Appendix

Table A1. Zebrafish model in ototoxic assessment (2009-2020). The complete dataset

Authors	Aim of the article	Additional models	Zebrafish details	Aim of using zebrafish	Sample sie	CIS concentration	Tested substances	Target	Molecular effect	Impact on cisplatin efficiency (based on the article)	Adversed effects (reported in the article)	Experimental model	Methods dedicated to zebrafish	Results	Conclusion
Monroe et al. 2020	to test if L-serine may reduce cisplatin-mediated ROS generation	HEI-OC1 cell line (in vitro)	≥6 mpf AB wild-type zebrafish (utricular tissue culture)	to investigate if cisplatin-mediated ROS generation in zebrafish utricular tissue culture	n= 3-6 (general)	100 µM	L-serine (the amino acid tereoisomer)	not applicable	present and functionally important in many proteins, source of one-carbon groups for the de novo synthesis of purine nucleotides and deoxythymidine monophosphate	L-serine did not alter the effect of cisplatin against the viability of HEI-OC1 cells	not stated	Experimental group: L-serine (100 µM) + CIS (100 µM) for 45 min; L-serine (100 µM) for 45 min; Control group: PBS	spectrophotometry, the fluorescent ROS detector dye (H2DCFDA)	Combination cisplatin (100 µM) and L-serine (100 µM) treatment causes ROS levels intermediate to cisplatin and L- serine alone and is not statistically different than control.	L-serine may act in auditory and vestibular tissues as an effective protectant against cisplatin-mediated toxicity.
Gu et al. 2020	to evaluate the potential antioxidant properties of Astaxanthin	HEI-OC1 cell line (in vitro), guinea pigs	5 dpf zebrafish (no data)	to evaluate the otoprotective role of Astaxanthin in cisplatin-induced hearing loss	n= 30 per group	60 µM	Astaxanthin (ATX) and ATX in lipid-polymer hybrid nanoparticles (ATX-LPN)	not stated	scavenging singlet oxygen and free radicals, preventing lipid peroxidation in biological membranes	not stated	not stated	Experimental group: ATX or ATX-LPN (1, 10 and 50 µg/ml) for 4 h then CIS (60 µM) for 24 h; Control group: EM	Assessment of neuromast hair cell damage (no specific staining)	Both ATX and ATX-LPN at concentrations of 1 and 10 µg/ml did not decrease the damage to hair cells induced by cisplatin. Only at the concentration of 50 µg/ml had an clear protective effect (32%).	ATX-LPN might be as a new therapeutic agent for the prevention of cisplatin-induced ototoxicity.
Wang et al. 2020	to examine the role of the sphingosine 1-phosphate receptor 2 (S1P2) in attenuating cisplatin-induced ototoxicity	S1P2 heterozygous mice (S1pr2+/−), Sprague-Dawley rats, cell lines (neural, breast, ovarin,prostate and liver cancer)	6 dpf zebrafish (no data)	to determine if CYM-5478 may be use in zebrafish	n= 24 per group	no data	CYM-5478 and NAC (an antioxidant)	S1P2 receptor agonist	cell proliferation, survival, and transcriptional activation	CYM-5478 did not affect cisplatin effect on tumor cells	not stated	Experimental group: CYM-5478 (20 µM) or NAC (1 mM) for 24h; Control group: vehicle	Assessment of neuromast hair cell damage (no specific staining)	Hair cell viability was improved by NAC and CYM-5478.	The S1P2 receptor might be a promising therapeutic approach for the treatment of cisplatin-induced ototoxicity.

Zheng et al. 2020	to assess the antioxidant and anti-apoptosis properties of Salvianolic acid B (Sal B)	HEI-OC1 cell line (in vitro)	4 dpf transgenic (Brn3C: EGFP) zebrafish	to investigate the protective effect of Sal B in vivo	no data	50 µM	Salvianolic acid B (Sal B)	Multiple pathways[57]	improving the energy metabolism, inhibiting TNF-alpha production	not stated	not stated	Experimental group: Sal B for 2h then CIS (50 µM) + Sal B (40 µM) for 24h; Sal B (40 µM) for 2 h; Control group: DMSO; CIS (50 µM) for 24h	TUNEL assay, FM1-43 assay (a marker for functional MET channels in hair cells)	Sal B pre-treatment group showed significantly reduced incidents of apoptosis compared those treatments with ototoxin alone. The FM1-43FX-positive cells did not show any significant differences between Sal B and control group.	Sal B reduces the generation of ROS in response to ototoxin damage.
Pang et al. 2018	to explore SIRT1's modulation of autophagy during cisplatin-induced ototoxicity	HEI-OC1 cell line (in vitro), C57BL/6 mice	5 dpf transgenic (ET-4) zebrafish	to confirm the role of autophagy in cisplatin-induced hearing loss (in vivo)	n= 6 per group	600 µM	Rapamycin (RA, an autophagy activator), SRT1720 (a new synthetic small molecule, the selective activator of SIRT1)	FKBP-12	inhibiting autophahy	not stated	not stated	Experimental group: RA (10 µM); RA (10 µM) for 1 h then CIS (600 µM) for 12 or 24h; CIS (600 µM)+ RA (10 µM) for 12 or 24h; SRT1720** (5 µM) for 1h; SRT1720 (5 µM) for 1 h+ CIS (600 µM) for 12 or 24h; CIS (600 µM) + SRT1720 (5 µM) for 12 and 24h; CIS (600 µM) for 12 and 24h; Control group: DMSO	Counting hair cells per 3 neuromast	12 h after cisplatin treatment, >50% hair cells were eliminated. Pre-treated with RA or SRT1720 reduce the damage. Although at 24 h after cisplatin exposure, nearly all hair cells were eliminated, while pre-treatment with RA or SRT1720 attenuated cisplatin-induced hair cell loss.	SIRT1 can be a potential therapeutic strategy for the treatment of cisplatin-induced hearing loss.

Kitcher et al. 2019	to explore the versatility and mechanisms of protection of ORC-13661 and determine the protective effect against cisplatin	wild-type CD-1 mice (cochlear culture)	5-7 dpf AB wild-type zebrafish	to assess the protective efficiency in zebrafish	n= 9-11 per group	25–200 µM	ORC-13661 (a new drug that was derived from PROTO-1)	mechanoelectrical transducer (MET) channel in outer hair cells	reducing ototoxis substances entry into hair cells through the MET channels	not stated	not stated	Experimental group: CIS (25–200 µM) for 24 h + ORC-13661 (0.10–8.33 µM)*; Control group: CIS (25–200 µM) for 24 h	Counting hair cells (SO1, SO2, O1, and OC1)	Cisplatin treatment caused lateral line hair cell loss across all concentrations tested. The HC_{50} for protection against 200 µM cisplatin was 2.2 µM.	ORC-13661 protects against cisplatin toxicity in a dose-dependent manner.
Monroe et al. 2019	to explore if the curcuminoids (CLEFMA and EF24) alter cisplatin efficiency and reduce cisplatin ototoxicity	lung cancer cell line	≥ male and female 6 mpf zebrafish (inner ear)	to determine if treatment with cisplatin, the curcuminoids, and the cisplatin–curcuminoid combinations alters hearing abilities	n= 6-8 per group	25 mg/kg	CLEFMA, EF24 (curcuminoids)	NF-κB and miRNA-21[58]	ROS reduction claim	curcuminoids are unable to potentiate the effect of cisplatin	not stated	Experimental group: (microinjection) CIS (25 mg/kg) 24h after CLEFMA or EF24 (5mg/kg); Control group: single injection of CLEFMA (5mg/kg); EF24 (5mg/kg); CIS (25 mg/kg)	ABR (0.1, 0.25, 0.4, 0.8, 1, 1.5 and 3 kHz) 24 and 48h after drug administration	The cisplatin caused a significantly greater threshold shift than the cisplatin–CLEFMA and cisplatin–DMSO treatments (the whole hearing range). Zebrafishes injected only CLEFMA and EF24 had similar auditory thresholds.	AEP results suggests that the curcuminoids may be able to reduce cisplatin-induced auditory threshold shifts.
Rocha-Sanchez et al. 2018	to describe the therapeutic effect of Quinoxaline (Qx) against ototoxic-induced hair cell death	-	5 dpf trangenic Tg(brn3c:GFP) zebrafish and wild-type zebrafish	n.a.	n= 20 per group	50-800 µM	Quinoxaline (Qx, the heterocyclic compound quinoxaline)	various tyrosine kinases[59]	not clear	not stated	Protective effect of 150 µM Qx depended on the incubation protocol.	Experimental group: Qx (50-300 µM) for 2h then CIS (400 µM) for 6h; Qx (50-300 µM) for 2 h, then 6h (400 µM) CIS+ Qx (50-300); Qx (300 µM) for 2h+ CIS (50-800 µM) +/- Qx for 6 h; CIS (50-800 µM) for 6h; Control group: DMSO	Recordings of microphonic potentials, TUNEL assay, proliferation assay, neuromast sassessment (IO4, OP1, M2, O1, O2, MI2)	Qx did not block the mechanotransduction channels based on FMI-43 and microphonic potentials. 300 µM Qx presented full protection. against CIS (50-400 µM).	Animals incubated with Qx are protected against the cisplatin.

Monroe et al. 2018a	to determine if the platinum(II) complexes caused hearing loss	-	≥6 mpf AB wild-type zebrafish (inner ear)	n.a.	n= 8-15 per group	-	-	-	-	-	-	Experimental group: (microinjection): phenanthriplatin, pyriplatin, Pt(Et2dien)Cl or Pt(dien)Cl (25mg/kg) and an equivalent volume of vehicle (0.9% NaCl); control group: EM	Hair cell quantification analysis (saccule and utricle), ABR (0.1, 0.25, 0.4, 0.8, 1, 1.5 and 3 kHz) 24 and 48h after drug administration	Phenanthriplatin treatment produced greater thresholds than cisplatin treatment At 24 hour post-injection, the compounds did not cause a significant effect on hair cell density in the saccule. After 48 hours, cisplatin did cause a reduction in hair cell density in three regions locations along the rostral-caudal axis, and phenanthriplatin reduced hair cell counts. In the utricle, hair cell density was not reduced compared to vehicle 24 and 48 h.	Both complex produced threshold shifts similar to cisplatin, unlike cisplatin, the monofunctional compounds produce virtually no effect on auditory hair cell density. It suggests that these complexes could act in a mechanistically different fashion than cisplatin (possibly through modulation of transcription or protein targets).
Monroe et al. 2018b	to test the effects of cisplatin and EF-24 treatment on intracellular ROS release	-	≥6 mpf AB wild-type zebrafish (saccular and utricular inner ear cultures)	n.a.	n= 3-4 per group	100-500 µM	EF-24 (a synthetic curcumin analog)	NF-κB and miRNA-2158	not clear	curcuminoid is not unable to potentiate the effect of cisplatin	not stated	Experimental group: (saccular and utricular tissue cultures) CIS (100-500 µM) for 45 min; EF-24 (100-500 µM); CIS (100-500 µM) for 45 min, after 3h EF-24 (100-500 µM); Control group: DMSO	A fluorescent spectrophotometric assay (18th h of the experiment)	Low (100 mM) and high (500 mM) cisplatin caused increased ROS release in both endorgan tissues relative to controls, but both low (100 µM) and high (500 µM) molarity EF-24 treatment either did not alter ROS release or reduced	The zebrafish inner ear tissue culture system may be a new technique for assessing novel substances.

														compared to controls.	
Vargo et al. 2017	to investigate the protective role of mdivi-1 against cisplatin toxicity	-	5-6 dpf: wild type (Tübingen strain), cdh23 tj264a mutant* and (transgenic pvalb3b:GFP) zebrafish *does not have functional MET in hair cells	n.a.	n= 10-17 per group	50-200 µM	Mdivi-1	mitochondrial fission protein Drp1 (dynamin-related GTPase) and dynamin I	changes in mitochondrial function	not stated	High doses of mdivi-1 (>10 µM) were toxic to the zebrafishes	Experimental group: CIS (50-200 µM) and/or mdivi-1* (1-10 µM) for 16 h; Control group: EM	Counting hair cells per neuromast, FM1-43 assay (3-4 neuromasts), recordings of microphonic potentials	Mdivi-1 (3-7 µM) protect hair cells against CIS (50 and 100 µM). Cisplatin did not affect MET in neuromast hair cells. Microphonic potentials records have been not affected after 100 µM cisplatin exposition. 50 µM CIS did not change the number of hair cell in neuromasts in mumant but in wild type.	Mitochondria protection prevents cisplatin-induced hair cell death in zebrafish.
Todd et al. 2017	to determine if anatomic recovery results in functional recovery of rheotaxis swimming behavior; to investigate if the created system can detect that functional recovery	-	5-6 dpf AB wild-type zebrafish	n.a.	n= 37 (general)	250-1000 µM	-	-	-	-	-	Experimental group: CIS (250, 500, 750, or 1000 µM) for 4 h, then rinsed 3x in EM; CIS (1000 µM) for 4h, then 3x rinsed in EM and raised in EM up to 72h post injury; Control group: EM	Neuromast selection (P3, P4, P5, P6, P7, P8, and P9), rheotaxis assessment (30 min to allow for dark adaptation, next the water flow is turned on and after 1 minute of acclimatization to the flow, recording 2 min of flow behavior)	The high-throughput behavioral assay produces RIs that decrease with higher dosages of cisplatin. Partial recovery of cisplatin-induced anatomic damage is also observed in the partial recovery of behavioral function.	Automated analysis techniques detect individual zebrafish, compute their orientation, and quantify the rheotaxis behavior of a zebrafish test population, producing a powerful, high-throughput behavioral assay.

Kruger et al. 2016	to screen a natural compound library to find the novel hair cell protectants	-	5 dpf wild-type (Ekkwill) zebrafish	n.a.	n= 8-15 per group	500 µM	E6 Berbamine (a synthetic derivative of bisbenzyl isoquinoline)	G-quadruplex DNA, MET channels	reducing ototoxis substances entry into hair cells through the MET channels	not stated	not stated	Experimental group: E6 berbamine (0.25, 0.5, 1, 10 µM)+ CIS (500 µM) for 6 h, CIS (500 µM); Control group: DMSO	Neuromast assessment (S01, S02, IO1-IO4, M2, MI1, MI2 and O2), recordings of microphonic potentials	E6 (1 and 10 µM) protected hair cell against cisplatin (500 µM).	E6 barbamine presents otoprotective properties against cisplatin-induced damage by direct interaction with MET channels.
Lee et al. 2015	to evaluate the protective effect of quercetin on cisplatin-induced haring loss	-	5 dpf trangenic (Brn3C:EGFP) zebrafish	n.a.	n= 10 per group	1000 µM	quercetin	stimulator of SIRT1 and a PI3K inhibitor	inhibiting the production of inflammation-producing enzymes (cyclooxygenase (COX) and lipoxygenase (LOX))	not stated	not stated	Experimental group: CIS (1 mM) + quercetin (10, 50, 100 and 200 µM) for 4 h; CIS (1 mM); Control group: EM	Neuromast assessment (SO1, SO2, 01, OC1), TUNEL assay	Hair cell damage was decreased by co-treatment of quercetin and cisplatin (100 µM). Apoptosis was decreased by quercin. The ultrastructure was preserved in zebrafish by the combination of 100 µM quercetin and cisplatin.	Quercin shows protective effects against (1 mM) cisplatin-induced toxicity in a zebrafish.
Niihori et al. 2015	to create a high-throughput platform for drug development assessing ototoxic and otoptotective compounds	-	4-5 dpf AB wild-type zebrafish	n.a.	n= 3-7 per group	250-1000 µM	Dexamethasone (DEXO)	glucocorticoid receptor	changes in gene expression leading to multiple downstream effects	not stated	not stated	Experimental group: CIS (0, 250, 500, 750, or 1000 µM) for 4 h, then rinsed 3x in EM; DEXO (5 µM) for 12 h + CIS (1000 µM) for 4 h, then rinsed 3x in EM; Control group: EM	Neuromast selection (P3, P4, P5, P6, P7, P8, and P9), rheotaxis assessment (30 minutes to allow for dark adaptation, next the water flow is turned on and after 1 min of acclimatization to the flow, recording 2 min of flow behavior)	DEXO pre-exposure partially reduced cisplatin induced damage and rheotaxis index (RI). The system demonstrated a linear correlation between 250 and 1000 µM of CIS and rheotactic swimming behavior.	The rescue swimming behavior can serve as a biomarker for rescued hair cell function. There is a dose response between cisplatin exposure, progressive hair cell damage, and reduced rheotaxis in zebrafish.

Thomas et al. 2015	to screen the ActiProbe 10K library of 10 000 small molecules to identify new protective molecules against cisplatin	human lung adenocarcinoma cells, NCI-H23	4-5 dpf AB wild-type zebrafish	to screen new molecules	n= 7-13 per group	20-200 µM	CHCP1 and CHCP2 (small molecules)	not stated	not stated	there was a small reduction in tumor kill with increasing doses of CHCP1 and CHCP2 . In addition, both CHCP1 alone and CHCP2 alone caused dose-dependent tumor cell death	CHCP1 above 100 µM began to be lethal to the zebrafish larvae	Initial Screen: YO-PRO1, then EM+ 1,5µl of compound for 1 h then co-treated with CIS (50µM) for 24h; Experimental group: pretreated with vehicle only (1.1% DMSO in EM) or the protective compound (50 µM); CHCP1 (0-100 µM) or CHCP2 (0-100 µM) for 1 h; CIS (20, 50, 75, 100, 200 µM) + CHCP1 or CHCP2 for 24h; CHCP1 or CHCP2 for 1 h, then, cotreated with 50 µM Rho-Pt for 1 h and rinsed in EM, then treated with SYTOX Green Control group: CIS (50 µM), EM	Neuromast selection, counting hair cells (SO1, SO2,O1 and OC1), Pho-Pt uptake (Sytox), immunohistochemistry	CHCP1 and CHCP2 caused dose-dependent reduction in cisplatin-induced hair cell death. Pretreatment with CHCP2 (100 µM) presented the maximum protection. Treatment with CHCP1, but not CHCP2 reduced hair cell uptake of Rho-Pt.	2 small molecules (CHCP1 and CHCP2) protect against cisplatin-induced hair cell death. CHCP2 maintained protection over a wider range of cisplatin doses. The protective effects of CHCP1 and CHCP2 are only partially maintained with increased cisplatin concentration.
Min et al. 2014	to investigate whether preconditioning by using Dexmedetomidine (DEX) decreased the occurrence of ischemia in inner cells of the ear	-	5 dpf transgenic (Brn3C: EGFP) zebrafish	n.a.	no data	1000 µM	Dexmedetomidine	Central alpha-2 Adrenergic receptors	Adrenergic alpha2-Agonist	not stated	not stated	Experimental group: DEX (0.1, 1, 10 µM) for 150 min, then CIS (1 mM) for 6h; DEX (10 µM) + YOH (100 µM) for 150 min then CIS (1mM) for 6 h; Control group: EM (control for 8 hours)	Neuromasts assessment (P1, P2, P3), hair cell counting from P3	Hair cell apoptosis by cisplatin was attenuated more significantly in the DEX preconditioning group than in the control group. The preconditioning effects were not blocked by yohimbine.	Hearing loss caused by vibration-induced noise could be reduced by using DEX. Preconditioning resulted in a reduction of ischaemic damage that was related to short ischaemia

															or drug administration prior to long ischaemia.
Hong et al. 2013	to evaluate the effects of Edaravone on cisplatin-induced ototoxicity in transgenic zebrafish	-	5 dpf transgenic (Brn3C) zebrafish	n.a.	n= 10 per group	1000 µM	Edaravone	free radicals	ROS reduction claim	not stated	not stated	Experimental group: CIS (1000 µM) + Edaravone (50, 100, 250, 500, 750, 1000 or 2000 µM) for 4 h, then rinsed 3x in EM; Control group: EM	Neuromasts assessment (SO1, SO2, O1, OC1), TUNEL assay	Edaravone (750 µM) protected the hair cell against cisplatin-induced (1000 µM) and decreased the TUNEL reaction.	Edaravone protects against cisplatin-induced hair cell loss by preventing apoptosis.
Thomas et al. 2013	to evaluate the mechanism of cisplatin-induced hair cell death (potential cisplatin uptake modulators)	-	5 dpf AB wild-type, mariner, sputnik, Tg(pou4f3:gap43-GFP) zebrafish	n.a.	n= 9-12 per group	0-200 µM	Quinine	Fe(II)-protoporphyrin IX; platelet glycoprotein IX; potassium channel	not stated	not stated	not stated	Experimental group: CIS (50-150, 250-500 µM) for 6 and 24 h; CIS (0-200 µM) and/or Rho-Pt for 24 h Cop* (0-0.25 µM) for 20 min, then CIS (50 µM) + Cop for 6 or 24h; CIM** (0-400 µM) for 1 h, then CIS (50 uM) + CIM for 6 or 24h; CIM (400 µM) + Cop (0.05-0.5 µM) + CIS (50 µM) for 24 h; Cop (0.25 µM) + CIM (400 µM) + CIS (100-500 µM) for 6 h; Quinine (10-100 µM) for 1 h, then Quinine + CIS (250-500 µM) for 24h; EGTA or quinine (5 mM) with no calcium for 20	Hair cell counting (SO1, SO2, O1 and OC1), immunohistochemistry, FM1-43 assay (in 5 neuromasts selected from SO3, O1, OC1, D1, MI2, MI1, O2, OP1, M2 or IO4)	Pretreatment with quinine (100 µM) resulted in significant protection against cisplatin (250 and 500 µM) for 6h. Low concentration copper and/or cimetidine treatment does not prevent cisplatin-induced hair cell death. Increasing concentrations of cimetidine did not significantly protect against cisplatin-induced hair cell death.	Functional mechanotransduction is required for cisplatin-dependent hair cell death. Blockade of mechanotransduction using quinine protects against cisplatin-induced hair cell death. Inhibition of Oct2 and Ctr1 do not significantly affect cisplatin induced hair cell death.

												min, then CIS (0-500 µM) or Rho-Pt (50 µM) for 4 h; Control group: EM *to inhibit Ctr1 ** to inhibit Oct2			
Uribe et al. 2013	to develop a cisplatin dose-response curve	-	5 dpf AB wild-type and transgenic (Brn3c mGFP) zebrafish	n.a.	n= 9-16 per group	250-1000 µM	Dimethyl sulfoxide (DMSO)	free radicals	reducing ROS claim	DMSO potentiates the effect of cisplatin	Co-treated with DMSO more than >0.01% resulted in killing more hair cell than cisplatin alone	Experimental group: CIS (0.25; 0.50; 0.75; 1.0 and 1.5 mM) co-treated with DMSO (0.001, 0.005, 0.01, 0.05 0.1, 0.5%) + CIS (1 mM) for 4 h; EtOH or MeOH or PEG 400 (0.75%) for 4 h; CIS (1mM) + 0.75% EtOH or 0.75% MeOH or 0.75% PEG 400; Control group: DMSO	Neuromast assessment, hair cell counting in O2	Co-treated with DMSO more than >0.01% resulted in killing more hair cell than cisplatin alone. 1 mM CIS noticeably fewer hair cells. PEG 400 (0.75%) were lethal for zebrafish during 4h incubation.	DMSO potentiates the effects of cisplatin and killed more sensory hair cells than treatment with cisplatin alone.
Shin et al. 2013	to evaluate the effectiveness of KR-22335 as an otoprotective agent against cisplatin-induced toxicity	HEI-OC1 cell line (in vitro)	4 dpf AB wild-type zebrafish	to confirm the efficiency of KR-22335 in vivo	n= 4-5 per group	50 µM	KR-22335 (a novel synthetic compound, 3-Amino-3-(4-fluoro-phenyl)-1H-quinoline-2,4-dione)	not stated	ROS reduction claim	KR-22335 did not interfere with the anti-cancer effect of cisplatin	not stated	Experimental group: KR-22335 (1, 10, 100 µg/ml) for 1 h, then CIS (50 µM) for 24 h; Embryos: CIS (50µM) + KR-22335 (50 or 100µg/ml); Control group: EM	Neuromasts assessment (SO1, SO2, O1, OC1), TUNEL assay in embryos	Neuromasts from cisplatin-treated larvae showed marked damage to hair cells in neuromasts. Exposure to 50 µM cisplatin for 1 h resulted in severe morphological damages, such as loss or fusion of kinocilium. Cotreatment with KR-22335 reduced cisplatin-induced hair	Cisplatin promotes apoptotic cell death in neuromasts of zebrafish. Cisplatin-induced apoptosis could be inhibited by KR-22335 pretreatment in vivo.

														cell loss compared with treatment with cisplatin alone. Exposure to 50 µM cisplatin for 24 h resulted in increased TUNEL positive cells. Co-treatment with KR-22335 reduced TUNEL positive cells compared with treatment with cisplatin alone.	
Coffin et al. 2013	to investigate cell death pathways activated in response to ototoxic substances such as neomycin, gentamycin and cisplatin	-	5-6 dpf AB wild-type zebrafish	n.a.	n= 7-12 per group	250-750 µM	FUT-175, Z-LLF-CHO, 3-MA , D-methionine, leupeptin	FUT-175 (Serine Protease), Z-LLF-CHO (proteases), 3-MA (PI3Kγ; Vps34), D-methionine (chelates heavy metals), Leupeptin (protease)	FUT-175: reducing eosinophil infiltration, mast cell activation	not stated	Z-LLF-CHO appeared mildly ototoxic during continuous exposure experiments; Z-LLF-CHO and D-methionine presented limited protection against cisplatin	Experimental group: inhibitor (10 µM) for 1h, then CIS (500 µM) for 6h; DMSO for 6h; CIS (500 µM) for 6h, 2x rinsed in EM; inhibitor* (10 µM) for 1h, then CIS (500 µM) + inhibitor for 6h; inhibitor (10 µM) for 1h, then CIS (250-1000 µM) for 6h; cycloheximide (1–100 lM) for 1h then cycloheximide + CIS (250-750 µM) * 61 pharmacological inhibitors were tested	Assessment of 10 head neuromasts, hair cell counting (SO1, SO2, IO1, IO2, IO3, OP1, and M2)	FUT-175, Z-LLF-CHO presented a narrow protection against cisplatin in comparison to 3-MA, D-methionine and leupeptin.	The pattern of protection seen in this cell death inhibitor library screen indicates that there is considerable overlap in the cell death pathways activated by cisplatin.

Mackenzie, Raible, 2012	to test if hair cell regeneration requires proliferation; to analyze the time of regeneration after treatment with different ototoxin and if proliferative regeneration is the predominant mechanism of hair cell regeneration	-	3-5 dpf AB wild-type and transgenic Et (krt4:EGFP) zebrafish	n.a.	n= 8 per group	50 µM	Flubendazole (Flu, microtubule assembly blocker)	tubulin	stop producing the new hair cells in zebrafish	not stated	Flubendazole shows a little toxicity to hair cells. Incubating larvae in flubendazole during recovery significantly blocked the addition of new hair cells	Experimental group: CIS (50 µM) for 24 h + Flu* (5µM); Control group: EM, CIS (50µM) for 30 min-10 h	FM 1-43FX assay (IO1-4, M2, MI1, MI2, O2, SO1, S02), proliferation assay	Regeneration after cisplatin is delayed in comparison to NEO or GEN regeneration until 96 hpt. Incubating larvae in Flu during regeneration significantly blocked the addition of new hair cells. Incubation in Flu preserved a little protection There was no dose-dependent effect of Flu on regeneration.	Inhibiting proliferation with Flu blocked the production of new hair cells. It indicates that proliferation has a dominant role during regeneration of lateral line.
Vlasits et. al. 2012	to screen of FDA-approved drug library reveals compounds that protect hair cells from cisplatin and determine the optimal concentration of protective compound	-	5-6 dpf AB wild-type zebrafish	n.a.	n= >9 per group	0-100 µM	Benzamil	epithelial sodium channel	inhibits sodium reabsorption in the distal convoluted tubules	not stated	not stated	Experimental group: 2 YO-PRO1 (2 µM) for 30 min then incubation in one of the library compounds (4 µg/ml) for 1 h and then + CIS (50 µM) for 24h; protective compounds (0, 0.5, 1, 5, 10, 50 µM) + CIS; CIS (0-100 µM) + benzamil (0, 5, 10, 25, 50, 100 µM) for 24h; Control group: CIS (50 µM) for 24h	Assessment of 10 neuromasts (IO 1-4, SO1-2, M2, MI1-2, O2)	Benzamil protected hair cells from cisplatin-induced hearing loss in zebrafish.	The protective compounds should be develop in studies in mammals.

Hirose et al. 2011	to screen a library of 88 anti- cancer drugs to identify drugs that damage hair cells of the zebrafish lateral line	-	5 dpf AB wild-type zebrafish	n.a.	n= 7-10 per group	50 µM	-	-	-	-	-	Experimental group: potential drug (0, 10, 50, 100, 200 and 400 µM) for 1 or 6 h; CIS (50 µM) + Dox or Vinc or Vino (1-100 µM) or 5-Fu (1-50 µM) for 6h; Control group: EM (150 µL)	Hair cell counting (SO1, SO2, O1 and OC1)	Carboplatin was not detected as a toxic. Anti-cancer drugs vincristine (Vinc), vinorelbine (Vino), doxorubicin (Dox) presented synergistic ototoxic effects.	Combination doses of drugs that individually causes no hair cell loss could cause significant hair cell death when used together.
Choi et al. 2011	to investigate the protective effects of apocynin on cisplatin-induced hair cell death	HEI-OC1 cells	5 dpf transgenic (Brn3C:EGFP) zebrafish	to study the effect of apocynin	n= 4 per group	1000 µM	Apocynin	nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase)	ROS reduction	not stated	not stated	Experimental group: CIS (1 mM)+ apocynin (125,250,500 and 1000 µM) for 6h, THEN 3x rinsed in EM; Control group: apocynin (125,250,500 and 1000 µM)	Hair cell counting (SO1, SO2, 01, OC1), TUNEL assay	Apocynin decreased the TUNEL reaction and prevent cisplatin-induced hair cell loss at the neuromasts at low concentration (125-250 µM).	Apocynin prevents cisplatin induced apoptotic cell death.

Abbreviations: CIS, cisplatin; RA, Rapamycin; SIRT1, Sirtuin 1; Qx, quinoxaline; COP, copper; dpf, days post-fertilization; mpf, months post-fertilization; GM, gentamicin; NM, neomycin; OPC, optimally protective concentration; h, hour; EM, embryo media; TL, total length; min, minutes; RI, Rheotaxis index; FF, fenofibrate; SnPPIX, tin protoporphyrin IX; TBBPA, tetrabromobisphenol-A; ROS, reactive oxygen species; DMSO, vehicle dimethyl sulfoxide; Rho-Pt, rhodamine-conjugated platinum reagent; HGF, hepatocyte growth factor; dexmedetomidine, DEX; YOH, yohimbine; N-acetylcysteine, NAC; Texas red-conjugated Neomycin, NRT; CIM, cimetidine; Flu, F