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Design and Development of a Macrocyclic Series Targeting Phosphoinositide 3-Kinase δ

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KEYWORDS. Macrocycle, PI3Kδ, Thermodynamics, Lipid kinase

ABSTRACT: A macrocyclization approach has been explored on a series of benzoxazine phosphoinositide 3-kinase δ inhibitors, resulting in compounds with improved potency, permeability and *in vivo* clearance, whilst maintaining good solubility. The thermodynamics of binding was explored *via* surface plasmon resonance and the binding of lead macrocycle **19** was found to be almost exclusively entropically driven, compared with progenitor **18** which demonstrated both enthalpic and entropic contributions. The pharmacokinetics of macrocycle **19** was also explored *in vivo*, where it showed reduced clearance when compared with the progenitor **18**. This work adds to the growing body of evidence that macrocyclization could provide an alternative and complementary approach to the design of small molecule inhibitors, with the potential to deliver differentiated properties.

Phosphoinositide 3-kinase δ (PI3Kδ) is a lipid kinase, which catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PIP_3 is a signaling molecule with roles in key processes such as cell growth, proliferation, survival and migration.[1-3] PI3Kδ is predominantly expressed in leukocytes,[1] and is an established target for diseases such as Chronic Obstructive Pulmonary Disease,[4-6] Oncology indications such as Chronic Lymphocytic Leukemia[7] and Sjögrens Syndrome.[8]

On-going work within our laboratories had identified the benzoxazine series of inhibitors (e.g. compound **1**) as a promising opportunity to provide clinical candidates. An analysis of the binding mode of compound **1** by X-ray crystallography highlighted the proximity of the 6-substituent of the benzoxazine core and the sulfonamide substituent from the pyridine, with room to accommodate a linker between them (Figure 1) and generate a macrocycle.

Macrocyclization is an established strategy for modulating properties[9-10] such as potency, by constraining the conformation close to the bioactive pose;[12-13] selectivity, by reducing the available binding modes;[14] permeability, by reducing the molecular volume;[12] and clearance, by restricting binding to metabolizing enzymes or by providing a steric block;[12] as well as offering structural novelty.[15] It was therefore of interest to explore the effect of macrocyclization within the emerging benzoxazine template for targeting PI3Kδ.

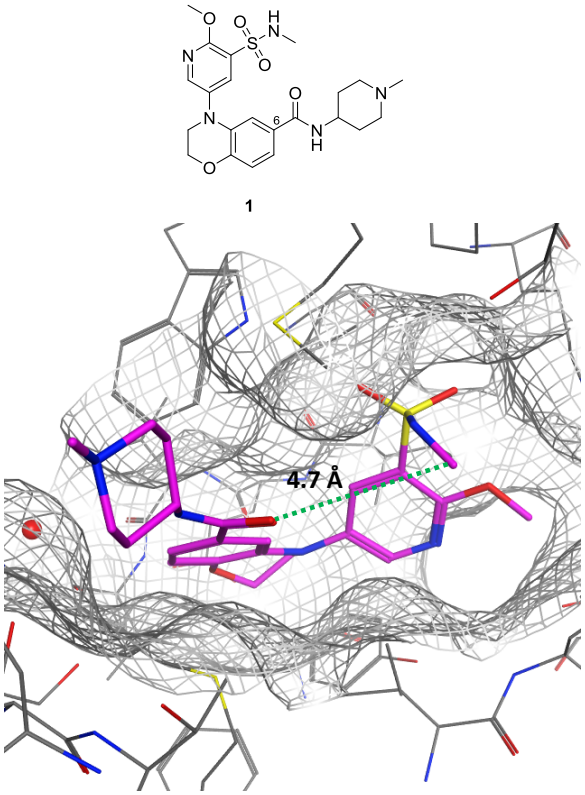


Figure 1. A protein bound X-ray crystal structure of compound **1** (XXXXX, 2.5 Å), with hinge binder located at the rear

of the image, highlighting the potential for macrocyclization within the template.

Initial efforts focused on the design and synthesis of analogues of the progenitor compound **2**, a simplified analogue of key compound **1** (Figure 2). Based on previous SAR from the template and computational modelling, both amine and amide linkers at the benzoxazine 6-position were selected. A variety of linker lengths as well as both forward and reverse sulfonamides on the pyridine were explored.

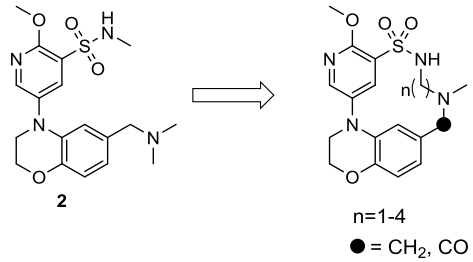


Figure 2. Progenitor compound and exemplar macrocyclic targets.

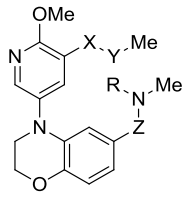
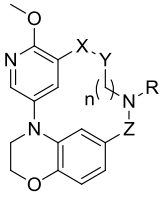
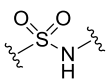
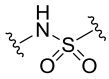
						
			Progenitor	**Macrocycle n = 2**	**Macrocycle n = 3**	**Macrocycle n = 4**
			PI3Kδ pIC_{50} (LE)			
X—Y = 	**Z = CH_2**	**R = H**	**Compound 3 5.9 (0.31)**	**ND**	**Compound 4 6.2 (0.32)**	**Compound 5 6.8 (0.33)**
		R = Me	**Compound 2 5.6 (0.29)**	**Compound 6 5.1 (0.26)**	**Compound 7 5.9 (0.29)**	**Compound 8 6.6 (0.31)**
	Z = CO	**R = H**	**Compound 9 7.4 (0.38)**	**ND**	**Compound 10 6.4 (0.31)**	**Compound 11 7.2 (0.34)**
		R = Me	**Compound 12 5.8 (0.28)**	**Compound 13 4.9[a] (0.24)**	**Compound 14 5.2 (0.24)**	**Compound 15 6.2 (0.28)**
X—Y = 	**Z = CH_2**	**R = H**	**Compound 16 5.8 (0.31)**	**ND**	**Compound 17 7.0 (0.36)**	**ND**
		R = Me	**Compound 18 6.2 (0.31)**	**Compound 19 7.7 (0.39)**	**ND**	**Compound 20 7.1 (0.33)**
	Z = CO	**R = H**	**Compound 21 7.3 (0.37)**	**ND**	**Compound 22 7.8 (0.38)**	**ND**
		R = Me	**Compound 23 5.5 (0.27)**	**Compound 24 6.9 (0.34)**	**ND**	**Compound 25 6.3 (0.29)**

Table 1. pIC_{50} data measured in a PI3Kδ enzyme assay; all data N≥2; SD≤0.45; [a]compound measured as < 4.5 once. ND = compound not selected for synthesis.

Analysis of the potency data showed that macrocyclic linkers were generally tolerated at the PI3Kδ binding site (Table 1). Within the aryl *S*-linked sulfonamide series (**2**-**15**), reductions in potency were observed with ethylene linked compounds **6** and **13**. We reasoned that this was a result of the short linker length, preventing optimal interactions at the core of the molecule. Hence, longer linkers were explored in more depth. With propylene and butylene linkers, only modest increases or small decreases in potency and ligand efficiency were observed. The compound with the largest potency increase was butyl-linked macrocycle **8**, which exhibited a 10-fold potency increase over its progenitor compound **2**. However, this linker is likely to have significant flexibility and therefore further optimization should be possible.

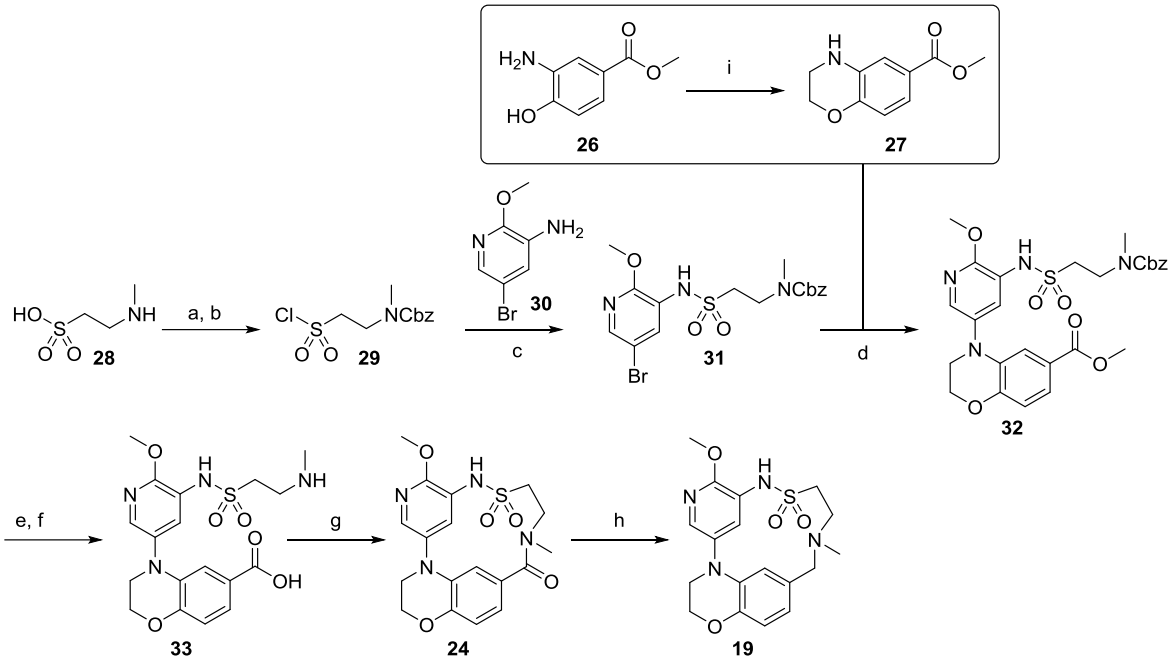
Alternative aryl *N*-linked sulfonamide compounds were also explored. It was proposed that differences in the sulfonamide orientation could provide a more favorable vector for cyclization and hence the requisite progenitor and macrocyclic compounds were designed and synthesized.

Within the aryl *N*-linked sulfonamide series (**16**-**25**), more dramatic increases in potency were observed, with all macrocyclic compounds having higher potency and efficiency than the corresponding progenitor compounds. The largest increases were observed for the ethylene linked compounds **19** and **24**. This contrasts with the *S*-linked sulfonamide series where these

were the least potent compounds, suggesting for the *N*-linked series, the conformational constraint is favorable for binding. Propylene and butylene linked compounds were found to have more modest increases in potency.

Macrocycle **19** and progenitor **18** were selected for further study due to the greater than 30-fold increase in potency and corresponding efficiency increase observed for these compounds. Macrocycle **19** was synthesized according to Scheme 1. Synthesis of other macrocycles and progenitor compounds is available in the supporting information using analogous synthetic procedures.

Scheme 1. Synthesis of key macrocycle compound 19.[a]



[a]Reagents and conditions: (a) CbzCl, aq. NaOH, 58%; (b) $SOCl_2$, toluene, 94%; (c) pyridine, 21%; (d) **27**, $Pd_2(dba)_3$, RuPhos, Cs_2CO_3, 25%; (e) LiOH, THF, H_2O, 69%; (f) H_2, Pd/C, THF, EtOAc, 64%; (g) MsCl, imidazole, DCM, 21%; (h) BH_3•DMS, THF, MeOH, 10%; (i) 1,2-dibromoethane, K_2CO_3, DMF, 85%

Macrocycle **19** could be accessed from commercially available starting material **28**. Carboxybenzyl protection[16] and sulfonyl chloride formation[17] yielded intermediate **29**. Sulfonamide formation with commercial amine **30** gave sulfonamide **31**. Buchwald-Hartwig amination[18] with benzoxazine **27**, synthesized by double alkylation of amino alcohol **26**, afforded cross-coupled product **32**. This was then deprotected by ester hydrolysis and hydrogenolysis to reveal amino acid **33**. Intramolecular cyclization with mesyl chloride furnished macrocycle **24**. Macrocyclization step generally proved challenging with only low to moderate yields obtained in most examples. Subsequent lactam reduction with borane gave macrocycle product **19**.

A number of strategies were explored to further rationalize the origin of the potency increase noted upon cyclization of progenitor **18** to macrocycle **19**. Firstly, protein bound X-ray crystal structures were generated for these compounds to explore the binding modes (Figure 2).

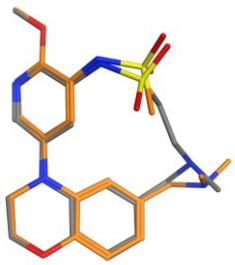


Figure 2. An overlay of protein bound X-Ray crystal structure of macrocycle **19** (XXXX, 2.2Å) and progenitor **18** (XXXX, 2.2Å); protein not shown.

Analysis of these structures revealed that the binding modes for the progenitor and macrocycle are almost identical; formation of the macrocycle does not negatively impact the key binding interactions from the conserved core structure. We therefore proposed the compound pair would have similar enthalpic contributions to binding, with the reduced conformational freedom of the macrocycle providing a reduced entropic penalty to binding and resultant increase in potency. This is supported by an unconstrained conformational search for compounds **18** and **19**, which indicate an increased preference for

sulfonamide conformations close to the bio-active conformation in the case of the macrocycle, **19**, compared with progenitor **18** (Figure in Supporting Information). Interestingly, analysis of the amine end of the macrocyclic linker indicated that the bioactive conformation was one of the least preferred observed dihedral angles in macrocycle **19**, indicating that this linker may still be sub-optimal.

To further investigate the thermodynamics of binding, the entropic and enthalpic contributions to binding were derived experimentally using surface plasmon resonance (SPR). The SPR data was used to construct a Van't Hoff analysis for both compounds[19, 20] and the entropy and enthalpy of binding were obtained (Table 2).

	Progenitor 18	Macrocycle 19
$\Delta G_{25\,°C}$ / kJ mol^{-1}	- 37.3	- 48.8
ΔH / kJ mol^{-1}	- 58.9	- 0.7
ΔS / J K^{-1} mol^{-1}	- 72.5	159.9
pK_d 25 °C	6.6	8.4

Table 2. Results of Van't Hoff analysis of SPR data.

The data obtained were consistent with the potency increase from progenitor to macrocycle; a more favorable Gibb's free energy change of binding and corresponding increase in pK_d were observed. Also, as expected, the Van't Hoff analysis showed the macrocycle had a reduced entropic penalty to binding, hypothesized, in part to be due to reduced conformational freedom in the unbound state. The magnitude of the positive entropic contribution and the reduction of the enthalpic contribution to close to zero, were surprising, however it is well precedented that accurately predicting the thermodynamics of ligand binding is challenging due to the complexity of the system (i.e. ligand, protein and solvent effects).[21-22]

Next, we wished to explore the effect of macrocyclization on biological and biophysical properties within the template (Table 3).

		Progenitor 18	Macrocycle 19
pIC_{50} PI3Kδ (LE, LipE) N≥3		6.2 (0.31, 4.6)	7.7 (0.39, 3.4)
pIC_{50} PI3K α, β, γ N≥3		4.9[a], 5.1, 4.6[b]	5.6, 5.9, 5.8
Whole blood pIC_{50}[c] N=3		5.8	6.5
Chrom $logD_{pH\,7.4}$		1.6	4.3
CLND solubility / µM		≥ 406	≥ 331
AMP / nm s^{-1}		140	530
HSA / AGP % binding		37 / 64	88 / 70
pK_{aH}		9.4	5.7
In vitro clearance / mL min^{-1} kg^{-1}	Mic (human, minipig, rat)	<0.40 1.63 0.48	<0.40 0.92 <0.46
	Hep (human, minipig, rat)	<0.45 <0.89 <0.80	<0.45 <0.89 <0.80

Table 3. A comparison of key developability properties between progenitor **18** and macrocycle **19**. [a]Compound measured as < 4.5 on one occasion; [b]compound measured as < 4.5 on three occasions; SD≤0.32 (enzyme assays); SD≤0.51 (cellular assay); [c] IFNγ inhibition in cytostim stimulated whole blood assay.

As previously stated, macrocycle **19** exhibits increased potency and ligand efficiency compared to progenitor **18**. This also translates into a more modest increase in whole blood potency. It was expected that macrocycle **19** would have similar selectivity over the closely related PI3K isoforms, however a trend towards slightly improved selectivity was observed. This is possibly due to the restricted conformations of the macrocycle.

Macrocycle **19** is more lipophilic than progenitor **18** and therefore has a lower lipophilic efficiency (LipE), probably due to decreased ability to hydrogen bond within the restricted template. This is reflected in the measured $pKa_{H,}$ with a significantly lower basicity for the cyclic amine compared with the acyclic analogue; a trend observed in multiple macrocycle/progenitor pairs, across all linker lengths (full data not shown). Given the magnitude of the difference, it is hypothesised that in addition to the inductive effect of the sulfonamide on the amine $pKa_{H,}$, that the macrocycle may also provide a steric block to the amine lone pair thereby reducing basicity further. This is supported by the lowest energy conformation orientating the lone pair towards the center of the ring (Figure 3).

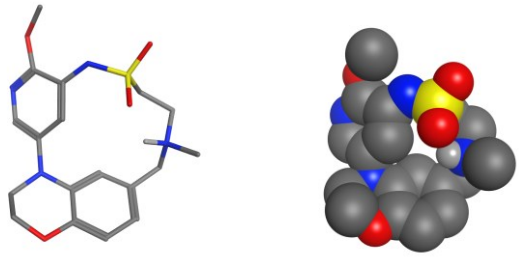


Figure 3. Lowest energy conformation of macrocycle **19**; protonated form shown to indicate directionality of lone pair.

The high lipophilicity results in highly permeable compounds, however aqueous solubility is also maintained. Additionally, the increase in lipophilicity is not reflected in higher clearance, with both compounds displaying *in vitro* clearances close to the lower limit of the hepatocyte assay threshold, across species. The lipophilicity does result in an increase in plasma protein binding, however this still falls within acceptable ranges for both compounds. In summary, although more lipophilic, with a lower LiPE, macrocycle **19** does not exhibit many of the associated poor developability properties.[23]

Given that *in vitro* clearance data showed low clearance, both the compounds were advanced into a rat *in vivo* PK study to further evaluate the differences in clearance (Table 4).

	Progenitor 18	**Macrocycle 19**
***In vivo* Cl / mL min^{-1} kg^{-1}**	**233**	**12.3**
Vd_{ss} / L kg^{-1}	**8.5**	**1.8**
Half-life / h	**0.60**	**1.5**
Predicted F_u (based on HSA, AGP and CMR)[24]	**0.40**	**0.06**
$Cl_{unbound}$ / mL min^{-1} kg^{-1}	**587**	**192**

Table 4. Intravenous rat PK data for compounds **18** and **19**.

The macrocycle displays much lower *in vivo* clearance in the rat compared to the progenitor compound, suggesting that the macrocycle could be suitable for oral delivery. Pleasingly, on calculating the unbound clearance using a validated biomimetic model for predicting human fraction unbound (F_u),[24] and assuming that F_u is species independent, it is clear this improvement results from a true modulation of intrinsic clearance and not just as a secondary effect of reducing the free fraction available to be cleared (Table 4). It is hypothesized that this reduced clearance is due to the macrocyclic restriction preventing access to conformations required to bind to some metabolizing enzymes.

In summary, it has been shown that macrocyclization within a series of PI3Kδ inhibitors, can provide differentiated compounds, with improvements in potency, permeability and *in vivo* clearance shown with key exemplars. Analysis of the thermodynamics of binding for one pair of compounds, also highlights the entropic advantage of macrocyclization. These data further support macrocyclization as an attractive strategy for lead optimization.

ASSOCIATED CONTENT

Supporting Information

Supporting information is available in PDF.

Synthetic procedures and characterization of PI3Kδ inhibitors, PI3Kδ assay data, conformational search results and SPR data for compounds **18** and **19**; and PI3Kδ crystallography methods and data statistics for compounds **1**, **18** and **19**.

Accession Codes

Coordinates have been deposited with the Protein Data Bank. Authors will release atomic coordinates and experimental data upon article publication.

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

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ABBREVIATIONS

PI3K, Phosphoinositide 3-kinase; LE, Ligand Efficiency; ND, Not Determined; IFNγ, Interferon Gamma; SPR, Surface Plasmon Resonance; CLND, Chemiluminescent Nitrogen Detection; AMP, Artificial Membrane Permeability; HSA, Human Serum Albumin; AGP, α-1-glycoprotein; h, human; mp, minipig; r, rat; RuPhos, 2-Dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl; PIP_2, Phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; Vd_{ss}, steady state volume of distribution; $Cl_{unbound}$, unbound clearance; CMR, calculated molar refractivity; DCM, dichloromethane; dba, Dibenzylideneacetone; PK, pharmacokinetics.

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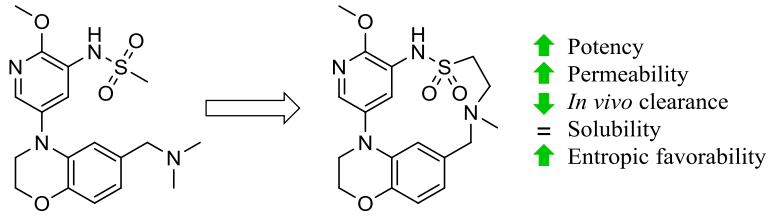

18
PI3Kδ pIC_{50} 6.2

19
PI3Kδ pIC_{50} 7.7