RESOLUTE

Functional Assays for Solute Carrier Transporters

Impedance-based transport assay for SLC22A3 using HEK293 JumpIn SLC22A3 OE cells

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Assay description

xCELLigence is a label-free, impedance-based, real-time and non-invasive assay with which whole cell responses can be detected upon cell stimulation. Cells are seeded in wells containing gold-plated electrodes. Changes in cell properties such as proliferation, viability, morphology and adhesion can be accurately measured with xCELLigence. Read-outs of this assay can be potency values (EC_{50}, IC_{50}), which are calculated using either the RTCA software or GraphPad Prism.
HEK293-JumpIn-OCT3 is an adherent human embryonic kidney cell line generated by RESOLUTE, which is transfected with the human Organic Cation Transporter 3 (OCT3) gene (SLC22A3) in a doxycycline-inducible expression system. This assay can be used to determine the potency (EC_{50}) of MPP^+ uptake by JumpIn-OCT3 cells. Furthermore, this assay can be used to study OCT3 inhibitors and determine their pIC_{50}.

Assay protocol

Label-free cellular impedance measurements were performed using an xCELLigence Real-Time Cell Analyzer (RTCA) SP system (ACEA biosciences, San Diego, CA, USA. The xCELLigence biosensor detects changes in cell morphology, adhesion, and proliferation through impedance of the electron flow. Baseline impedance of the E-plate was determined in 40 µL culture medium with or without 1 µg/mL dox to induce OCT expression. HEK293-JI-OCT3 (60,000 cells/well) were seeded in 50 µL culture medium and cells were allowed to settle in the E-plate for 30 min at RT. Next, the E-plate was placed in the xCELLigence station at 37 °C, 5% CO2, and cell growth was monitored every 15 min for 22 h to reach confluency. Hereafter, cells were treated with 5 µL inhibitor at increasing concentrations or vehicle (i.e., PBS supplemented with 0.1% DMSO), which were added to the E-plate utilizing a VIAFLO 96 handheld electronic 96 channel pipette (Integra Bioscience, Tokyo, Japan). Inhibitor responses were monitored for 1 h at 1 min intervals. Transporter activity was subsequently induced by addition of 5 µL pre-warmed MPP^+ (substrate) or PBS (vehicle) using the VIAFLO 96. For MPP^+ dose-response curves cells were stimulated with MPP^+ concentrations ranging from 10^{-3}M to 10^{-7} M. For inhibitor studies cells were stimulated with a submaximal concentration of MPP^+, which corresponds to 100 µM for OCT3. Changes in cell morphology were monitored every 15 s for 30 min, followed by every minute for 30 min and finally every 5 min for 1 h. For cytotoxicity experiments, this measurement schedule was extended by monitoring every 15 min up to a total of 52 h.

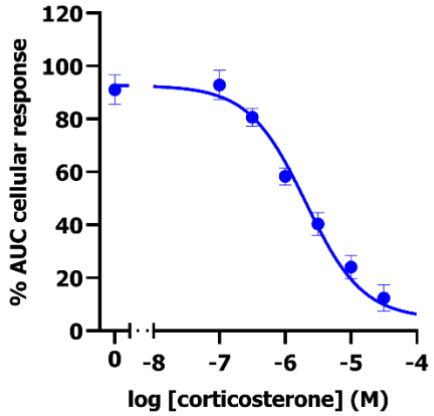
Data Analysis: For xCELLigence experiments, RTCA software v2.0 (ACEA Biosciences) was used to normalize the CI to the time-point prior to substrate addition. Next, to correct for sub-strate-independent effects the vehicle response was removed from all data points. For in-hibitor experiments, data were also corrected for inhibitor-induced effects when the inhib-itor produced a substantial cellular response in absence of MPP^+. Vehicle and inhibitor corrected data was analyzed by calculating the net AUC over the first 60 min for OCT1 and OCT3 or 120 min for OCT2 to reach a maximal peak response for all concentrations after stimulation with MPP^+. The 1 mM MPP^+-induced response (AUC) in presence of dox was taken as the maximum response (E_{max}) and set to 100%. Notably, conditions in which the MPP^+ response in presence of inhibitor after correction for vehicle and inhibitor-induced effects obtained a negative net AUC were omitted from analyses as this could indicate inhibitor-induced toxic effects, as observed for Nilotinib. Concentration-response curves were fitted using a non-linear regression three parameter response model to obtain pEC_{50} and pIC_{50} values.

Additional information

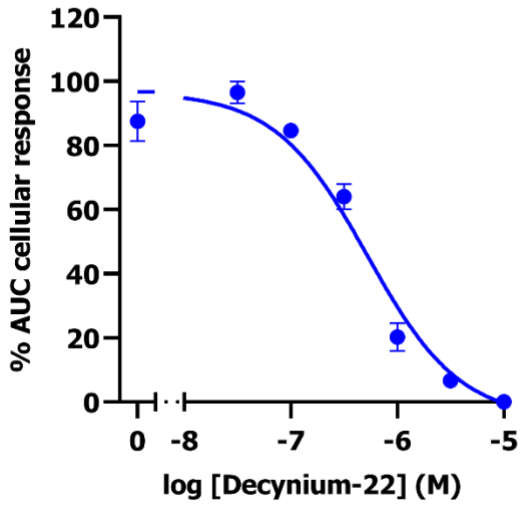
Target data

SLC	SLC22A3
Synonyms	organic cation transporter 3 (OCT3), Extraneuronal monoamine transporter (EMT)
SLC sub-family	Solute Carrier Family 22 (organic cation transporters)
UniProt ID	Canonical form (O75751-1)
RESOLUTE Cell ID	CE028P-M (HEK-SLC22A3-WTOE-p1)

Assay data

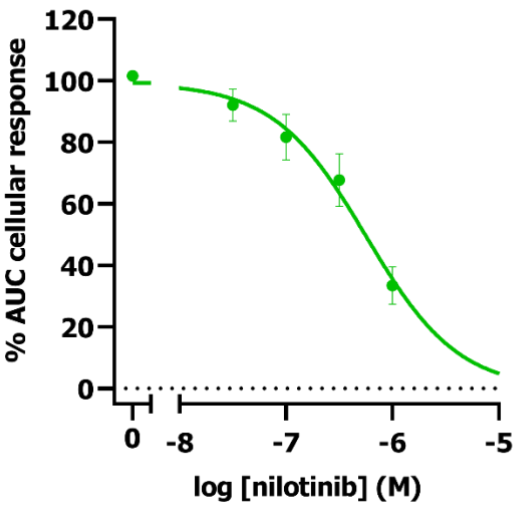
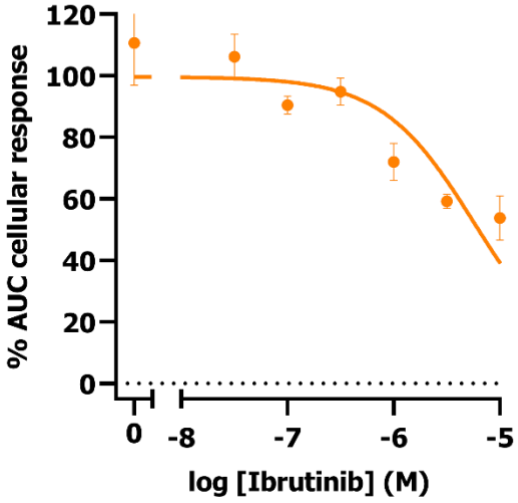
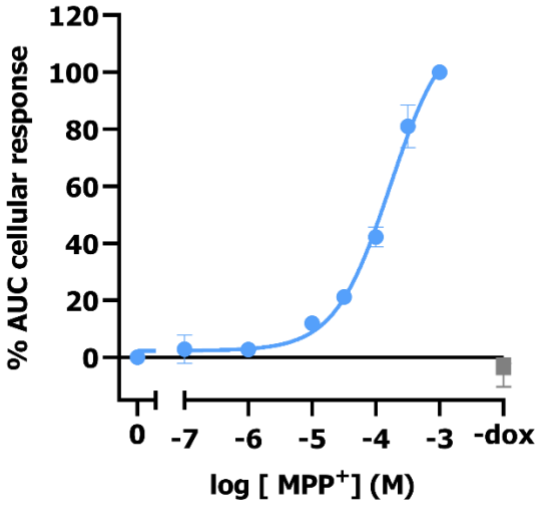


Compound name (1)	corticosterone
PubChem CID	5753
Vendor (catalogue #)	Sigmaaldrich: 27840
Mode of action	Inhibitor
Standard value type (i.e EC50, PoC, etc)	IC_{50} = 1.6 µM



Compound name (2)	Decynium-22
PubChem CID	5484462
Vendor (catalogue #)	Sigmaaldrich: #323764
Mode of action	Inhibitor
Standard value type (i.e EC50, PoC, etc)	IC_{50} = 0.4 µM

Compound name (3)	Nilotinib

	PubChem CID	
	Vendor (catalogue #)	Medchemexpress: #HY-10159
	Mode of action	Inhibitor
	Standard value type (i.e EC50, PoC, etc)	IC_{50} = 0.5 µM
	**Compound name (4)**	Ibrutinib
	PubChem CID	24821094
	Vendor (catalogue #)	Medchemexpress: #HY-10997
	Mode of action	Inhibitor
	Standard value type (i.e EC50, PoC, etc)	IC_{50} = 7.9 µM
	**Compound name (5)**	MPP^+ iodide
	PubChem CID	10924457
	Vendor (catalogue #)	Sigma #D048
	Mode of action	substrate
	Standard value type (i.e EC50, PoC, etc)	EC_{50} =199 µM

Discussion

Add additional information related to Assay Data

Cross references

- Report at Zenodo.
- https://doi.org/10.3390/ijms23031203