ESHELMAN SCHOOL
OF PHARMACY

NSP13 helicase unwinding assay and ATPase screening results

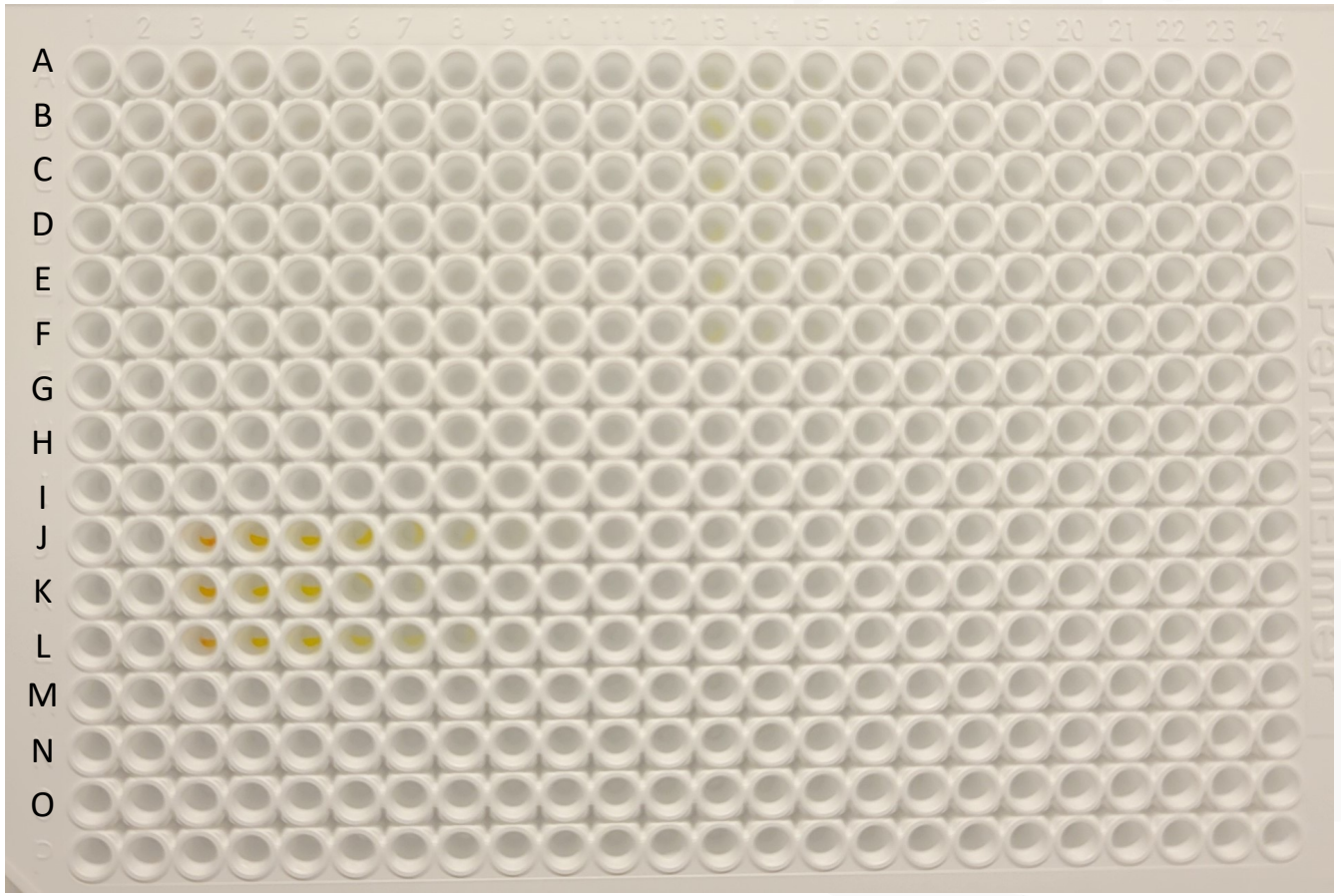
Plate layout

- Starting concentration 200 µM
- Dilution 3-fold, 10 points total

Compound	Column	Rows	SMILES	Molecular weight
SSYA10-001	3	A	C=CCN1C(=NNC1=S)CSC2=CC=CC=C2[N+](=O)[O-]	308.38
SSYA10-001	3	B	C=CCN1C(=NNC1=S)CSC2=CC=CC=C2[N+](=O)[O-]	308.38
SSYA10-001	3	C	C=CCN1C(=NNC1=S)CSC2=CC=CC=C2[N+](=O)[O-]	308.38
LicoflavoneC	3	D	CC(=CCC1=C2C(=C(C=C1O)O)C(=O)C=C(O2)C3=CC=C(C=C3)O)C	338.35
LicoflavoneC	3	E	CC(=CCC1=C2C(=C(C=C1O)O)C(=O)C=C(O2)C3=CC=C(C=C3)O)C	338.35
LicoflavoneC	3	F	CC(=CCC1=C2C(=C(C=C1O)O)C(=O)C=C(O2)C3=CC=C(C=C3)O)C	338.35
Ox-ATP	3	G	[Na].Nc1ncnc2n(cnc12)[C@H](O[C@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)C=O)C=O	505.17
Ox-ATP	3	H	[Na].Nc1ncnc2n(cnc12)[C@H](O[C@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)C=O)C=O	505.17
Ox-ATP	3	I	[Na].Nc1ncnc2n(cnc12)[C@H](O[C@H](COP(O)(=O)OP(O)(=O)OP(O)(O)=O)C=O)C=O	505.17
RA-0003626-01	3	J	O=C(/C=C(C(OCC)=O)\O)/C=C/C1=CN(CC2=CC=CC=C2)C3=CC=CC=C31	375.42
RA-0003627-01	3	K	O=C(/C=C(C(O)=O)\O)/C=C/C1=CN(CC2=CC=CC=C2)C3=CC=CC=C31	347.37
RA-0003628-01	3	L	O=C(/C=C(C(OCC)=O)\O)/C=C/C1=CN(C2=CC=C(F)C=C2)C3=CC=CC=C31	379.39
ADP	3	M	Nc1ncnc2n(cnc12)[C@@H]3O[C@H](COP(O)(=O)OP(O)(O)=O)[C@@H](O)[C@H]3O	427.2
ADP	3	N	Nc1ncnc2n(cnc12)[C@@H]3O[C@H](COP(O)(=O)OP(O)(O)=O)[C@@H](O)[C@H]3O	427.2
ADP	3	O	Nc1ncnc2n(cnc12)[C@@H]3O[C@H](COP(O)(=O)OP(O)(O)=O)[C@@H](O)[C@H]3O	427.2

Plate visualization

SSYA10-001	A
SSYA10-001	B
SSYA10-001	C
LicoflavoneC	D
LicoflavoneC	E
LicoflavoneC	F
Ox-ATP	G
Ox-ATP	H
Ox-ATP	I
RA-0003626-01	J
RA-0003627-01	K
RA-0003628-01	L
ADP	M
ADP	N
ADP	O



Protocols

- Compound pre-dispensed into either white (ATPase assay) or black (unwinding assay) 384-well plates
- 5µLs, 2X NSP13 stock in assay buffer dispensed into each well
- Plates incubated at RT for 30 minutes before starting assay
- Each compound except for UCL compounds were performed in triplicate per plate (see plate layout)
    - UCL compound stocks were limited so only per plate, there was only one row for each compound but each assay condition was performed in duplicate to ensure reproducibility was maintained
- Each assay with and without NSP13 was performed in duplicate within the same plate and within the same day for consistency

No enzyme assay conditions

- Unwinding assay:
  - 5 µLs assay buffer added to pre-plated compounds
  - Plate incubated at RT for 30 minutes
  - 5 µLs, 2X substrate mix added
  - Plate incubate for reaction time
  - Results visualized on Envision instrument
- ATPase assay:
  - 5 µLs assay buffer added to pre-plated compounds
  - Plate incubated at RT for 30 minutes
  - 5 µLs, ATP:ADP mix added
  - Plate incubate for reaction time
  - Results visualized on Envision instrument

Unwinding assay conditions

Assay buffer: 20 mM HEPES (pH 7.5), 20 mM NaCl, 0.01% BSA, 0.003 Twen-20, 0.1 mM TCEP

NSP13: 1 nM

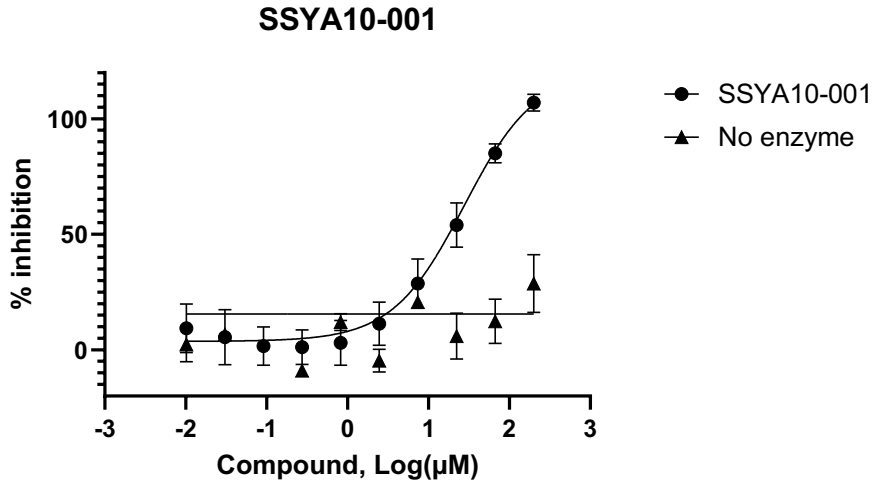
ATP: 0.5 mM

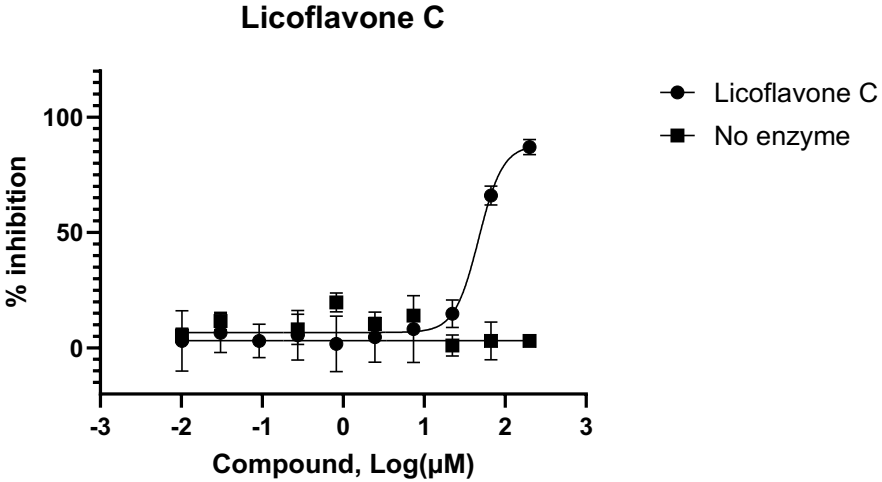
dsDNA: 0.5 µM

ssDNA: 2.5 µM

Time: 45 minutes

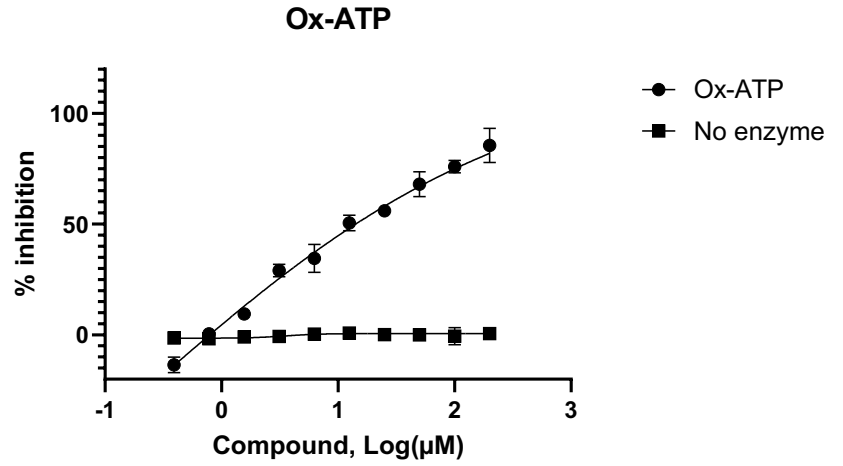
Literature controls

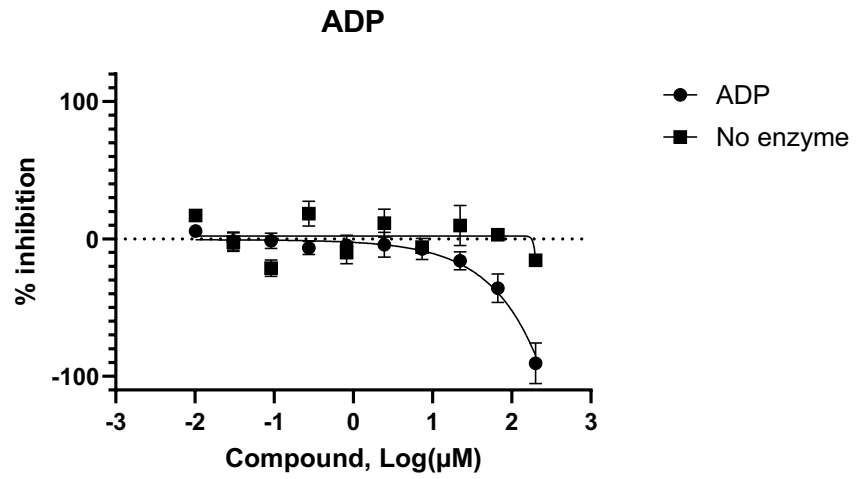




Compound ID	IC_{50} (μM)	Hill slope
SSYA10-001	27.56	0.98
Licoflavone C	47.13	2.92

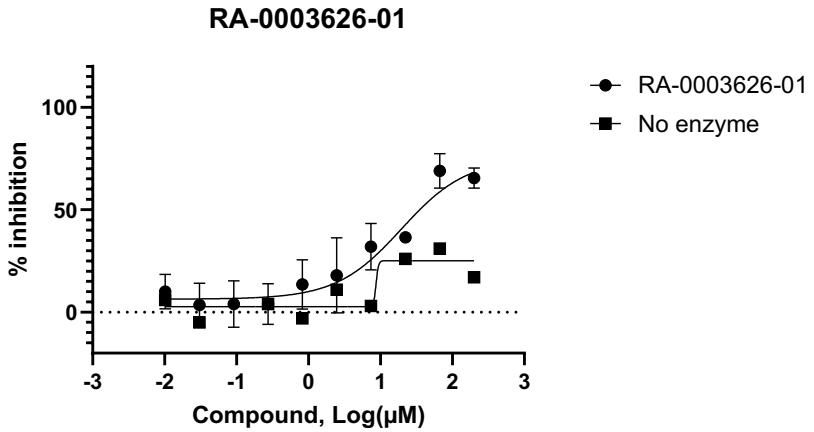
Control compounds

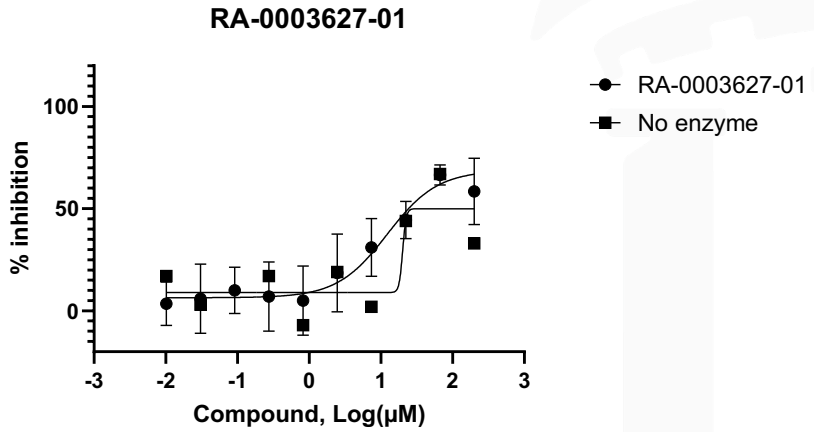


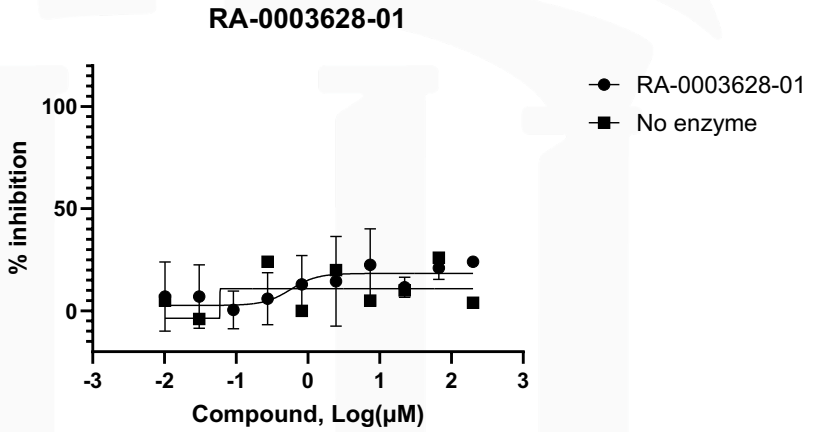


Compound ID	IC_{50} (µM)	Hill slope
Ox-ATP	0.24	0.27
ADP	ND	-0.71

UCL compounds







Compound ID	IC_{50} (μM)	Hill slope
RA-0003626-01	19.6	0.96
RA-0003627-01	12.31	1.25
RA-0003628-01	ND	2.36

ATPase assay

Reaction buffer: 50 mM HEPES (pH 7.5), 5 % glycerol, 5 mM DTT, 0.01 % BSA, 0.02 % Tween-20, 2 mM magnesium acetate

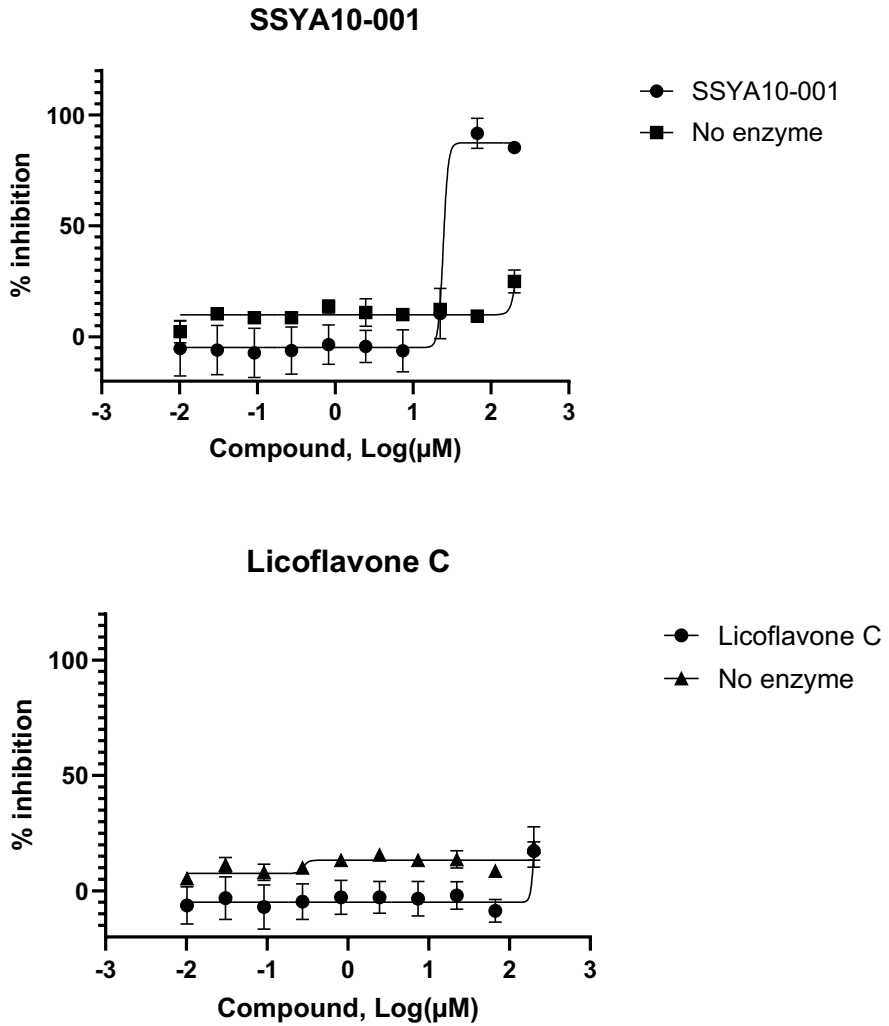
NSP13: 0.125 nM

ATP: 1 µM

PolyT ssDNA: 4 nM

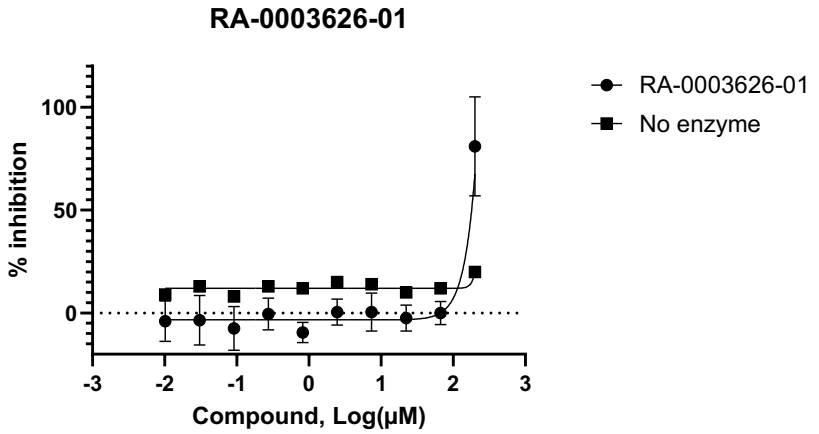
Time: 1 hour

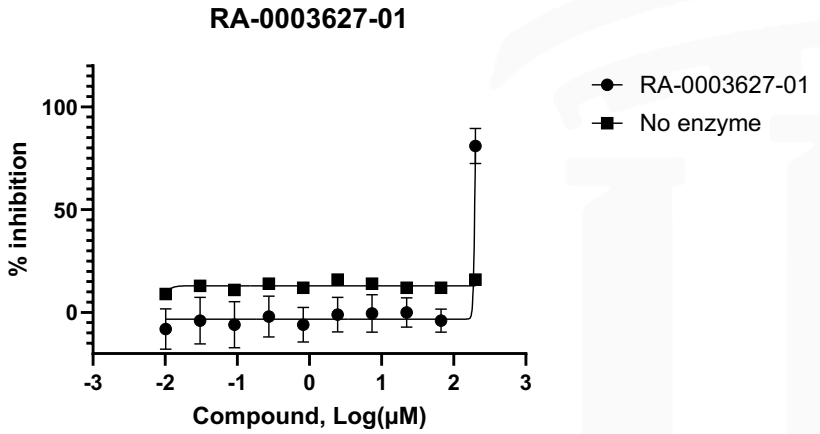
Literature compounds

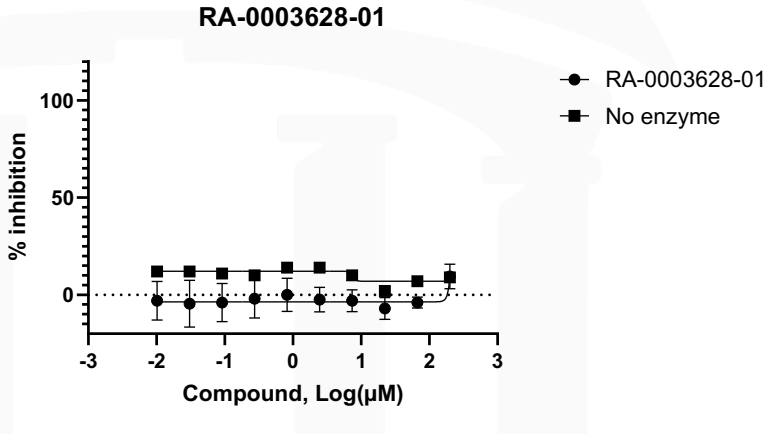


Compound ID	IC_{50} (μM)	Hill slope
SSYA10-001	24.45	13.89
Licoflavone C	>200	19.79

UCL compounds

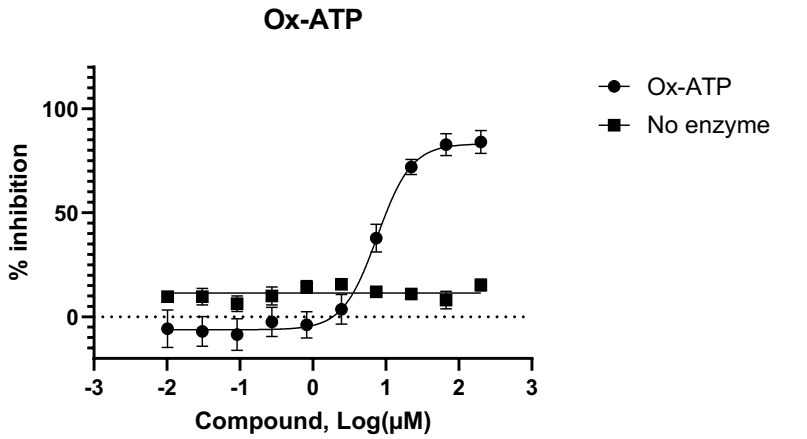


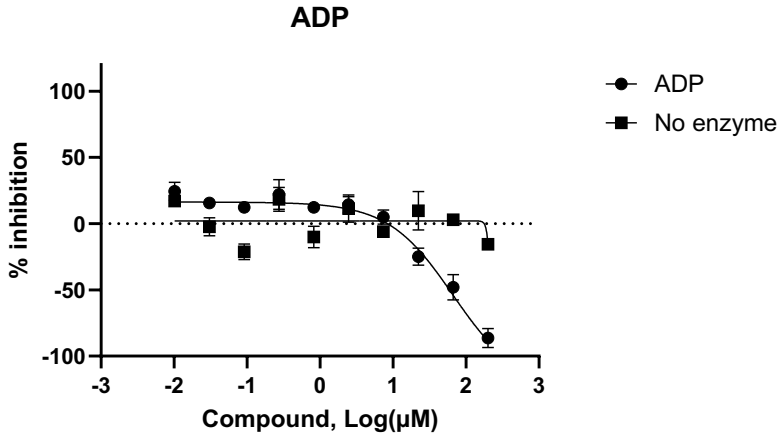




Compound ID	IC_{50} (μM)	Hill slope
RA-0003626-01	>200	2.78
RA-0003627-01	>200	33.66
RA-0003628-01	>200	14.05

Control compounds





Compound ID	IC_{50} (μM)	Hill slope
Ox-ATP	7.6	1.84
ADP	66.14	-0.98