OPM-116, a highly potent and specific PI3Kγ inhibitor to enhance antitumor immunity

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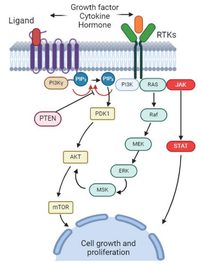
Introduction

PI3Kγ, characterized by its distinct expression pattern and biological functions, has long been scrutinized as a therapeutic target for various disorders, including cancer. Inhibiting PI3Kγ effectively reprograms M2 macrophages into an M1 phenotype within the tumor microenvironment, thereby enhancing activated T cell expansion by alleviating macrophage-mediated suppression. Recent clinical evidence underscores the compelling role of PI3Kγ in combination with PD-L1 in anti-tumor activity, regardless of PD-L1 status.

However, achieving selectivity for PI3Kγ has been challenging due to the high sequence homology among class 1 PI3K isoforms (Drew SL, et al 2020). Macrocyclization of linear small molecules substantially reduces their conformational flexibility, altering various physicochemical and biological characteristics. The unexpected selectivity observed among closely related target molecules is attributed to the conformationally constrained structure (Ma et al, 2022).

Here, we present the discovery of **OPM-116**, a potent and selective ATP-competitive inhibitor of PI3Kγ. This macrocyclic compound exhibits over 300-fold selectivity for PI3Kγ over other class I isoforms. We demonstrate its selectivity in biochemical and cellular assays, supported by predictions of a novel and unique hinge binding mode compared to known PI3K inhibitors, providing a reliable binding mode prediction



Class I PI3Ks (phosphoinositide 3-kinases) are composed of two parts: a p110 catalytic module and an adaptor subunit, forming obligate heterodimers. Depending on the type of adaptor and the activating membrane receptor, Class I PI3Ks can be divided into two groups: class IA and class IB. Class IA, which includes PI3Kα, β, and δ, is linked to a p85 regulatory subunit and activated by tyrosine kinase receptors (RTKs). In contrast, the sole member of class IB, PI3Kγ, binds to either p101 or p87. These adaptors connect p110γ to the Gβγ subunit of G protein-coupled receptors (GPCRs), ensuring full enzyme activation

Material and Methods

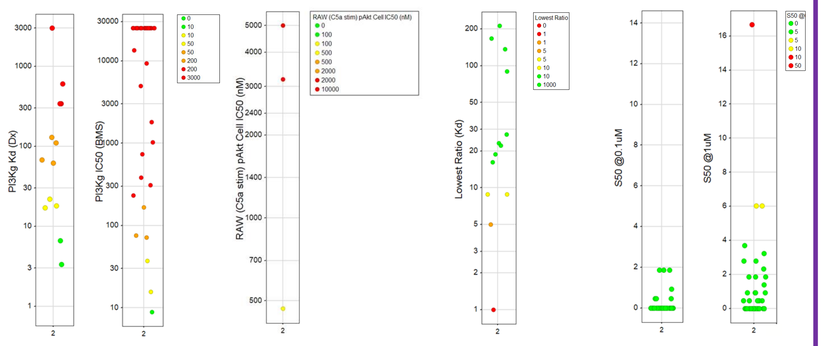
- The biochemical assays presented were done by binding assays at DiscoverX. The specificity was done at Eurofins (radiometric assay).
- Cell potency was evaluated in-vitro on Raw cells stimulated with C5a . pAKT was evaluated to measure compound activity. IC50 are presented in nM.
- In vitro metabolism, permeability and protein binding were evaluated at Cyprotex, UK.
- PBS and FaSSIF (Fasted State Simulated Intestinal Fluid) solubility are presented in µM values
- The metabolic stability, solubility, PK study, protein binding & CYP inhibition were done at Oncodesign Services
- hERG Patch Clamp Assays was done at Reaction biology
- Molecular modeling was conducted utilizing Schrodinger Maestro, release 2024-1

Bibliography

- Drew S.L., Thomas-Tran R., Beatty J.W., Fournier J., Lawson K.V., Miles D.H., Mata G., Sharif E.U., Yan X., Mailyan A.K. Discovery of potent and selective PI3Kγ inhibitors. J. Med. Chem. 2020;63:11235–11257. doi: 10.1021/acs.jmedchem.0c01203
- Ma J., Sanchez-Duffhues G., Caradec J., Benderitter P., Hoflack J., Dijke P.T. Development of small macrocyclic kinase inhibitors. Futur. Med. Chem. 2022;14:389–391. doi: 10.4155/fmc-2021-0342. - DOI – PubMed
- Miller MS, Thompson PE, Gabelli SB. Structural Determinants of Isoform Selectivity in PI3K Inhibitors. Biomolecules. 2019 Feb 26;9(3):82. doi: 10.3390/biom9030082. PMID: 30813656; PMCID: PMC6468644.

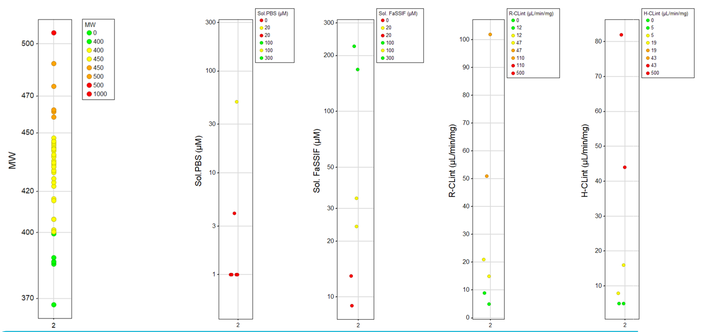
Results

Biochemical Potency | Cell Potency | Selectivity vs PI3K family (Kd) | Specificity vs kinome



PI3Kγ series library showing some examples with outstanding potency and selectivity against target.

MW | Solubility | Microsomal stability



Compounds display MW slightly over 400, examples of good solubility and microsomal stability

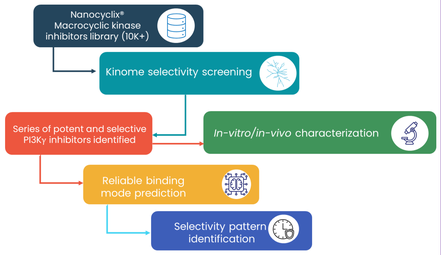
OPM-116 displays high potency, selectivity and specificity with drug-like properties. Initial SAR identified within Series 2

	Series 2	OPM-116
MW/cLogD/TPSA	**348-528 / 0.1-6.1 / 58-123**	**401 / 3.1 / 74**
PI3Kγ Kd / Biochem. IC_{50} (nM)	3-3000 (n=13) / 9-3000 (n=41)	**3 / 9**
Specificity (Kd) vs PI3K family (lowest ratio)	x1-x212 (n=13)	**x90**
Selectivity (S_{50}) vs 216 kinases	0-16.7% at 1µM	**3.2% at 1µM**
Cell assay IC_{50} (RAW pAkt)	0.47-5µM (n=3)	**0.47µM**
Solubility PBS/FaSSIF	1-50µM / 9-225µM (n=6)	4µM / 225µM
Microsomal Clint mouse/rat/human (µl/min/mg)	162 (n=1) / 2-102 (n=6) / 5-82 (n=6)	162 / 51 / 82
Fu rat/human (%)	0.6 / 0.5 (n=1)	0.6 / 0.5
CYP inhibition IC_{50} (5 isoforms)	>10µM (n=1)	**>10µM**
hERG IC_{50}	>10µM (n=1)	**>10µM**
PK Rat IV at 1mg/kg		
Cl	12 (n=1)	12
$DNAUC_{(0-inf)}$ (h*kg*ng/mL/mg)	1402 (n=1)	**1402**
T1/2 (h)	1.6 (n=1)	1.6
Vd (L/kg)	1.6 (n=1)	1.6

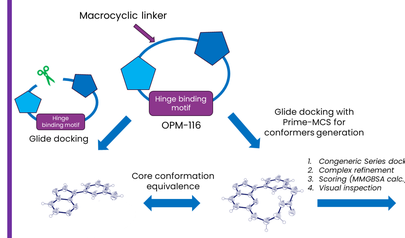
Binding Mode Prediction

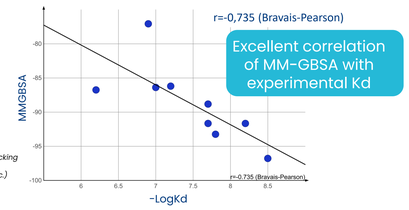
A schematic flowchart illustrates the workflow for Hit/Lead identification and prediction of binding modes in multiple PI3K isoforms. The process consists of 6 principal steps that start with our exclusive Nanocyclix® Diversity library containing more than 10 000 macrocyclic kinase inhibitors.

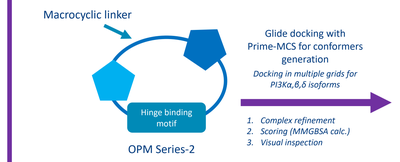
During modelling the macrocycle linker was removed in order to compare conformations of open and cyclized molecules. Conformation and binding modes matched for both type of compounds. Complex refinement provided excellent correlation of experimental Kd with MM-GBSA values.



PI3Kγ-OPM Series - Reliable Binding Mode Prediction



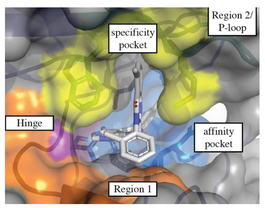




Interaction scheme

		Affinity pocket	Hinge		Region 1	
PI3Kγ	Trp812 ✓	Tyr867 ✓	Val882	✓✓	Lys890	✓
PI3Kα	Trp780 ✓	Tyr836 ✗*	Val851	✓✓	Gln859	✓
PI3Kβ	Trp781 ✗	Tyr833 ✗	Val848	✗	Asp856	✗
PI3Kδ	Trp760 ✗*	Tyr813 ✗	Val851	✓✓	Asn836	✗

✓ strong interaction ✓ weak interaction
✓✓ bidentate interaction ✗ no interaction
* Limited number of compounds



From Miller MS, et al 2019 (PMID: 30813656)

Structural determinants of PI3K isomorph selectivity

- Specific interactions with non-conserved residues
- Restricting flexibility in affinity and specificity pocket
- Modification of the hinge binding moiety

Key structural elements that contributes to high isoform selectivity of OPM PI3Kγ inhibitors:

- Novel and unique hinge binding motif comparing to known PI3K inhibitors
- Restricted flexibility of the molecules due to macrocyclization
- Productive interactions with non-conserved Lys890 in Region 1
- Bidentate Interaction with Val882 and disruption of internal H-bond between Val882 and non-conserved Ser885 (PI3Kγ numbering) in α, β, δ isoforms

Conclusions

- **OPM-116 is a first in class macrocyclic inhibitor of PI3Kγ with a unique hinge binding motif comparing to known PI3Kγ inhibitors**
- **OPM-116 has excellent potency and specificity for PIK3γ, with drug-like characteristics, making it a good option for clinical development**

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