Discovery and Optimization of 2-Arylquinazolin-4-ones into a Potent and Selective Tankyrase Inhibitor Modulating Wnt Pathway Activity

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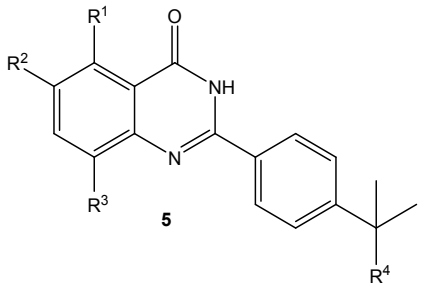
Supplementary information

Table 1. Assay data of the best compounds with SD / SEM for the values



Cpd	R^1	R^2	R^3	R^4	TNKS1	TNKS2	PARP1	DLD1 axin2
					IC_{50} (nM)[a]			EC_{50} (nM)[b]
5d	H	F	H	OH	12.5 ± 6	10.5 ± 4	349 ± 50	614 ± 253
5j	F	F	H	OH	7 ± 0.6	7.5 ± 0.9	512 ± 87	456 ± 107
5k	H	F	F	OH	7 ± 1.5	9.2 ± 0.8	782 ± 44	319 ± 104
5m	H	F	CH_3	OH	0.8 ± 0.1	0.5 ±0.3	60 ± 2	37 ± 7
XAV-939					12 ± 4	4 ± 1	155 ± 5	1011 ± 519

[a]All values are based at least on duplicates.

[b]All values are based at least on biological triplicates.

Figure 1. Biochemical assay IC_{50} determination for **5k**

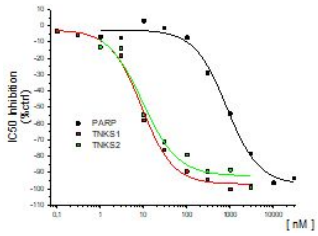
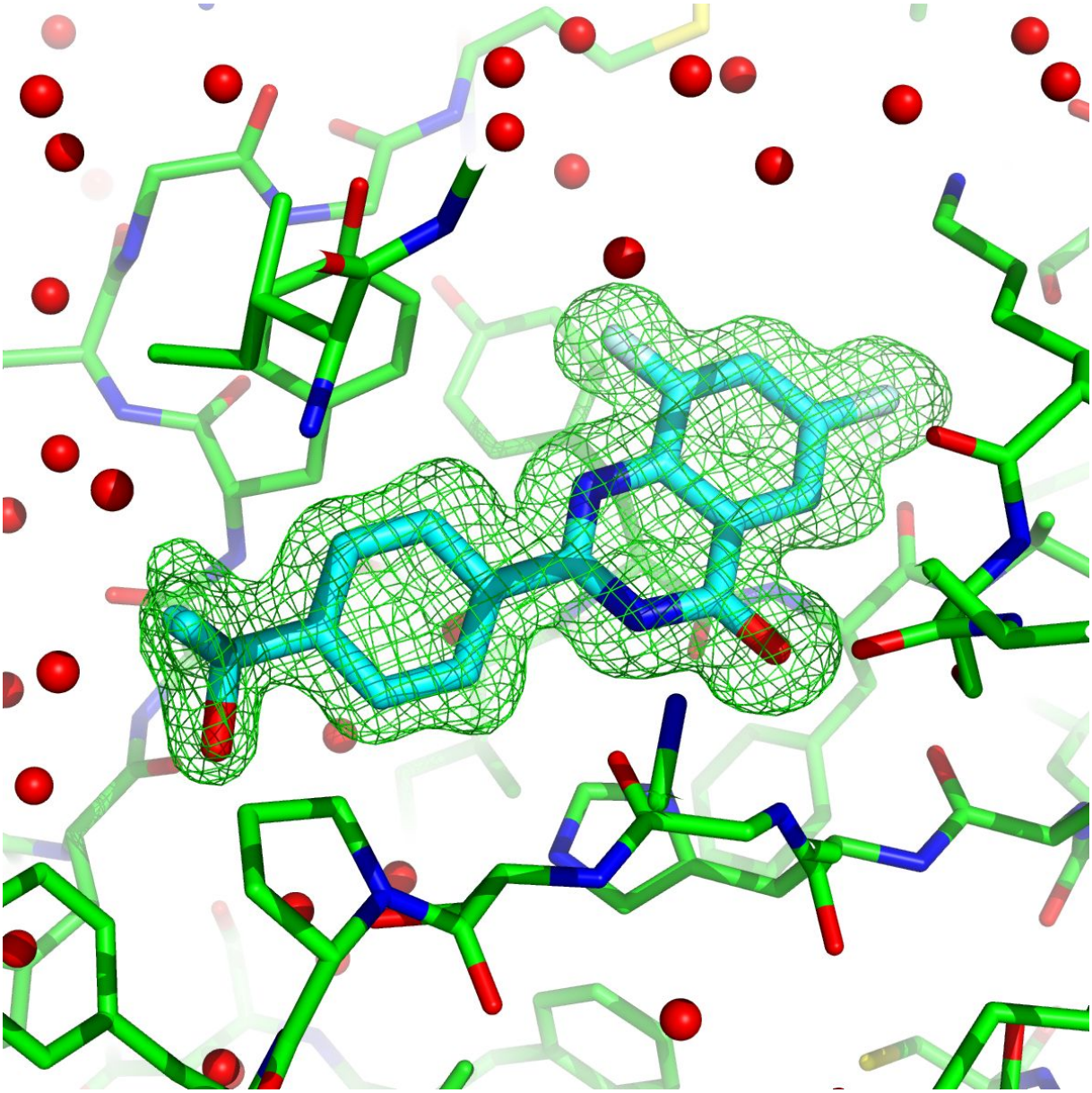


Figure 2. Ligand Fo-Fc omit map at 2.5 σ cutoff



Determination of microsomal stability (intrinsic clearance)

A microsomal stability assay is used to measure *in vitro* clearance (Clint). The assay involves measuring the rate of disappearance of a compound due to its intrinsic attitude to be metabolized ("intrinsic" meaning that the disappearance is not affected by other properties like permeability, binding etc. that play a role when quantifying *in vivo* clearance). The microsomal stability (intrinsic clearance, Clint) and thus metabolic stability is generally given as µl/min/mg protein. It can be visualized as the volume of solution that 1 mg of microsomes is able to clear of the compound in one minute.

Instrumentation

A Tecan Genesis workstation (RSP 150/8) was used for to perform the microsomal incubations. Analysis was carried out using a Waters ACQUITY UPLC system coupled to an ABSciex API3000 mass spectrometer. Data analysis was performed using Assay Explorer (Symyx).

UPLC conditions

Column: Acquity UPLC BEH C18, 2.1 x 50mm, 1.7 µm (Waters)

Mobile phases: A = 0.1 % formic acid in water; B = acetonitrile

Gradient Time	% A	% B
initial	90	10
0.47	5	95
0.65	5	95
0.66	90	10

Flow rate: 0.750 mL/min; Detection: ESI, MRM; Injection: 10 µL; Column temperature: 50°C

Chemicals

- Potassium phosphate buffer: 0.05 M potassium phosphate buffer pH 7.4 containing 1 mM $MgCI_2$
- NADPH (nicotinamide adenine dinucleotide phosphate): 22.5 mg NADPH-Na_4 in 1.8 ml potassium phosphate buffer
- Acetonitrile: 50 Vol% acetonitrile (1 volume acetonitrile, 1 volume water)
- DMSO: 20 Vol% DMSO in water
- Stock solution of 20 mg/ml human or mouse liver microsomes (protein)/ml in phosphate buffer
- Stock solution of 10 mM compound in 100% DMSO

Determination of Kv11.1 (hERG) ion channel activity

In this assay, a potential in vitro effect of test compounds on the Kv11.1 (hERG) ion channel current is investigated which mediates the rapidly activating, delayed rectifier cardiac potassium current (IKr). The assay is performed with a stable Kv11.1 (hERG) transfected human embryonic kidney cell line (HEK293) by whole cell patch clamp technique carried out at room temperature. The Kv11.1 (hERG) ion channel blocker quinidine is used as reference compound. The effects of the test compounds and quinidine are normalized to the corresponding vehicle control. The whole cell recordings are carried out with an automated patch clamp device. (PatchlinerTM, Nanion Technologies, Munich). Hereby the Patch Clamp measurements run on silicate-coated chips with a hole of a defined diameter. Solutions, cell suspension and compounds are applied by a Teflon-laminated pipette needle through microfluidic silicate-laminated channels. Commercial patch clamp amplifiers (EPC10, HEKA Elektronik Dr. Schulze GmbH, Germany) are used for the patch clamp recordings.

HEK293 cells stably expressing the hERG gene are held at -80 mV. Steady-state inhibition of Kv11.1 (hERG) potassium current due to test/reference compound application is measured using a pulse pattern with fixed amplitudes: 51 ms / -80 mV, 500 ms / +40 mV, 500 ms / -40 mV, 200 ms / -80 mV. The hERG-specific voltage protocol is repeated at 10 s intervals. The leak current is subtracted by a P4 leak subtraction.

Cells are resuspended in extracellular patch clamp solution (EC) and applied into the chip. After trapping the cell, the EC is exchanged by seal enhancer solution (SE) to improve the sealing procedure. When the whole cell configuration is attained, seal enhancer is washed out by the application of EC. The recording is started in EC for 1.5 min. Afterwards DMSO (vehicle control, 0.1% DMSO) is applied and the control current is measured for 3 min. Following control steady-state current, test compound is applied twice at the same concentration and the tail current is measured for 3.5 min each. For the determination of a concentration-relationship, the test compound is applied as a cumulative concentration-response curve and each concentration is measured for 5 min. The reference compound quinidine is treated in the same way.

The effect on Kv11.1 (hERG) ion channel activity is judged from the tail current amplitude monitored at -40 mV (current of interest, COI). Results are calculated from the last recorded current traces. Changes in Kv11.1 (hERG) ion channel activity between control value, defined as 100 % Kv11.1 (hERG) ion channel activity, application of test compound and application of quinidine is reported as percent change of control value of COI.

An aliquot of test compound is collected for concentration verification during the recording. The sample is immediately measured by HPLC and the final compound concentration within the assay is calculated according to a calibration curve.

Caco-2 Papp determination

Caco-2 cells were maintained in DMEM in an atmosphere of 5% CO_2. For transport experiments, 0.125×10^6 cells/well were seeded on polycarbonate filter inserts and allowed to grow and differentiate for 21 days before the cell monolayers were used for experiments. Drug transport experiments were carried out using a cocktail approach in a 4-dimensional setting. Apparent permeability coefficients were determined for A > B and B > A directions with and without the presence of cyclosporine A as a transporter inhibitor. Up to five test items and reference compounds were dissolved in Hank's balanced salt solution at pH 7.4 to yield a final concentration of 1 μM. The assays were performed in HBSS containing 25 mM HEPES (pH 7.4) in an atmosphere of 5% CO_2 at 37 °C. Prior to the study, the monolayers were washed in prewarmed HBSS. At the start of the experiments, prewarmed HBSS containing the test items was added to the donor side of the monolayer and HBSS without test items was added to the receiver side. The plates were shaken at 150 rpm at 37 °C during the experiment. After 2 h, the transwell insert containing the monolayer was carefully removed and placed in a new plate, and aliquots of both the receiver and donor sides were taken and diluted with an equal volume of ACN containing the internal standard. The mixture was centrifuged, and the supernatant analyzed by LC–MS/MS. The apparent permeability coefficients (Papp) were calculated using the formula $Papp = (Vrec/A \times C_0donor) \times dCrec/dt \times 10^6$ with dCrev/dt being the change in concentration in the receiver compartment with time, Vrec the volume of the sample in the receiver compartment, C_0donor the concentration in the donor compartment at time 0, and A the area of the compartment with the cells.