Supplemental Figure 1: Zebrafish assays for potential otoprotective compounds.

(A, B) Assay testing the ability of ToCRIScreen library compounds to block FM1-43FX from entering hair cells of zebrafish lateral line neuromasts. (A) Control larvae treated with 3 μM FM1-43FX, (B) larvae treated with 100 μM of compound 13143 and 3 μM FM1-43FX. (C, D) Assay testing the ability of compounds to block loading of 25 μM of Texas Red-conjugated neomycin (TR-Neo). (C) Control larvae treated with 25 μM TR-Neo, (D) larvae treated with 100 μM of compound 13143 and 25 μM TR-Neo. (E-H) Assay testing the ability of compounds to protect against neomycin-induced cell death. Hair cells were pre-loaded with 3 μM Yo-Pro-1. (E) Neuromast of a control larva, (F) neuromast of a larva treated with 6.25 μM of neomycin, (G) neuromast of a larva treated with 25 μM of compound 13143 and 6.25 μM of neomycin, (H) neuromast of a larva treated with 25 μM of compound 13218 and 6.25 μM of neomycin. Compound 13218 provides full protection; compound 13143 is partially protective. Representative neuromasts from the trunk (posterior) lateral line are shown in each panel. Images are representative of n = 3 independent experiments with approximately 3 fish per well. Scale bar = 25 μm.
Supplemental Figure 2: Percentage survival of hair cells for each cochlea treated with an otoprotective compound. Black circles represent cochleae treated with 5 µM gentamicin and red squares represent cochleae treated with 5 µM gentamicin and either 10 or 50 µM of otoprotective compound. Data are cell counts plotted as a percentage of control. Dotted line represents the threshold above which a compound is considered protective. Number of independent experiments detailed in Supplemental Table 1.
Supplemental Figure 3: Compounds providing protection against gentamicin-induced hair-cell loss in mouse cochlear cultures at a concentration of 10 µM. Cochlear cultures from P2 pups were treated for 48 h with (A) 0.5% DMSO (n = 67), (B) 5 µM gentamicin and 0.5% DMSO (n = 67) or (C-O) 5 µM gentamicin and 10 µM of compounds (C) 13087 (n = 6), (D) 13097 (n = 6), (E) 13104 (n = 7), (F) 13142 (n = 8), (G) 13143 (n = 8), (H) 13150 (n = 6), (I) 13154 (n = 5), (J) 13170 (n = 5), (K) 13190 (n = 6), (L) 13196 (n = 5), (M) 13218 (n = 7), (N) 13222 (n = 10) and (O) 13228 (n = 7). Cultures were labelled with TRITC-phalloidin and images were acquired from the basal coil. Samples are representative. A compound was considered protective if it protected in ≥ 60% of tests. Asterisks identify compounds that damage hair bundles (only compound 13170 in this assay) while arrows indicate specific examples of some of the damaged bundles. Scale bar = 50 µm.
Supplemental Figure 4: Effects of compounds (50 µM) on mouse cochlear hair cells in the absence of gentamicin. Cultures prepared from P2 pups were treated for 48 h with either (A) 0.5% DMSO (n = 2) or (B-N) 50 µM compound as indicated (n = 2 for all compounds). Cultures were labelled with TRITC-phalloidin and images were acquired from the basal coil. Asterisks identify compounds that damage hair bundles. Scale bar = 50 µm.
Supplemental Figure 5: Antimicrobial activity of gentamicin in the presence of otoprotective compounds. Box-whisker plots of percentage difference in survival of (A) *Pseudomonas aeruginosa*, (B) *Staphylococcus aureus* and (C) *Klebsiella pneumoniae* (measured by ATP luminescence) in the presence of 2.2 µM gentamicin and 11 µM of the 13 otoprotective compounds (ratio of 1:5) compared to compound-free gentamicin control. Each compound was tested with 3 technical replicates and 3 independent biological replicates. Thick line = median, boxes = interquartile range (IQR), whiskers = an additional 1.5x IQR; means shown as filled circles, outliers shown as open circles.
Supplemental Figure 6: Reduction of gentamicin-induced hair-cell loss in zebrafish larvae with otoprotectants at a concentration of 50 µM. Zebrafish larvae (4 dpf) were treated for 5 h with either E3 control (A), 10 µM gentamicin (B), or (C-L) 10 µM gentamicin and 100 µM of compound as indicated. Neuromasts were pre-labelled with 3 µM Yo-Pro-1. n = 3 independent experiments with 3 or more fish per well. Representative images of individual neuromasts shown. Scale bar = 25 µm.