8. Publications and Posters

List of Publications

1. Rajesh Bahekar, **Bhushan Dave**, Shubhangi Soman, Dipam Patel, Rajendra Chopade, Radhika Funde, Jeevan Kumar, S. Sachchidanand, Poonam Giri, Abhijit Chatterjee, Jogeswar Mahapatra, Purvi Vyas, Krishnarup Ghoshdastidar, Debdutta Bandyopadhyay, Ranjit C. Desai. Discovery of 1,3-dihydro-2H-imidazo[4,5-c]quinolin-2-ones based novel, potent and PI3Kδ selective inhibitors. *Bioorganic & Medicinal Chemistry Letters* 29 (**2019**) 1313–1319.

List of Posters

1. Bhushan Dave, Rajendra Chopade, Dipam Patel, Jignesh Pethani, Hardik shah, Amitgiri Goswami, Abhijit Chatterjee, Purvi Vyas, Debdutta Bandyopadhyay, Jeevan Kumar, Mukul Jain, Rajesh Bahekar, Ranjit Desai, Shubhangi Soman. Design and Synthesis of Benzofuran based pyrazolo[3,4-*d*]pyrimidinamine derivatives as potent and selective PI3Kδ Inhibitor. Poster presented in **8th RBF international Symposium, Advances in New Drug Discovery Technologies and Translational Research at Zydus Research Centre, Ahmedabad on Feb 2-4, 2017**.

2. Bhushan Dave, Rajendra Chopade, Dipam Patel, Jignesh Pethani, Hardik shah, Amitgiri Goswami, Abhijit Chatterjee, Purvi Vyas, Debdutta Bandyopadhyay, Jeevan Kumar, Sachchidanand S, , Mukul Jain, Rajesh Bahekar, Rajiv Sharma, Ranjit Desai, Shubhangi Soman. Design and Synthesis of 2,4-Disubstitued quinoline based pyrazolo [3,4-*d*] pyrimidinamine derivatives as potent and selective PI3Kδ inhibitors. Poster presented in **9th RBF international Symposium, Advances in New Drug Discovery and Development at Zydus Corporate Park, Ahmedabad on Feb 6-8, 2020**.

3. Bhushan Dave, Rajendra Chopade, Dipam Patel, Amitgiri Goswami, Abhijit Chatterjee, Purvi Vyas, Debdutta Bandyopadhyay, Sachchidanand S, Jeevan Kumar, Mukul Jain, Rajesh Bahekar, Rajiv Sharma, Ranjit Desai, Shubhangi Soman. Design, Synthesis and Pharmacological evolution of Imidazo-Quinolinyl derivatives as PI3Kdelta-Selective Inhibitors. Poster presented in **Advances in Chemistry of Bioactive Molecules (ACBAM-2020) at M.S. University, Baroda on Jan 17-18, 2020**. Above poster presentation was awarded 1st prize.

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Discovery of 1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-ones based novel, potent and PI3Kδ selective inhibitors☆

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ABSTRACT

PI3Kδ is implicated in various inflammatory and autoimmune diseases. For the effective treatment of chronic immunological disorders such as rheumatoid arthritis, it is essential to develop isoform selective PI3Kδ inhibitors. Structure guided optimization of an imidazo-quinolinones based pan-PI3K/*m*-TOR inhibitor (Dactolisib) led to the discovery of a potent and orally bioavailable PI3K*δ* isoform selective inhibitor (**10h**), with an improved efficacy in the animal models.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovitis and joint destruction. Treatment with biologic agents such as tumor necrosis factor (TNF) inhibitors has improved outcomes, but in general, biologics are expensive, injectable and many patients have inadequate responses. Thus, orally bioavailable small molecule inhibitors that target signal transduction and regulate innate and adaptive immune responses in RA have emerged as potential alternatives to expensive biologics.[1]

Phosphoinositide-3-kinases (PI3Ks) constitute central signaling hub that mediates diverse and crucial cell functions, including cell growth, proliferation, differentiation and survival.[2,3] PI3Ks have been classified into three classes (I, II and III) based on substrate specificity, sequence homology and 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 class IA comprises three closely related kinases, PI3K-*α*, *β* and *δ*, while the class IB contains only one member PI3K-γ.[4] The PI3Kα and β are expressed in a wide variety of tissues and organs. PI3Kγ is found mainly in leukocytes, while expression of PI3Kδ is restricted to spleen, thymus, hematopoietic cells and peripheral blood leukocytes.[5] PI3Kγ and PI3Kδ are mainly expressed in rheumatoid arthritis (RA) synovium and regulate innate and adaptive immune responses.[6]

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.[7–9] Knowing the potential side effects associated with PI3K*α* and *β* isoforms inhibition (due to universal expression), recently, efforts are directed towards the development of PI3K*δ* selective inhibitors, for the effective treatment of hematological malignancy and inflammatory disorders.[10–12]

Over the past decades, several structurally diverse PI3K inhibitors were identified containing quinolines and imidazoquinolinones as promising scaffold (Fig. 1). The Omipalisib[13,14] and Dactolisib[14], discovered by GlaxoSmithKline and Novartis respectively, as dual PI3K and mTOR inhibitors are under clinical trials. PI3Kδ selective inhibitors, Idelalisib[15] (ZYDELIG®, Gilead Sciences, in 2014) is available for the treatment of hematologic malignancies. Recently, US-FDA approved Duvelisib[16,17] (COPIKTRA®, Verastem, Inc) for the treatment of chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL). There is still requirement to develop safe and highly potent PI3Kδ

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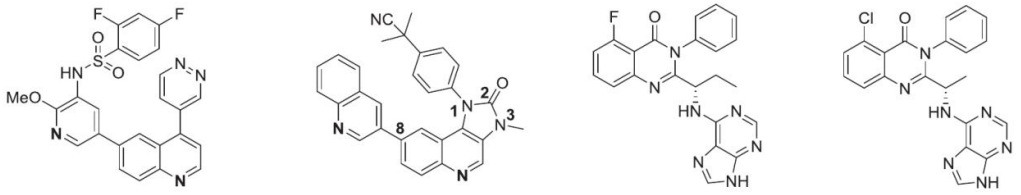


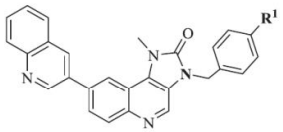
Fig. 1. The structures of quinoline and imidazoquinolinone-based PI3K inhibitors.

selective inhibitors, for the effective management of chronic inflammatory and autoimmune disorders, such as rheumatoid arthritis (RA) and hematological malignancy.

We aim to discover novel, potent and orally bioavailable PI3K*δ* selective inhibitors, mainly by favoring the suitable accommodation of designed molecules, in the specificity pocket, to achieve PI3Kδ selectivity.[18] Considering imidazoquinolinone moiety as a starting point, appropriate structural modifications were carried out in the Dactolisib (pan-PI3K/mTOR inhibitor), to improve PI3Kδ selectivity. Two set of compounds were designed (as listed in Tables 1 and 2). Initial modifications on imidazole ring (of Dactolisib), involves positional changes of methyl (N1 to N3) and phenyl (N3 to N1) groups and introduction of a carbon spacer (phenyl to benzyl) at N3 position. Further modifications were carried out at the *p*-position of benzyl ring (Set-1, Table 1) to obtain the single digit nM potency (**9c**). Compound **9c** was found to be potent but showed moderate isoform selectivity (Table 3). In the second set (Set-2, Table 2), changes were carried out on the 8th position of **9c** to improve isoform selectivity and in vivo efficacy.

Herein, we report, design, synthesis and biological evaluation of novel 1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-ones based PI3K*δ* selective inhibitors. Test compounds were screened in vitro for PI3Kδ inhibitory activity and most potent compounds from each set were tested for in vitro PI3K isoform selectivity (α, β & γ) and mTOR inhibitory activity.[19] Based on the in vitro results, highly potent and selective compound **10h** was selected for in vivo PK and PD (anticancer and anti-inflammatory activities) studies.

Table 1
PI3K*δ* inhibitory activity of 4-substituted-(benzyl)-8-quinolinyl-imidazo[4,5-*c*]quinolinone (**9a-g**).



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.

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, following the modified literature procedure.[20] Treatment of bromo anthranilic acid (**1**) with nitro methane gives nitro vinyl anthranilic acid (**2**), which was cyclized using potassium acetate in acetic anhydride to get the bromo nitro quinolinol (**3**). Compound **3** was converted to reactive chloro derivate (**4**) using $POCl_3$, followed by nucleophilic substitution with methylamine to get the compound **5**. Nitro group of **5** was reduced, using $SnCl_2$ to get the compound **6,** which was cyclized using diphosgene in the presence of base, to obtain the imidazoquinoline (**7**). Alkylation of **7** using strong base and aryl halides furnished compounds **8a-g**, which were converted to **9a-g** and **10a-m**, using $PdCl_2(PPh_3)_2$, potassium bicarbonate and aryl or heteroaryl boronic acids.

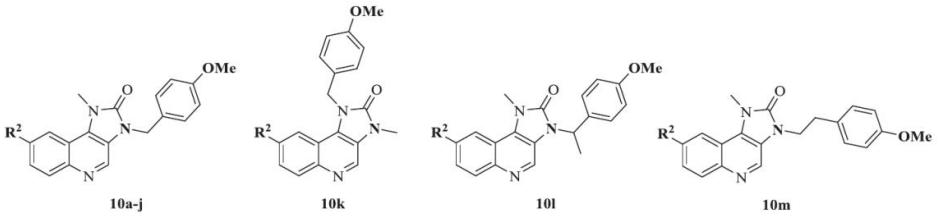
Overall, 20 compounds (**9a-g** and **10a-m**) were prepared in good yield (60 to 80%), under the mild reaction condition. Spectral data of compounds were found to be in conformity with the structures assigned, which ensure the formation of the compounds **9a-g** and **10a-m** (see supporting information for analytical and spectral data).

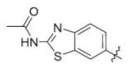
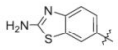
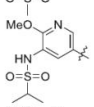
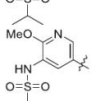
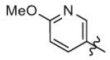
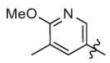
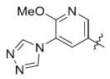
For in vitro PI3Kδ inhibitory activities, Idelalisib and Dactolisib were used as a positive control. In Set-1 (Table 1), 4-substituted-(benzyl)-8-quinolinyl-imidazo[4,5-*c*]quinolinone (**9a-g**) analogues displayed varying degree of PI3Kδ inhibitory activities at 100 nM concentration. Compound **9a** (2-methyl-propanenitrile substitution on a benzyl ring) showed moderate PI3Kδ inhibitory activity (56% PI3Kδ inhibition at 100 nM), while replacement of the nitrile group (Compound **9b**: 73% inhibition) exhibited enhanced PI3Kδ inhibitory activity (IC_{50}: 28.3 nM). Replacement of isopropyl (**9b**) with methoxy (**9c**, IC_{50}: 9.5 nM) and methyl (**9d**, IC_{50}: 18.4 nM) demonstrated higher PI3Kδ inhibitory activity, whereas replacement with an electron withdrawing NO_2 (**9e**, 62% inhibition), electronegative F (**9f**: 51% inhibition) and the unsubstituted compound **9g** (22% inhibition) displayed weak PI3Kδ activity.

In Set-1, compound **9c** (methoxy) showed most potent PI3Kδ inhibitory activity, which was found to be similar to Dactolisib (IC_{50}: 8 nM; Table 1) and 4.5 fold less potent compared to Idelalisib (IC_{50}: 2.1 nM). Thus, in the Set-1, positional changes of methyl (N1 to N3) and phenyl (N3 to N1) groups and introduction of a carbon spacer (phenyl to benzyl) at N3 position, followed by substitution on *p*-position of benzyl ring led to the single digit nM potent compound (**9c**) with moderate isoform selectivity (Table 3).

To improve PI3Kδ isoform selectivity, modifications were carried out in **9c** as a primary lead. In the second set (Table 2), we found that the PI3K*δ* inhibitory activity was retained by doing modifications at 8th position of quinoline in **9c**. As listed in Table 2 (Compounds **10a-m**), replacement of quinoline moiety in **9c** with benzothiazole (**10a** and **10b** (acylated **10a**)) showed 50% PI3Kδ inhibition at 100 nM. Phenyl

Table 2
Influence of modification of C8 position of Quinoline moiety on PI3K*δ* isoform selectivity and mTOR selectivity.



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
10 g		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.

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

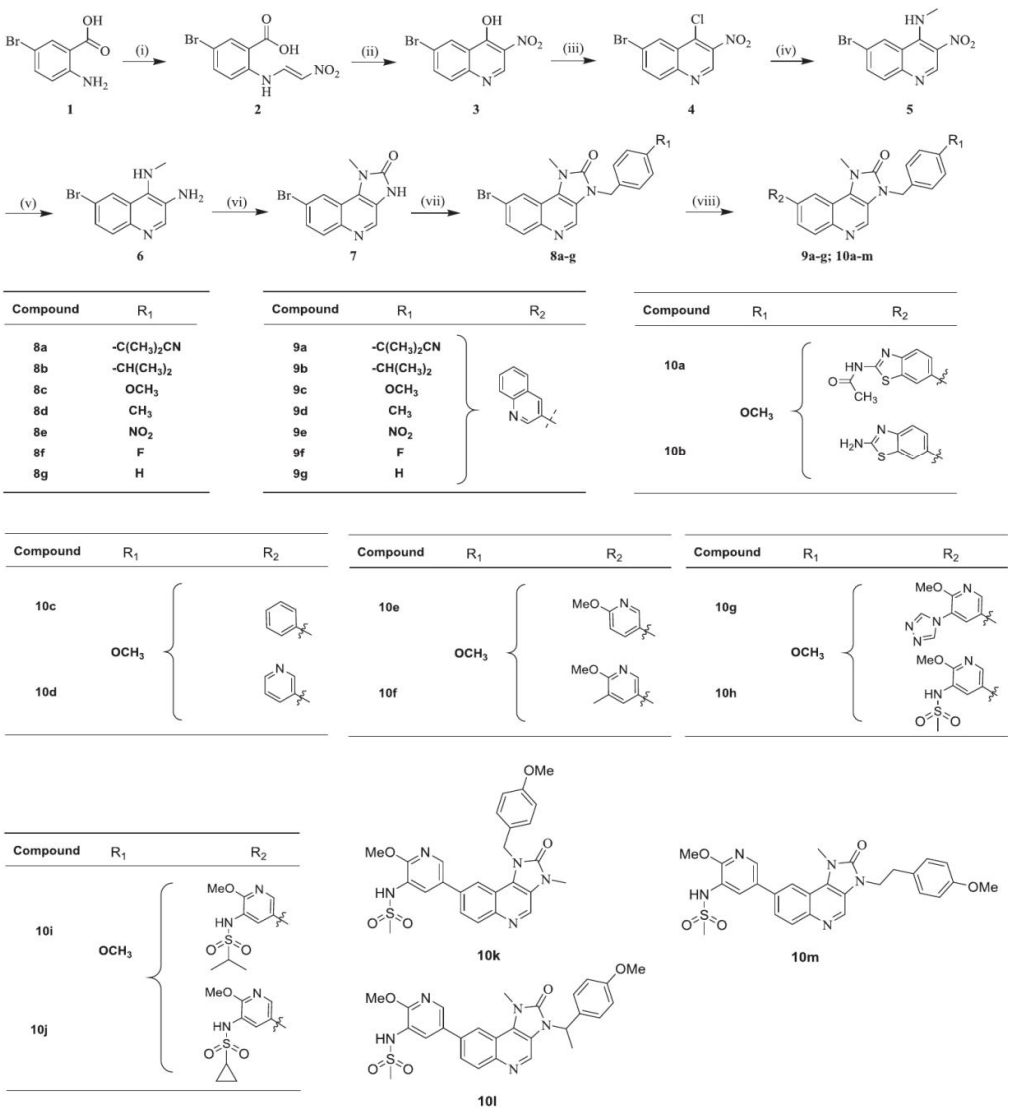
[a]The IC_{50} values are shown as the mean for at least two experiments. [b]PI3K inhibitory activity assay Kit (Millipore) was used to screen the test compounds.

derivative (**10c**) was found to be less potent (PI3K*δ* IC_{50}: 29.4 nM) compared to **9c**, while Pyridyl derivatives (**10d**) showed some improvement in the potency (IC_{50}: 20 nM). Introduction of methoxy (**10e**) and 3-methyl-2-methoxy substituents (**10f**) were found to be equipotent (IC_{50}: ~11 nM). Triazole motif (**10g**) showed less activity compared to **9c**, while *m*-methanesulfonamide derivative (**10 h**) was found to be the most potent (IC_{50}: 1.9 nM) among all. Replacement of methyl group in **10h**, with isopropyl (**10i**) and cyclopropyl (**10j**) showed three fold less activity compared to **10h**.

Compound **10K**, a regioisomer of **10h**, wherein benzyl and methyl groups are interchanged on imidazole ring showed two fold less active compared to **10h**. Similarly, racemic compound **10l**, and **10m** (one carbon homologs at N3 with respect to **10h**) were found to be less active.

Most potent compounds (**9c**, **10h** and **10k**) were evaluated for their selectivity against PI3K isoforms (α, β and γ) and mTOR. As shown in the Table 3, initial hits (**9c** and **10k**) showed moderate selectivity against 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. Moreover, it was noted that selectivity of **10h** against all the three isoforms was higher than standard compounds. In general, it was observed that the potency and selectivity of imidazoquinolinone-based PI3Kδ inhibitors can be modulated using suitable substituents at R^1 and R^2 positions.

In vitro kinase profiling study of **10h** was carried out @ 1 μM concentration, against 140 kinases and % inhibition was found to be < 20% at 1 μM concentration. Compound **10h** was tested for its anti-



Scheme 1. Synthesis of compounds **9a-g** and **10a-l**. Reagents and conditions: i) conc. HCl, water, 26 °C, 6h, then nitro methane, NaOH, water, Conc. HCl, 26 °C, 16 h, 95%; ii) KOAc, acetic anhydride, 120–125 °C, 4h, 60%; iii) $POCl_3$, 120 °C, 4 h, 80%; iv) Me-NH_2, TEA, DCM, 26 °C, 12 h, 94%; v) $SnCl_2.2(H_2O)_2$, EtOAc, 26 °C, 60%; vi) Diphosgene, TEA, DCM, 0–26 °C, 65%; vii) R_1-Ar-CH_2-X (Cl or Br), NaH, THF, 0–26 °C, 65–75%; viii) R_2-$B(OH)_2$, $PdCl_2(PPh_3)_2$, $KHCO_3$, DMF, H_2O, 90–95 °C, 1.5 h, 60–80%.

proliferative activities against TMD-8 cell lines[21] with Idelalisib as a reference compound. 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[22] (< 10% CYP inhibition at 10 μM concentration, for CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4) and hERG liabilities (IC_{50}: > 30 μM), while Idelalisib showed moderate CYP3A4 inhibition.[23]

A comparative single dose (3 mg/kg, po and 1 mg/kg, iv) 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). In PK study, **9c** showed moderate

Table 4
Pharmacokinetic study parameters[a] 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

[*]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.
[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.

AUC and clearance, which resulted into overall low bioavailability (~15%). Compound **10h** showed rapid Tmax, higher AUC (~5 fold, compared to standard), extended $t_{1/2}$ (~3.5 hr) and good oral bioavailability (%F ~69 over standard, 38%). Compound **10 h** showed extended $t_{1/2}$ and higher AUC, which could be due to its low clearance compared to standard (8.24 vs 72.5 ml/min/kg, iv).

Considering low bioavailability of **9c**, in PD models, only **10h** was evaluated. Collagen Induced Arthritis (CIA) mice model was used to check anti-arthritic efficacy of test compounds.[17] Arthritis was developed in male DBA1j mice, using collagen mixture and mice were recruited for the study once clinical signs were visible. Eight animals were assigned in each of the four groups [vehicle, positive control (Dactolisib, 60 mg/kg dose was selected considering low oral bioavailability, compared to **10h**) and two doses of test compound **10h** (10 and 30 mg/kg) was selected considering 89% plasma protein binding and $t_{1/2}$ of ~3.5 h]. Treatment was continued for four weeks and percentage inhibition in clinical score was recorded.

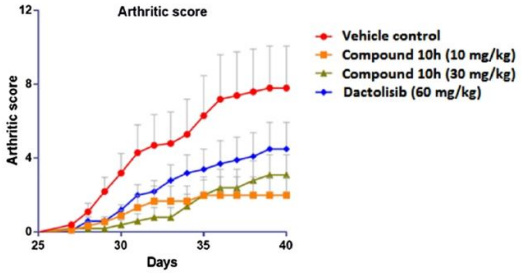


Fig. 2a. Effect of Compound **10h** and **Dactolisib** in CIA mice model.

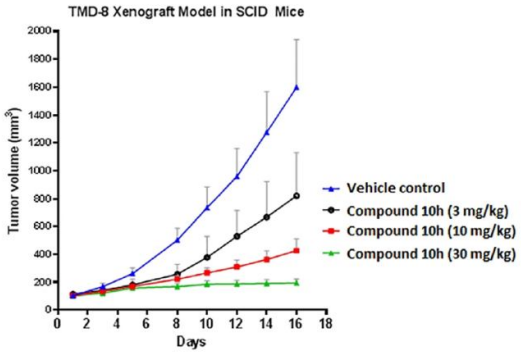


Fig. 2b. In vivo anti-tumor activity of Compound **10h** in SCID mice xenograft model.

As shown in the Fig. 2a, standard and **10h** showed good 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). Inhibition of tumor volume compared to vehicle control (untreated group) was considered as efficacy end point. As shown in Fig. 2b, three doses (3, 10 and 30 mg/kg/day, orally) 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.

The structure of mPI3Kδ co-crystallised with Idelalisib (PDB ID: 4XE0)[18] was prepared using protein preparation wizard of Schrodinger Suite 2018 at pH 7.4, which was used for generating the docking grid. The Glide docking was performed using Glide SP with default parameters. Ligand molecules (Idelalisib, Dactolisib, **9c**, **10h** and **10k**) were prepared using LigPrep module of Schrodinger Suite 2018.[24]

The results of docking has been summarized in Fig. 3 where it is clear from the docking poses of the docked ligands that specificity pocket was fully occupied by **10h** and **10k**, however in case of Dactolisib and **9c**, the specificity pocket was not occupied and this explains why **10h** and **10k** were more selective towards PI3Kδ.

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 imidazoquinoline 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 results of docking study showed that specificity pocket was fully occupied by **10h**, which explains its more selective towards PI3Kδ. Overall pre-clinical data suggest that the development of a potent and selective PI3Kδ inhibitor could be viable therapeutic option for the effective management of rheumatoid arthritis and hematological malignancy.

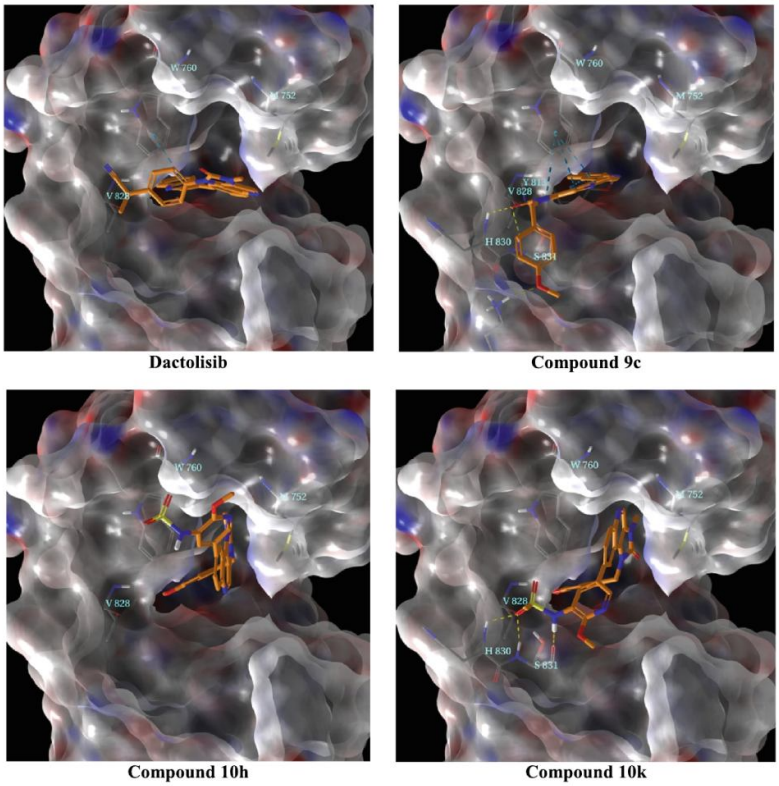


Fig. 3. The Glide docking studies of Compounds **9c, 10h**, **10k** and **Dactolisib** into the site of PI3Kδ (PDB ID: 4XE0). Compounds are shown as sticks. Hydrogen bonds are shown as yellow dashed lines.

Acknowledgments

Authors thank the management of Zydus Research Centre, Cadila Healthcare Ltd. for support and encouragement and the analytical department for providing analytical data.

Notes

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.04.007.

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Design and Synthesis of Benzofuran based pyrazolo[3,4-*d*]pyrimidinamine perivatives as potent and selective PI3Kδ inhibitors

Bhushan Dave*$, Jignesh Pethani*, Hardik shah*, Amitgiri Goswami*, Abhijit Chatterjee*, Purvi Vyas*, Debdutta Bandyopadhyay*, Jeevan Kumar*, Mukul Jain*, Rajesh Bahekar*, Ranjit Desai* and Shubhangi Soman$

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$ Department of Chemistry, Faculty of Science, M.S. University of Baroda, Vadodara 390002, India

Abstract

Phosphoinositide-3-kinases (PI3Ks) mediates diverse and crucial cell functions. The class IA PI3Ks comprises PI3K-α, β and δ, while the class IB contains PI3K-γ. Knowing the potential side effects associated with PI3K-α and β isoforms inhibition (due to universal expression), recent efforts are focused towards the development of potent and PI3K-δ selective inhibitors for the treatment of hematologic malignancies and inflammatory diseases. In the present investigation, we report synthesis and in vitro screening of benzofuran based pyrazolo[3,4-*d*]pyrimidinamine derivatives (**10a-j**) as potent and selective PI3Kδ inhibitors. Further test compounds were screened for mTOR selectivity and compounds **10a-c** & **10b-j** exhibited better PI3Kδ selectivity over mTOR.

Introduction

PI3K constitute central signaling hub that mediates many diverse and crucial cell functions, including cell growth, cell proliferation, metabolism and survival.[1] Isoforms, p110α and p110β are ubiquitously expressed, while p110δ and p110γ are expressed predominantly in hematopoietic cells, especially, PI3Kδ are expressed in leukocytes and considered as useful target for the treatment of hematologic malignancies and inflammatory diseases.[2] Several groups are working for identifying selective inhibitors of PI3Kδ.[3] Currently a number of selective PI3Kδ inhibitors are discovered, which are in various stages of clinical development for diverse indications such as cancer, asthma and inflammation.[4]

Mechanism of Action

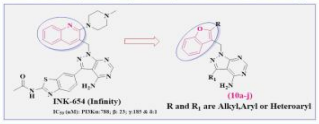
PI3Ks are lipid kinases that catalyze the phosphorylation of 3'-hydroxyl group of phosphatidylinositol and phosphatidylinositides in response to extracellular stimuli. The 3-phosphorylated phospholipids (PIP3s) generated by PI3K act as second messengers, recruiting kinases with lipid binding domains, such as Akt and PDK1. Binding of Akt to membrane PIP3s causes the translocation of Akt to the plasma membrane and its activation.[5]

Results and Discussion

Title compounds **10a-j** were designed as a bioisosteric replacement of quinoline ring of **INK-654** with benzofuran ring system (**Figure 1**). The synthesis of titled compounds **10a-j** were carried out as depicted in **Scheme 1**, followed by *in vitro* testing in PI3K ELISA assays of p1108/p85α. Among the compounds tested, **10a-c** & **10b-j** showed good *in vitro* PI3Kδ inhibitory activity (>50% inhibition at 10 nM). The test compounds were also screened for mTOR selectivity and compounds **10a-c** & **10b-j** exhibited better PI3Kδ selectivity over mTOR. Additionally, molecular docking studies were carried out for **10j** and the docking results of **10j** correlates with its potent in vitro PI3Kd activity.

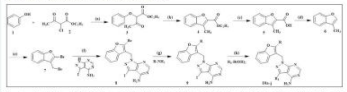
Figure 1: PI3K activation pathways

Figure 2: Bioisosteric replacement of quinoline ring of **INK-654** with benzofuran ring system



Experimental

Scheme 1: Synthesis of title compounds **10a-j**.



Reagents and Conditions: (a) K_2CO_3, Acetone, Reflux, 5 h.; (b) Conc. H_2SO_4, 5 to 0 °C; (c) 10% KOH, 2 h; (d) 280 °C, 2 h; (e) NBS, CCl_4, Reflux, 5h; (f) t-BuOK, DMF, 20°C, 15h.; (g) Secondary amine, 1,4-Dioxane, Reflux, 12h.; (h) Aryl boronic acid, $PdCl_2(PPh_3)_2$, $KHCO_3$, DMF:H$_2$O, 80 °C, 2h.

Chemistry: Overall, 10 compounds (**10a-j**) were prepared in good yield, under the mild reaction condition (**Scheme 1**) and were characterized with their physical, analytical and spectral data (1H NMR, ESI MS). The percentage yield in the final step was found to be in the range of 60-80%. The ESI MS and NMR spectral data of all the synthesized compounds were also found to be in conformity with the structures assigned and ensure the formation of the compounds **10a-j**. The representative spectral data of 6-(4-amino-1-((2-(4-ethylpiperazin-1-yl)benzofuran-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-amine (**10l**) is listed below.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.04 (m, 3 H), 2.21 (s, 3 H), 2.30-2.35 (m, 2 H), 2.38-2.40 (m, 4 H), 3.52 (m, 4 H), 5.61 (s, 2 H), 7.08-7.09 (m, 2 H), 7.32-7.34 (d, *J=8 Hz*, 1 H), 7.49-7.51 (d, *J=8 Hz*, 1 H), 7.63-7.65 (m, 1 H), 7.83-7.85 (d, *J=12 Hz*, 1 H), 8.19 (s, 1 H), 8.39 (s, 1H), 12.43 (s, 1H). **ESI-MS** (relative intensities): 568.1 (M+H)+ 100% (+ve mode).

In vitro* Assay: *PI3K ELISA Assays of p1108/p85α:

Procedure: PI3K Kinase assay Kit from Millipore was used to screen inhibitors of human PI3-kinases p1108. The kinase reaction was setup with 25ng of the PI3K enzyme (δ), 10μM PIP2 substrate & 1X kinase reaction buffer in 25 μL/well. Different conc. of the NCEs were added to the reaction mixture & incubated for 1h at 37°C. 25 μL/well of Biotinylated-PIP3/EDTA working solution was added to the reaction well followed by 50μl of GRP1 working solution to all wells. The reaction was incubated for 1h at 26°C & washed 4 times with 200 μL/well 1XTBST. SA-HRP working solution (50μl) is added & again incubated at 26°C for 1h. 100μL of the Substrate TMB is added to develop the color in the dark for 5-20 minutes and stopped by adding 100 μL of the stop solution. The absorbance measured at 450nm & relative percentage of inhibition is calculated with respect to the positive control. In vitro PI3Kδ (at 10, 100 & 1000 nM) and mTOR inhibitory activity (at 1000 nM) for test compounds (**10a-j**) are reported in **Table 1**.

Docking Study: As a representative compound from this series, molecular docking studies of **10j** was carried out by using (Glide version 6.7) Schrodinger software to study an interaction of **10j** with PI3Kδ receptor. It was found that core benzofuran ring was oriented in the specificity pocket, N-ethyl piperazine ring aligned towards hydrophobic region and substitution at R_1 position was found to be directed towards affinity pocket. Thus, docking results of **10j** correlates with its potent *in vitro* PI3Kδ activity.

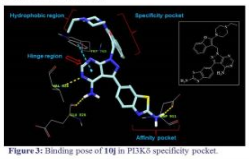


Figure 3: Binding pose of **10j** in PI3Kδ specificity pocket.

Table 1: *In vitro* PI3Kδ and mTOR inhibitory activity data of **10a-j**

Sr. No.	Structure		% inhibition PI3k δ at 3 conc.			% inhibition mTOR
	R	R_1	1nM	10nM	100nM	1000nM
10a*			**68**	90	102	15
10b*			**70**	88	100	12
10c*			**55**	78	98	16
10d			30	50	78	41
10e			10	45	53	18
10f			11	55	68	22
10g			40	58	78	38
10h*			**69**	90	99	13
10i*			**70**	95	108	11
10j*			**90**	98	112	10
INK 654	Std compound		**59**	88	110	10

(*Compounds will be screened for selectivity against other isoform using PI3K Kinase assay).

Summary and Conclusion:

- Based on *invitro* results, we can summarized that among ten novel compounds tested for PI3Kδ and mTOR activities, compound **10a-c, 10b-j** were found to be potent and selective PI3Kδ inhibitors over mTOR.
- 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.
- Further ADME and in vivo profiling, followed by structure guided optimization of these lead molecules may likely to provide potent and PI3K-δ selective inhibitor for the treatment of hematologic malignancies and inflammatory diseases.

Acknowledgment

We are grateful to the management of Zydus Group for encouragement and the analytical department for providing analytical support.

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ZRC communication No: 523

Poster No.: 181

Design and Synthesis of 2, 4-Disubstitued Quinoline based pyrazolo [3, 4-d] pyrimidinamine derivatives as potent and selective PI3Kδ inhibitors

Bhushan Dave*$, Rajendra Chopade*, Dipam Patel*, Jignesh Pethani*, Hardik shah*, Amitgiri Goswami*, Abhijit Chatterjee*, Purvi Vyas$, Debdutta Bandyopadhyay$, Jeevan Kumar*, Sachchidanand S*, Mukul Jain#, Rajesh Bahekar*, Rajiv Sharma*, Ranjit Desai* and Shubhangi Soman$

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$ Department of Chemistry, Faculty of Science, M.S. University of Baroda, Vadodara 390002, India

Abstract

Phosphoinositide-3-kinases (PI3Ks) mediate diverse and crucial cell functions. The class IA PI3Ks comprises PI3K-α, β and δ, while the class IB contains PI3K-γ. Knowing the potential side effects associated with PI3K-α and β isoforms inhibition (due to universal expression), recent efforts is focused towards the development of potent and PI3K-δ selective inhibitors for the treatment of hematologic malignancies and inflammatory diseases. Earlier we have reported benzofuran[1] based pyrazolo [3,4-d]pyrimidinamine derivatives as potent and selective PI3Kδ inhibitors. In the present investigation, we report synthesis and in vitro screening of 2, 4-Disubstituted Quinoline based pyrazolo [3, 4-d] pyrimidinamine derivatives (**7a-j**) as potent and selective PI3Kδ inhibitors[2].

Introduction

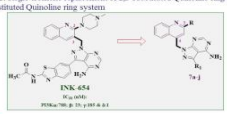
PI3K constitute central signaling hub that mediates many diverse and crucial cell functions, including cell growth, cell proliferation, metabolism and survival[3]. Isoforms, p110α and p110β are ubiquitously expressed, while p110δ and p110γ are expressed predominantly in hematopoietic cells, especially, PI3Kδ are expressed in leukocytes and considered as useful target for the treatment of hematologic malignancies and inflammatory diseases[4]. Several groups are working for identifying selective inhibitors of PI3Kδ. Currently a number of selective PI3Kδ inhibitors are discovered, which are in various stages of clinical development for diverse indications such as cancer, asthma and inflammation[5,6].

Mechanism of Action

PI3Ks are lipid kinases that catalyze the phosphorylation of 3'-hydroxyl group of phosphatidylinositol and phosphatidylinositides in response to extracellular stimuli. The 3-phosphorylated phospholipids (PIP3s) generated by PI3K act as second messengers, recruiting kinases with lipid binding domains, such as Akt and PDK1. Binding of Akt to membrane PIP3s causes the translocation of Akt to the plasma membrane and its activation[7].

Figure 1: PI3K Pathway

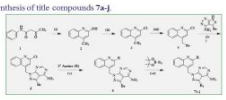
Figure 2: Regio isomeric replacement of 2,3-substituted Quinoline ring of **INK-654** with 2,4-substituted Quinoline ring system



Chemistry

Synthesis of 2, 4-Disubstituted Quinoline based pyrazolo [3, 4-d] pyrimidinamine derivatives (**7a-j**) was carried out as depicted in **Scheme 1**, following the modified literature procedure [8]. 4-methylquinolin-2-ol (**2**) was prepared using well known Knorr quinoline synthesis by treating aceto-acetanilide (**1**) with conc. H_2SO_4 acid which was converted to chloro derivate (**3**) using $POCl_3$. Free radicle bromination at benzylic methyl was carried out using NBS and Catalytic Benzoyl peroxide to give reactive benzyl bromide derivative (**4**). Next reaction was double nucleophilic displacement reaction first was Nucleophilic Substitution (**SN2**) using 3-bromo-1H-pyrazolo[3,4-d]pyrimidin-4-amine to get the Chloro pyrazolo[3,4-d]pyrimidine compound (**5**) and second was Nucleophilic Aromatic substitution (**SNAr**) using various cyclic secondary amine followed by Suzuki coupling reaction with various aryl/hetroaryl boronic acid to furnished compounds (**7a-j**).

Scheme 1: Synthesis of title compounds **7a-j**.



Reagents and Conditions: (i) Conc.H_2SO_4, 110°C, 4 hr. (ii) $POCl_3$, 120°C, 5 hr. (iii) NBS, Benzoyl Perfox, 5 hr. (iv) t-BuOK, DMF, 20°C, 15 hr. (v) Secondary amine, 1,4-Dioxane, Reflux, 12 hr. (vi) Aryl /Hetroaryl boronic acid, $PdCl_2(PPh_3)_2$, $KHCO_3$, DMF:H$_2$O, 80 °C, 2 hr.

Chemistry: Overall, 10 compounds (**7a-j**) were prepared in good yield, under the mild reaction condition (**Scheme 1**) and were characterized with their physical, analytical and spectral data (1H NMR, ESI MS). The percentage yield in the final step was found to be in the range of 60-80%. The ESI MS and NMR spectral data of all the synthesized compounds were also found to be in conformity with the structures assigned and ensure the formation of the compounds **7a-j**. The representative spectral data of 6-(4-amino-1-((2-(4-methylpiperazin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-amine (**7b**) is listed below.

^{1}H NMR (400 MHz, **MeOD**) δ ppm:- 2.82 (s, 3H), 3.13-3.70 (m, 4H), 3.60-3.70 (m, 4H), 5.99 (s, 2H), 7.05 (s, 1H),7.31-7.34 (m, 1H), 7.50-7.60 (m, 3H), 7.71-7.73 (m, 1H),7.88-7.90 (m, 1H), 8.14-8.16 (m, 1H), 8.33 (s, 1H). **ESI-MS** (relative intensities): 523.2 (M+H)+ 100% (+ve mode).

In vitro* Assay: *PI3K ELISA Assays of p1108/p85α:

Procedure: PI3K Kinase assay Kit from Millipore was used to screen inhibitors of human PI3-kinases p1108. The kinase reaction was setup with 25ng of the PI3K enzyme (δ), 10μM PIP2 substrate & 1X kinase reaction buffer in 25 μL/well. Different conc. of the NCEs were added to the reaction mixture & incubated for 1h at 37°C. 25 μL/well of Biotinylated-PIP3/EDTA working solution was added to the reaction well followed by 50μl of GRP1 working solution to all wells. The reaction was incubated for 1h at 26°C & washed 4 times with 200 μL/well 1XTBST. SA-HRP working solution (50μl) is added & again incubated at 26°C for 1h. 100μL of the Substrate TMB is added to develop the color in the dark for 5-20 minutes and stopped by adding 100 μL of the stop solution. The absorbance measured at 450nm & relative percentage of inhibition is calculated with respect to the positive control. In vitro PI3Kδ (at 10, 100 & 1000 nM) and mTOR inhibitory activity (at 1000 nM) for test compounds (**7a-j**) are reported in **Table 1**.

Docking Study: As a representative compound from this series, molecular docking studies of **7a** was carried out by using (Glide version 6.7) Schrodinger software to study an interaction of **7a** with PI3Kδ receptor (PDB id-**2wxk**)[10]. It was found that core Quinoline ring was oriented in the specificity pocket, N-methyl piperazine ring aligned towards hydrophobic region and substitution at R_1 position was found to be directed towards affinity pocket. Thus, docking results of **7a** correlates with its potent *in vitro* PI3Kδ activity.

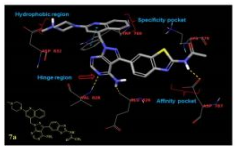


Figure 3: Binding pose of **7a** in PI3Kδ specificity pocket.

Table 1: *In vitro* PI3Kδ and mTOR inhibitory activity data of **7a-j**

Sr. No.	Structure		% inhibition PI3k δ at 3 conc.			% inhibition mTOR
	R	R_1	1nM	10nM	100nM	1000nM
7a*			**66**	**82**	**110**	**10**
7b*			**62**	**84**	**105**	**12**
7c*			**50**	**75**	**94**	**14**
7d			45	52	60	30
7e			35	44	52	35
7f			42	58	70	28
7g			32	54	70	35
7h*			**70**	**92**	**108**	**10**
7i*			**72**	**95**	**105**	**12**
7j*			**68**	**94**	**98**	**15**
INK 654	**Reference Compound**		**59**	**88**	**110**	**10**

(*Compounds will be screened for selectivity against other isoform using PI3K Kinase assay).

Summary and Conclusion:

- Based on *invitro* results, we can summarized that among ten novel compounds tested for PI3Kδ and mTOR activities, compound **7a-c, 7h-j** were found to be potent and selective PI3Kδ inhibitors over mTOR.
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- Further ADME and in vivo profiling, followed by structure guided optimization of these lead molecules may likely to provide potent and PI3K-δ selective inhibitor for the treatment of hematologic malignancies and inflammatory diseases.

Acknowledgment

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Poster No. :

Communication No.:

Design, Synthesis and Pharmacological evolution of Imidazo-Quinolinyl derivatives as PI3Kdelta-Selective Inhibitors

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Abstract

Phosphoinositide-3-kinases (PI3Ks) mediate diverse and crucial cell functions. The class IA PI3Ks comprises PI3K-α, β and δ, while the class IB contains PI3K-γ. Knowing the potential side effects associated with PI3K-α and β isoforms inhibition (due to universal expression), recent efforts is focused towards the development of potent and PI3K-δ selective inhibitors for the treatment of hematologic malignancies and inflammatory diseases. In the present investigation, we report synthesis and in vitro screening of Imidazo-Quinolinyl derivatives (**10a-h**) as potent and selective PI3Kδ inhibitors[1].

Introduction

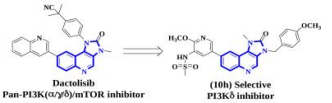
PI3K constitute central signaling hub that mediates many diverse and crucial cell functions, including cell growth, cell proliferation, metabolism and survival [2]. Isoforms, p110α and p110β are ubiquitously expressed, while p110δ and p110γ are expressed predominantly in hematopoietic cells, especially, PI3Kδ are expressed in leukocytes and considered as useful target for the treatment of hematologic malignancies and inflammatory diseases[3]. Several groups are working for identifying selective inhibitors of PI3Kδ. Currently a number of selective PI3Kδ inhibitors are discovered, which are in various stages of clinical development for diverse indications such as cancer, asthma and inflammation[4,5].

Mechanism of Action

PI3Ks are lipid kinases that catalyze the phosphorylation of 3'-hydroxyl group of phosphatidylinositol and phosphatidylinositides in response to extracellular stimuli. The 3-phophorylated phospholipids (PIP3s) generated by PI3K act as second messengers, recruiting kinases with lipid binding domains, such as Akt and PDK1. Binding of Akt to membrane PIP3s causes the translocation of Akt to the plasma membrane and its activation[6].

Figure 1: PI3K activation pathways

Figure 2: Structural modification on Imidazo-Quinolinyl system



Dactolisib
Pan-PI3K(α/γδ)/mTOR inhibitor

(10h) Selective
PI3Kδ inhibitor

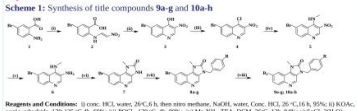
Chemistry

Synthesis of 1,3-dihydro-2*H*-imidazo[4,5-c]quinolin-2-one derivatives (**9a-g** and **10a-h**) was carried out as depicted in **Scheme 1**, following the modified literature procedure[7]. Treatment of bromo anthranilic acid (**1**) with nitro methane gives nitro vinyl anthranilic acid (**2**), which was cyclized using potassium acetate in acetic anhydride to get the bromo nitro quinolinol (**3**). Compound **3** was converted to reactive chloro derivate (**4**) using $POCl_3$, followed by nucleophilic substitution with methylamine to get the compound **5**. Nitro group of **5** was reduced, using $SnCl_2$ to get the compound **6**, which was cyclized using diphosgene in the presence of base, to obtain the imidazo quinolinyl (**7**). Alkylation of **7** using strong base and aryl halides furnished compounds **8a-g**, which were converted to **9a-g** and **10a-m**, using $PdCl_2(PPh_3)_2$, potassium bicarbonate and aryl or heteroaryl boronic acids.

All compounds (**9a-g,10a-h**) were prepared in good yield, under the mild reaction condition (**Scheme 1**) and were characterized with their physical, analytical and spectral data (1H NMR, ESI MS). The percentage yield in the final step was found to be in the range of 60-80%. The ESI MS and NMR spectral data of all the synthesized compounds were also found to be in conformity with the structures assigned and ensure the formation of the compounds **10a-j**.

Experimental

Scheme 1: Synthesis of title compounds **9a-g** and **10a-h**



Reagents and Conditions: i) conc. HCl, water, 26°C,6 h, then nitro methane, NaOH, water, Conc. HCl, 26 -5°C,16 h, 95%; ii) KOAc, acetic anhydride, 120-125 °C,4h, 60%; iii) $POCl_3$, 120 °C, 4h, 80%; iv) M-NH_2, TEA, DCM, 20°C, 12h, 94%; v) $SnCl_2.2(H_2O)_2$, EtOAc, 26 °C , 60%; vi) Diphosgene, TEA, DCM, 0-26°C, 65%; vii) R, Ar-CH$_2$ X (Cl or Br), NaH, THF, 0-26 °C, 6h – 75 %; viii) R_2-$B(OH)_2$,$PdCl_2(PPh_3)_2$, $KHCO_3$, DME, H_2O, 90-95 °C, 1.5 h, 60 - 80%.

In vitro* Assay: *PI3K ELISA Assays of p110δ/p85α:

Procedure: PI3K Kinase assay Kit from Millipore was used to screen inhibitors of human PI3-kinases p1108[8]. The kinase reaction was setup with 25ng of the PI3K enzyme (δ), 10μM PIP2 substrate & 1X kinase reaction buffer in 25 μL/well. Different conc. of the NCEs were added to the reaction mixture & incubated for 1h at 37°C. 25 μL/well of Biotinylated-PIP3/EDTA working solution was added to the reaction well followed by 50μl of GRP1 working solution to all wells. The reaction was incubated for 1h at 26°C & washed 4 times with 200 μL/well 1XTBST. SA-HRP working solution (50μl) is added & again incubated at 26°C for 1h. 100μL of the Substrate TMB is added to develop the color in the dark for 5-20 minutes and stopped by adding 100 μL of the stop solution. The absorbance measured at 450nm & relative percentage of inhibition is calculated with respect to the positive control. In vitro PI3Kδ (at 10, 100 & 1000 nM) and mTOR inhibitory activity (at 1000 nM) for test compounds (**10a-j**) are reported in **Table 1**.

Table 1: *In vitro* PI3Kδ inhibitory activity data of **9a-g, 10a-h.**

9a-g, 10a-h

Comp.	R_1	R_2	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]	Comp.	R_1	R_2	PI3Kδ inhibition (%)[a,b]	PI3Kδ IC_{50} (nM)[c]
9a			56	ND	9b			73	28.3
9c			98	9.5	9d			80	18.4
9e			62	ND	9f			51	ND
9g			22	ND	10a			52	ND
10b			58	ND	10c			72	29.4
10d			81	20	10e			87	11.4
10f			88	11.1	10g			62	ND
10h			99	1.9	Dactolisib			56	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.				

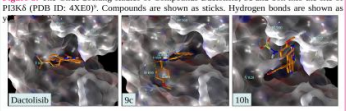
Table 2: Isoform selectivity of compounds against PI3K (α, β, δ, and γ) and mTOR activities

Most potent compounds (**9c and 10h**) were evaluated for their selectivity against PI3K isoforms (α, β and γ) and mTOR. As shown in the **Table 2**, initial hits **(9c)** showed moderate selectivity against PI3K isoforms and mTOR over PI3Kδ. Compound **10h** (IC50: 1.9 nM) demonstrated 469, 310, and 59-fold selectivity over PI3Kα, β and γ respectively. Moreover, it was noted that selectivity of **10h** against all the three isoforms was higher than standard compounds. In general, it was observed that the potency and selectivity of imidazo-quinolinonyl based PI3Kδ inhibitors can be modulated using suitable substituents at R and R^2 positions.

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
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.

Figure 3: The Glide docking studies of Compounds Dactolisib, 9c and 10h into the site of PI3Kδ (PDB ID: 4XE0)[9]. Compounds are shown as sticks. Hydrogen bonds are shown as yellow dashed lines.



Summary and Conclusion:

- we have synthesized and evaluated novel series of imidazo-quinolinyl derivatives as selective PI3Kδ inhibitors. In first, appropriate modifications were carried out in the imidazo-quinolyl ring, which led to an identification of a single digit nM potent PI3Kδ inhibitor (**9c**), with moderate isoform selectivity. 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.
- The results of docking has been summarized in Fig. 3 where it is clear from the docking poses of the docked ligands that specificity pocket was fully occupied by **10h**, however in case of Dactolisib and **9c**, the specificity pocket was not occupied and this explains why **10h** was more selective towards PI3Kδ.

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