Development of a selective HPPD inhibitor without an off-target liability

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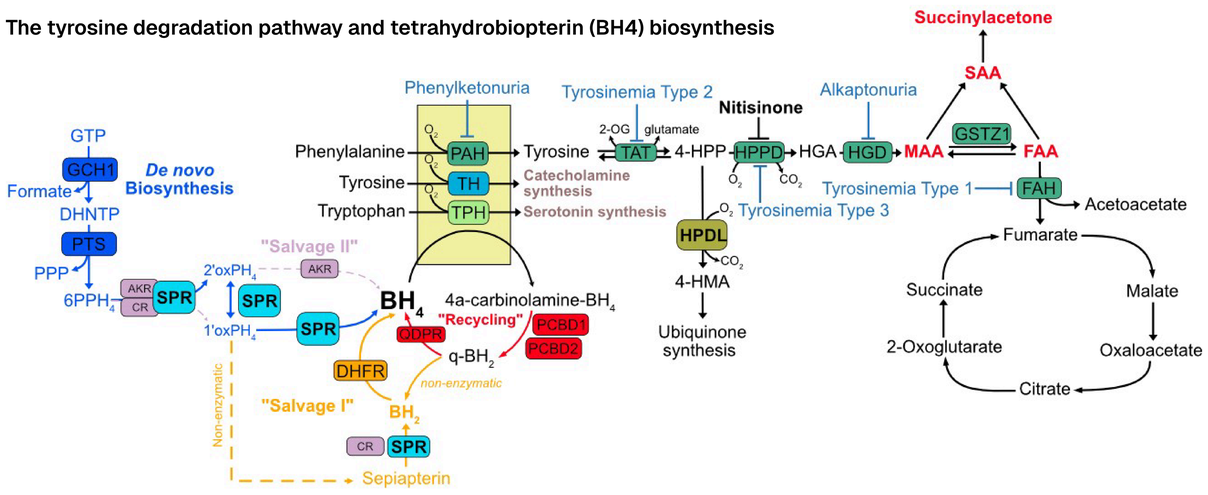
Inborn errors in tyrosine metabolism and their interaction with catecholamine metabolism

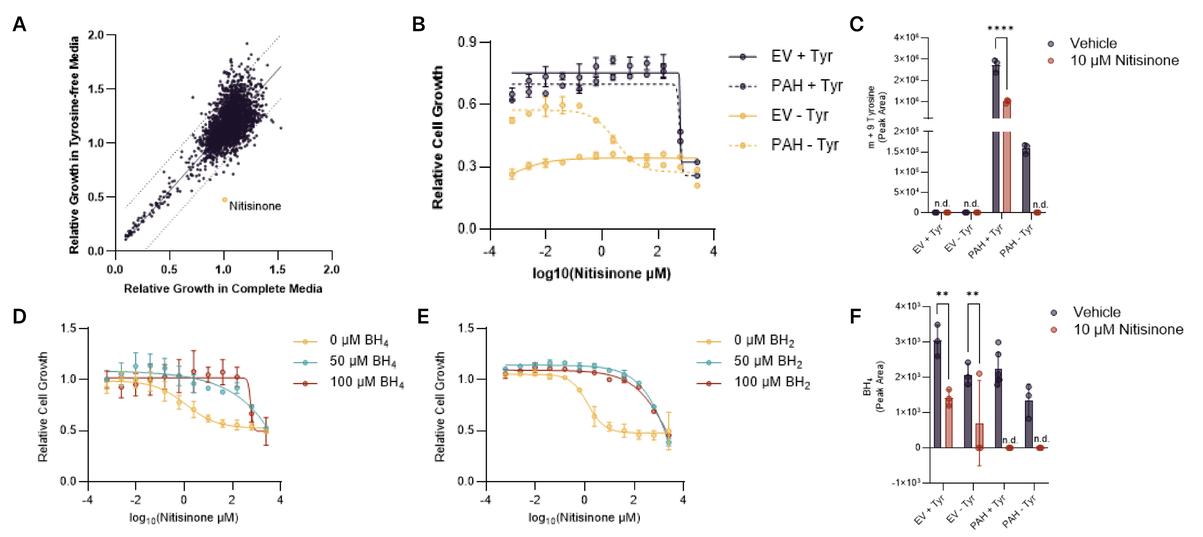
The tyrosine degradation pathway and tetrahydrobiopterin (BH4) biosynthesis



Nitisinone, a HPPD inhibitor, is an approved treatment for inborn errors of metabolism Tyrosinemia Type I (TT1) and Alkaptonuria (AK). An on-label warning/precaution of nitisinone is "Variable degrees of intellectual disability and developmental delay", with several clinical research studies finding that, unfortunately, neurocognitive impairment and decline are consistently observed in TT1 patients treated with nitisinone (Masurel-Paulet et al., 2008, De Laet et al., 2011, Thimm et al., 2011, Thimm et al., 2012, Bendadi et al., 2014, van Ginkel et al., 2016, Garcia et al., 2017, van Vliet et al., 2019, van Vliet et al., 2022).

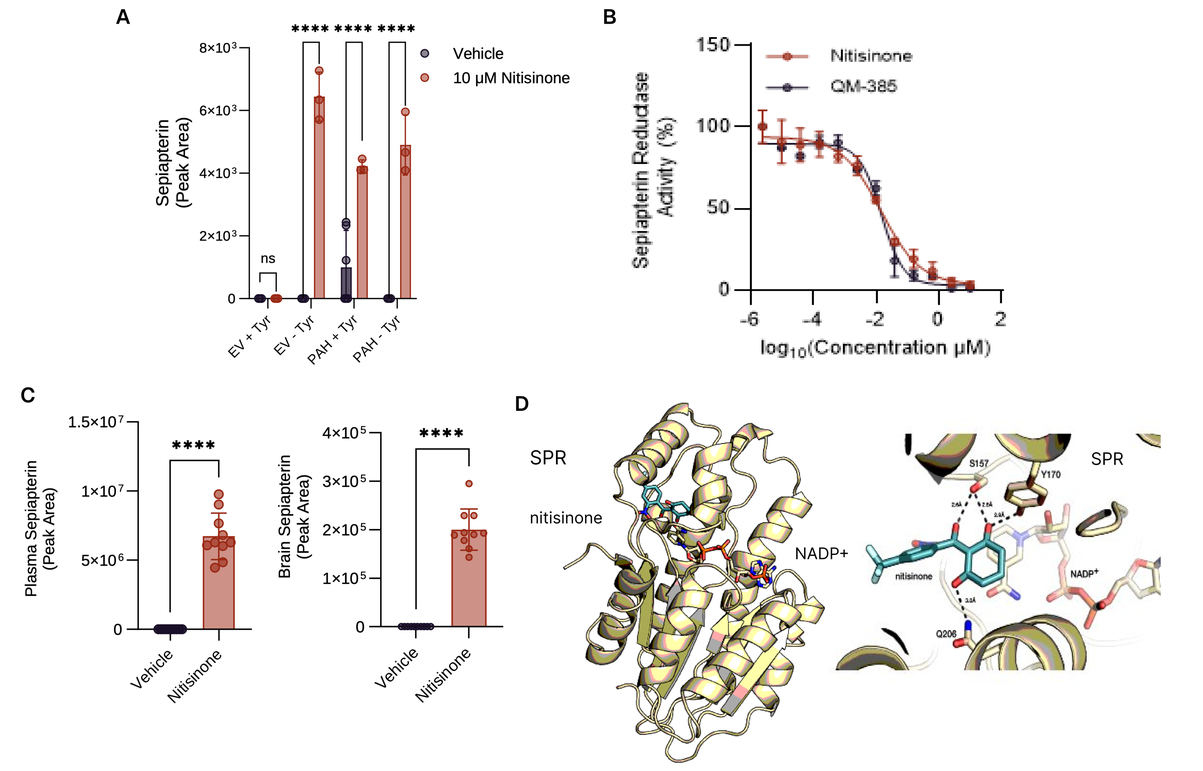
GCH1 = GTP cyclohydrolase 1, PTS = 6-pyruvoyl tetrahydrobiopterin synthase, SPR = sepiapterin reductase, AKR = aldoketoreductase, CR = carbonyl reductase, DHFR = dihydrofolate reductase, QDPR = dihydropteridine reductase, PCBD1/2 = pterin-4-alpha-carbinolamine dehydratase 1/2 , PAH = phenylalanine hydroxylase, TH = tyrosine hydroxylase, TPH, = tryptophan hydroxylase TAT = tyrosine aminotransferase, HPDL = HPPD-like protein, HPPD = 4-hydroxyphenylpyruvate dioxygenase, HGD = homogentisate dioxygenase, GSTZ1 = maleylacetoacetate isomerase, FAH = fumarylacetoacetate hydrolase, MAA = maleylacetoacetate, SAA = succinylacetoacetate, FAA = fumarylacetoacetate, 4-HPP = 4-hydroxyphenylpyruvate, HGA = homogentisate, 4-HMA = 4-hydroxymandelate.

Nitisinone Inhibits PAH-dependent Tyrosine Biosynthesis by Compromising BH4 Availability



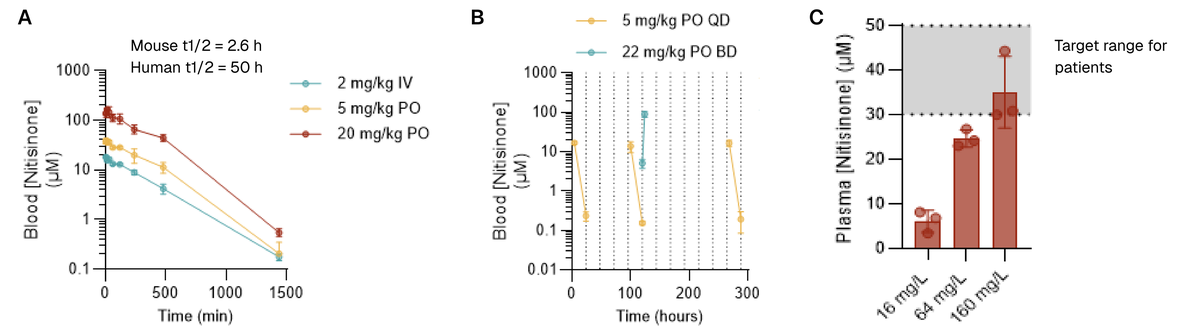
A. Phenotypic screen of a chemical library in DLD1-PAH cells cultured with and without tyrosine reveals that nitisinone causes tyrosine auxotrophy. B. Growth of DLD1-EV/PAH cells treated with increasing concentrations of nitisinone in media with and without tyrosine. C. Intracellular levels of m+9 tyrosine in DLD1-EV/PAH cells cultured with 13C9-phenylalanine in the presence or absence of tyrosine. D,E. Growth of DLD1-PAH cells treated with increasing concentrations of nitisinone in tyrosine-free media supplemented with BH4 or BH2. F, Intracellular BH4 levels in DLD1-EV/PAH cells treated with or without nitisinone in the presence or absence of tyrosine. n.s. not significant, ** p<0.01, **** p<0.0001, two-way ANOVA. n.d. not detected. Data are mean +/- standard deviation.

Nitisinone Inhibits Sepiapterin Reductase (SPR) in vitro and in vivo



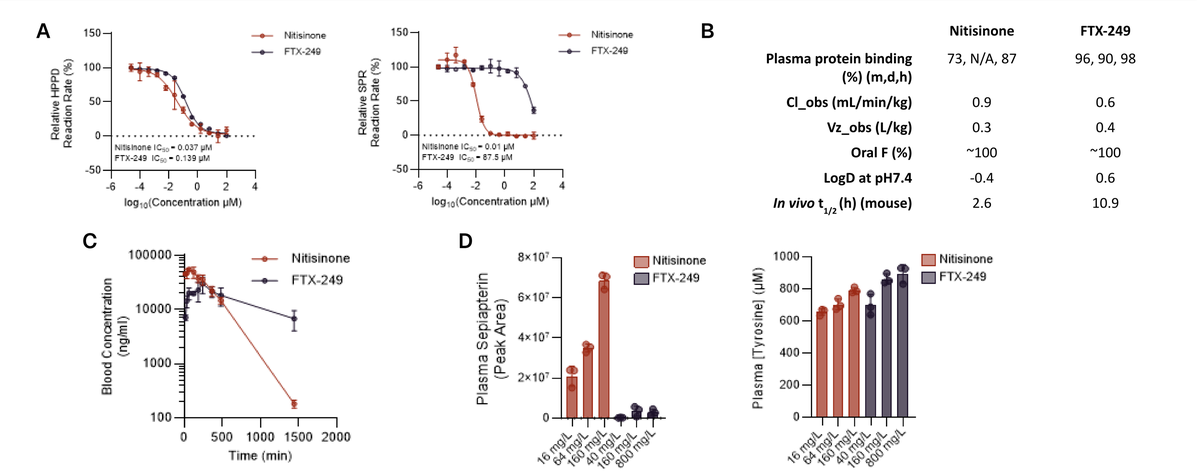
A. Intracellular sepiapterin levels in DLD1-EV/PAH cells treated with or without nitisinone in the presence or absence of tyrosine. B. Biochemical activity assay of human SPR with increasing concentrations of nitisinone or QM-385. C. Brain and plasma levels of sepiapterin in Balb/c mice treated orally with 4 mg/kg nitisinone QD, n=10 per group (**** p<0.0001, t-test). D. X-ray crystallographic structure of SPR bound to nitisinone shows nitisinone binding in the pterin active site via an extensive hydrogen bonding network.
n.s. not significant, **** p<0.0001, two-way ANOVA (a). Data are mean +/- standard deviation.

Nitisinone Pharmacokinetics Differ Significantly Between Humans and Mice



Significantly lower half life of nitisinone in mice than in humans means that mice require dosing via drinking water at 160 mg/L to replicate high and stable human exposures.
A. Blood nitisinone levels after a single dose in wild type C57/Bl6 mice, n=3 per group. B. Blood nitisinone levels after 12-day repeat dosing in wild type C57/Bl6 mice, n=3 per group. Horizontal dashed lines denote time of each dose. C. Plasma nitisinone levels in wild type C57/Bl6 mice treated with nitisinone via drinking water for 5 days, n=3 per group. The shaded area indicates exposures of nitisinone in HT-1 patients. n=3 per group. Data are mean +/- standard deviation.

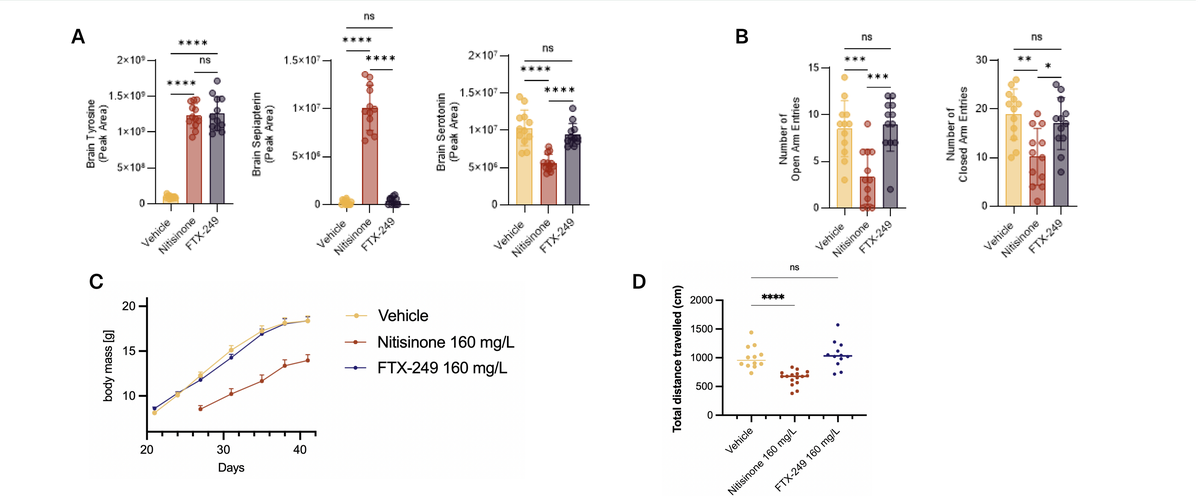
Selective HPPD inhibitor development



	Nitisinone	FTX-249
Plasma protein binding (%) (m,d,h)	73, N/A, 87	96, 90, 98
Cl_obs (mL/min/kg)	0.9	0.6
Vz_obs (L/kg)	0.3	0.4
Oral F (%)	~100	~100
LogD at pH7.4	-0.4	0.6
In vivo $t_{1/2}$ (h) (mouse)	2.6	10.9

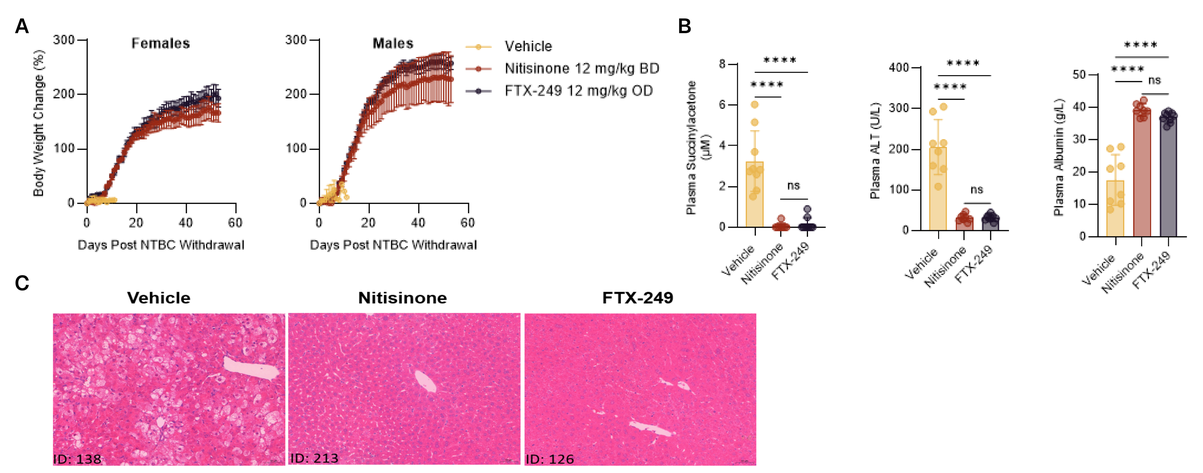
FTX-249 is a selective HPPD inhibitor with excellent ADME and PKPD properties.
A. Biochemical assay of HPPD and SPR activity with increasing concentrations of nitisinone or FTX-249. B. Key ADME and PK parameters. C. Direct comparison of blood concentration of nitisinone and FTX-249 in C57/Bl6 mice, following a 20 mg/kg single oral dose of each drug, n=3 per group D. Plasma tyrosine and sepiapterin levels in wild type C57/Bl6 mice treated with nitisinone or FTX-249 via drinking water for 5 days, n=3 per group.

Nitisinone, but not Tyrosine Elevation, Induces Developmental Delay and Behavioural Changes in Mice



Nitisinone changes brain serotonin levels, delays development and induces kinematic and behavioural changes in mice.
A. Brain tyrosine, sepiapterin and serotonin levels in wild type Balb/c mice treated orally with vehicle, 12 mg/kg nitisinone BD or 12 mg/kg FTX-249 QD, n=12 per group. B. Elevated plus maze results show number of open and closed arm entries of wild type Balb/c mice treated orally with vehicle, 12 mg/kg nitisinone BD or 12 mg/kg FTX-249 QD in the elevated plus maze test, n=12 per group. C. Development of C57/Bl6 pups treated with FTX-249 or nitisinone via drinking water dosing for dams at indicated levels from day 3. D. Total distance travelled in an elevated plus maze experiment for pups treated with FTX-249 or nitisinone from day 3.

FTX-249 Effectively Treats Tyrosinaemia Type 1 in Mice



A. Growth of Fah-/- mice treated with vehicle, 12 mg/kg nitisinone BD or 12 mg/kg FTX-249 QD, n=9 per group. B. Plasma succinylacetone, ALT and albumin levels in Fah-/- mice treated with vehicle, 12 mg/kg nitisinone BD or 12 mg/kg FTX-249 QD, n=9 per group. C. Representative H&E sections of livers from Fah-/- mice treated with vehicle, 12 mg/kg nitisinone BD or 12 mg/kg FTX-249 QD, n=9 per group.
n.s. not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, one-way ANOVA. Data are mean +/- standard deviation.

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