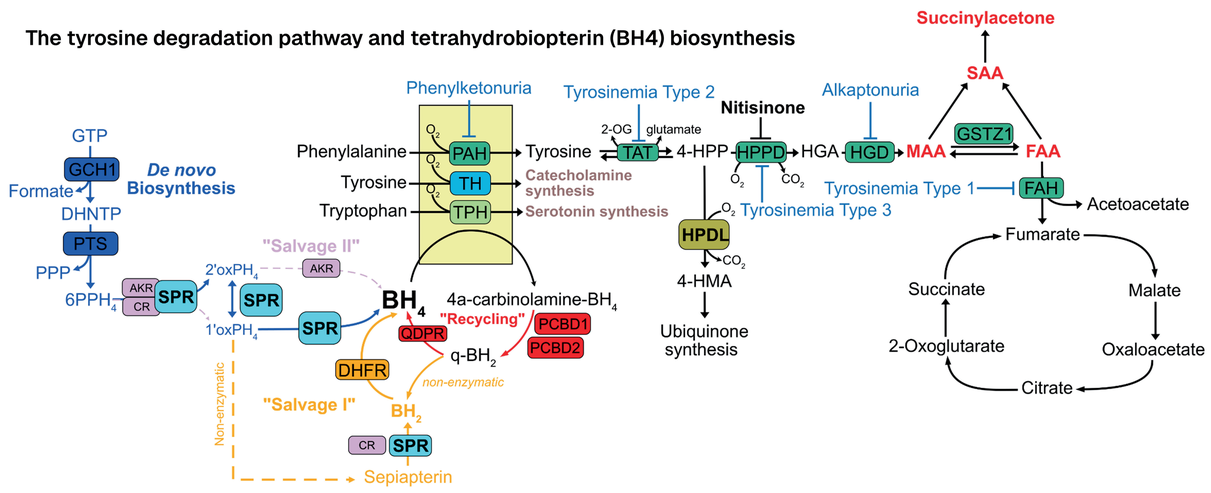
Development of a Selective HPPD Inhibitor Without an Off-target Liability

Petros A. Tyrakis*, Domen Kampjut*^, H. Jonathan G. Lindström, Nigel Ramsden and Oliver D. K. Maddocks

Faeth Therapeutics R&D, Cambridge Research Institute, Robinson Way, Cambridge, CB2 0RE, UK / Faeth Therapeutics, 701 Tillery Street, Austin TX 78702, USA

*Equal contribution, ^Presenting author

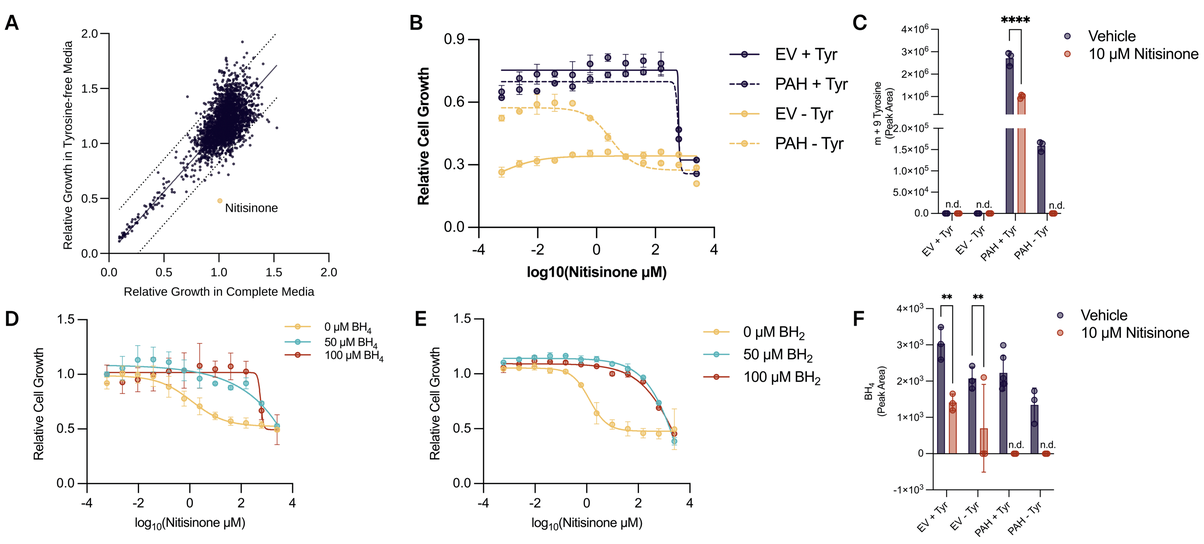
Inborn Errors in Tyrosine Metabolism and Their Interaction With Catecholamine Metabolism



Nitisinone, a HPPD inhibitor, is an approved treatment for two inborn errors of metabolism, hereditary tyrosinemia type I (HT-1) and alkaptonuria (AKU). 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 HT-1 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).

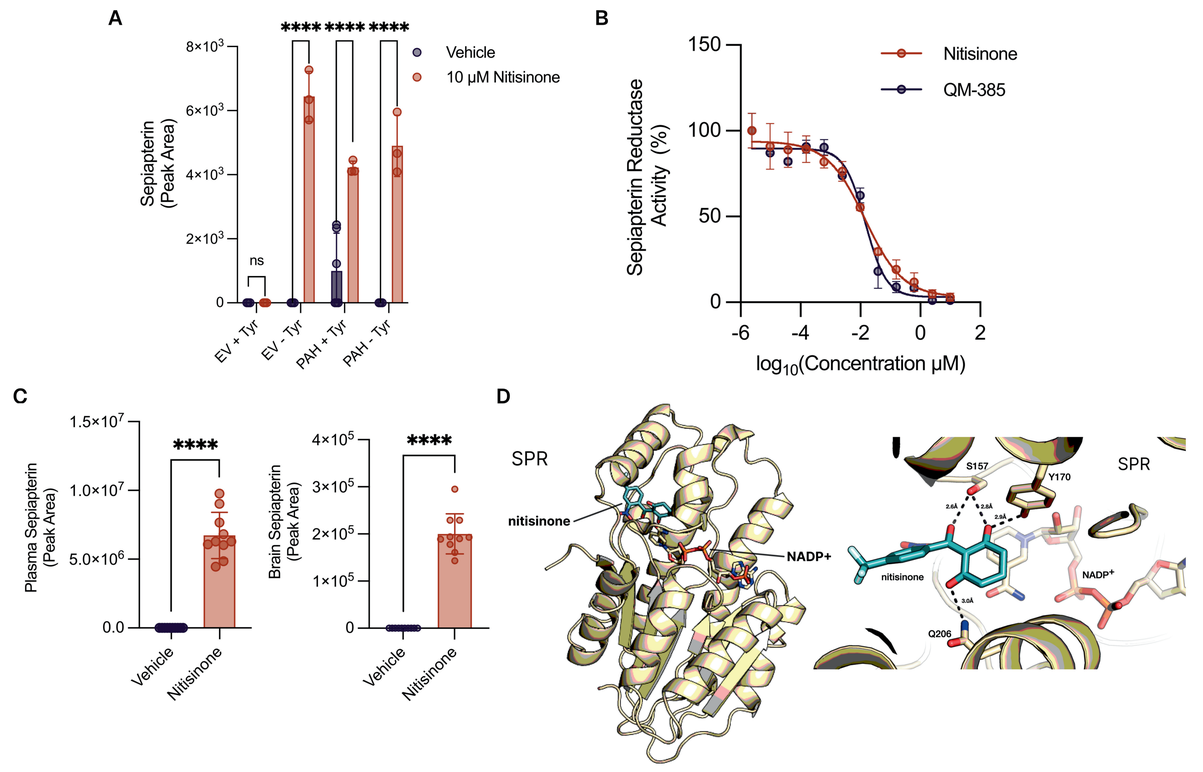
GCH1 = GTP cyclohydrolase 1, PTS = 6-pyruvoyl tetrahydrobiopterin synthase, SPR = sepiapterin reductase, AKR = aldoketoreductase, CR = carbonyl reductase, DHFR = dihydrofolate reductase, QDPR = dihydropteridine reductase, PCBD1/2 = pterin-4-alpha-carbinolamine dehydratase 1/2 , PAH = phenylalanine hydroxylase, 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 BH_4 Availability



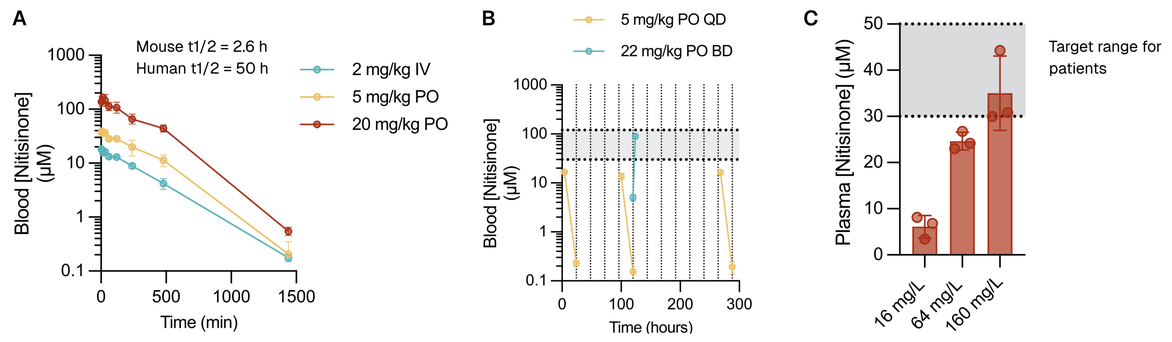
A, Phenotypic screen of a chemical library in DLD1-PAH cells cultured with and without tyrosine reveals that nitisinone causes tyrosine auxotrophy. **B,** Growth of DLD1-EV/PAH cells treated with increasing concentrations of nitisinone in media with and without tyrosine. **C,** Intracellular levels of m+9 tyrosine in DLD1-EV/PAH cells cultured with $^{13}C_9$-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 BH_4 or BH_2. **F,** Intracellular BH_4 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.

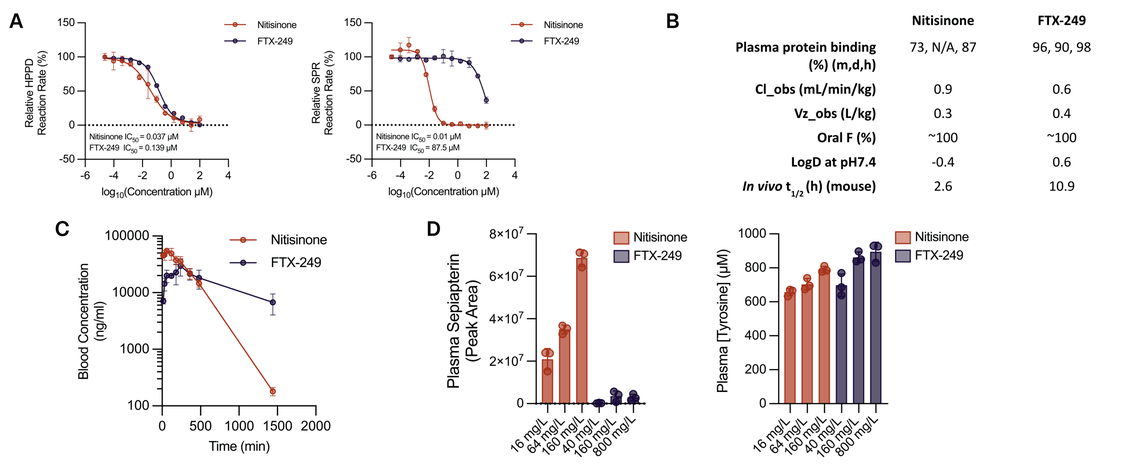
Nitisinone Pharmacokinetics Differ Significantly Between Humans and Mice



The significantly faster half life of nitisinone in mice than in humans means mice require dosing via drinking water at high doses to replicate 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 the indicated dose of nitisinone via drinking water for 5 days, n=3 per group. The shaded area in B and C indicates exposures of nitisinone in HT-1 patients. n=3 per group. Data are mean +/- standard deviation.

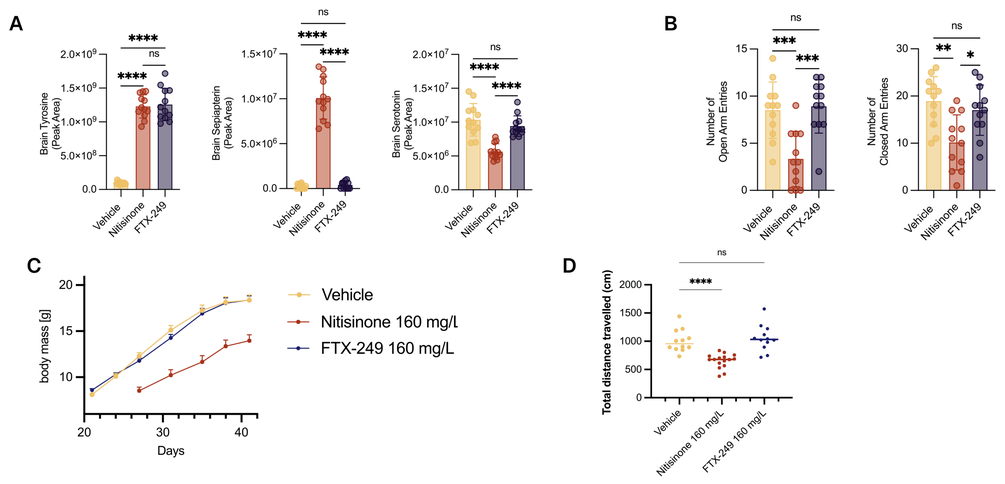
Selective HPPD Inhibitor Development



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.

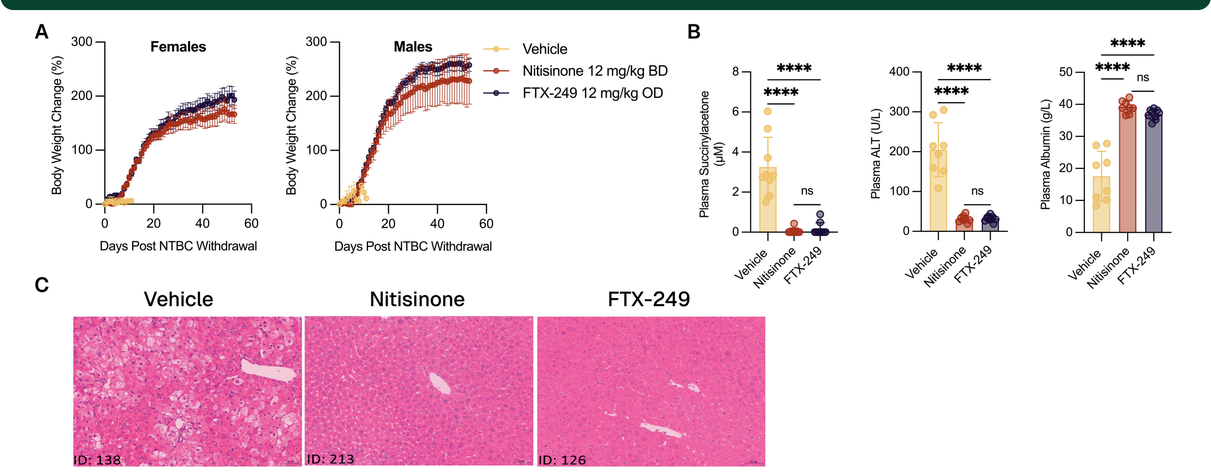
Nitisinone, but not Tyrosine Elevation, Causes Slower Postnatal Growth and Behavioural Changes in Mice



Nitisinone lowers brain serotonin levels, reduces postnatal growth with persistently lower body weight 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,** 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,** Growth of C57/Bl6 pups treated with FTX-249 or nitisinone via drinking water dosing for dams at indicated levels from P3 to weaning, and then in drinking water of the mice after weaning. **D,** Total distance travelled in an elevated plus maze experiment for 4.5 week old C57/Bl6 mice treated with FTX-249 or nitisinone as in C.

FTX-249 Effectively Treats Tyrosinemia 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.

Drug Label, https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021232s019lbl.pdf
Bendadi, F., de Koning, T. J., Visser, G., Prinsen, H. C., de Sain, M. G., Verhoeven-Duif, N., . . . van Hasselt, P. M. (2014). Impaired cognitive functioning in patients with tyrosinemia type I receiving nitisinone. J Pediatr, 164(2), 398-401. doi:10.1016/j.jpeds.2013.10.001
De Laet, C., Munoz, V. T., Jaeken, J., Francois, B., Carton, D., Sokal, E. M., . . . Goyens, P. J. (2011). Neuropsychological outcome of NTBC-treated patients with tyrosinaemia type 1. Dev Med Child Neurol, 53(10), 962-964. doi:10.1111/j.1469-8749.2011.04048.x
Garcia, M. I., de la Parra, A., Arias, C., Arredondo, M., & Cabello, J. F. (2017). Long-term cognitive functioning in individuals with tyrosinemia type 1 treated with nitisinone and protein-restricted diet. Mol Genet Metab Rep, 11, 12-16. doi:10.1016/j.ymgmr.2017.01.016
Masurel-Paulet, A., Poggi-Bach, J., Rolland, M. O., Bernard, O., Guffon, N., Dobbelaere, D., . . . Touati, G. (2008). NTBC treatment in tyrosinaemia type I: long-term outcome in French patients. J Inherit Metab Dis, 31(1), 81-87. doi:10.1007/s10545-008-0793-1
Thimm, E., Herebian, D., Assmann, B., Klee, D., Mayatepek, E., & Spiekerkoetter, U. (2011). Increase of CSF tyrosine and impaired serotonin turnover in tyrosinemia type I. Mol Genet Metab, 102(2), 122-125. doi:10.1016/j.ymgme.2010.11.003
Thimm, E., Richter-Werkle, R., Kamp, G., Molke, B., Herebian, D., Klee, D., . . . Spiekerkoetter, U. (2012). Neurocognitive outcome in patients with hypertyrosinemia type I after long-term treatment with NTBC. J Inherit Metab Dis, 35(2), 263-268. doi:10.1007/s10545-011-9394-5
van Ginkel, W. G., Jahja, R., Huijbregts, S. C., Daly, A., MacDonald, A., De Laet, C., . . . van Spronsen, F. J. (2016). Neurocognitive outcome in tyrosinemia type 1 patients compared to healthy controls. Orphanet J Rare Dis, 11(1), 87. doi:10.1186/s13023-016-0472-5
van Vliet, K., van Ginkel, W. G., Jahja, R., Daly, A., MacDonald, A., De Laet, C., . . . van Spronsen, F. J. (2019). Emotional and behavioral problems, quality of life and metabolic control in NTBC-treated Tyrosinemia type 1 patients. Orphanet J Rare Dis, 14(1), 285. doi:10.1186/s13023-019-1259-2
van Vliet, K., van Ginkel, W. G., Jahja, R., Daly, A., MacDonald, A., Santra, S., . . . van Spronsen, F. J. (2022). Neurocognitive outcome and mental health in children with tyrosinemia type 1 and phenylketonuria: A comparison between two genetic disorders affecting the same metabolic pathway. J Inherit Metab Dis, 45(5), 952-962. doi:10.1002/jimd.12528.

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