CHAPTER IV

4. Design, Synthesis and Biological evaluation of 2,4-disubsitued quinoline pyrazolo-pyrimidine derivatives as PI3Kδ inhibitors.

Design strategy

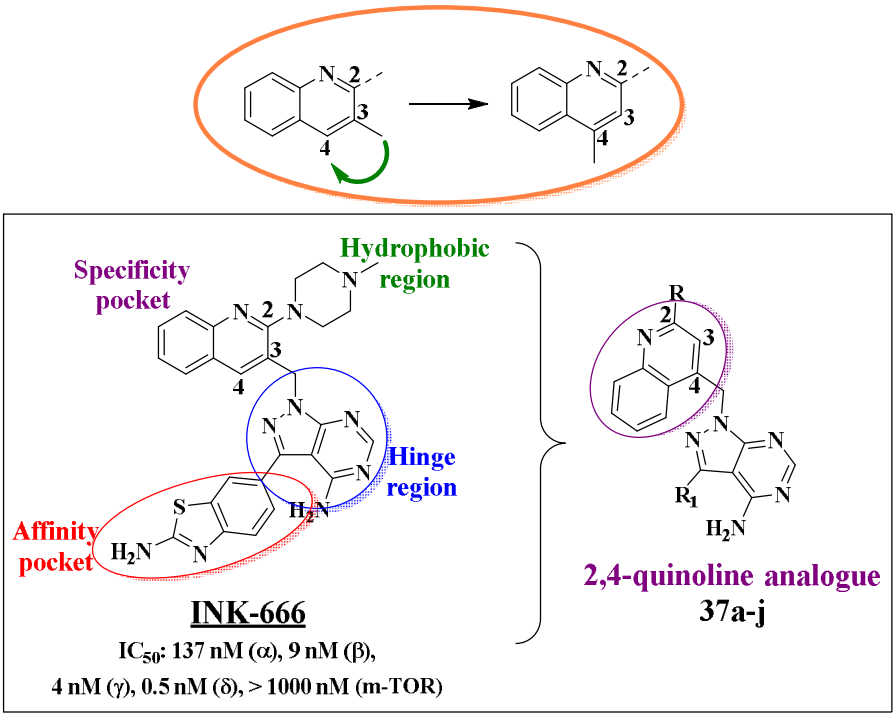


Figure 39: Designing strategy for 2,4-disubstituted quinoline

As discussed in previous chapter, we have developed a novel, potent and selective benzofuran pyrazolo-pyrimidine based PI3Kδ selective inhibitor using INK-666 scaffold. From chapter 2, we knew the importance of pyrazolo pyrimidine ring and substitution on it (benzthiazole or 3-hydroxy phenyl ring), hence in this chapter, we have reported 2,4-disubsitued quinoline pyrazolo-pyrimidine derivatives as PI3Kδ inhibitors by shifting position of substituent on quinoline ring from 3rd to 4th position of reference compounds (INK-666/654), **Figure 39**.

Ren A et al. reported SAR for 2,3-disubstituted quinoline based PI3Kδ inhibitors in their invention **[58]**. In this chapter, we have synthesised 2,4-disubstituted quinoline as PI3Kδ selective inhibitors to explore SAR. Initially, we did *in silico* studies and docking overlay pose analysis for newly designed compounds with INK-654 in PI3Kδ ATP binding pocket (PDBID: **2WXK**) and docking results were promising. Newly designed compounds were exactly overlapping with reference compounds **(Figure 40)** and it was observed that all the key interactions for PI3K inhibitions were retained in the hinge region (Val_{828}) and affinity pocket.

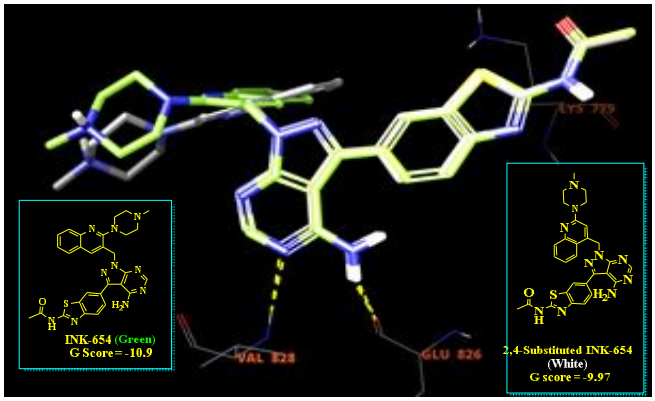


Figure 40: Overlay of INK-654 and 2,4-disustituted quinoline derivatives (PDBID: **2WXK**)

Overall 10 compounds were synthesised in this series, in which hinge binder pyrazolo pyrimidine and 2,4-disubstituted quinoline ring were kept constant. Modifications were done on R (N-methyl piperazine, piperidine, morpholine and pyrrolidine) and R_1 (3-hydroxy phenyl and benzthiazole derivatives). All compounds were evaluated for PI3Kδ inhibitory activity.

4.1. Chemistry

4.1.1. Materials and Methods

All reagents used were obtained from Sigma Aldrich and were used without further purification. Solvents were purchased from a commercial source and used after distilling or drying according to the known methods. All the air and/or moisture sensitive reactions were carried out in dry solvents, under the nitrogen atmosphere. Melting points were recorded in open glass capillaries, using a scientific melting point apparatus (Mettler Toledo, Switzerland) and are uncorrected.

The ^{1}H NMR spectra were recorded on a Bruker Avance-400 (400MHz) spectrometer, Switzerland. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS (tetramethylsilane), either in $CDCl_3$, CD_3OD or DMSO- d_6. Signal multiplicities are represented as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiple). ^{13}C NMR spectra were recorded on Bruker Avance-400 at 100 MHz either in $CDCl_3$ or DMSO-d_6.

Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer, Japan. Elemental analyses were carried out, using a Perkin-Elmer 2400 CHN analyser, UK. UPLC analysis were carried out at λmax 220nm, using column YMC-Triart C18 (100*2.0 mm) on Water acquity UPLC, Europe (Austria).

Progress of the reactions was monitored by TLC, using precoated TLC plates (E. Merck Kiesegel 60 F254, Germany) and the spots were visualized by UV and/or iodine vapours. The chromatographic purification was

performed on silica gel (230-400 mesh). Few compounds directly used for the next step without purification and analysis.

In the next section we highlighted some positional isomeric substitution examples, the importance of quinoline as a pharmacophore and possible routes for the synthesis of quinoline.

4.1.2. Positional isomeric substitution examples

Example 1

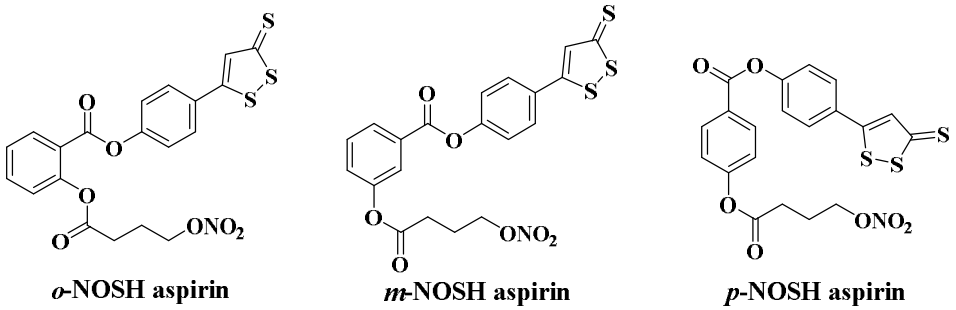


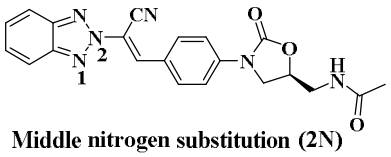
Figure 41: *ortho, m*eta and *para* NOSH aspirin.

Vannini F et al. reported effect of NOSH aspirin [Nitric oxide (NO) and hydrogen sulfide (H_2S) releasing agents] positional isomers as anti-cancer agent and COX (Cyclooxygenase inhibitor) inhibitor. NOSH aspirin, *ortho*, *meta* and *para* isomer showed IC_{50}: 0.042±0.011 μM, 0.24±0.11 μM and 0.46±0.17 respectively, in HT-29cell line, **Figure 41**. All three isomer were equally potent in COX-1(% inhibition 40-50 %) and COX-2 (% inhibition 20-27 %) inhibitory assay, with selectively inhibiting COX-1 over COX-2 isoenzyme **[99]**.

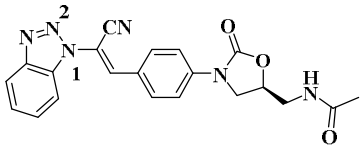
Example 2

Das J et al. reported two positional isomers of benzotrizole (*1N* and *2N*) as antibacterial agents. In first isomer the attachment were on middle

nitrogen (2N) and in the second isomer, the attachment was on terminal nitrogen (1N). Both isomers were equally potent against three different strains of *Staphylococcus aureus* (gram positive), **Figure 42 [100]**.



Middle nitrogen substitution (2N)



Terminal nitrogen substitution (1N)

MIC (μg/mL) in *Staphylococcus aureus (S.a)*		
S.a 29213	*S.a 49951*	*S.a 33591*
8	**8**	**8**
16	**16**	**16**

Figure 42: Benzotrizole (*1N* and *2N*) derivatives as antibacterial agents.

4.1.3. Quinoline as therapeutic agent

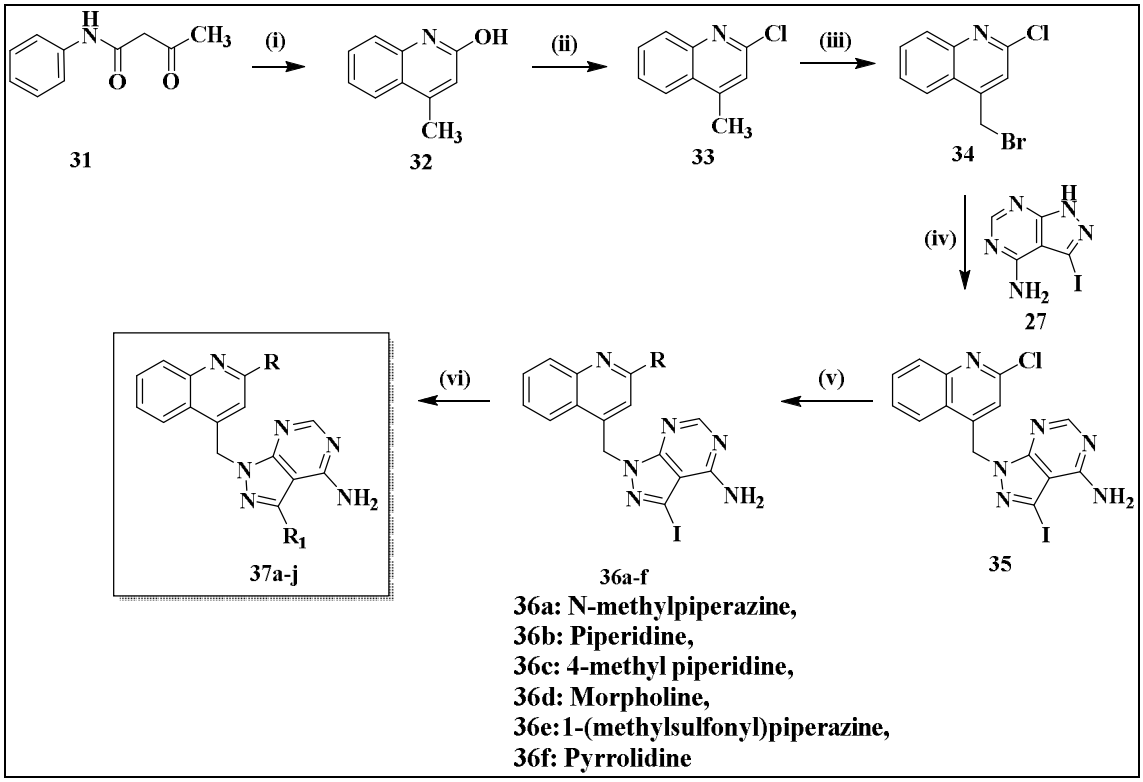
Quinoline based compounds has multiple medical benefits, but mainly quinoline is used as anti-malarial, anti-cancer, anti-diabetic and anti-microbial agents **[101-102]**. The best example of quinoline as anti-malarial agent is Quinine, Chloroquine, Mefloquine, and Amodiaquine **[103]**. Recently, various quinoline-based compounds had shown anti-inflammatory and anti-cancer activity by inhibition of various kinases **[104]**, such as Epidermal growth factor receptor (EGFR) inhibitor **[105-109]**, Mitogen-activated protein kinase (MAPK) inhibitors **[110-115]**, Anaplastic lymphoma kinase 5 (ALK5) inhibitors **[116-119]**, Proteasome inhibitors **[120-122]**, Src (Sarcome genes) inhibitor **[123-127]** etc.

Total 10 compounds were designed and general procedure for the synthesis of compounds **37a-j** and protocol for biological evaluation is described below.

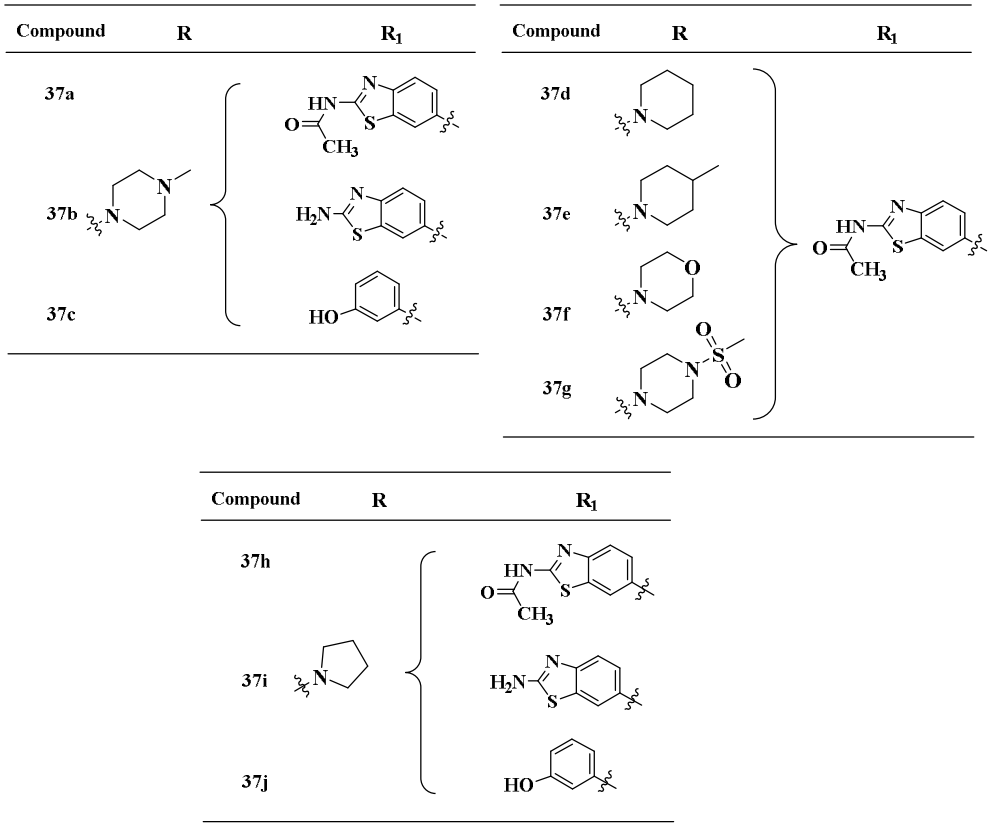
4.1.4. General procedure of the synthesis of title compounds 37a-j.

The compounds **37a-j** were synthesized as described in **Scheme 5**. Aceto acetanilide **31** was cyclised in conc. sulphuric acid to give 2-hydroxy-4-methyl quinoline **32**, followed by chlorination using phosphorus oxychloride to 2-chloro-4-methyl quinoline **33 [128]**. Free radical bromination at benzlic methyl was carried out using N-bromosuccinimide (NBS) and catatalytic benzoyl peroxide in CCl_4 to give bromo benzyl quinoline **34**, which was reacted with 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **27** to iodo chloro compound **35**. Compound **35** was reacted with secondary amine (R) in 1,4-dioxane to compound **36a-f**. The compound **36a-f** was subjected to suzuki coupling reactions with benzthiazole boronate ester or (3-hydroxyphenyl)boronic acid to furnish compound **37a-j.**

Scheme 5: Synthesis of 2,4- disubstituted quinoline derivatives **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.



4.1.4.1. Experimental procedure for Synthesis of 2, 4-disubsitued quinoline pyrazolo–pyrimidine compounds 37a-j (Scheme 5).

Step I: Preparation of 4-methylquinolin-2-ol **32.**

Acetoacetanilide (6 g, 23.14 mmol) was suspended in H_2SO_4 (20 ml) and heated at 100-110 ^oC for 1 h. Reaction was cooled up to room temperature and dumped in cold water (150 ml), stirred for 30 min. The white precipitates was filtered and washed with cold water (2 X 100 ml) followed by drying under vacuum to give the 4-methylquinolin-2-ol **32** white solid (4.2 g yield: 77.8 %).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.41 (s, 3H), 6.39 (s, 1H), 7.17-7.21 (m, 1H), 7.29-7.31 (m, 1H), 7.47-7.51 (m, 1H), 7.69 (d, J = 7.2 Hz, 1H), 11.58 (s, 1H). **ESI-MS:** 159.8 $(M)^+$. **Purity** (UPLC): 99.12%.

Step II: Preparation of 2-chloro-4-methylquinoline **33.**

4-methylquinolin-2-ol (4.2 g, 26.41 mmol) was suspended in $POCl_3$ (4.84 g, 31.69 mmol). Above suspension was heated at120 ^oC for 5 h. Reaction mixture was allowed to cool and was quenched with cold water (120 ml). The precipitates was filtered and washed with cold water (2 X 50 ml) to give white solid which was dried in vacuum to give title 2-chloro-4-methylquinoline **33** as off white solid (4 g yield: 85 %).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.70 (s, 3H), 7.50 (s, 1H), 7.66-7.70 (m, 1H), 7.80-7.84 (m, 1H), 7.94 (dd, J_1 = 8.4 Hz, J_2 = 0.4 Hz, 1H), 8.12 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H). **ESI-MS:** 177.8 $(M+H)^+$. **Purity** (UPLC): 98.84 %.

Step III: Preparation of 4-(bromomethyl)-2-chloroquinoline **34.**

2-chloro-4-methylquinoline (4.0 g, 22.59 mmol) was dissolved in CCl_4 (100 ml). NBS (7.60g, 33.85 mmol) and benzoyl peroxide (0.545 g, 0.22 mmol) were added to above solution and reaction mixture was refluxed for 18 h. Reaction mixture was cooled and solid was filtered, washed with cold CCl_4 (50 ml). Filtrate was concentrated and evaporated to dryness to provide the desired 4-(bromomethyl)-2-chloroquinoline **34** as light orange oil (4 gm**,** yield: 70%), which was used as such without purification for next reaction.

Step IV: Preparation of 1-((2-chloroquinolin-4-yl)methyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine **35**.

3-iodo-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine **27 (**2.36 g, 9.04 mmol) was dissolved in DMF (40 ml), Potassium tert-butoxide (1.02 g, 9.04 mmol) was added. Reaction mixture was stirred for 30 min at room temperature. 4-(bromomethyl)-2-chloroquinoline **34** (3 gm**,** 11.30 mmol) dissolved in DMF (20 ml)) was added to above suspension and reaction mixture was stirred for 18 h at

room temperature. Reaction mixture was quenched by adding cold water (150 ml) to give crude product, which was filtered and washed with water (2 X 50 ml). The crude solid was purified using column chromatography to give pure 1-((2-chloroquinolin-4-yl)methyl)-3-iodo-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine **35** as white solid (0.9 g, yield: 18%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 6.03 (s, 2H), 7.16 (s, 1H), 7.68-7.72 (m, 1H), 7.84-7.88 (m, 1H), 7.99-8.02 (m, 1H), 8.29-8.31 (m, 2H). **ESI-MS:** 436.7 $(M+H)^+$. **Purity** (UPLC): 95.40 %.

Step V: Preparation of **36a-f**.

1-((2-chloroquinolin-4-yl)methyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine **35** (0.75 g, 1.72 mmol) was suspended in secondary amine (10 ml, 100 mmol) reaction mixture was refluxed for 18 h. After completion of reaction the solution was cooled and poured into ice cooled water (50 ml). Solid was filtered and washed with cold water (2 X 25 ml) to give pure **(36a-f)** (Yield: 50-60%).

4.1.4.2. Spectral data of intermediates 36a-f.

3-iodo-1-((2-(4-methylpiperazin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 36a.

36a was prepared by following the general procedure described in section 4.1.4.1 (Step V) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.33 (s, 3H), 2.42 (t, J = 4.8 Hz, 4H), 3.64 (t, J = 4.4 Hz, 4H), 5.86 (s, 2H), 7.15-7.19 (m, 2H), 7.48-7.52 (m, 1H), 7.59 (d, J = 8 Hz, 1H), 7.91(d, J = 8 Hz, 1H), 8.32 (s, 1H). **ESI-MS**: 500.9 $(M+H)^+$. **Purity** (UPLC): 96.80 %.

3-iodo-1-((2-(piperidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 36b.

36b was prepared by following the general procedure described in section 4.1.4.1 (Step V) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.80-1.88 (m, 6H), 3.50-3.60 (m, 4H), 5.80 (s, 2H), 6.68 (s, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.40-7.45 (m, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.80-7.84 (m, 1H), 8.28 (s, 1H). **ESI-MS**: 485.9 $(M+H)^+$. **Purity** (UPLC): 96.10 %.

3-iodo-1-((2-(4-methylpiperidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 36c.

36c was prepared following the general procedure described in section 4.1.4.1 (Step V) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.92 (d, *J* = 6.4 Hz, 3H), 1.08-1.11 (m, 2H), 1.64-1.68 (m, 3H), 2.86-2.92 (m, 2H), 4.40-4.44 (m, 2H), 5.80 (s, 2H), 7.09 (s, 1H), 7.12-7.06 (m, 1H), 7.45-7.50 (m, 1H), 7.51-7.56 (m, 1H), 7.88 (d, *J* = 8 Hz, 1H), 8.31 (s, 1H). **ESI-MS** m/z 499.9 $(M+H)^+$. **Purity** (UPLC): 95.50 %.

3-iodo-1-((2-(4-methylpiperidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 36d.

36d was prepared following the general procedure described in section 4.1.4.1 (Step V) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.55 (t, *J* = 4.2 Hz, 4H), 3.70 (t, J = 4 Hz, 4H), 5.81 (s, 2H), 6.66 (s, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.41-7.46 (m, 1H),

7.53 (d, *J* = 7.6 Hz, 1H), 7.81-7.85 (m, 1H), 8.29 (s, 1H). **ESI-MS**: 488.0 $(M+H)^+$. **Purity** (UPLC): 97.50 %.

3-iodo-1-((2-(4-(methylsulfonyl)piperazin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 36e.

36e was prepared following the general procedure described in section 4.1.4.1 (Step V) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.70 (s, 3H), 3.10-3.18 (m, 4H), 3.70-3.78 (m, 4H), 5.85 (s, 2H), 6.68 (s, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 7.40-7.46 (m, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.80-7.83 (m, 1H), 8.30 (s, 1H). **ESI-MS**: 565.0 $(M+H)^+$. **Purity** (UPLC): 97.20 %.

3-iodo-1-((2-(pyrrolidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 36f.

36f was prepared following the general procedure described in section 4.1.4.1 (Step V) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.9-1.9 (m, 4H), 3.43-3.46 (m, 4H), 5.83 (s, 2H), 6.61 (s, 1H), 7.12 (t, *J* = 8.4 Hz, 1H), 7.40-7.42 (m, 1H), 7.45-7.49 (m, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 8.30 (s, 1H). **ESI-MS**: 472.00 $(M+H)^+$. **Purity** (UPLC): 97.80 %.

Step VI: Preparation of compound **37a-j**

A mixture of (1.00 mmol) of **R (1-methyl piperazine, piperidine, 4-methyl piperidine, morpholine, 1-(methyl sulfonyl) piperazine**) substituted iodo quinoline (**36)** and (0.01 mmol) bistriphenylphosphine palladium dichloride [$PdCl_2$ $(PPh_3)_2$] in DMF (10 ml) was heated to 80-85 ^oC. To this mixture (1.00 mmol) Substituted boronic acid/ester was added (dissolved in 9 ml of DMF) and

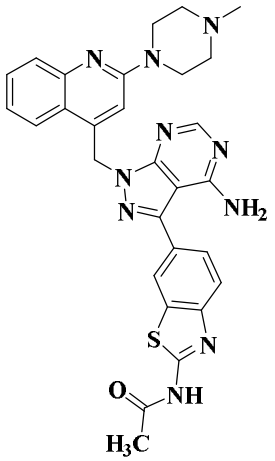
(6.00 mmol) of potassium bicarbonate ($KHCO_3$) (dissolved in 10 ml water). The mixture was heated for 2 h. at 95 ^oC. The mixture was cooled to 0-5 ^oC; water (50ml) was added and stirred for 1h. The crude product was filtered and washed with water, dried. Crude product was purified employing flash chromatography using 0-10% MeOH: DCM mobile phase to give the titled compound. (Yield: 65-80 %).

4.1.4.3. Spectral data of final compounds 37a-j.

All final compounds were characterised using ^1H NMR, ESI-MS, and CHN analysis, melting point are also reported (uncorrected). Most potent compounds **34b** and **37i** were fully characterised using ^1H NMR, ^{13}C NMR, ESI-MS and IR. Purity of all compounds was checked using UPLC (ultra-performance liquid chromatography).

N-(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-yl)acetamide 37a.

37a was prepared by following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.

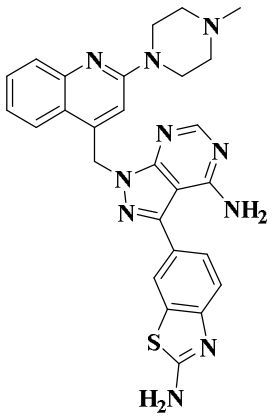


37a

MP: 241-242°C, **Yield:** 50%. **^{1}H NMR** (MeOD-d_1, 400 MHz) δ ppm: 2.28 (s, 3H), 2.56 (s, 3H), 2.80-2.95 (m, 4H), 3.70-3.90 (m, 4H), 6.00 (s, 2H), 7.02 (s, 1H), 7.28-7.32 (m, 1H), 7.55-7.59 (m, 1H), 7.69-7.73 (m, 2H), 7.88-7.90 (m, 1H),), 8.13-8.17 (m, 2H), 8.35 (s, 1H). **Analysis (CHNS):** Calculated for $C_{29}H_{28}N_{10}OS$: C, 61.69; H, 5.00; N, 24.81; S, 5.68; Found: C, 61.75; H, 5.12; N, 24.95; S, 5.72. **ESI-MS**: 565.3 $(M+H)^+$. **Purity** (UPLC): 97.80%.

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 37b.

37b was prepared following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.



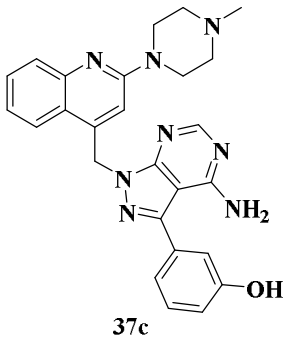
37b

MP: 227-228°C, **Yield:** 52%. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 2.22 (s, 3H), 2.41(bs, 4H), 3.32 (bs, 4H), 5.89 (s, 2H), 7.09 (s, 1H), 7.19-7.23 (m, 1H), 7.42 (d, J = 1.2 Hz, 2H), 7.49-7.53 (m, 1H), 7.54-7.59 (m, 1H), 7.62 (s, 2H), 7.86 (t, J = 1.2 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 8.34 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6): 44.69, 45.91, 47.83, 54.66, 97.76, 110.88, 118.44, 121.26, 121.54, 122.89, 123.81, 125.56, 126.33, 127.21, 129.97, 132.35, 143.51, 145.28, 148.10, 153.80, 154.98, 156.54, 157.09, 158.78, 168.03. . **IR (KBr):** 3061, 1649, 1614, 1529, 1431, 1280, 1001, 758. **Analysis (CHNS):** Calculated

for $C_{27}H_{26}N_{10}S$: C, 62.05; H, 5.01; N, 26.80; S, 6.13; Found: C, 62.14; H, 5.14; N, 26.55; S, 6.18. **ESI-MS** (relative intensities): 523.1 $(M+H)^+$. **Purity** (UPLC): 99.05%.

3-(4-amino-1-((2-(4-methylpiperazin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenol 37c.

37c was prepared following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.

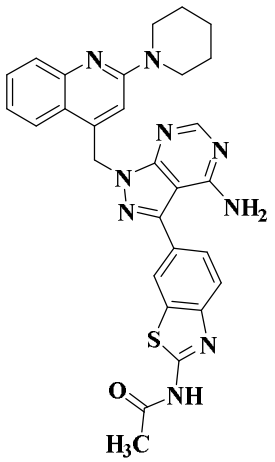


37c

MP: 218-219°C, **Yield:** 59%. **^{1}H NMR** (MeOD-d_1, 400 MHz) δ ppm: 2.34 (s, 3H), 2.55-2.57 (m, 4H), 3.60-3.80 (m, 4H), 5.97 (s, 2H), 6.87 (s, 1H), 6.91-6.94 (m, 1H), 7.06-7.07 (m, 1H), 7.10-7.12 (m, 1H), 7.27-7.30 (m, 1H), 7.35-7.39 (m, 1H), 7.54-7.56 (m, 1H), 7.67-7.69 (m, 1H), 8.09-8.11 (m, 1H), 8.32 (s, 1H). **Analysis (CHNS):** Calculated for $C_{26}H_{26}N_8O$: C, 66.94; H, 5.62; N, 24.02; Found: C, 67.14; H, 5.72; N, 24.10. **ESI-MS**: 467.1 $(M+H)^+$. **Purity** (UPLC): 97.85%.

N-(6-(4-amino-1-((2-(piperidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-yl)acetamide 37d.

37d was prepared following the general procedure described in section 4.1.4.1(Step VI) as a white solid.

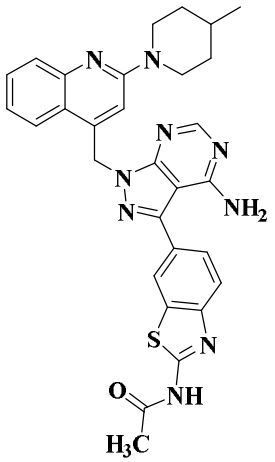


37d

MP: 230-231°C, **Yield:** 52%. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 1.48-1.55 (m, 4H), 1.61-1.62 (m, 2H), 2.21 (s, 3H), 3.62-3.64 (m, 4H), 5.91 (s, 2H), 7.03 (s, 1H), 7.16-7.18 (m, 1H), 7.49-7.56 (m, 2H), 7.62-7.64 (dd, J_1 = 8 Hz, J_2 = 1.6 Hz, 1H), 7.82-7.84 (d, J = 8Hz, 1H), 8.02-8.04 (d, J = 7.6Hz, 1H), 8.02-8.04 (d, J = 7.6Hz, 1H), 8.18-8.19 (m, 1H), 8.35 (s, 1H), 12.42 (s, 1H). **Analysis (CHNS):** Calculated for $C_{29}H_{27}N_9OS$: C, 63.37; H, 4.95; N, 22.93; S, 5.83 Found: C, 63.48; H, 5.10; N, 23.02, S; 5.88. **ESI-MS**: 550.1 $(M+H)^+$. **Purity** (UPLC): 97.65%.

N-(6-(4-amino-1-((2-(4-methylpiperidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-yl)acetamide 37d.

37d was prepared following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.

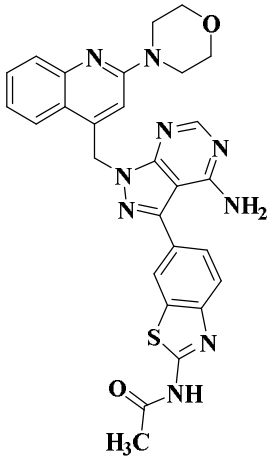


37e

MP: 245-246°C, **Yield:** 54%. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 0.89-0.91 (d, J = 6.4Hz, 3H), 1.23 (m, 2H), 1.66-1.69 (m, 3H), 2.21 (s, 3H), 2.80-2.92 (m, 2H), 4.38-4.41 (m, 2H), 5.91 (s, 2H), 7.04 (s, 1H), 7.15-7.25 (m, 1H), 7.49-7.51 (m, 1H), 7.55-7.56 (m, 1H), 7.62-7.64 (dd, J_1 = 8.4 Hz, J_2 = 2 Hz, 1H), 7.82-7.84 (d, J = 8Hz, 1H), 8.02-8.04 (d, J = 7.6Hz, 1H), 8.18 (s, 1H), 8.35 (s, 1H), 12.41 (s, 1H). **Analysis (CHNS):** Calculated for $C_{30}H_{29}N_9OS$: C, 63.90; H, 5.19; N, 22.36; S, 5.69 Found: C, 64.08; H, 5.25; N, 22.45, S; 5.74. **ESI-MS**: 564.0 $(M+H)^+$. **Purity** (UPLC): 98.10%.

N-(6-(4-amino-1-((2-morpholinoquinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-yl)acetamide 37f.

37f was prepared following the general procedure described in section 4.1.4.1(Step VI) as a white solid.

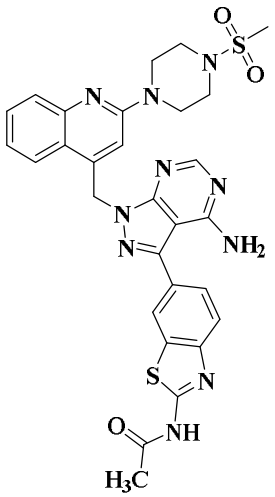


37f

MP: 233-234^{o}C, **Yield:** 53%. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 2.21 (s, 3H), 3.56-3.59 (t, *J* = 4.8Hz, 4H), 3.69-3.71 (t, *J* = 4Hz, 4H), 5.93 (s, 2H), 7.07 (s, 1H), 7.22-7.26 (m, 1H), 7.51-7.55 (m, 1H), 7.60-7.64 (m, 2H), 7.82-7.84 (d, *J* = 8.4Hz,1H), 8.07-8.09 (d, *J* = 7.2Hz, 1H), 8.18 (s, 1H), 8.35 (s, 1H), 12.41 (s, 1H). **Analysis (CHNS):** Calculated for $C_{28}H_{25}N_9O_2S$: C, 60.97; H, 4.57; N, 22.85; S, 5.81 Found: C, 61.12; H, 4.65; N, 22.94, S; 5.88. **ESI-MS**: 552.1 $(M+H)^+$. **Purity** (UPLC): 98.30%.

N-(6-(4-amino-1-((2-(4-(methylsulfonyl)piperazin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-yl)acetamide 37g.

37g was prepared following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.

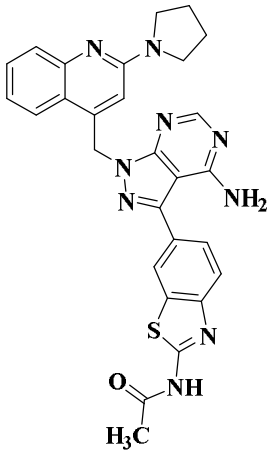


37g

MP: 250-251°C, **Yield:** 48%. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 2.67 (s, 3H), 2.82 (s, 3H), 3.15-3.22 (m, 4H), 3.70-3.80 (m, 4H), 5.95 (s, 2H), 7.21 (s, 1 H), 7.27- 7.30 (m, 1H), 7.55-7.58 (m, 1H), 7.62-7.68 (m, 2H), 7.83-7.85 (d, *J* = 8 Hz, 1H), 8.09-8.11 (d, *J* = 8Hz, 1H), 8.18-8.19 (d, *J* = 4 Hz, 1H), 8.39 (s, 1H), 12.42 (s, 1H). **Analysis (CHNS):** Calculated for $C_{29}H_{28}N_{10}O_3S_2$: C, 55.40; H, 4.49; N, 22.28; S, 10.20. Found: C, 55.52; H, 4.55; N, 22.35, S; 10.32. **ESI-MS** m/z 628.8 $(M+H)^+$. **Purity** (UPLC): 97.20%.

N-(6-(4-amino-1-((2-(pyrrolidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-yl)acetamide 37h.

37a was prepared following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.

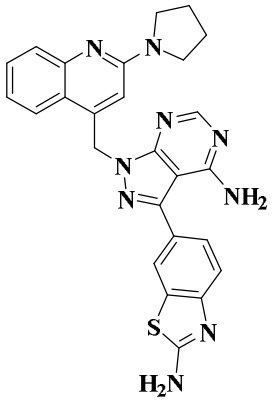


37h

MP: 244-245°C, **Yield:** 58%**. ^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 1.91-1.93 (m, 4H), 2.21 (s, 3H), 3.38-3.43 (m, 4H), 5.93 (s, 2H), 6.57 (s, 1H), 7.16 (t, *J* = 7.2 Hz, 1 H), 7.49 (t, *J* = 8.4 Hz, 1H), 7.55-7.57 (m, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 8.04-8.07 (m, 1H), 8.21-8.23 (m, 1H), 8.34 (s, 1H), 12.34 (s, 1H). **Analysis (CHNS):** Calculated for $C_{28}H_{25}N_9OS$: C, 62.79; H, 4.70; N, 23.54; S, 5.99. Found: C, 62.88; H, 4.79; N, 23.60, S; 6.04. **ESI-MS**: 536.1 $(M+H)^+$. **Purity** (UPLC): 98.80%.

6-(4-amino-1-((2-(pyrrolidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-amine 37i.

37i was prepared following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.

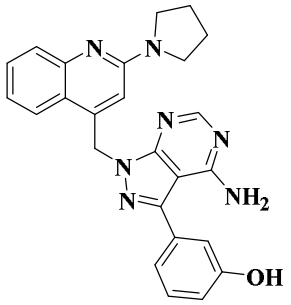


37i

MP: 230-231°C, **Yield:** 45%. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 1.93 (t, *J* = 6.4 Hz, 4H), 3.40 (t, *J* = 12 Hz, 4H), 5.91 (s, 2H), 6.56 (m, 1H), 7.14-7.18 (m, 1H), 7.44-7.51 m, 3H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.63 (s, 2H), 7.90 (s, 1H), 8.05 (d, *J* = 8 Hz, 1H), 8.32 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6): 25.46, 46.86, 47.65, 97.74, 110.56, 118.56, 120.91, 121.27, 121.71, 123.85, 125.61, 126.33, 126.76, 129.82, 132.37, 143.00, 145.24, 148.85, 153.80, 155.06, 155.31, 156.53, 158.77, 168.04. **IR (KBr) cm^{-1}:** 3371, 1618, 1529, 1431, 1271, 800. **Analysis (CHNS):** Calculated for $C_{26}H_{23}N_9S$: C, 63.27; H, 4.70; N, 25.54; S, 6.50. Found: C, 63.35; H, 4.79; N, 25.61, S; 6.54. **ESI-MS**: 494.2 $(M+H)^+$. **Purity** (UPLC): 98.10%.

3-(4-amino-1-((2-(pyrrolidin-1-yl)quinolin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenol 37j.

37j was prepared following the general procedure described in section 4.1.4.1 (Step VI) as a white solid.



37j

MP: 218-219°C, **Yield:** 52%. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ ppm: 1.91-1.94 (m, 4H), 3.39-3.43 (m, 4H), 5.91 (s, 2H), 6.54 (s, 1H), 6.86 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.01-7.05 (m, 2H), 7.16 (d, J = 7.2 Hz, 1H), 7.32 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 7.2 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 8.32 (s, 1H), 9.70 (s, 1H). **Analysis (CHNS):** Calculated for $C_{25}H_{23}N_7O$: C, 68.63; H, 5.30; N, 22.41. Found: C, 68.77; H, 5.39; N, 22.50. **ESI-MS**: 438.2 $(M+H)^+$. **Purity** (UPLC): 97.98%.

4.2. Biological evaluation

All the synthesized compounds **(37a-j)** were assessed for their PI3Kδ inhibitory activities in order to establish structure activity relationship (SAR). Some additional profiling studies (CYP, hERG) of selected compounds were also carried out. Selected compounds were docked in PI3Kδ ATP binding pocket to see the orientation and interaction in hinge region, affinity pocket, selectivity pocket and hydrophobic region. A brief assay protocol used for various biological studies is as follows.

4.2.1. *In-vitro* PI3K inhibitory activity assay

For PI3K inhibitory activity assay, kit from Millipore was used to screen inhibitory activity of test compounds. The assay was carried out using experimental protocol described in the section **2.2.1,** in the chapter II.

4.2.2. *In-vitro* CYP inhibition study assay

The assay was carried out using experimental protocol described in the section **2.2.7,** in the chapter II.

4.2.3. hERG inhibition (Rb efflux assay)

The assay is carried out using experimental protocol described in the section **2.2.8,** in the chapter II.

4.2.4. Docking protocol

Docking protocol was carried using experimental protocol described in the section **2.2.10,** in the chapter II.

4.3. Result and Discussion

In this section, we summarized results and discussion of 2,4-disubstituted quinoline pyrazolo pyrimidine based PI3Kδ inhibitors.

- *In vitro* PI3Kδ inhibitory activity, selectivity and SAR
- *In vitro* CYP inhibition study and hERG liabilities
- Docking studies

4.3.1. *In vitro* PI3Kδ inhibitory activity, selectivity and SAR

All the synthesized compounds were assessed for their PI3Kδ inhibitory activity; INK-666 was employed as the positive control, **Table 14**. It was found that the majority of 2,4-disubstituted quinoline **(37a-j)** analogues displayed varying degree of PI3Kδ inhibitory activity at 100 nM concentration.

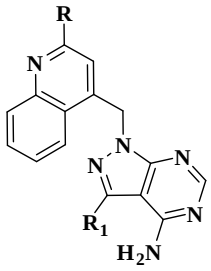
Initial set of compounds **37a, 37b** and **37c** bearing an *N*-methyl piperazine substitution **(R)** on a quinoline ring showed excellent PI3Kδ

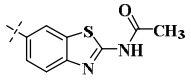
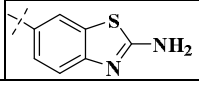
inhibitory activity. Compound **37a** with acylated N-(benzo[d]thiazol-2-yl) derivative, compound **37b** deacylated N-(benzo[d]thiazol-2-yl) derivative and compound **37c** showed excellent inhibitory activity with an IC_{50}: 0.60 nM, 0.52 nM and 0.82 nM respectively.

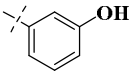
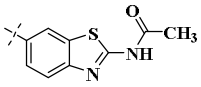
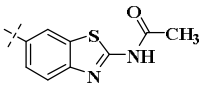
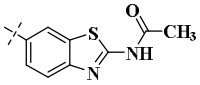
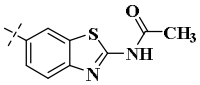
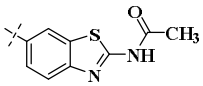
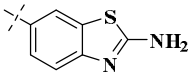
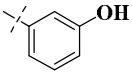
We further prepared few compounds with different cyclic amine such as piperidine (**37d**), 4-methyl piperidine (**37e**) and morpholine (**37f**) keeping benzthiazole ring constant. Compounds **37d**, **37e** and **37f** were found to be less potent as compared to N-methyl piperazine derivative. We further replaced methyl of N-methyl piperazine ring with methane sulphonyl group (compound **37g**) and checked its inhibitory activity, It showed moderate PI3K inhibition (70% inhibition, IC_{50}: 9.85 nM).

In the second set of compounds, where in *N*-methyl piperazine was replaced with pyrrolidine (R) on a quinoline ring. Compound **37h**, **37i** and **37j** showed excellent PI3Kδ inhibitory activity. Compound **37h**, **37i** and **37j** were most potent, showed IC_{50}:0.65 nM, 0.60 and 0.85 nM respectively.

Table 14: *In vitro* PI3Kδ inhibitory activity data of 2,4-disubstituted quinoline pyrazolo pyrimidine derivatives.



Sr. No.	R	R_1	PI3Kδ inhibition (%)[a, b]	PI3Kδ IC_{50} (nM)[c]
37a			110	0.60
37b			105	0.52

37c			**94**	**0.82**
37d			60	ND
37e			52	ND
37f			70	9.85
37g			70	10.1
37h			**108**	**0.65**
37i			**105**	**0.60**
37j			**98**	**0.85**
INK-666			**110**	**0.50**

[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.

4.3.2. CYP (Cytochrome) inhibition and hERG liabilities for compound 37a-j

Additional profiling studies of compounds **37a**, **37b**, **37c**, **37h**, **37i** and **37j** was carried out and it was found to be devoid of CYP (<10% CYP inhibition at 10 μM concentration, for CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4) and hERG liabilities (IC_{50}: > 30 μM).

4.3.3. Molecular modelling study

Molecular docking studies of compound **37a** and **37h** were carried out by using (Glide version 6.7) Schrodinger software to study an interaction of test compound with PI3Kδ receptor (PDB ID: **2WXK**). **Figure 43,** report docking image for compound **37a** and **37h.** The active site was defined to include residues within 10 A^{o} and the key amino acid residues in binding and specificity pockets are highlighted in respective figures.

As described in designing section, INK-666 and INK-654 interacts effectively with PI3Kδ ATP binging site. Similarly, docking studies illustrate that **37a** and **37h** interact closely with the key residues of PI3Kδ ATP-binding pockets, Pyrazolo pyrimidine ring served as the hinge binder and it forms key hydrogen bonds with Val_{828} and Glu_{826}. Both compounds, **Figure 43** adopts propeller-shaped conformation, where in the 2,4-disustituted quinoline ring was found to be sandwiched into the induced hydrophobic specificity pocket between Trp_{760} and Met_{752} in PI3Kδ specificity pocket. In compound **37a** and **37h** benzthiazole ring interacts with the affinity pocket through hydrogen bond with Asp_{787}, N-methyl piperazine and pyrrolidine ring were projected towards hydrophobic region. Thus, docking study results, confirms potent PI3Kδ inhibitory activity of 2,4-disustituted quinolines analogue.

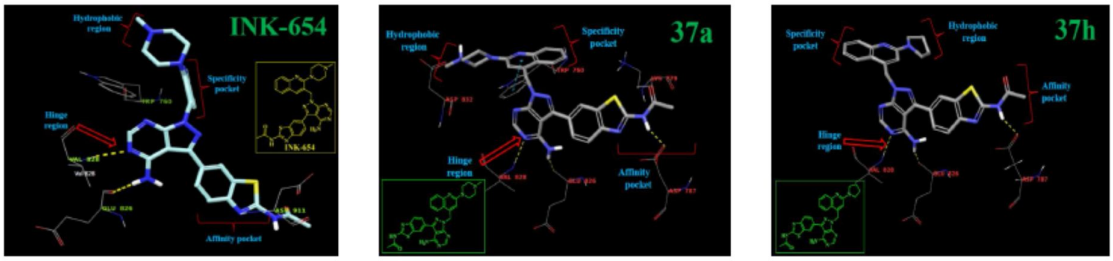


Figure 43: Docking image of INK-654, **37a** and **37h** (PDB ID: **2WXK**)

4.4. Conclusion

Based on *in vitro* study results, we can summarize that among twenty novel compounds tested for PI3Kδ inhibitory activity in this series, compounds **37a**, **37b**, **37c**, **37h**, **37i** and **37j** were found to be potent PI3Kδ inhibitors. *In vitro* activity results and docking study further validates our hypothesis of designing 2,4-disustituted quinoline based novel and potent PI3Kδ inhibitors.

Additional profiling studies of selected compounds was carried out and it was found to be devoid of CYP and hERG liabilities (IC_{50} :> 30 μM), while INK 666 showed moderate CYP3A4 inhibition. Docking results of **37a** and **37h** correlates with its potent *in vitro* PI3Kδ inhibitory activity.

Further, compound **37a**, **37b**, **37c**, **37h**, **37i** and **37j** will be subjected for PI3K isoform selectivity. Based on selectivity results, most potent and selective compound will be subjected for PK profiling in mice followed by efficacy evaluation, using xenograft model to check in anticancer activity and CIA model to check in anti-inflammatory activity, in mice.