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In vitro **Toxicology**

Lysosomal Trapping (Lysosomotropism)

Background Information

'Although there is a limited understanding of how lysosomotropism contributes to toxicity, an association between the two has been observed and therefore assays that identify lysosomal impairment compounds are desired.'

[2]Nadanaciva S, Lu S, Gebhard DF, Jessen BA, Pennie WD, Will Y (2011) *Toxicology In Vitro* **25**; 715-723

- Lysosomes are essential for the degradation of old organelles and engulfed microbes and also play a role in programmed cell death[1].
- Lysosomotropic agents e.g., chloroquine, accumulate preferentially in the lysosomes of cells in the body.
- These agents tend to have both lipophilic or amphiphilic compounds with basic moieties. Once inside the acidic environment of the lysosome, the drug becomes protonated and trapped in the organelle[2].
- Trapping of drugs in the lysosome may be one mechanism leading to drug-induced phospholipidosis for cationic amphiphilic drugs (CAD).
- Cyprotex's lysosomal trapping assay uses high content screening to identify both lysosomotropism and cytotoxicity according to a recently published method[2].

Protocol

Test System
High content screening using lysosomal and nuclear dyes

Cell Line
HepG2, human liver carcinoma cell line (other cell lines available on request, e.g., rat cardiomyocyte derived H9c2 cells)

Test Article Concentrations
8 point dose response curve with top concentration based on cell loss or solubility limit

Quality Controls
Vehicle control

Negative control = piroxicam

Positive control = chloroquine

Test Article Requirements
50 µL of a 30 mM DMSO solution or equivalent amount in solid compound.

Data Delivery
Summary report

Minimum effective concentration (MEC) and AC_{50} value for each cell health parameter (cell loss, nuclear morphology, DNA fragmentation and lysosomotropism)

Graphical representation of data

To find out more contact enquiries@cyprotex.com

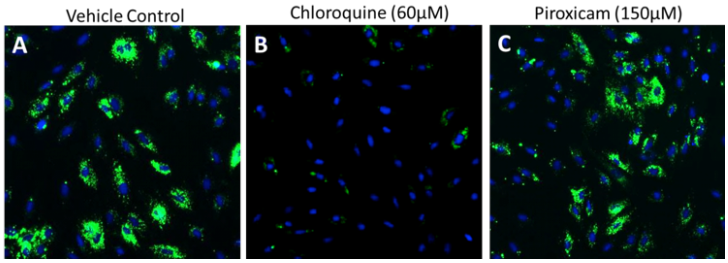
'Weak bases in their non-ionized state permeate membranes and accumulate in the acidic interior of lysosomes where they are protonated and thus become unable to diffuse back into the cytosol.[3]'

Lysosomal Trapping (Lysosomotropism)

Known lysosomotropic and non-lysosomotropic agents were screened in the lysosomal trapping assay. Data generated were compared to those published in the literature.

Figure 1

Representative high content screening images for cells treated with (A) vehicle control (B) 60µM chloroquine (a lysosomotropic agent) and (C) 150µM piroxicam (a non-lysosomotropic agent) over a 4 hr period.



Drugs which are lysosomotropic such as chloroquine competitively inhibit uptake of the lysosomal dye into the lysosomes.

Table 1

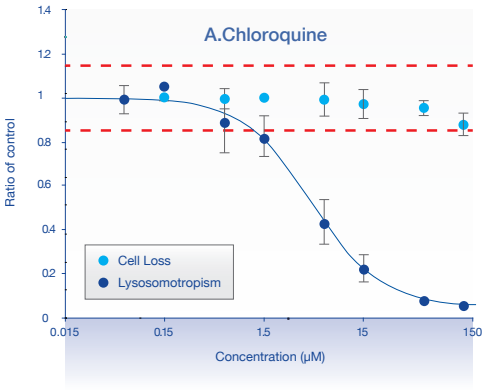
Lysosomal trapping and cytotoxicity data for a set of 12 compounds with comparison against literature data[2,4].

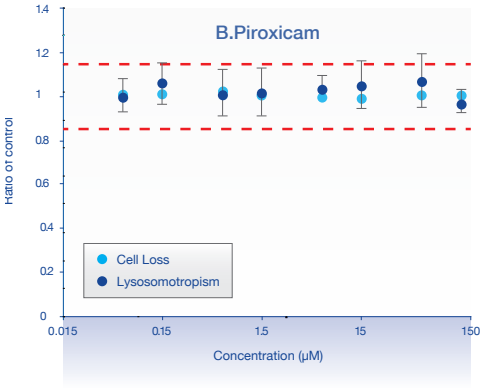
Compound	cLogP	Basic pK_a	Cyprotex decrease in lysosomal staining IC_{50}(µM) in HepG2	Cyprotex decrease in lysosomal staining IC_{50}(µM) in H9c2	Literature decrease in lysosomal staining IC_{50}(µM)	Cyprotex cytotoxicity IC_{50}(µM) in HepG2	Cyprotex cytotoxicity IC_{50}(µM) in H9c2	Literature cytotoxicity IC_{50}(µM)
Acetaminophen	0.51	9.46	>4000	>4000	>500	>4000	>4000	>500
Diclofenac	4.98	-2.1	>500	>500	>500	>500	>500	>500
Rosuvastatin	1.47	-2.8	>150	>150	>500	>150	>150	>500
Piroxicam	2.2	3.79	>300	>300	>150	>300	>300	>150
Amiodarone	7.24	8.47	6.34	7.14	3.8	144	75	12.6
Chloroquine	5.28	10.37	16.4	6.38	11.1	>150	>150	148.3
Chlorpromazine	5.18	9.2	9.1	16.5	5.8	59.2	27.5	20.9
Desipramine	4.02	10.02	35.6	59.2	4.6	>150	41.7	10.7
Fluoxetine	4.09	9.8	22.7	27.9	6.5	81.1	27.7	13.9
Paroxetine	3.1	9.77	19.2	20.6	5.3	67.7	18.2	10.7
Sertraline	5.06	9.85	6.43	14.2	6.6	42.3	11.3	12.2
Tamoxifen	5.93	8.76	15.2	22.2	3.8	70.4	24	6.6

Cyprotex data correlates well with data generated in the literature. It has been suggested that compounds with a cLogP > 2 and a basic pK_a of between 6.5 and 11 exhibit a higher propensity for lysosomotropism[2].

Figure 2

Graphical representation of lysosomal trapping data for chloroquine (a lysosomotropic agent) and piroxicam (a non-lysosomotropic agent).





Chloroquine causes a concentration dependent decrease in lysosomal staining compared to vehicle control treated cells. No effect was observed for piroxicam. No cytotoxic effects were observed for either compound at the concentration range tested. Data represents mean of triplicate incubations ± standard deviation.

References

[1] Turk B and Turk V (2009) *J Biol Chem* **284**, 21783-21787
[2] Nadanaciva S *et al.*, (2011) *Toxicology In Vitro* **25**; 715-723
[3] Daniel WA and Wojcikowski J (1997) *Pharmacology & Toxicology* **80**, 62-68
[4] Kazmi F *et al.*, (2013) *Drug Metab Dispos* **41**, 897-905