Synopsis

of

The Thesis Entitled

"Design, Synthesis and Biological Evaluation of Novel PI3Kδ Inhibitors for the Treatment of Leukemia and Inflammatory Diseases"

To be submitted to The Maharaja Sayajirao University of Baroda

For the Degree

Of

DOCTOR OF PHILOSOPHY

In Chemistry

By

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Under guidance of

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Name of the student: Mr. Dave Bhushan Naresh

Faculty: Science

Subject: Chemistry

Name of Guide: Prof. Shubhangi S Soman (M.Sc. PhD).

Name of Co-Guide: Dr Rajesh H Bahekar (M. Pharm. PhD).

Title of the Thesis : "Design, Synthesis and Biological Evaluation of Novel PI3Kδ Inhibitors for the Treatment of Leukemia and Inflammatory Diseases"

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Place of Work : Department of Medicinal Chemistry,

Zydus Research Centre,

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(Gujarat, India)

CHAPTER 1

Introduction

1.1 Inflammation and Rheumatoid Arthritis (RA)

Inflammation is derived from the Latin word *inflammare* which means *"to set on fire with passion."*. Inflammation is part of the body's defence mechanism through which the immune system recognizes and removes harmful foreign particle and begins the healing process. Sometimes, the immune system triggers an inflammatory response inappropriately, which causes acute autoimmune diseases. Key symptoms of Inflammation are redness *(rubor)*, swelling *(tumor)*, heat *(calor)*, pain *(dolar)*, and loss of tissue function, which result from inflammatory cell responses to infection or injury. (1)(2).

Worldwide, 3 out of 5 people are dying due to inflammatory diseases like stroke, chronic respiratory diseases, heart disorders, cancer, obesity, and diabetes. There are many external factors such as allergic reactions, chemical irritants, burns, infections, wounds, injury and internal factors such as growth hormone, cytokine and stimuli which account acute inflammation. Untreated acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases like Cardiovascular disease, Autoimmune disease, Rheumatoid Arthritis, Cancer etc.(3)

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease, which means our immune system attacks healthy cells in your body by mistake, causing inflammation (painful swelling) in the affected parts of the body usually wrist and small joints of hands and feet. Around 0.25 to 1% population in the world is affected by Rheumatoid Arthritis (RA). Risk for women is higher (8.4%) as compared to men (5.1%). (Arthritis Rheum, 2011,63(3), 633-639). In a joint with RA, the lining of the joint becomes inflamed, causing damage to joint tissue. This tissue damage can cause long-lasting or chronic pain, unsteadiness (lack of balance), and deformity (misshapenness). (4)

Signs and symptoms of RA are Pain or aching in more than one joint, Stiffness in more than one joint, Tenderness and swelling in more than one joint, Weight loss, Fever, Fatigue, or tiredness, Weakness. RA can also affect other tissues throughout the body and cause problems in organs such as the lungs, heart, and eyes.

1.2. Current therapies for treatment of RA

Sr. No	Class	Drug name	Side effect
1	Disease-modifying anti-rheumatic drugs (DMARDs)	Methotrexate, Leflunomide, Hydroxychloroquine, Sulfasalazine	Feeling sick, loss of appetite, a sore mouth, diarrhoea, headaches, hair loss
2	Biological treatments	Etanercept , Infliximab	Skin reactions at the site of the injections, infections, feeling sick, a high temperature, headaches
3	JAK inhibitors	Tofacitinib, Baricitinib	Nasopharyngitis, Diarrhea, Infection, Lymphoma, Risk of blood clots
4	**PI3Kδ inhibitors**	**Idelisib, Leniolisib, AMG319, Duvelisib, Copanlisib, Umbralisib**	**Hyperglycaemia, Fatigue, Dry skin, weight loss**
5	Non-steroidal anti-inflammatory drugs (NSAIDs)	Ibuprofen, Diclofenac	Heartburn, abdominal pain, gas, omitting, constipation
6	Steroids	Prednisone, Dexamethasone, Triamcinolone	weight gain, osteoporosis(weakening of the bones) easy bruising, muscle, weakness thinning of the skin

The current therapies for RA are disease-modifying anti-rheumatic drugs (DMARDs) biological treatments, Selective Inhibition of signal transduction cascades (JAK, PI3K), non-steroidal anti-inflammatory drugs (NSAIDs) and Steroids. Other operative therapies are Surgery, Arthroscopy (remove inflamed joint tissue), and Joint replacement**.** (5)

RA treatment are well tolerated for short periods, but long-term administration may result in persistent adverse events such as Feeling sick, loss of appetite, sore mouth, diarrhea, headaches, hair loss, Skin reactions at the site of the injections, infections, a high temperature, Nasopharyngitis, Lymphoma, Risk of blood clots, Hyperglycemia, Fatigue, Dry skin, weight loss, Heartburn, abdominal pain, gas, omitting, constipation, weight gain, osteoporosis(weakening of the bones) easy bruising, muscle. New therapies available have

significantly improved the clinical situation, but suffer with adverse side effects such as organ toxicity, less selective and metabolically less stable. Need to explore the new molecular targets and strategies to develop novel Anti Inflammatory agents and one of the most advances and upcoming target for treatment of Rheumatoid arthritis and Leukemia is inhibition of Phosphoinositide 3-Kinase delta inhibitor (PI3Kδ inhibitor).

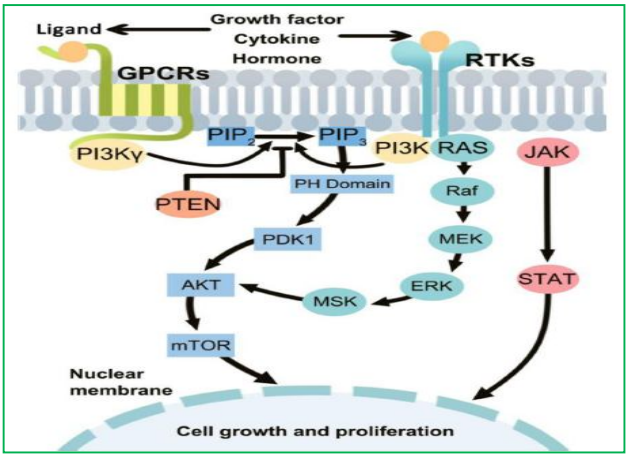
1.3. Phosphatidylinositol-3-kinase

Phosphoinositide-3-kinases (PI3Ks) constitute central signalling hub that mediates diverse and crucial cell functions, including cell growth, proliferation, differentiation, motility and survival.(6) (7) PI3Ks have been classified into three classes (I, II and III) based on substrate specificity, sequencing homology and types of regulatory subunits. The class I PI3Ks consists of four kinases (PI3K-*α*, *β*, *δ* and *γ)* and further grouped into two sub-classes: class IA and class IB. The PI3Kα and β are expressed in a wide variety of tissues and organs. PI3Kγ is found mainly in leukocytes, while expression pattern of PI3Kδ is restricted to spleen, thymus, hematopoietic cells and peripheral blood leukocytes. PI3Kγ and PI3Kδ are mainly expressed in rheumatoid arthritis (RA) synovium and regulate innate and adaptive immune responses. (8)

1.3.1 PI3K activation pathways:

PI3K is normally activated by a variety of extracellular stimuli, such as growth factors, cytokines, and hormones. Upon activation, PI3K catalyzes the phosphorylation of PtdIns(4,5) P2(PIP2) to produce PtdIns(3,4,5) P3(PIP3), which act as second messenger for other signaling proteins, such as kinases AKT and PDK1 can bind to the lipid products of PI3K and thereby localize to the cell membrane to activate cell growth and cell survival pathways. (9)

Figure 1: PI3K activation



1.3.2. Significance for targeting PI3Kδ isoform:

Inhibition of PI3Ks is considered as one of the most interesting targets. Earlier attempts were mainly focused on developing the broad-spectrum (pan) inhibitors of the PI3K (*α, β, γ* and *δ*) isoforms, as potential oncology therapeutics. However, knowing the potential side effects associated with PI3K*α* and *β* isoforms inhibition (due to universal expression), recently, more efforts are directed towards the development of isoform selective inhibitors, particularly PI3K*δ* selective inhibitors, for the safe and effective treatment of hematological malignancy and inflammatory disorders.

1.3.2. Current Scenario of PI3K Inhibitors

Compound	Generic Name	Development Phase	Indication	Originator
IPI 145 (δ/γ)	Duvelisib Copiktra™	Approved September 2018	Chronic lymphocytic leukemia, small lymphocytic lymphoma, inflammatory Diseases	Infinity/Verastem
CAL 101 (δ)	Idelalisib Zydelig™	Approved July 2014	Chronic lymphocytic leukemia, small lymphocytic lymphoma	Gilead Science
BAY 80-6946 (α/δ)	Copanlisib Aliqopa™	FDA Approved 2019	hematological and solid malignancies, relapsed follicular lymphoma	Bayer
CDZ173 (δ)	Leniolisib	Late III	activated PI3Kδ syndrome COPD, Asthma	Novartis
RP5264 / GR 1202 (δ)	Umbralisib	Late III	marginal zone lymphoma, COPD, Asthma	TG Therapeutics

BYL719 (α)	Alpelisib Piqray™	Approved May 2019	advanced or metastatic breast cancer	Novartis
GDC 0032	Taselisib	Late III	metastatic breast cancer	Roche
AMG319 (δ)	-	II	Rheumatoid arthritis	Amgen
IBI-376 (δ)	Parsaclisib	II	hematological malignancies; Solid tumors	Incyte Corporation
INK 1117 (α)	Serabelisib	II	Breast cancer; Endometrial cancer; Renal cell carcinoma	Takeda
RP 6530 (δ/γ)	Tenalisib	II	Relapsed/Refractory Indolent Non-Hodgkin's Lymphoma	Rhizen

1.3.3. Ultimate needs

Currently available class of drugs have significantly improved the clinical situation, but exhibits adverse side effects such as organ toxicity, less selective and metabolically less Anti Inflammatory agents stable. So there is unmet need to explore a new molecular targets and strategies. One of the most advances and upcoming target for treatment of Rheumatoid arthritis and Leukemia is inhibition of Phosphoinositide 3-Kinase delta inhibitor (PI3Kδ inhibitor)

1.3.4. Objectives of my Research

Our objective was to develop Novel, Orally active, selective PI3Kδ inhibitor as Anti-Inflammatory agents for treatment of Rheumatoid arthritis and Leukemia to overcome the existing side effect. Synthesis of newly designed PI3Kδ inhibitors containing heterocyclic ring such as imidazo-quinoline, benzofuran and quinoline and their characterization using analytical techniques. In vitro screening of all the synthesized compounds for PI3Kδ inhibitory activity followed by selectivity study of most potent compound against other Kinases and PI3K isoform. Further it will be explore for metabolic stability and docking study in PI3Kδ crystal structure.

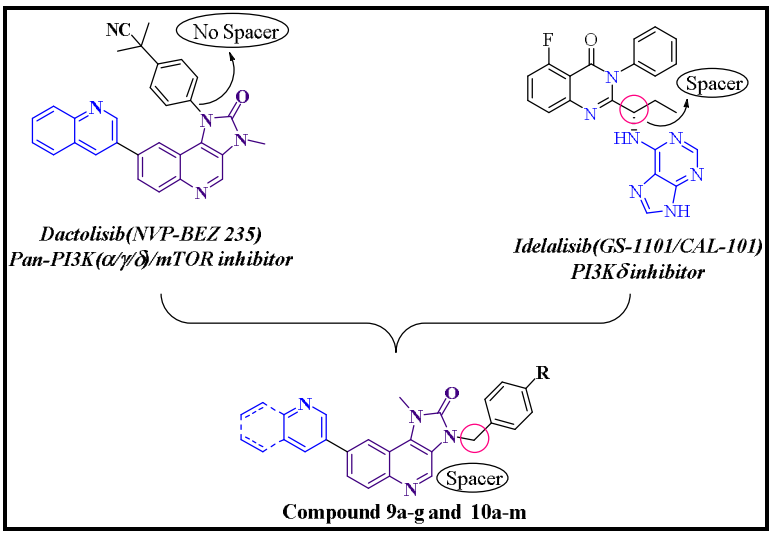
CHAPTER 2

Designing strategy and Synthesis of compound based on Imidazo-quinoline derivatives.

2.1. Designing strategy

Dactolisib (NVP-BEZ235) is a potent dual PI3K-mTOR inhibitor. Considering the structural homology of Idelalisib (GS-1101/CAL-101, Selective PI3Kδ inhibitors), Dactolisib lack spacer in its homology (Figure 1). Hence spacer analogue of it's would show high degree of selectivity over m-TOR and other PI3K isoform. (10)

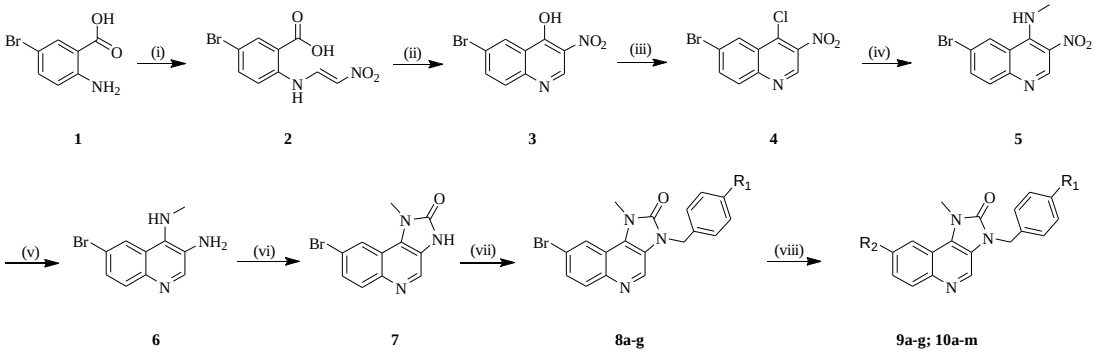
Figure 2: Designing strategy of compound based on Imidazo-quinoline derivatives



2.2. Synthesis of compound based on Imidazo-quinoline derivatives.

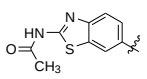
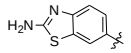
Synthesis of 1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one derivatives (**9a-g** and **10a-m**) was carried out as depicted in Scheme 1.

Scheme 1:

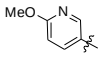
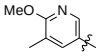


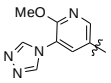
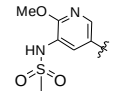
Compound	R_1
8a	$-C(CH_3)_2CN$
8b	$-CH(CH_3)_2$
8c	OCH_3
8d	CH_3
8e	NO_2
8f	F
8g	H

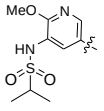
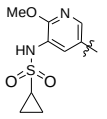
Compound	R_1	R_2
9a	$-C(CH_3)_2CN$	
9b	$-CH(CH_3)_2$	
9c	OCH_3	
9d	CH_3	
9e	NO_2	
9f	F	
9g	H	

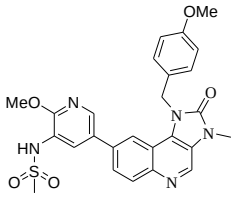
Compound	R_1	R_2
10a	OCH_3	
10b		

Compound	R_1	R_2
10c	OCH_3	
10d		

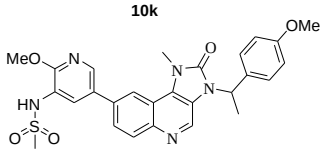
Compound	R_1	R_2
10e	OCH_3	
10f		

Compound	R_1	R_2
10g	OCH_3	
10h		

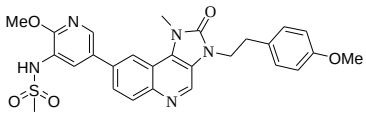
Compound	R_1	R_2
10i	OCH_3	
10j		



10k



10l

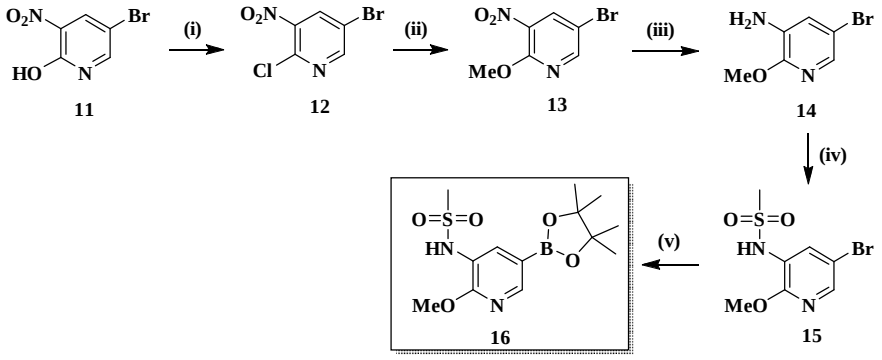


10m

Reagents and conditions: i) conc. HCl, water, 26^{o}C,6 h, then nitro methane, NaOH, water, Conc. HCl, 26^{o}C,16 h,ii) KOAc, acetic anhydride, 120-125^{o}C,4h, iii) $POCl_3$, 120 ^{o}C, 4h, iv) Me-NH_2, TEA, DCM, 26^{o}C, 12h, v) $SnCl_2.2(H_2O)$, EtOAc, 26^{o}C vi) Diphosgene, TEA, DCM, 0-26^{o}C, vii) R1-Ar-CH_2-X (Cl or Br), NaH, THF, 0-26^{o}C viii) R_2-$B(OH)_2$,$PdCl_2(PPh_3)2$, $KHCO_3$, DMF, H_2O, 90-95^{o}C, 1.5 h.

2.2.1. Synthesis of intermediate pyridyl boronate ester (16):

Scheme 2:



Reagents and conditions:(i) $POCl_3$, DIPEA, 80-85^oC, 4 hr; (ii) NaOMe, MeOH, 0-26^oC, 4 hr; (iii) Fe, Conc. HCl, EtOH, 26^oC, 16 hr; (iv) Mesyl-Chloride, Pyridine, 26^oC, 24 hr; (v) Bis PIN, $PdCl_2$(dppf), (1,4-Dioxane, Reflux, 24 hr.

2.3. *In vitro* PI3Kδ inhibitory activity data of Imidazo-quinoline derivatives at R_1 position:

Table 1: PI3Kδ inhibitory activity of 4-substituted-(benzyl)-8-quinolinyl-imidazo[4,5-c]quinolinone **(9a-g)**

Comp.	R_1	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]
9a		56	ND
9b		73	28.3
9c		**98**	**9.5**
9d	-CH_3	80	18.4
9e	-NO_2	62	ND
9f	-F	51	ND
9g	-H	22	ND
Dactolisib	-	92	8
Idelalisib	-	96	2.1

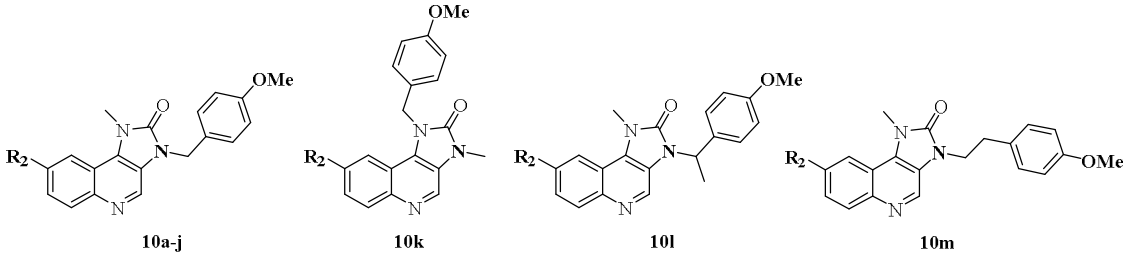
[a]All the data are shown as the mean for at least two experiments. [b]PI3Kδ inhibition at the concentration of 100 nM using PI3K Kinase Activity/Inhibitor assay Kit from Millipore. [c]The IC_{50} values for PI3Kδ inhibition.ND: not detected

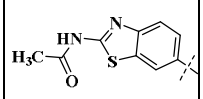
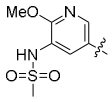
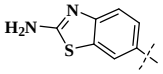
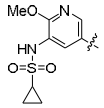
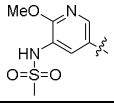
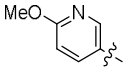
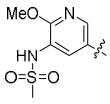
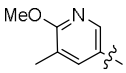
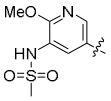
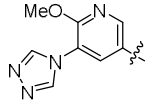
Compound **9c** bearing methoxy group afforded the most potent PI3Kδ inhibitory activity, which was found to be similar to Dactolisib (IC_{50}: 8 nM; Table 1) and approximately 4.5 fold

less potent compared to Idelalisib (IC_{50}: 2.1 nM). Further to improve PI3Kδ isoform selectivity, modeling studies were carried out, considering compound **9c** as primary lead. In table 2, various substitutions were carried out on the **R_2** position of **9c** and their PI3Kδ inhibitory activity is reported.

2.3.1. *In vitro* PI3Kδ inhibitory activity data of Imidazo-quinoline derivatives of 9c at R_2 position:

Table 2: Influence of modification of R_2 position of Quinoline moiety on PI3Kδ isoform.



Comp.	R_2	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]	Comp.	R_2	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]
10a		52	ND	**10h**		**99**	**1.9**
10b		58	ND	**10i**		91	6.2
10c		72	29.4	**10j**		93	5.9
10d		81	20	**10k**		98	4.1
10e		87	11.4	**10l**		60	ND
10f		88	11.1	**10m**		56	ND
10g		62	ND	**Idelalisib**		96	2.1

[a]All the data are shown as the mean for at least two experiments. [b]PI3Kδ inhibition at the concentration of 100 nM using PI3K Kinase Activity/Inhibitor assay Kit from Millipore. [c]The IC_{50} values for PI3Kδ inhibition.ND: not detected.

It was found that the PI3K*δ* inhibitory activity was preserved to a large extent by making modification at R_2 position of quinoline in **9c**. As listed in Table 2 (Compounds **10a-m**), substitution of quinoline moiety in **9c** with Pyridyl derivatives (**10h**) was found to be the most potent (IC_{50}: 1.9 nM) among all, presumably due favourable orientation and interactions in the affinity pocket and hinge regions.

2.3.2. Selectivity of the new PI3Kδ inhibitors

Based on the above preliminary PI3Kδ inhibitory activity results, most potent compounds (**9c**, **10h** and **10k**) were evaluated for their selectivity against PI3K isoforms (α, β and γ) and mTOR (mammalian target of rapamycin). As shown in Table 3, Initial hit (**9c**) and **10k** showed moderate selectivity against other three PI3K isoforms and mTOR over PI3Kδ. Compound **10h** (IC_{50}: 1.9 nM) demonstrated 469, 310, and 59-fold selectivity over PI3Kα, β and γ respectively.

Table 3: Isoform selectivity of compounds against PI3K (α,β,γ, and δ) and mTOR activities.

Comp.	Biochemical IC_{50} [nM][a]				
	PI3Kα[b]	PI3Kβ[b]	PI3Kγ[b]	PI3Kδ[b]	mTOR[b] (p70S6K)
9c	421	342	38	9.5	676
10h	**891**	**589**	**112**	**1.9**	**>1000**
10k	289	241	42	4.1	580
Dactolisib	5	79	6	8	14
Idelalisib	831	571	92	2.1	>1000

[a]The IC_{50} values are shown as the mean for at least two experiments. [b]PI3K inhibitory activity assay Kit (Millipore) was used to screen the test compounds.

2.3.3. Anti-proliferative activities, CYP (Cytochrome) inhibition and hERG (human ether-a-go-go-related gene) liabilities for Compound 10h:

In anti-proliferative in vitro assay, **10h** and Idelalisib exhibited potent anti-proliferative activity with an IC_{50} value of 340 and 795 nM respectively. Additional profiling studies of compound **10h** was carried out and it was found to be devoid of CYP and hERG liabilities (IC_{50}: > 30 μM), while Idelalisib showed moderate CYP3A4 inhibition. (11)

2.4. In vivo pharmacokinetic (PK) and pharmacodynamics (PD) studies of compound 10h:

A comparative single dose 3 mg/kg, po (Per os / Oral administration) and 1 mg/kg, iv (intravenous injection) PK profile of compounds **9h**, **10h** and Dactolisib was evaluated in male C57BL/6J mice (n =6) and the various PK parameters (Tmax, Cmax, $t_{1/2}$, Cl, AUC and %F) were recorded (Table 4). (12)

Table 4: Pharmacokinetic study parametersa of **9c**, **10h** and **Dactolisib** in C57 mice

Compd	Tmax (h)	Cmax (μg/ml)	$t_{1/2}$ (h)	Cl (ml/min/kg), iv	AUC (0-α) h μg/ml	%F*
9c	0.38	127.61	2.31	22.9	329.08	14.77
10h	0.25	1278.49	3.48	8.24	3831.48	68.91
Dactolisib	0.21	273.83	1.88	72.5	243.64	37.82

[a]In male C57BL/6J mice (n=6), compounds were administered orally (p.o) at 3 mg/kg dose and plasma concentration was analyzed by LC-MS, values indicate Mean ± SD.* Oral bioavailability (%F) was calculated wrt to iv AUC. Compounds **9c**, **10h** and Dactolisib administered at 1 mg/kg dose, iv AUC: 742.56, 1852.95 and 215.14 respectively.

Considering low bioavailability of **9c**, in PD models, only **10h** was evaluated. Collagen Induced Arthritis (CIA) mice model was used to assess anti-arthritic efficacy of test compounds. As shown in the **Figure 2a**, standard and **10h** showed significant reduction in the arthritic score, compared to vehicle control (untreated group). Two fold higher dose of a standard compound was used, considering two fold difference in the mice oral bioavailability. At 30 mg/kg dose, compound **10h** showed superior activity compared to standard compound (dose 60 mg/kg). Body weights of the animals were also recorded 3 times a week as a measure of treatment related side effect and **10h** showed no significant reduction in the body weight, even at 30 mg/kg dose, while Dactolisib exhibited reduction in body weight.

Additionally, in vivo anti-tumor activity of **10h** was checked in male SCID mice xenograft model (inoculated with TMD-8 cells). As shown in **Figure 2b**, three doses (3, 10 and 30

mg/kg/day, orally for 28 days) of **10h** were administered and it showed dose dependent reduction in the tumor volume. At 30 mg/kg dose, **10h** showed complete inhibition of tumor volume compared to vehicle control. Body weights of the animals were also recorded and **10h** showed no significant reduction in the body weight, even at 30 mg/kg dose. Thus improved PK of **10h** justifies its potent in vivo activity in both the animal models.

Figure 2a: Effect of Compound 10h and **Dactolisib** in CIA mice model.

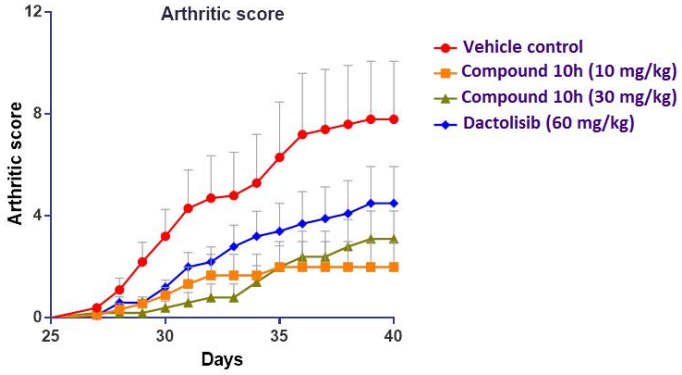
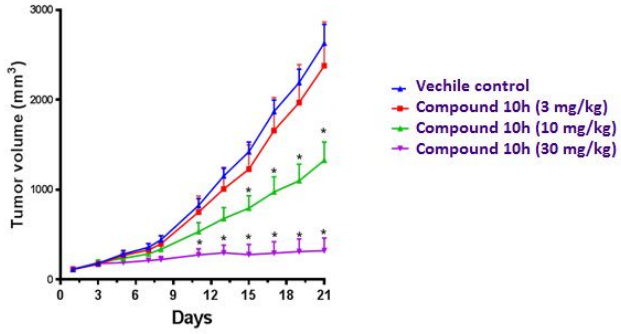


Figure 2b: In vivo anti-tumor activity of Compound **10h** in SCID mice xenograft model.



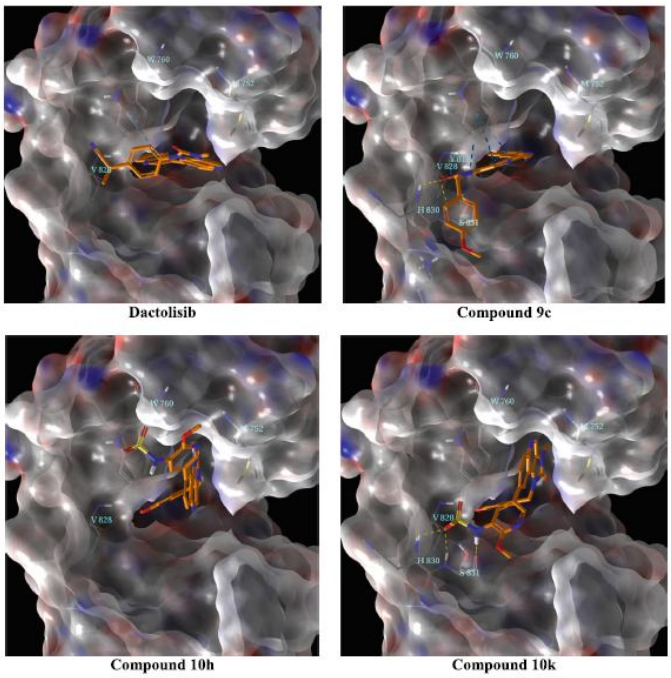
2.5. Molecular modeling study

To understand the potent and selective PI3Kδ inhibitory activity of **10h**, docking studies were carried out using Glide 5.6 for compounds **10h**, **10k** and Dactolisib (Figure 3). The PI3Kδ crystal structure was retrieved from the RCSB Protein Data Bank (PDB entry 4XE0).

As shown in the Figure 3, all the three compounds showed favorable interactions with the PI3Kδ ATP-binding pockets, imidazoquinoline ring served as the hinge binder and it forms key hydrogen bonds with Val_{828} and Glu_{826}. However, the Dactolisib makes no interaction

with W_{760}, key residue in specificity pocket. Compound **10h** adopts propeller-shaped conformation where the *p*-methoxy-benzyl-imidazo moiety was found to be sandwiched into the induced hydrophobic specificity pocket between Trp_{760} and Met_{752} and methoxy-benzyl group forms π-π stacking interactions with W_{760} in PI3Kδ selectivity pocket, thereby, force the inhibitor to adopt an extended conformation. Also, propeller-shaped conformation of **10h** favors to accommodate methanesulfonamide pyridyl ring of **10h**, in the affinity pocket. It appears that an additional hydrogen bonding of **10h** with W_{760} and propeller shape orientation contributes towards improved PI3Kδ selectivity and inhibitory activity in this case. While compound **10k** showed weak interaction with W_{760}. (13)

Figure 3: The Glide docking studies of Compounds 9c, 10h, 10k and Dactolisib into site of PI3Kd (PDB ID:4XE0). Compounds are shown as sticks. Hydrogen bonds are shown as yellow dash lines.



3. Conclusion

In conclusion, we have synthesized and evaluated two sets of novel series of 1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one derivatives as selective PI3Kδ inhibitors. In first set, appropriate modifications were carried out in the imidazo quinoline ring, which led to an identification of a single digit nM potent PI3Kδ inhibitor (**9c**), with moderate isoform selectivity. In set 2, further structure-activity relationship (SAR) studies on the 8th position of

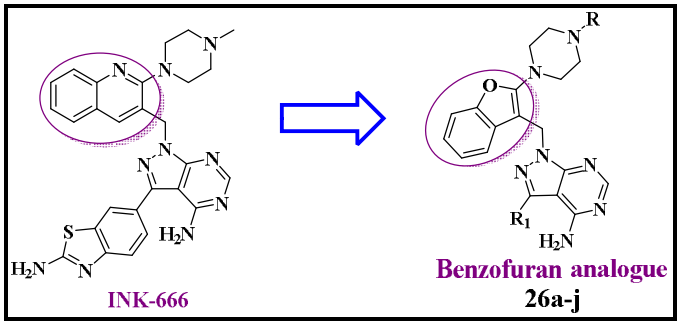
9c resulted in to the discovery of *N*-(2-methoxy-5-(3-(4-methoxybenzyl)-1-methyl-2-oxo-2,3-dihydro-1H-imidazo[4,5-*c*]quinolin-8-yl)pyridin-3-yl)methanesulfonamide (**10h**) that showed improved isoform selectivity, PK profile and good efficacy in a CIA and xenograft animal models. The molecular docking study of compound **10h** indicated key hydrogen bonding interactions, which justifies its selective PI3Kδ inhibitory activity. Overall pre-clinical data suggest that the development of a potent and selective PI3Kδ inhibitor could be viable therapeutic option for the safe and effective treatment of rheumatoid arthritis.

CHAPTER 3

Designing strategy and Synthesis of compound based on Benzofuran pyrazolo -pyrimidine derivatives.

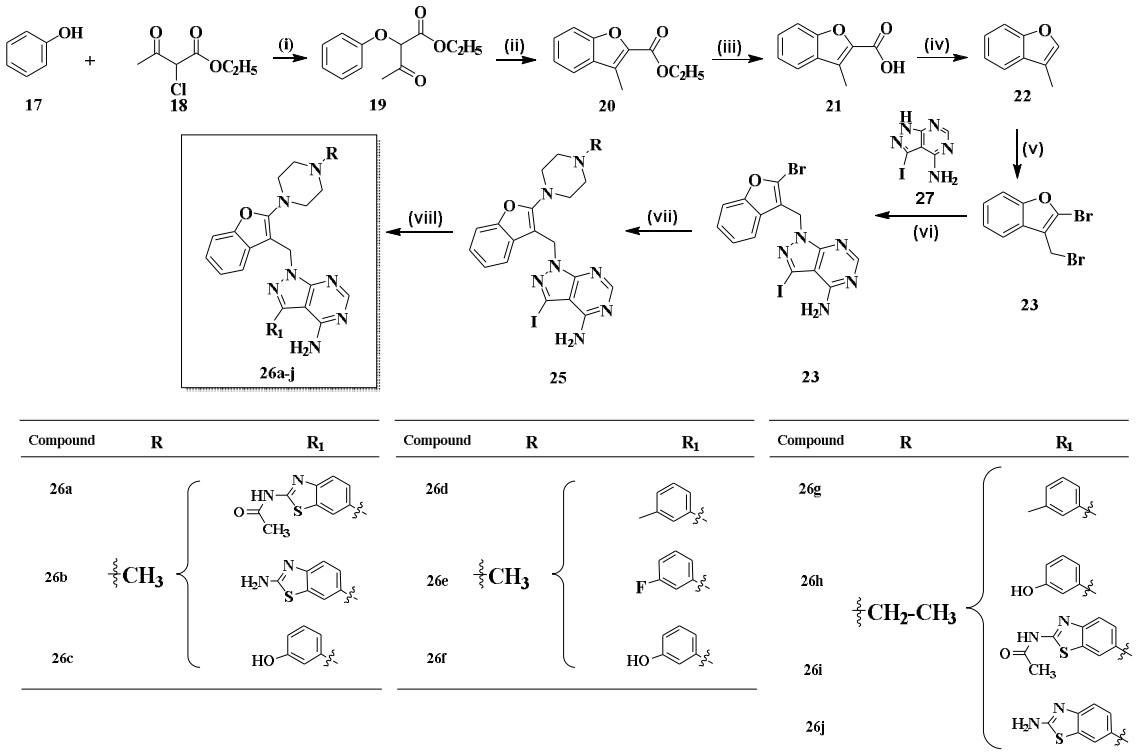
3.1. Designing strategy

Intellikine PI3K inhibitor possess high degree of selectivity over other isoform (INK-666 IC_{50} for PI3Kδ: 0.5 nM; PI3Kα: 137 nM, PI3Kβ: 9 nM; PI3Kγ: 4 nM).Using the Intellikine scaffold a new series has been proposed where 2, 3- disubstituted quinoline ring is bioisosterically replaced with Benzofuran ring to get novel, potent and selective PI3Kδ inhibitors benzofuran analogue compound **10a-j.**



3.2. Synthetic scheme for Benzofuran pyrazolo pyrimidine derivatives:

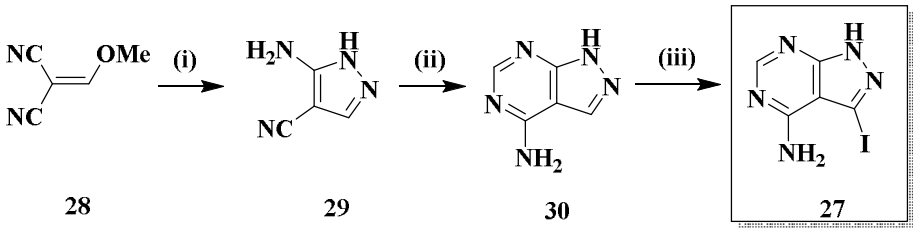
Scheme 3: Synthesis of Benzofuran pyrazolo pyrimidine compounds **26a-j**.



Reagents and conditions:(i) K_2CO_3, Acetone, Reflux, 5 hr; (ii) Conc.H_2SO_4, 0-5^oC, 4 hr; (iii) 10% KOH, 26^oC, 2 hr; (iv) 280^oC, 2 hr; (v) NBS, CCl_4, Reflux, 5 hr; (vi) t-BuOK, DMF, 26^oC, 15 hr; (vii) **R**- sub. Piperazine, (1,4)-Dioxane, Reflux, 12 hr; (viii) Aryl boronic acid (**R_1**), $PdCl_2$ $(PPh_3)_2$, $KHCO_3$, DMF: H_2O, 90^oC, 2 hr.

3.2.1. Synthetic scheme for preparing Iodo (4) compound

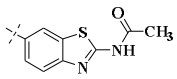
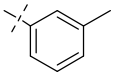
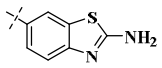
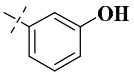
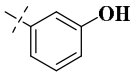
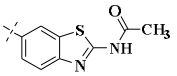
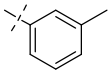
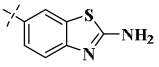
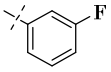
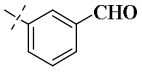
Scheme 1: Synthesis of Iodo (4) compounds



Reagents and conditions:(i) NH_2-NH_2, 80-85^oC, 5 hr; (ii) Formamide, 180-185^oC, 4 hr; (iii) NIS, DMF, 80-85^oC, 16 hr.

In vitro PI3Kδ inhibitory activity data Benzofuran pyrazolo -pyrimidine derivatives:

Sr No.	R	R_1	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]	Sr No.	R	R1	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]

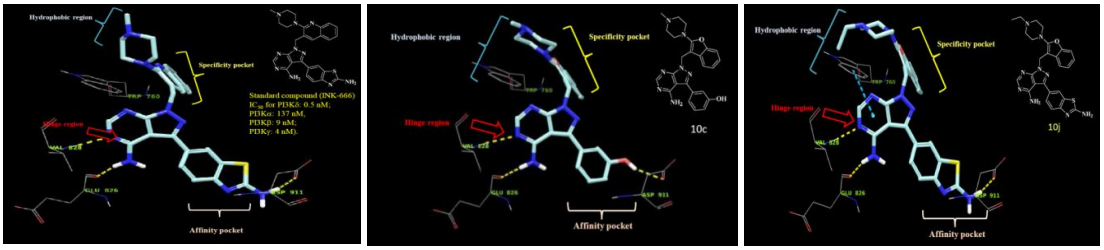
26a	Methyl		102	0.68	**26g**	Ethyl		79	ND
26b			100	0.60	**26h**			99	0.90
26c			99	0.95	**26i**			108	0.60
26d			78	10.1	**26j**			112	0.58
26e			53	ND	**INK 654**			110	0.50
26f			68	ND					

[a]All the data are shown as the mean for at least two experiments. [b]PI3Kδ inhibition at the concentration of 100 nM using PI3K Kinase Activity/Inhibitor assay Kit from Millipore. [c]The IC_{50} values for PI3Kδ inhibition. ND: not detected.

3.3. Molecular modeling study

Molecular docking studies of compound **26a-j** were carried out by using (Glide version 6.7) Schrodinger software to study an interaction of INK666 with PI3Kδ receptor (PDB ID: **2WXK**). **Figure A** is the Docking image for Standard compound (INK-666), **Figure B** and **Figure C** are Docking image for compound **26c** and **26j** respectively.(10)

Fig A **Fig B** **Fig C**



4. Conclusion

Based on invitro results, we can summarized that among ten novel compounds tested for PI3Kδ and mTOR activities, compound **26a-c**, **26h-j** were found to be potent and selective PI3Kδ inhibitors over mTOR. All synthesized compounds were of highest purity (>99%) and characterized using ^{1}HNMR, ESI-MS, and IR. In vitro results validate our hypothesis of

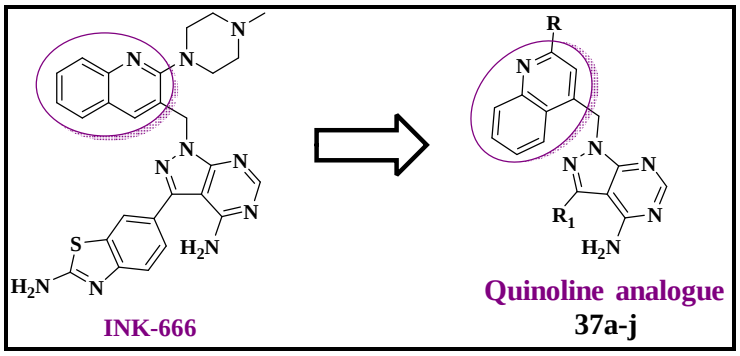
designing benzofuran based novel, potent and selective PI3Kδ inhibitors as a bioisosteric replacement of quinoline ring of INK-654 with benzofuran ring system. Additional profiling studies of compound **26a-c** and **26h-j** was carried out and it was found to be devoid of CYP22 (< 10% CYP inhibition at 10 µM concentration, for CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4) and hERG liabilities (IC50:>30 µM), while INK 654 showed moderate CYP3A4 inhibition. Docking results of **26a-j** correlates with its potent in vitro PI3Kδ activity.

CHAPTER 4

Designing strategy and Synthesis of compound based on 2,4-disubsitued quinoline pyrazolo -pyrimidine derivatives.

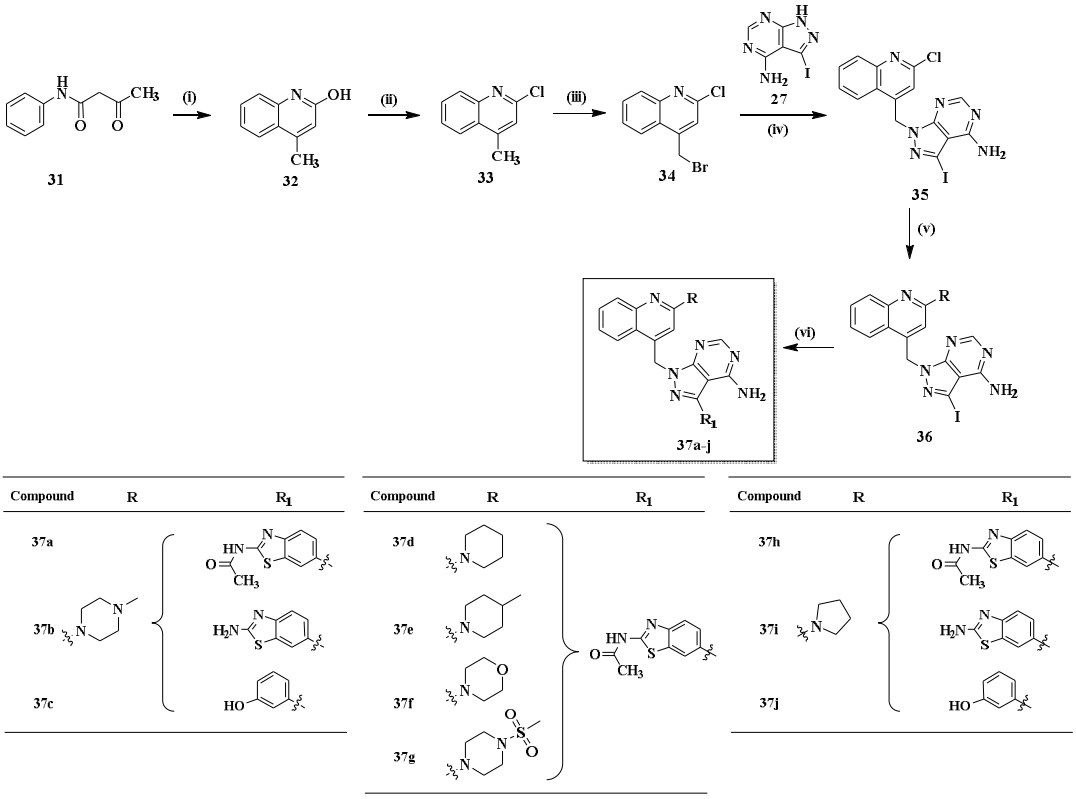
4.1. Designing strategy

Using the Intellikine scaffold two new series has been proposed one of them is Benzofuran analogue with ring modification and other is positional analogue of quinoline.



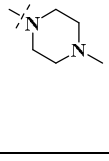
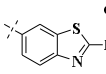
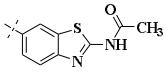
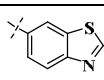
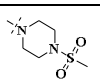
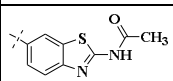
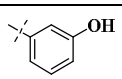
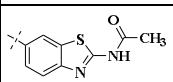
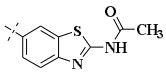
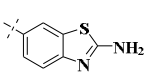
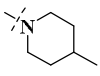
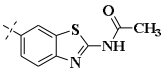
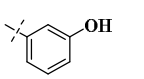
4.2. Synthetic scheme for 2, 4- disubstituted quinoline pyrimidine derivatives:

Scheme 1: Synthesis of 2, 4- disubstituted quinoline pyrimidine **37a-j**.



Reagents and conditions:(i) Conc.H_2SO_4, 110^oC, 4 hr; (ii) $POCl_3$, 120^oC, 5 hr; (iii) NBS, Benzene, Reflux, 5 hr; (iv) t-BuOK, DMF, 26^oC, 15 hr; (v) Secondary amine (R), (1,4)-Dioxane, Reflux, 12 hr; (vi) Aryl boronic acid (R_1), $PdCl_2(PPh_3)_2$, $KHCO_3$, DMF:H_2O, 90^oC, 2 hr.

4.3. *In vitro* PI3Kδ inhibitory activity data 2, 4- disubstituted quinoline pyrimidine derivatives:

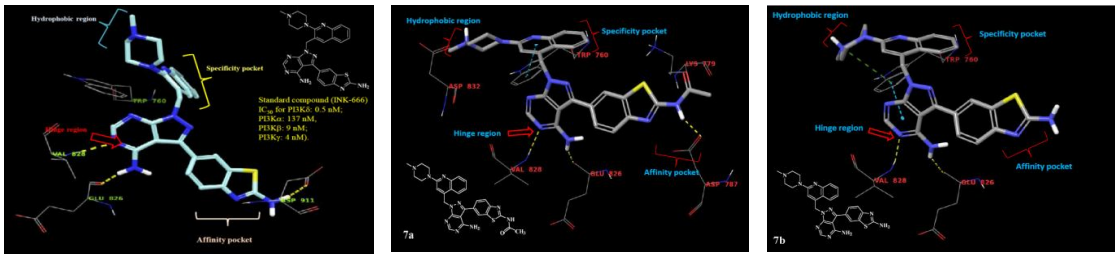
Sr No.	R	R_1	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]	Sr No.	R	R_1	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]
37a			110	0.60	37f			70	9.85
37b			105	0.52	37g			70	10.1
37c			94	0.82	37h			108	0.65
37d			60	ND	37i			105	0.60
37e			52	ND	37j			98	0.85

INK 654 **Standard compound**	**110**	**0.50**	

4.4. Molecular modeling study

Molecular docking studies of compound **37a-j** were carried out by using (Glide version 6.7) Schrodinger software to study an interaction of INK666 with PI3Kδ receptor (PDB ID: **2WXK**). Figure A is the Docking image for Standard compound (INK-666), Figure B and Figure C are Docking image for compound **37a** and **37b** respectively.(10)

Fig A **Fig B** **Fig C**



5. Conclusion

Based on invitro results, we can summarized that among ten novel compounds tested for PI3Kδ and mTOR activities, compound **37a-c, 37h-j** were found to be potent and selective PI3Kδ inhibitors over mTOR. All synthesized final compounds were of highest purity (>99%) and characterized using 1HNMR, ESI-MS, and IR. In vitro results validate our hypothesis of designing 2,4-substituted quinoline based novel, potent and selective PI3K inhibitors by regio isomeric replacement of quinoline ring of INK-654. Additional profiling studies of compound **37a-c** and **37h-j** was carried out and it was found to be devoid of CYP22 (< 10% CYP inhibition at 10 µM concentration, for CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4) and hERG liabilities (IC50:>30 µM), while INK654 showed moderate CYP3A4 inhibition. Docking results of **37a-b** correlates with its potent in vitro PI3Kδ activity.

CHAPTER 5

Protocol for biological studies, Experimental procedure for intermediate and final compound along analytical data of selected intermediate and final compound will be written in this chapter.

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