Metabolic stability and biological activity of spiro[3.3]heptane analogues of Sonidegib

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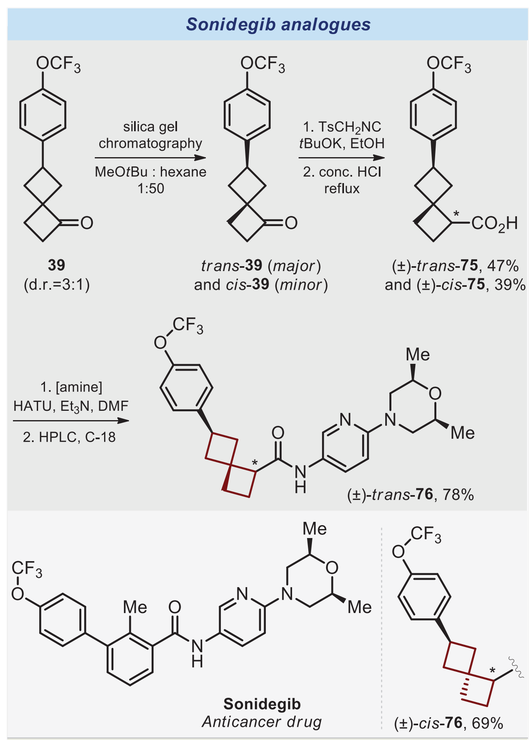
Introduction and Aim

The phenyl ring is the most popular ring in natural products, bioactive compounds, and drugs. It was shown that a replacement of the central phenyl ring in a bioactive compound can improve physicochemical properties and retain bioactivity. Spiro[3.3]heptane core is often used in medicinal chemistry as a unique 3D-shaped scaffold and has never been used as a benzene bioisostere before.
In this work, we have investigated how spiro[3.3]heptane incorporation into an anticancer drug Sonidegib effects its physicochemical properties and biological activity.

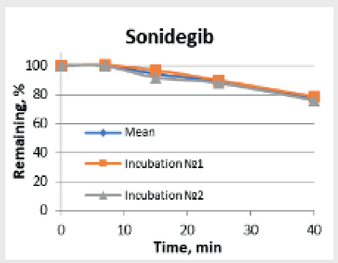


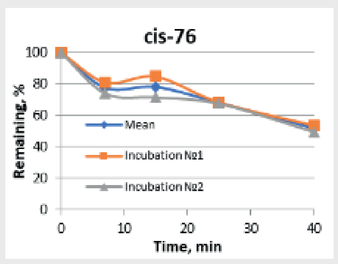
Scheme 1. Synthesis and properties of Sonidegib analogues - compounds (±) trans-76 and (±)-cis-76.

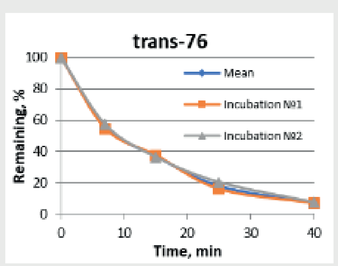
Methods

Synthesis of two saturated analogues of Sonidegib, ***trans-76*** and isomeric ***cis-76***, was commenced from ketone 39 as indicated in Scheme 1.
To determine physicochemical properties, the test and reference compounds were assessed for kinetic solubility in phosphate-buffered saline (PBS, pH 7.4); the experimental distribution coefficient (LogD) was determined in n-octanol – PBS.
Metabolic stability testing was performed using human liver microsomes (HLM) at five time points over 40 minutes using HPLC-MS/MS; half-life (t1/2), and intrinsic clearance (Clint) were calculated. The permeability of the compounds was tested in the bidirectional Caco-2 assay.
To compare the biological activity, the experimental inhibition of the Hedgehog signaling pathway in Gli-Luc reporter NIH3T3 cell line was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience, #60690-1). The CellTiter-Glo Luminescent Cell Viability Assay was used to determine the number of viable cells in culture by quantifying ATP, which indicates the presence of metabolically active cells after treatment for 25h.

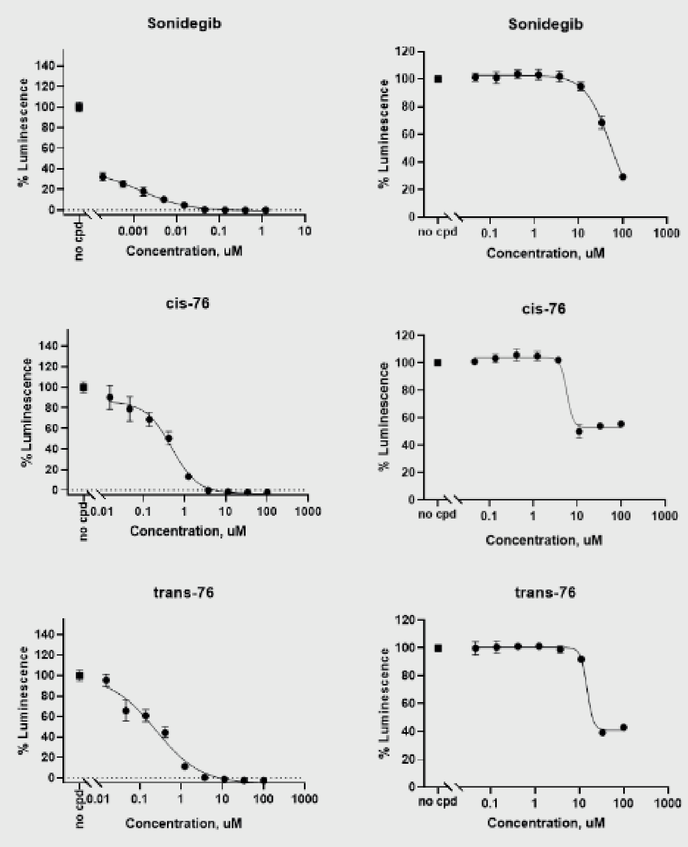
Results





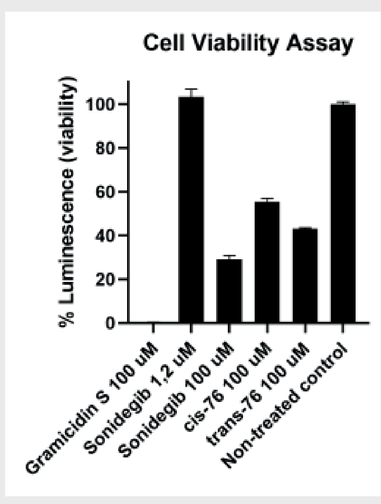


Stability data for Sonidegib, cis-76 and trans-76 analogues in human liver microsomes.



Inhibition of the Hedgehog signalling pathway in cell-based Gli-Luc reporter system (0 % luminescence - cells without activation and 100 % luminescence – activated cells (no compound).

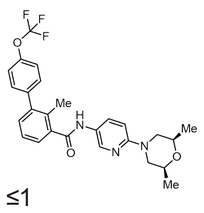
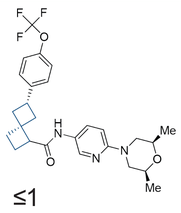
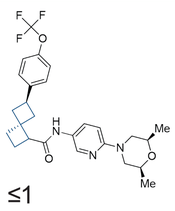
The viability (% luminescence) of Gli Reporter – NIH3T3 cells after treatment with Sonidegib, cis-76 and trans-76 for 25h in CellTiter-Glo Luminescent Cell Viability Assay.



Cells viability (%) of Gli Reporter – NIH3T3 cells after treatment with compounds for 25h. The data were presented as mean ± SD (n=3).

Replacement of the meta-substituted phenyl ring in Sonidegib did not affect its water solubility and only slightly decreased calculated lipophilicity and chemical stability values. However, this structural modification significantly influenced the metabolic stability of the drug (see Table 1).
Compounds trans-76 and cis-76 were found to be two orders of magnitude less active in Hedgehog signaling pathway assay, compared to the original drug Sonidegib. Nevertheless, both saturated analogues demonstrated relatively high micromolar inhibition. Both cis-76 and trans-76 exhibited higher cytotoxicity on NIH3T3 cells compared to Sonidegib in Luminescent Cell Viability Assay. Tested analogues fully inhibited the Hedgehog signaling pathway at concentrations at least 3-10 times lower than needed for partial cytotoxic effect.

Table 1. Experimental data for Sonidegib, ***trans-76*** and ***cis-76*** analogues

Compound	Sonidegib	**cis-76** analogue	**trans-76** analogue
Structure			
PBS solubility, pH 7.4, μM	≤1	≤1	≤1
LogD, pH 7.4	3.68 ± 0.06	3.61 ± 0.003	3.55 ± 0.2
CL_{int}, ul/min/mg, HLM	18	36	156
$t_{1/2}$, min, HLM	92.7	46.7	10.7
Caco-2 permeability, P_{app} (AB) 10^{-6} cm/s	2.8 ± 0.1	2.7 ± 0.4	2.3 ± 0.3
Caco-2 efflux ratio (P_{app}B-A/P_{app}A-B)	0.3	0.4	0.4
Hedgehog signaling inhibition (IC50, uM)	0.001462	0.476	0.242
NIH3T3 cells viability (IC50, uM)	53.5	5.8	14,9

Conclusions

In this study, we showed that the incorporation of spiro[3.3]heptane instead of the meta-substituted phenyl ring of Sonidegib retain cytotoxici but decreases the metabolic stability and biological activity (inhibition of the Hedgehog signaling pathway) of the drug.

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References

1. R. D. Taylor, M. MacCoss, A. D. G. Lawson. Rings in Drugs. *J. Med. Chem.* **2014**, *57*, 5845-5859.
2. Prysiazhniuk K, Datsenko O, *et al. Spiro[3.3]heptane as a Non-collinear Benzene Bioisostere. ChemRxiv. Cambridge: Cambridge Open Engage*; **2023**.