclofenac in reducing pain in the contralateral paw, the effect of test compounds on the pain pathway is worth exploring in detail.

4. As mast cells are important players in several inflammatory conditions (Kalesnikoff and Galli, 2008, Galli, 1993), the mechanism of multimodal mast cell stabilizing activity of DMFO in antigen and non-antigen-induced degranulation may be studied in detail.

5. The mechanism of non-ulcerogenicity needs to be investigated further. Co-administering these test compounds with traditional NSAIDs might yield a synergistic combination with greater anti-inflammatory efficacy and diminished ulcerogenicity. This needs to be evaluated further.

6. Pharmacokinetic evaluation of test compounds may probably explain the lower efficacy in-vivo as compared to in-vitro/cell-based studies

7. Patent-filed molecule DMPI may be suitably modified pharmaceutically for enhanced efficacy.

8. Structural identities/peculiarities of the test compounds may underlie the observed pleiotropism. Structural innovation on a much larger scale may yield a library of this novel class of non-ulcerogenic anti-inflammatory agents. Such a library of compounds could be subject to multitarget SAR, enabling us to optimise therapeutic potential and minimise toxicity.
9. The COX2 and HO1 expression studies suggest inhibition of NFκB and Nrf2 activation. However direct evidence is lacking. This is a limitation of the present work.

10. The T_h cell modulatory activity, COX2/HO1 expression and AMPK activation have to be evaluated in-vivo. This limitation warrants further studies in this direction.

11. Pleiotropism arising from structural peculiarities may most likely underlie the non-uniform biological activity of the test compounds. However, the large SEM in iv-vivo studies is a limitation of the present work, and has to be investigated further.

6.3 References


