Metabolic stability of Sonidegib saturated analogues

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Introduction and Aim

Sonidegib is a small molecule that acts as a selective smoothened (SMO) receptor antagonist. This drug is characterized by low solubility and high LogD value. Thus, the search for new bioisosteric analogs that have improved ADME properties is of interest. In this study, we compared the metabolic stability of *cis*- and *trans*- spiro[3.3]heptane (1, 2), and 7-oxa-azaspiro[3.5]nonane (3, 4) analogs of Sonidegib (Fig. 1).

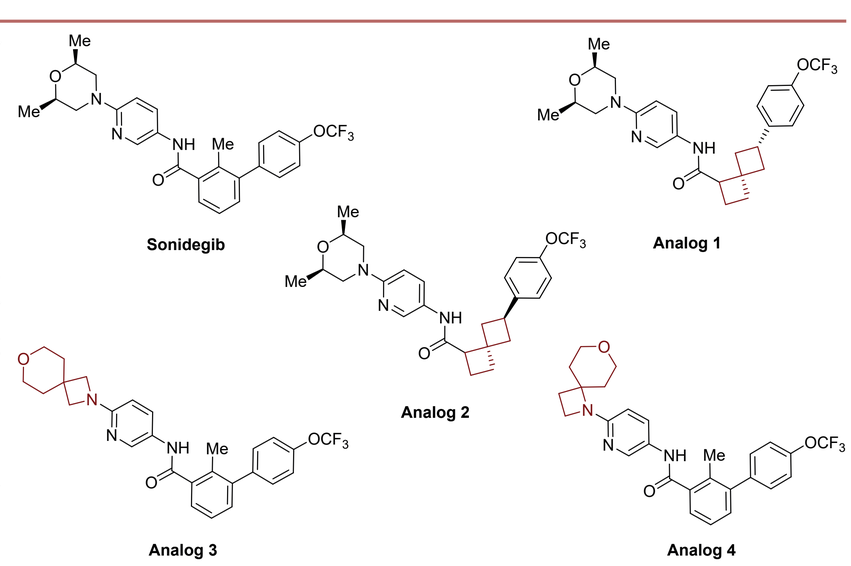

Figure 1. Structure of Sonidegib and analogues 1-4.

Methods

Metabolic stability of the Sonidegib and analogues was evaluated using human liver microsomes (HLM) and cryopreserved human hepatocytes (HS). The elimination constant (kel), half-life (t1/2), and intrinsic clearance (Clint) were determined. Cytochromes P450 Inhibition Assay (panel of 5: CYP3A4, 2D6, 1A2, 2C19, and 2C9) was performed in HLM. Samples were analyzed by LC-MS/MS.

Metabolites of the analogs were identified based on high-resolution mass spectrometry (HRMS) with AutoMS/MS acquisition mode obtained using the Agilent 6550 iFunnel Q-TOF system.

Kinetic solubility of the compounds in phosphate-buffered saline (PBS, pH=7.4; 2% final DMSO), and $LogD_{7.4}$ in *n*-octanol/PBS were experimentally measured. Plasma protein binding was tested by equilibrium dialysis with 100% and 10% human plasma. A unidirectional permeability assay in Caco-2 cells was performed using a standard protocol.

Results

Table 1. ADME parameters of Sonidegib analogues

Name	CLint, µL/min/mg protein (HLM)	Predicted *in vivo* CLint, mL/min/kg	$T_{1/2}$, min (HLM)	Clint, µl/min/ 10^6 cells (HS)	Predicted *in vivo* CLint, mL/min/kg	$T_{1/2}$, min (HLM)	Solubility in PBS (pH=7.4), µM	LogD (pH=7.4)	PPB (100% human plasma), %	PPB (10% human plasma), %	Caco-2 Papp (AB), 10^{-6} cm/s
Sonidegib	16	8.49	104	15.8	13.7	87.9	4.4	>4.5	99	99	2.8
Analog 1	**36**	12.63	46.7	15.1	13.5	91.8	<1	3.61	99.7	99.5	2.7
Analog 2	**156**	18.04	11	39.3	16.9	35	<1	3.55	99.7	99	2.3
Analog 3	**23**	10.35	72	23	15.2	60	9	3.9	99.7	96	-
Analog 4	**106**	17.01	16	6.6	9.6	210	26	>4.5	99.8	98	-

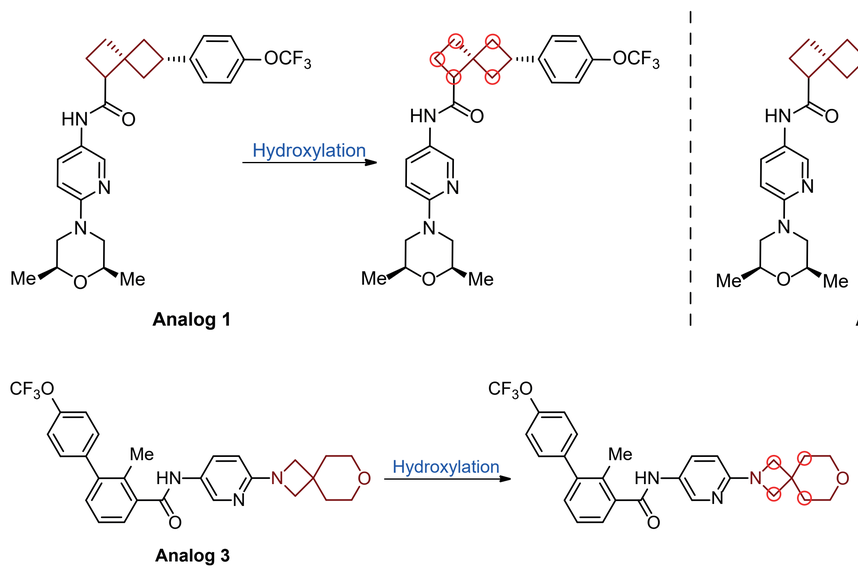

Figure 2. Main metabolites of analogues 1-4 in HLM.

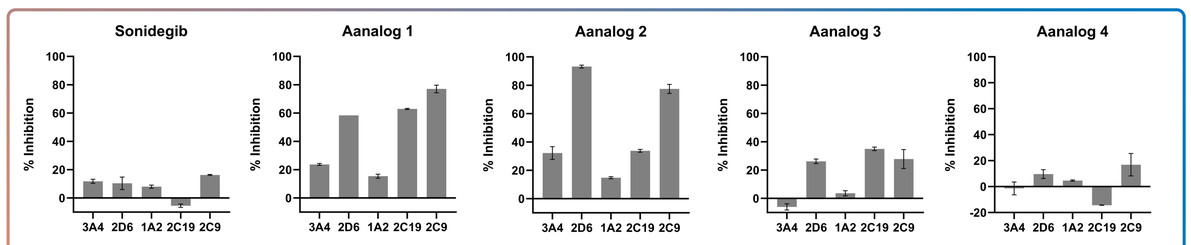

Figure 3. Bar graphs representing CYP450 inhibition profile for Sonidegib and analogues at the concentration of 10 µM. The data are represented as average inhibition ± SE

Replacing the *meta*-substituted phenyl ring with spiro[3.3]heptane (analogs **1** and **2**) in the structure of Sonidegib had minimal impact on logD, plasma protein binding, solubility in PBS, and permeability in Caco-2. However, the metabolic stability of analogs was notably lower and differed significantly between *trans*- and *cis*-isomers.

Replacement of the 2,6-dimethylmorpholine with 7-oxa-2-azaspiro[3.5]nonane and 7-oxa-1-azaspiro[3.5]nonane (analogs **3** and **4**) resulted in slightly increased solubility but decreased metabolic stability for analog **4**.

Metabolite profiling data showed hydroxylated product as the main metabolite for the analogs. For *cis*-isomer two-step hydroxylation in HLM was observed. It should be noted that oxidation primarily occurred in the bioisosteric fragments that have been substituted. Analogs **3** and **4**, similarly to Sonidegib, exhibited no inhibition of major CYPs, while analogs **1** and **2** demonstrated substantial inhibition of CYP2D6, 2C9 isoforms, and analog **1** also inhibited CYP2C19.

Conclusions

These data indicate that *cis*- and *trans*- spiro[3.3]heptane, and 7-oxa-azaspiro[3.5]nonanes can be useful structural elements in drug discovery projects. Metabolic stability of the obtained molecules differ significantly among *trans*- and *cis*-isomers or 7-oxa-2-azaspiro[3.5]nonane and 7-oxa-1-azaspiro[3.5]nonane analogs warranting metabolic stability evaluation after inserting them into the structure of drug candidates.

Further testing of specific enzymes involved in the metabolism of these compounds is to be performed.

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