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DILIsym® User Training – Parameterizing a Saturable Electron Transport Chain Inhibitor

December 2017

DILIsym® Development Team

Goal for This Training Session

Participants should understand the following general concepts:

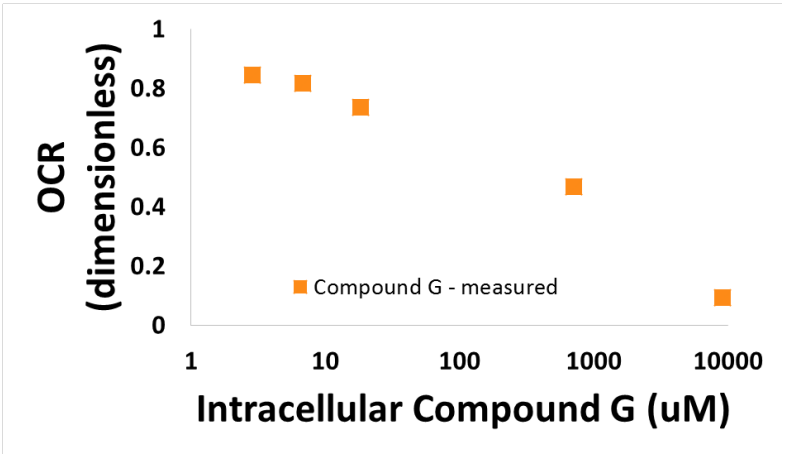
- Notable updates to toxicity parameters in DILIsym v7A as compared to v5A
- Methods used to parameterize and to simulate mitochondrial dysfunction in DILIsym
- DILIsym team recommendations for the use of saturable ETC inhibition

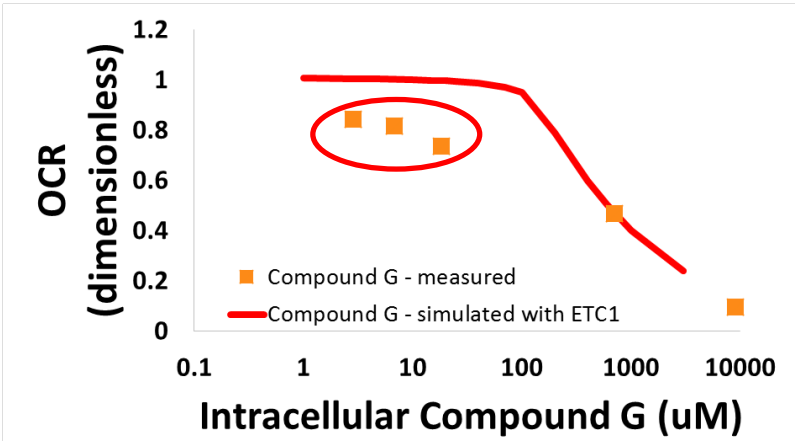
Toxicity Pathways Have Been Added for DILIsym v6A

- Several saturable toxicity pathways have been added for DILIsym v6A
  - Two saturable electron transport chain inhibition pathways (ETC-3 and ETC-4)
  - Two saturable reactive oxygen species production pathways (ROS-4 and ROS-5)
- Parameterization of these pathways has not been covered in a previous training session
  - Video instructions for parameterization of standard ETC inhibition and ROS production pathways are on the DILIsym website
  - Example of parameterization for saturable ETC inhibition follows

Saturable ETC Inhibition Can Be Used In Combination With Non-Saturable ETC Inhibition

- Some compounds exhibit OCR decline that appears to occur slowly at increasing concentrations
  - Compound G example – ETC inhibition clearly goes to completion but data cannot be fit using normal ETC-1 alone
- These compounds can be represented as a combination of both saturable ETC inhibition (ETC-3 or 4) and traditional, non-saturable inhibition (ETC-1 or 2)
- Parameterization of such compounds can be a challenge due to lack of exemplar compound for saturable ETC inhibition





Preclinical Data

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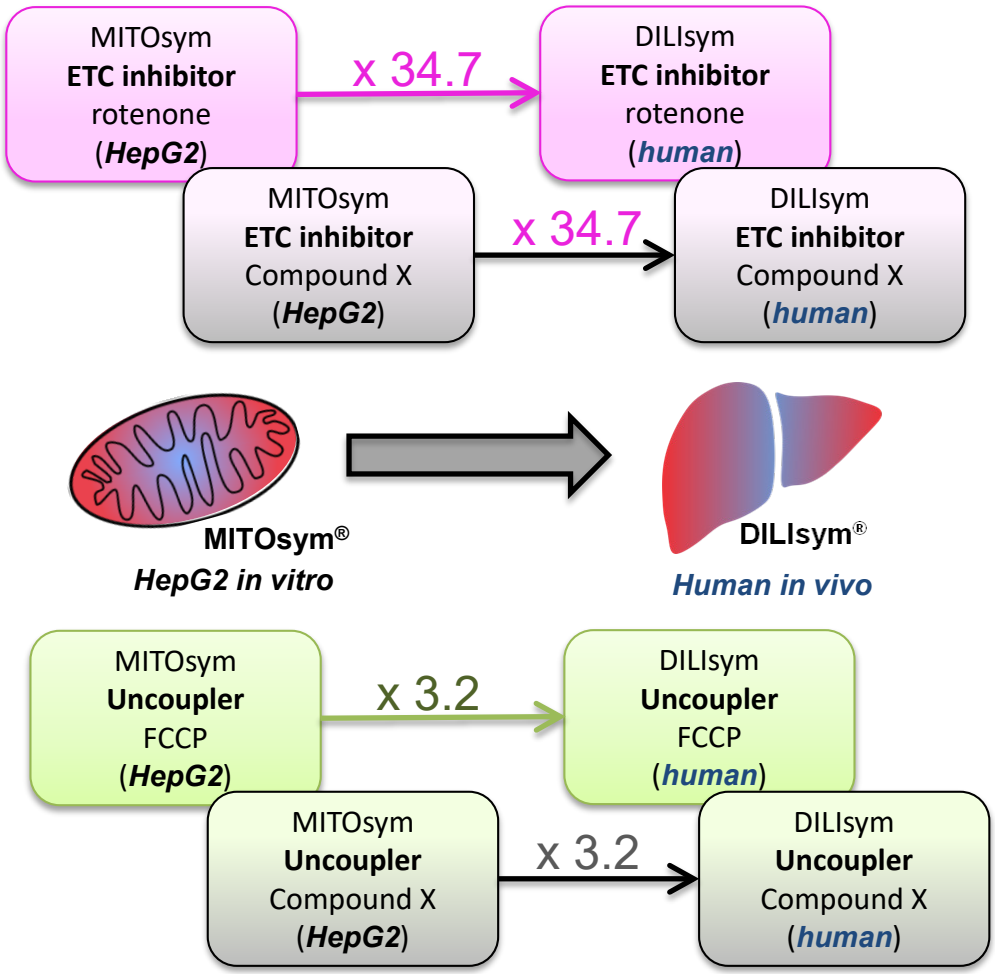
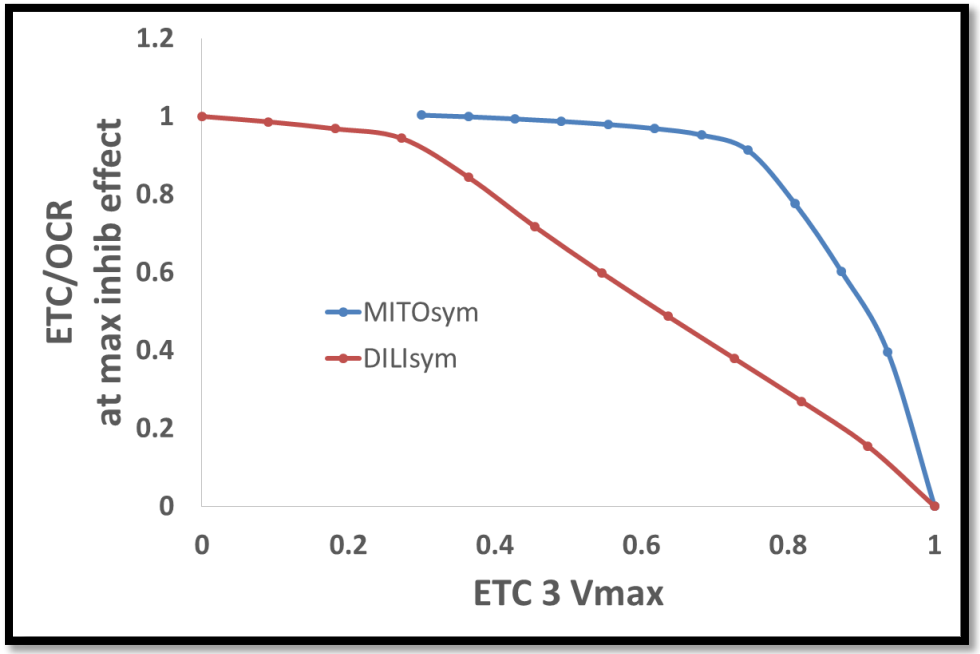
ETC Inhibition Coefficient Values Are Designed To Be Translated from MITOsym

- Traditionally, ETC and uncoupler coefficient values are calculated in MITOsym and translated into DILIsym using coefficients determined by experience with exemplar compounds
  - Approximates difference between *in vitro* and *in vivo* environments
  - Rotenone is the exemplar for ETC inhibitors
- Saturable ETC inhibition does not have an exemplar compound
- Coefficient can be translated directly by assuming similarity with rotenone, but what about V_{max} value?
  - Unclear what the difference between V_{max} values should be between *in vitro* and *in vivo* situations



Saturable ETC Inhibition V_{max} Values Do Not Directly Translate from MITOsym to DILIsym

- MITOsym and DILIsym have different behavior when saturable ETC inhibition is implemented
  - When concentration of inhibitor is much larger than the inhibition coefficient (i.e. inhibition is working at maximum capacity), MITOsym responds less than DILIsym
  - Unclear what is driving the difference in responses
- Direct translation of V_{max} from MITOsym to DILIsym will lead to substantially larger responses in DILIsym
  - This is plausible but unrealistic in the view of the DILIsym team

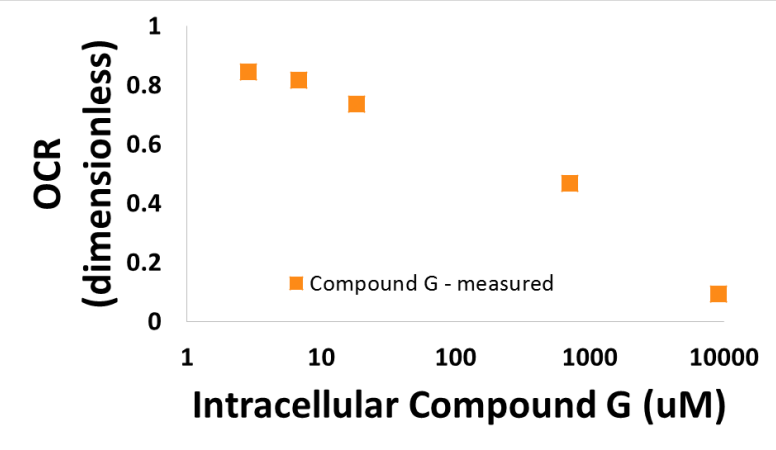


Recommended Method for Parameterization of Saturable ETC Inhibitors

- Electron transport chain constant (Km) values have a known exemplar and the conversion from MITOsym to DILIsym is well understood
- The conversion for saturable ETC V_{max} values does not have an exemplar; the differences between *in vitro* and *in vivo* are less certain
- When using saturable ETC inhibition to reproduce slowly declining OCR activity, we recommend the following:
  1. The curve should be reproduced in MITOsym and ETC3/4 (and ETC1/2, if using) coefficients should be translated over using the rotenone-derived conversion factor
  2. The curve should also be reproduced in DILIsym (with *in vitro* like setup) and the V_{max} from that simulation should be used in place of the V_{max} from MITOsym
  3. **The resulting parameters should be a hybrid of the coefficient from MITOsym and the V_{max} from DILIsym**

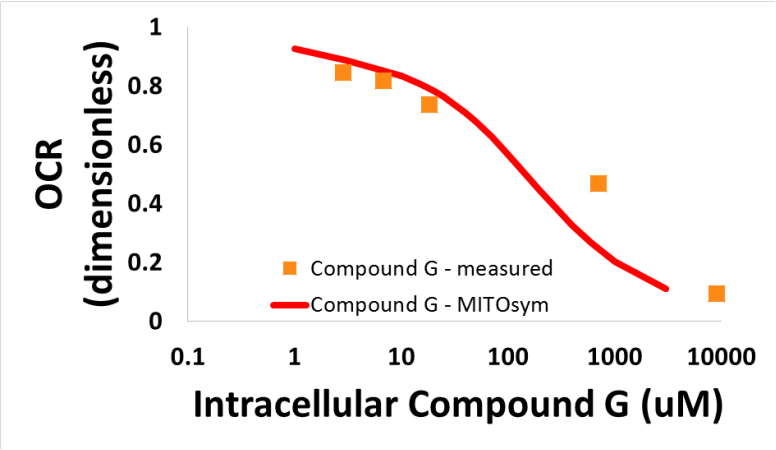
Parameterization Example: Combination of Saturable and Non-Saturable ETC Inhibition

- Compound G: demonstrates a steady decrease in OCR over time in HepG2 cells
  - Cannot be represented with ETC-1 alone



Parameterization Example: Combination of Saturable and Non-Saturable ETC Inhibition

- Compound G: demonstrates a steady decrease in OCR over time in HepG2 cells
  - Cannot be represented with ETC-1 alone
- Compound G fit in MITOsym yields a set of DILIsym parameters after translation



DILIsym Parameter	MITOsym parameter values	MITOsym parameters after translation		Units
Coefficient for ETC Inhibition 1	100	3470		μM
Coefficient for ETC Inhibition 3	0.054	1.89		μM
Max inhibitory effect for ETC inhibition 3	0.77	0.77		dimensionless

Preclinical Data and Simulation Results

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- Compound G: demonstrates a steady decrease in OCR over time in HepG2 cells
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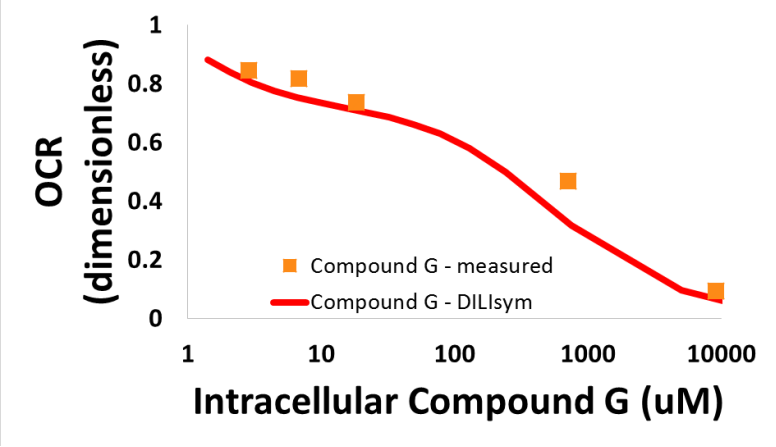
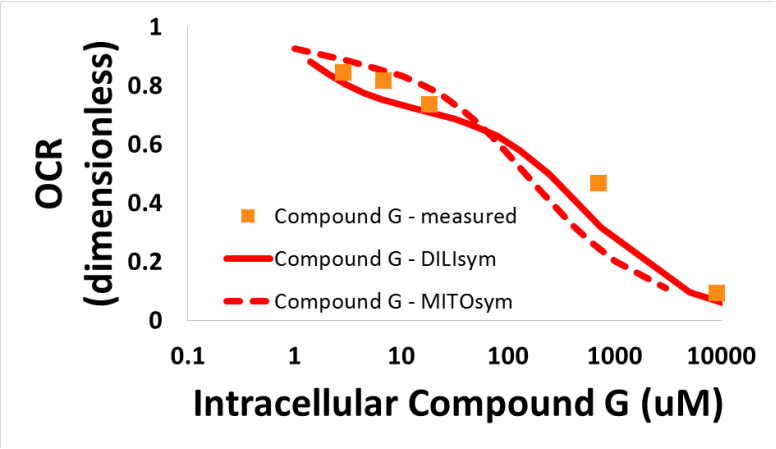
- Compound G fit in MITOsym yields a set of DILIsym parameters after translation
- DILIsym fit also yields set of parameters



DILIsym Parameter	MITOsym parameter values	MITOsym parameters after translation	DILIsym parameter values	Units
Coefficient for ETC Inhibition 1	100	3470	510	μM
Coefficient for ETC Inhibition 3	0.054	1.89	0.5	μM
Max inhibitory effect for ETC inhibition 3	0.77	0.77	0.45	dimensionless

Parameterization Example: Combination of Saturable and Non-Saturable ETC Inhibition

- Compound G: demonstrates a steady decrease in OCR over time in HepG2 cells
  - Cannot be represented with ETC-1 alone
- Compound G fit in MITOsym yields a set of DILIsym parameters after translation
- DILIsym fit also yields set of parameters
- **Parameter set to be used is combination of the two parameter sets**
  - **Coefficients from MITOsym (with translation factors applied)**
  - **V_{max} from DILIsym (no translation factors required)**



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