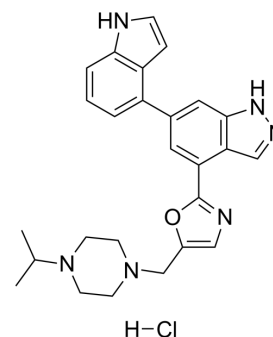


Nemiralisib hydrochloride

Cat. No.:	HY-19535
CAS No.:	1254036-77-5
Molecular Formula:	C ₂₆ H ₂₉ ClN ₆ O
Molecular Weight:	477
Target:	PI3K
Pathway:	PI3K/Akt/mTOR
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description	Nemiralisib hydrochloride (GSK2269557) is a potent and highly selective PI3K δ inhibitor with a pK _i of 9.9.			
IC₅₀ & Target	PI3K δ 9.9 (pKi)	PI3K γ 5.2 (pIC ₅₀)	PI3K α 5.3 (pIC ₅₀)	PI3K β 5.8 (pIC ₅₀)
In Vitro	Nemiralisib hydrochloride is highly selective for PI3K δ , with >1000-fold selectivity over the closely related isoforms PI3K α (pIC ₅₀ =5.3), PI3K β (pIC ₅₀ =5.8) and PI3K γ (pIC ₅₀ =5.2). Nemiralisib hydrochloride inhibits IFN γ in the peripheral blood mononuclear (PBMC) assay with an pIC ₅₀ of 9.7 ^[1] . MCE has not independently confirmed the accuracy of these methods. They are for reference only.			
In Vivo	To assess the suitability of the series for inhaled delivery clearance data in rat microsomes and subsequently in vivo pharmacokinetic data from Sprague Dawley male rats is obtained. Compounds (e.g., Nemiralisib hydrochloride) are administered by the oral or intravenous routes, at a dose level of 3 and 1mg/kg respectively (n=2 rats/route). Nemiralisib hydrochloride is active in a disease relevant brown norway rat acute OVA model of Type 2 helper T-cells (Th2)-driven lung inflammation ^[1] . MCE has not independently confirmed the accuracy of these methods. They are for reference only.			

PROTOCOL

Kinase Assay ^[1]

Inhibition of PI3Kinase enzymatic activity is determined using a homogeneous time resolved fluorescence (HTRF) kit assay format. Reactions are performed in assay buffer containing 50 mM HEPES, pH 7.0, 150 mM NaCl, 10 mM MgCl₂, <1 % cholate (w/v), <1 % CHAPS (w/v), 0.05 % sodium azide (w/v) and 1 mM DTT. Enzymes are preincubated with compound, serially diluted 4-fold in 100 % DMSO, for 15 mins prior to reaction initiation upon addition of substrate solution containing ATP at K_m for the specific isoform tested (PI3K α at 250 μ M, PI3K β at 400 μ M, PI3K δ at 80 μ M and PI3K γ at 15 μ M), PIP2 at either 5 μ M (PI3K δ) or 8 μ M (PI3K α , PI3K β and PI3K γ) and 10 nM biotin-PIP3. Assays are quenched after 60 mins by addition of a quench/detection solution prepared in 50 mM HEPES pH 7.0, 150 mM NaCl, <1 % cholate, <1 % Tween 20, 30 mM EDTA, 40 mM potassium fluoride and 1 mM DTT containing 16.5 nM GRP-1 PH domain, 8.3 nM Streptavidin-APC and 2 nM Europium-anti-GST, and are left for a further 60 mins in the dark to equilibrate prior to reading using a BMG RubyStar plate reader. Ratio data are normalised to high (no compound) and low (no enzyme) controls prior to fitting using a logistical four parameter equation to determine IC₅₀^[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Animal
Administration** ^[1]

Rats^[1]

In vivo pharmacokinetics is tested in Sprague Dawley male rats. Compounds (e.g., GSK2269557) are administered discretely by the oral or intravenous routes, at a dose level of 3 and 1 mg/kg respectively (n=2 rats/route). Compounds (e.g., GSK2269557) are formulated as a solution in DMSO:PEG200:water (5:45:50 v/v/v) at a dose volume of 6 (oral) and 2 (intravenous) mL/kg. All animals are serially bled from the tail vein and blood samples collected over a time-course of 0-7 h are submitted to LC-MS/MS analysis for the quantification of the parent compound. The main pharmacokinetic parameters are estimated by non-compartmental analysis.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Down K et al. Optimization of Novel Indazoles as Highly Potent and Selective Inhibitors of Phosphoinositide 3-Kinase δ for the Treatment of Respiratory Disease.

Caution: Product has not been fully validated for medical applications. For research use only.

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