



by  
Boehringer Ingelheim

# PI3 kinase $\gamma$ (PI3K) inhibitor

BI-9222

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## Summary

BI-9222 is a potent and selective Phosphoinositide-3-kinase gamma (PI3K $\gamma$ ) inhibitor for *in vitro* and *in vivo* studies, selectively targeting GPCR-dependent immune signaling pathways that regulate immune cell migration, inflammation, and effector functions. BI-9183 is available as a negative control.

## Chemical Structure

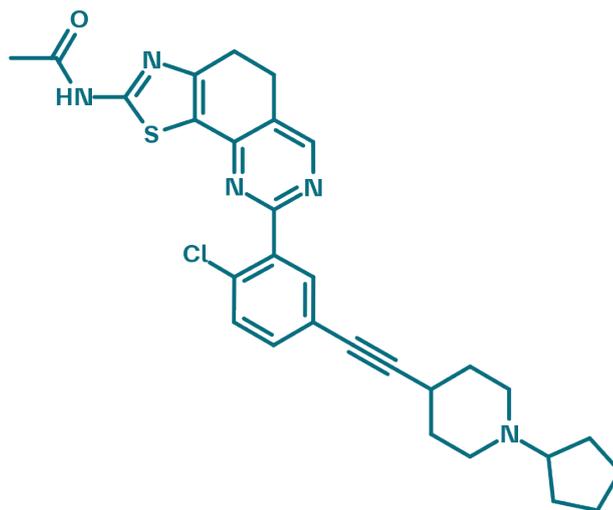


Figure 1 : 2D structure gamma of BI-9222

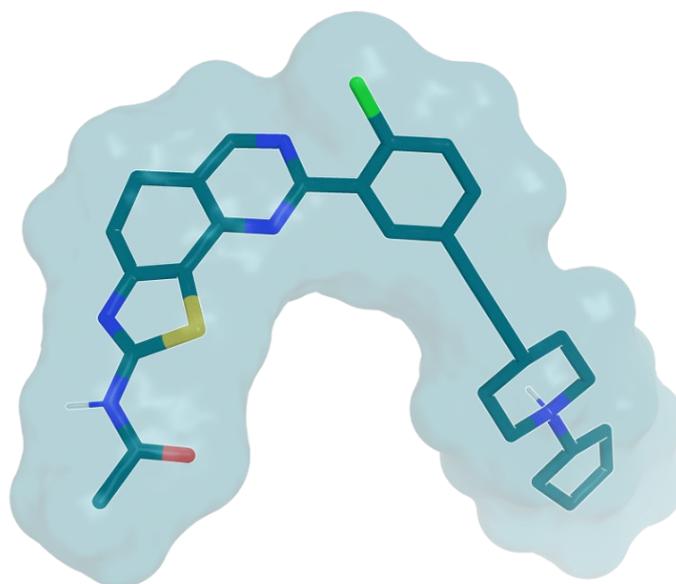


Figure 2 : 3D structure of BI-9222, as observed in the X-ray structure of the complex with PI3K $\gamma$

## Highlights

BI-9222 is a selective phosphoinositide-3-kinase gamma (PI3K $\gamma$ ) inhibitor<sup>1</sup>. PI3K $\gamma$  is a class I phosphoinositide 3-kinase that produces PI-(3,4,5)-P3 from PI-(4,5)-P2 in response to activation of GPCRs, thereby regulating intracellular signaling pathways. It is primarily expressed and active in immune cells, controlling processes such as cell migration, respiratory burst, inflammation, and immune responses. BI-9183 can be used as the negative control<sup>2</sup>.

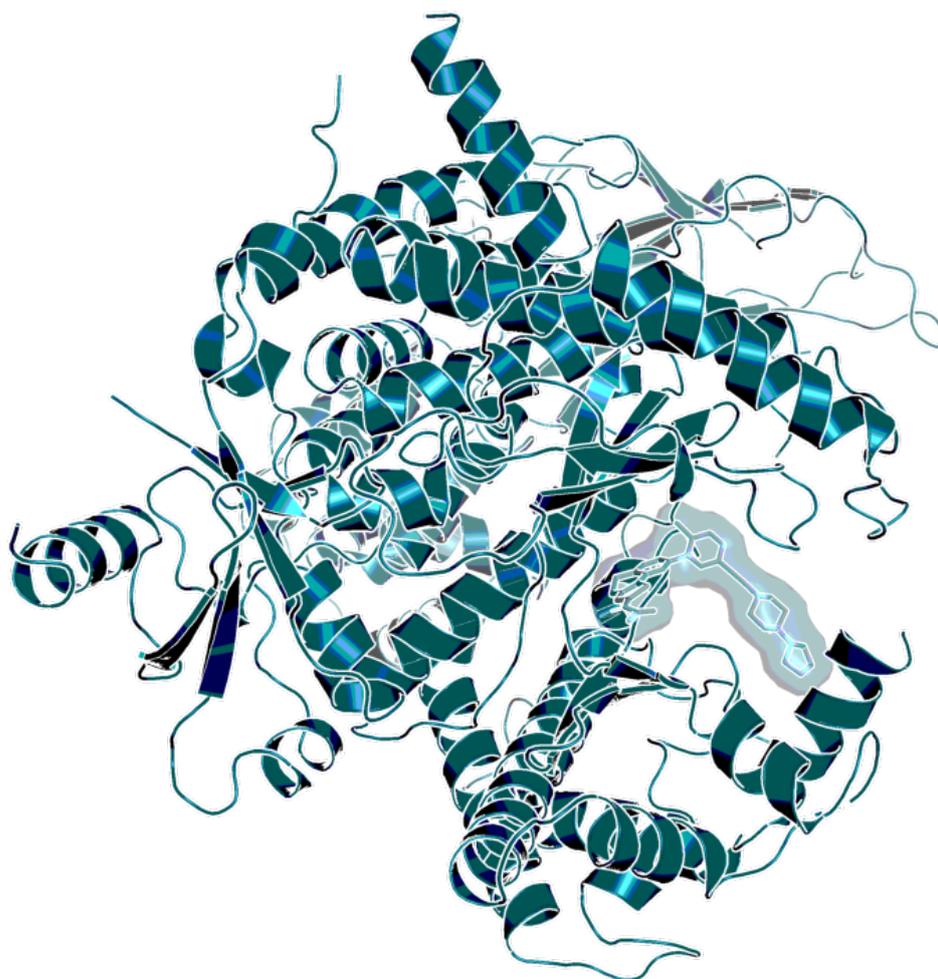
## Target information

Phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases that phosphorylate phosphatidylinositol-(4,5)-bisphosphate on the 3' position, generating signaling molecules that regulate critical cellular processes such as growth, survival, metabolism, and immune responses. The PI3 kinase (PI3K) pathway starts with the activation of cell surface receptors like receptor tyrosine kinases (RTKs), G-protein-coupled receptors (GPCRs), or immune receptors<sup>1</sup>.

PI3Ks are categorized into three classes (Class I, II, and III)<sup>4,5</sup>, with Class I being the most extensively studied due to its role in diseases like cancer, inflammation, and autoimmune disorders. Class I PI3Ks consists of four isoforms: PI3K $\alpha$ , PI3K $\beta$ , PI3K $\gamma$  and PI3K $\delta$ , each with distinct tissue distribution and functions.

PI3K $\alpha$  and PI3K $\beta$  are ubiquitously expressed and are involved in general cellular processes<sup>6</sup>, including proliferation and metabolism. In contrast, PI3K $\gamma$  and PI3K $\delta$  are predominantly expressed in leukocytes, making them critical regulators of the immune system.

PI3K $\gamma$  is primarily active in innate immune cells, such as macrophages and neutrophils, where it regulates cell migration, respiratory burst, inflammation, and immune responses expressed in immune cells, including macrophages, neutrophils, and T-cells, where it governs critical functions such as immune cell activation, migration, and trafficking. Activation of PI3K $\gamma$  occurs downstream of G-protein-coupled receptors (GPCRs) through interactions with G $\beta\gamma$  subunits and Ras proteins, leading to the generation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3), a key secondary messenger involved in cellular signaling. Dysregulation of PI3K $\gamma$  activity has been implicated in the pathogenesis of several diseases, including chronic inflammation, cardiovascular disorders, and cancer, highlighting its role as a central mediator of immune and inflammatory responses. Targeting PI3K $\gamma$  has therapeutic potential in treating inflammatory and autoimmune diseases by modulating these processes<sup>7</sup>.



**Figure 3: Protein Ligand Complex: X-ray structure of PI3K-gamma in complex with BI-9222, structure determined at Boehringer Ingelheim**

## ***In vitro* activity**

BI-9222 is a potent and selective inhibitor of PI3K $\gamma$ , showing sub-nanomolar activity and reduced potency against the other class I PI3K isoforms.

Probe name / Negative control	BI-9222	BI-9183
MW [Da] <sup>a</sup>	532.1	401.3
PI3 Kinase $\gamma$ (IC <sub>50</sub> ) [nM] <sup>b</sup>	0.6	120
PI3 Kinase $\delta$ (IC <sub>50</sub> ) [nM]	62 <sup>b</sup>	2067 <sup>c</sup>
PI3 Kinase $\alpha$ (IC <sub>50</sub> ) [nM] <sup>b</sup>	273.2	-
PI3 Kinase $\beta$ (IC <sub>50</sub> ) [nM] <sup>b</sup>	96.1	-

<sup>a</sup> For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs

<sup>b</sup> assay conditions: assay conditions apply to all measured isoforms<sup>2</sup>

<sup>c</sup> Assay condition <sup>8</sup>: HEK293T cells were transiently co-transfected with a NanoLuc-PIK3CD fusion vector and a PIK3R1 expression vector at a 1:9 ratio and incubated for approximately 20 h. Cells were then pre-treated with NanoBRET Tracer K-3 (150 nM) and test compounds for 2 h. NanoBRET signals were measured using a PHERAstar plate reader (BMG Labtech) equipped with luminescence filter pairs of 450 nm band-pass (donor) and 610 nm long-pass (acceptor). IC<sub>50</sub> values were determined, and dose-response curves were generated using GraphPad Prism 8 by fitting the data with a three-parameter log(inhibitor) versus response model using the following equation:  $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(X - \text{LogIC}_{50})})$ .

## In vitro DMPK and CMC parameters

The *in vitro* DMPK and CMC data for BI-9222 show a well-balanced ADME profile, characterized by good hepatocyte and microsomal stability, moderate solubility, and high permeability with a low efflux ratio, supporting efficient cellular uptake.

Probe name / Negative control	BI-9222	BI-9183
logD @ pH 2, 7.4, 11	1.8, 4.4, 4.8	4.3, n.a., 3.3
Solubility @ pH 7 [µg/mL]	4	<1
Caco-2 permeability AB @ pH 7.4 [ $\times 10^{-6}$ cm/s]	35.3	18
Caco-2 efflux ratio	0.5	0.5
Microsomal stability (human/rat) [% Q <sub>H</sub> ]	66 / 57	<23 / 49
Hepatocyte stability (human/rat) [% Q <sub>H</sub> ]	20 / 43	<4 / 70
Plasma Protein Binding (human/rat) [%]	>99.8 / 98.3	100 / 99.9
CYP 3A4 (IC <sub>50</sub> ) [µM]	>10	>50
CYP 2C9 (IC <sub>50</sub> ) [µM]	>10	30
CYP 2C19 (IC <sub>50</sub> ) [µM]	4.3	>50
CYP 2D6 (IC <sub>50</sub> ) [µM]	3.7	25.4
CYP 1A2 (IC <sub>50</sub> ) [µM]	>10	2.6

## In vivo DMPK parameters

The *in vivo* DMPK data for BI-9222 show low clearance (12% QH) and a long mean residence time of 12 hours after intravenous dosing. BI-9222 also demonstrates moderate oral bioavailability.

BI-9222	Rat <sup>a</sup>
Clearance [% Q <sub>H</sub> ]	12
Mean residence time after <i>i.v.</i> dose [h]	12
t <sub>max</sub> [h]	3.7
C <sub>max</sub> [nM]	335
F [%]	31
V <sub>ss</sub> [L/kg]	8.1

<sup>a</sup> *i.v.* dose: 0.8 mg/kg, *p.o.* dose: 5.6 mg/kg

## Negative control

BI-9183, a structurally close analog, can be used as negative control.

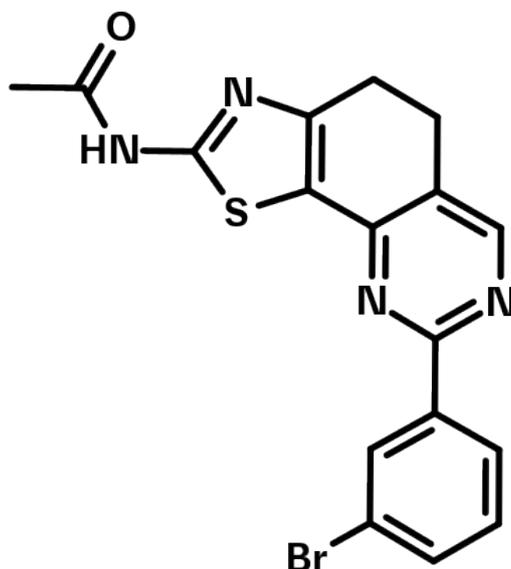


Figure 4: BI-9183 which serves as a negative control

## Selectivity

BI-9222 inhibited 2 out of 48 tested kinases (NEK2A, DYRK1A) by greater than 50% at a 10 $\mu$ M concentration and 1 (M2/H) out of 7 tested in SafetyScreen44<sup>TM</sup> by greater than 50% @ 1 $\mu$ M. Negative control BI-9283 inhibited 7 out of 44 tested kinases (DATRANS/HU, CCKA/H, 5HT2AH\_AGO, NEUP/H, DELTA2/H, A2A/H and 5HT2B/H AG) in SafetyScreen44<sup>TM</sup> by greater than 50% @ 10 $\mu$ M concentration.

Selectivity data available	BI-9222	BI-9183
SafetyScreen44™ with kind support of  eurofins	Yes	Yes
Invitrogen®	Yes	No
Dundee	Yes	No

## Supplementary data

2D structure files can be downloaded free of charge from [opnMe](#).

## References

1. Brandl T., Maier U., Hoffmann M., Scheuerer S., Joergensen A., Pautsch A., Breitfelder S., Grauert M., Hoenke C., Erb K., Pieper M., Pragst I. Thiazolyl-Dihydro-Quinazoline 2007 [WO 2007115929 A1](#).
2. Breitfelder S., Maier U., Brandl T., Hoenke C., Grauert M., Pautsch A., Hoffmann M., Kalkbrenner F., Joergensen A., Schaenzle G., Peters S., Buettner F., Bauer E. PI3-Kinasen. 2006 [WO 2006040279 A1](#).
3. Stark A.-K., Srisantharajah S., Hessel E. M., Okkenhaug K. Pi3k inhibitors in inflammation, autoimmunity and cancer *Curr Opin Pharmacol* **2015**, 23, 82–91. [DOI: 10.1016/j.coph.2015.05.017](#), [PubMed: 26093105](#).
4. Classification of PI3Ks into Classes I, II, and III: Fruman D. A., Chiu H., Hopkins B. D., Bagrodia S., Cantley L. C., Abraham R. T. The PI3K Pathway in Human Disease *Cell* **2017**, 170(4), 605–635. [DOI: 10.1016/j.cell.2017.07.029](#), [PubMed: 28802037](#).
5. Tissue Distribution and Functions of Class I Isoforms: Okkenhaug K., Vanhaesebroeck B. Pi3k in lymphocyte development, differentiation and activation *Nat Rev Immunol* **2003**, 3(4), 317–330. [DOI: 10.1038/nri1056](#), [PubMed: 12669022](#).
6. Overview of PI3Ks and Their Roles in Cellular Processes: Vanhaesebroeck B., Stephens L., Hawkins P. Pi3k signalling: The path to discovery and understanding *Nat Rev Mol Cell Biol* **2012**, 13(3), 195–203. [DOI: 10.1038/nrm3290](#), [PubMed: 22358332](#).
7. PI3K Delta and Gamma in Immune System Regulation: Rommel C., Camps M., Ji H. Pi3k delta and PI3K gamma: Partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Immunol* **2007**, 7(3), 191–201. [DOI: 10.1038/nri2036](#), [PubMed: 17290298](#).
8. We would like to acknowledge Susanne Müller-Knapp and Yufeng Pan (Structural Genomics Consortium Frankfurt) for their invaluable contribution in developing the assay and generating the scientific data that supported this study.