5. Overall summary and Future plans

5.1. Overall summary of current investigation

Inflammation is a part of body's defence mechanism involving activation of immune system, which recognizes and eliminates the harmful foreign particles. Acute inflammatory condition which are unsolved by innate immune system progress to chronic inflammatory condition; this may lead to developing various inflammatory diseases and cancer.

Current medications for RA and cancer include traditional therapies, biological agents and small molecule targeted therapies. There are many limitations associated with the traditional therapies, such as non-selectivity for selected therapeutic targets and its side effects. Similarly, biological agents have many drawbacks such as high cost and stability issue (needs low temperature storage conditions and specialised logistic vehicles). Targeted therapies have been proven with approved drugs such as Tofacitinib, Baricitinib (JAK inhibitors), which are safe for the treatment of RA. Idelalisib (PI3Kδ inhibitor) is currently approved for chronic lymphatic leukemia (CLL) and follicular lymphoma (FL). Due to adverse side effects associated with Idelalisib, it is not approved for the treatment of inflammatory diseases. We found development of PI3Kδ selective inhibitor could be an attractive target based therapy for safe and effective treatment of RA and cancer.

In the present investigation altogether three series of PI3Kδ inhibitors were designed. In the first series, imidazo-quinoline based PI3Kδ inhibitors, in the second series, benzofuran based PI3Kδ inhibitor and in the third series, 2,4-disubsitued quinoline based PI3Kδ inhibitors were prepared. Altogether, 49 compounds were synthesized, purified, characterized and

subjected for *in vitro* PI3Kδ inhibitory activity. Most potent PI3Kδ inhibitors from each series were further subjected for the *in vitro* selectivity over other PI3K isoforms. From each series, the most potent and selective compounds were subjected for the *in vivo* anti-inflammatory and anti-cancer activity followed by PK and toxicology studies.

In the first series (imidazo-quinoline based PI3Kδ inhibitor) compound **9c (**IC_{50}: 9.5 nM) was identified as primary lead compound. Compound **9c** was evaluated for selectivity study against PI3K isoforms (α, β, γ, δ) and m-TOR. In selectivity assay, **9c** showed moderate isoform selectivity. Thus, further modifications were carried out in compound **9c**, in order to enhance the PI3Kδ inhibitory activity as well as isoform selectivity. Modification at 8th position on compound **9c** resulted in to the discovery of compound **10h** (IC_{50}: 1.9 nM), with improved PI3Kδ inhibitory activity and isoform selectivity. In PK study, **9c** showed moderate AUC, high clearance and low bioavailability (~15%). Compound **10h** showed higher AUC, $t_{1/2}$ (~3.5 hr) and good oral bioavailability (%F ~69). **10h** showed no CYP inhibition up to 100μM concentration and hERG liabilities (IC_{50}: > 30 μM), **Figure 44**.

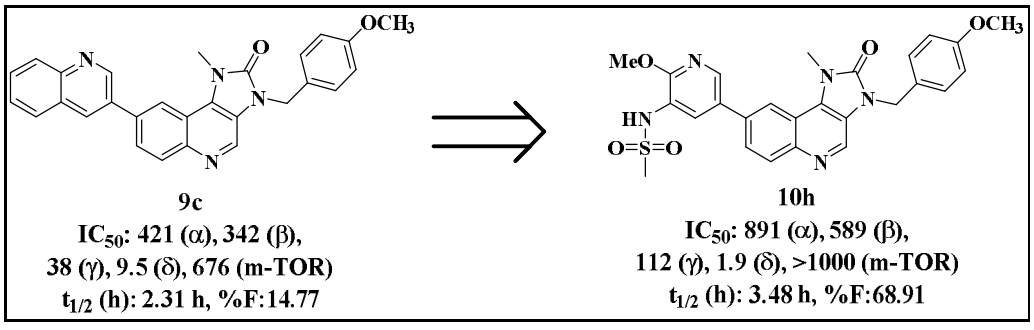
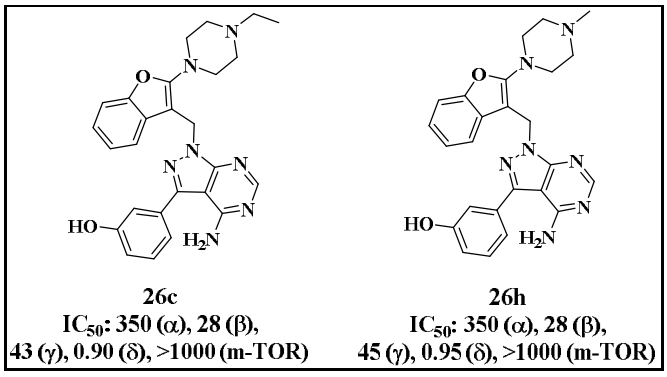


Figure 44: Hit to lead optimization in imidazo-quinoline series (Chapter II)

Compound **10h** showed extended $t_{1/2}$ (3.48 h), which translates to the promising efficacy in animal models of RA (CIA) and cancer (Xenograft). In the repeat dose acute toxicity study, **10h** showed no adverse changes related to gross pathology, clinical signs and liver toxicity. In the docking studies, **10h** showed all the favourable interaction, in the hinge region, affinity pocket, specificity pocket and hydrophobic region.

In benzofuran based series, compounds **26a**, **26b**, **26c**, **26f**, **26g** and **26h** showed excellent PI3Kδ inhibitory activity and were found to be comparable with standard compound (INK-666). These six compounds were further evaluated for PI3K isoform selectivity. **26a**, **26b**, **26f**, **26g** and INK-666 showed similar isoform selectivity. Compound **26c** and **26h** were more selective towards PI3Kδ isoform as compared to INK-666. Further, **26c** and **26h** showed no CYP inhibition up to 10 μM concentration and hERG liabilities (IC_{50}: > 30 μM). Compound **26c** and **26h** will be further subjected for PK profiling in mice followed by efficacy evaluation using xenograft model to check their anticancer and anti-inflammatory activity in suitable animal models. In docking studies, **26b**, **26c, 26g** and **26h** interact closely with the key residues of PI3Kδ ATP-binding pockets by adopting propeller shape orientation, **Figure 45**.



26c
IC_{50}: 350 (α), 28 (β), 43 (γ), 0.90 (δ), >1000 (m-TOR)

26h
IC_{50}: 350 (α), 28 (β), 45 (γ), 0.95 (δ), >1000 (m-TOR)

Figure 45: Lead compounds form benzofuran series (Chapter III)

In 2,4-disubstituted quinoline based series, initial set of compounds with N-methyl piperazine derivative, **37a (**IC_{50}: 0.60 nM)**, 37b** (IC_{50}: 0.52 nM) and **37c** (IC_{50}: 0.82) nM showed excellent PI3kδ inhibitory activity. In second set, we tried to replace N-methyl piperazine ring with similar cyclic amine. We prepared few compounds which showed moderate PI3Kδ inhibition as compared to INK-666. In the third set, pyrrolidine derivative compound, **37h (**IC_{50}: 0.65 nM)**, 37i** (IC_{50}: 0.60 nM) and **37j** (IC_{50}: 0.85) nM were equally potent as compared to N-methyl piperazine derivative and reference compound INK-666. Compounds **37a**, **37b**, **37c**, **37h**, **37i** and **37j** showed no CYP inhibition up to 10 μM concentration and hERG liabilities (IC_{50}: > 30 μM). In docking study, compounds **37a** and **37h** retained that all key interactions with PI3Kδ ATP binding pocket. Further, **37a**, **37b**, **37c**, **37h**, **37i** and **37j** will be assessed for PI3K isoform selectivity. Most potent and selected compound will be subjected for PK profiling followed by efficacy evaluation using suitable animal models.

5.2. Future plans

Compound **10h** showed excellent PI3Kδ inhibitory activities (*in vitro* and *in vivo*), the PK and safety profile of **10h** was found to be satisfactory with respect to the standard compound and therefore **10h** represents a promising candidate for further exploration. Future work includes some additional pre-clinical studies before it can be taken for clinical development. Compound **10h** will be subjected for long term efficacy studies and for chronic toxicological evolution, along with its PK profiling in higher animals, such as dog or monkey. If all the results of biological studies will be satisfactory, then we will proceed with the IND enabling studies of compound **10h** as a novel, potent, selective

PI3Kδ inhibitor for the safe and effective treatment of inflammatory diseases such as RA and cancer such as CLL.

During the development of compound **10h**, it has been observed that compounds from this series accommodate more flexibility with the aromatics substitutions (R_1) on imidazo-quinoline ring in the affinity pocket of PI3Kδ enzyme. Thus, on the basis of learning developed during the discovery of compound **10h,** a new series can be designed by retaining all the key interactions in the PI3Kδ ATP binding site, **Figure 46**. Aryl ring of imidazo-quinoline can be saturated to piperidine in order to enhance PK profile. The new designed compound **10n** may lead to change in molecular orientation with more effective binding interactions with the PI3Kδ enzyme. Main focus of developing new series will be towards improving the PK profile to get highly bioavailable PI3Kδ inhibitor.

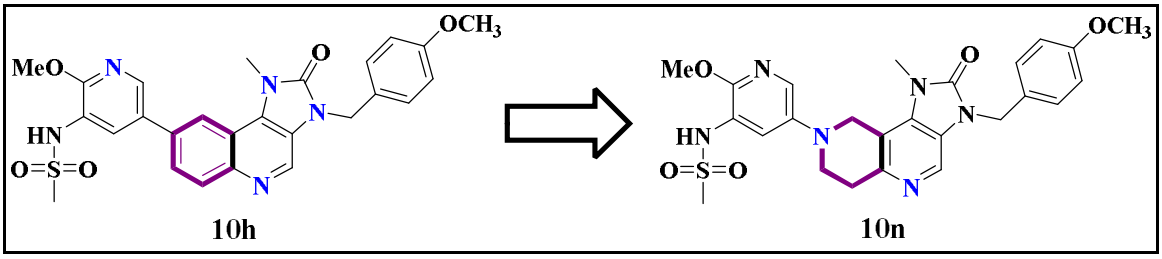


Figure 46: Proposed imidazo-quinoline based, orally bioavailable PI3Kδ selective inhibitors