**Supplementary File 2a**

|  |  |  |
| --- | --- | --- |
| **Proposed MOA** | **Tested Compounds (abbrv.)** | **References** |
| **Antioxidant** | N-Acetyl-L-cysteine (NAC) | (Lee et al., 2011; Schimel et al., 2011) |
| N-tert-Butyl-α-(2-sulfophenyl)nitrone | (Mandal et al., 2011) |
| **Hormones and analogs** | 17β-Estradiol | (Nonaka et al., 2000) |
| D-(-)-Norgestrel | (Doonan et al., 2011) |
| **PPAR agonist** | Rosiglitazone | (Doonan et al., 2009) |
| **Hsp90 inhibitor** | 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG) | (Tam et al., 2010) |
| **Ceramide synthesis inhibitor** | Myriocin | (Strettoi et al., 2010) |
| **α2-adrenergic agonist** | Clonidine | (Chao and Osborne, 2001) |
| **RTK inhibitor** | Sunitinib | (Latham et al., 2015) |
| **Necrosis inhibitor** | Necrostatin-1 | (Dong et al., 2012) |
| **β-adrenoceptor antagonist** | Levobetaxolol | (Agarwal et al., 2002) |
| **Calpain inhibitor** | Calpastatin peptide | (Paquet-Durand et al., 2010) |
| Calpeptin | (Das et al., 2006) |
| **HDAC inhibitor** | Vorinostat (SAHA) | (Berner and Kleinman, 2016; Zhang et al., 2015) |
| Romidepsin (FK228) | (Zhang et al., 2015) |
| Panobinostat (LBH589)  | (Zhang et al., 2015) |
| Entinostat (SNDX-275) | (Zhang et al., 2015) |
| **Antioxidant cocktail** | Alpha-tochopherol | (Komeima et al., 2007, 2006) |
| Ascorbic acid |
| Alpha-lipoic acid |
| MnTBAP |

**Compounds tested as positive controls**

List of 17 compounds previously reported as neuroprotectants in RP models tested for survival effects in *rho:YFP-NTR* zebrafish larvae using the primary screening protocol.

**Supplementary File 2b**

|  |  |
| --- | --- |
| **Autofluorescent Compounds****(27)** | Acriflavine (acriflavinium hydrochloride)Acriflavine hydrochlorideAklavine hydrochlorideAlexidine hydrochlorideAromatic cascara fluid extractBenserazideDanthronDithiazanine iodide (3,3 diethylthiadicarbocyanine iodide)FluoresceinGreen tea polyphenolsJuniper tarMerbrominNitroxoline (8-hydroxy 5-nitroquinoline)NogalamycinOftasceine (calcein)Paroxetine hydrochloridePhenylmercuric acetateProflavine hemisulfate salt hydrateProtamine chloridePyronin YPyrvinium pamoateQuinalizarinRiboflavinRiboflavin tetrabutyrateRose BengalRutilantinSafranin O |
| **Lethal Compounds****(6)** | Cetylpyridinium (Pyrisept)ClosantelHexadimethrine bromideIodineMethyl violet 2BPolymyxin B sulfate |

**List of eliminated compounds**

Compounds that were autofluorescent (precluding YFP signal detection) or lethal at the concentrations tested (10 mM to 0.625 mM).

**Supplementary File 2c**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Leads** | **Drug Name** | **SSMD** | **Conc.** | **Effect****subtype** | **Dose-dependent trend** |
|  | Aluminum chloride hexahydrate | 2.05;1.16 | 4;2 | Strong | Yes |
|  | Myrrh oil | 1.85 | 4 | Fairly strong | No |
| ● | Miconazole | 1.71; 1.77 | 2; 1 | Fairly strong | No |
|  | Digoxin | 1;1.67 | 4;0.25 | Fairly strong | No |
| ● | Dihydroartemisinin | 1.59 | 4 | Moderate | Yes |
| ● | Ciclopirox olamine | 1.56;1.28;1.05 | 4;2;0.5 | Moderate | Yes |
|  | Isopropamide Iodide | 1.54;1.27 | 2;0.125 | Moderate | No |
|  | Lactulose | 1.52 | 0.25 | Moderate | Yes |
| ● | Warfarin | 1.51;1.05 | 4;2 | Moderate | Yes |
|  | Ethohexadiol | 1.5 | 4 | Moderate | No |
|  | 6,7-Dihydroxyflavone | 1.12;1.04;1.48 | 1;0.25;0.125 | Moderate | No |
|  | Thiostrepton | 1.48 | 4 | Moderate | Yes |
| ● | Cloxyquin  | 1.47 | 4 | Moderate | Yes |
|  | Peruvian balsam | 1.46 | 0.5 | Moderate | No |
|  | Almond oil from prunus dulcis | 1.41 | 0.25 | Moderate | No |
|  | Danthron | 1.41 | 0.25 | Moderate | Yes |
|  | 2-Amino-5-(4-nitro-phenylsulfonyl)thiazole | 1; 1.4; 1.06 | 2; 0.5; 0.25 | Moderate | No |
|  | Indomethacin | 1.38 | 0.125 | Moderate | Yes |
|  | Escitalopram oxalate | 1.34 | 0.125 | Moderate | No |
|  | Naproxen sodium | 1.34 | 0.25 | Moderate | No |
|  | Isoxsuprine hydrochloride | 1.31 | 0.25 | Moderate | No |
| ● | Zinc pyrithione  | 1.31 | 2 | Moderate | Yes |
|  | Diazepam | 1.3 | 0.125 | Moderate | Yes |
| ● | Calcimycin | 1.3 | 1 | Moderate | Yes |
|  | Amantadine Hydrochloride | 1.28 | 0.25 | Moderate | No |
|  | Nipecotic acid | 1.27 | 0.125 | Fairly moderate | No |
|  | Butoconazole nitrate | 1.27 | 2 | Fairly moderate | No |
|  | Pilocarpine hydrochloride | 1.26 | 2 | Fairly moderate | No |
|  | Hydroquinone | 1.24;1.02 | 2;0.5 | Fairly moderate | No |
|  | Acacetin | 1.12;1.24 | 4;0.25 | Fairly moderate | No |
|  | Eupatorin | 1.24 | 4 | Fairly moderate | Yes |
|  | Neohesperidin dihydrochalcone | 1.24 | 0.125 | Fairly moderate | No |
|  | Pseudoephedrine, (1S,2S)-(+)- | 1.24 | 0.25 | Fairly moderate | Yes |
|  | 5-fluoro-5'-deoxyuridine | 1.22 | 0.25 | Fairly moderate | No |
|  | 2-[N-(3-Phenylpropyl)thiocarbamoyl]-L-cysteine | 1.22 | 4 | Fairly moderate | Yes |
|  | Rathyronine | 1.21 | 0.25 | Fairly moderate | No |
|  | Chlorquinaldol  | 1.21 | 4 | Fairly moderate | No |
|  | Streptomycin sulfate | 1.03;1.2 | 4;1 | Fairly moderate | No |
|  | Orthothymotinic acid | 1.19 | 1 | Fairly moderate | No |
|  | Compactin  | 1.19 | 2 | Fairly moderate | Yes |
|  | Quinoline | 1.19 | 0.25 | Fairly moderate | No |
|  | Ampicillin sodium salt | 1.19 | 0.25 | Fairly moderate | Yes |
|  | Cyclocreatine | 1.17 | 1 | Fairly moderate | No |
|  | Succimer  | 1.17 | 0.5 | Fairly moderate | No |
|  | Rose oil | 1.17 | 0.25 | Fairly moderate | Yes |
|  | Bopindolol maleate | 1.16 | 0.25 | Fairly moderate | No |
|  | Pentolinium | 1.16 | 0.25 | Fairly moderate | Yes |
|  | Oxytetracycline hemicalcium salt | 1.16 | 4 | Fairly moderate | Yes |
|  | Dantrolene sodium | 1.14 | 0.125 | Fairly moderate | No |
|  | Methylatropine nitrate  | 1.14;1 | 4;0.5 | Fairly moderate | No |
|  | Magnesium gluconate | 1.14 | 4 | Fairly moderate | Yes |
|  | Leucovorin Calcium | 1.1;1;1.13 | 4;0.5;0.125 | Fairly moderate | No |
|  | Lobeline sulfate | 1.13;1.1 | 0.25;0.125 | Fairly moderate | Yes |
|  | Mercaptamine hydrochloride  | 1.12;1.05 | 1;0.5 | Fairly moderate | No |
|  | Chlordiazepoxide | 1.07;1.12;1.11 | 4;1;0.125 | Fairly moderate | No |
|  | Panthenol  | 1.12 | 4 | Fairly moderate | No |
|  | 1,2-bis(trimethylsilyloxy)ethane | 1.11 | 0.25 | Fairly moderate | No |
|  | Methyl orange | 1.11 | 4 | Fairly moderate | Yes |
|  | Dyclonine hydrochloride | 1.1 | 0.5 | Fairly moderate | No |
|  | 3-Hydroxybenzylhydrazine dihydrochloride | 1.1;1.07 | 0.25;0.125 | Fairly moderate | Yes |
|  | NCS-382 | 1.1;1.04 | 2;0.5 | Fairly moderate | No |
|  | 1,3 Diethyl 2 thiobarbituric acid | 1.1 | 4 | Fairly moderate | No |
| ● | Cortexolone  | 1.1 | 0.125 | Fairly moderate | Yes |
|  | 3-Amino-4-methoxybenzoic acid | 1.1 | 0.125 | Fairly moderate | No |
|  | Hydroxyquinoline benzoate  | 1.1 | 2 | Fairly moderate | No |
|  | Iodoquinol | 1.09 | 0.25 | Fairly moderate | No |
|  | Rofecoxib | 1.09 | 0.125 | Fairly moderate | Yes |
| ● | Sulindac | 1.09 | 0.25 | Fairly moderate | Yes |
|  | Terephthalic acid | 1.09 | 4 | Fairly moderate | No |
|  | 4-Hydroxy-6-Methylpyran-2-One  | 1.08 | 0.125 | Fairly moderate | No |
|  | D-Galactosamine hydrochloride | 1.08 | 0.25 | Fairly moderate | Yes |
|  | Strophanthin K | 1.08 | 0.125 | Fairly moderate | No |
|  | Lincomycin hydrochloride | 1.07 | 4 | Fairly moderate | No |
|  | Ethaverine hydrochloride | 1.07 | 4 | Fairly moderate | No |
|  | Hydroxypropyl cellulose  | 1.07 | 1 | Fairly moderate | No |
|  | Strychnine | 1.06 | 0.25 | Fairly moderate | Yes |
|  | Pyrantel | 1.06 | 0.5 | Fairly moderate | No |
|  | Bamethan sulfate  | 1.06 | 1 | Fairly moderate | No |
|  | Deoxycytidylic acid  | 1.06 | 0.25 | Fairly moderate | No |
|  | Belladonna tincture | 1.06 | 0.125 | Fairly moderate | Yes |
| ● | Artemisinin | 1.06 | 0.5 | Fairly moderate | No |
|  | Frequentin | 1.05 | 0.25 | Fairly moderate | No |
|  | O-Phenanthroline | 1.05 | 0.5 | Fairly moderate | No |
|  | Hexamethylene glycol | 1.05 | 1 | Fairly moderate | No |
|  | Dichlorisone acetate | 1.05 | 0.25 | Fairly moderate | No |
|  | β-Estradiol 3-benzoate | 1.05 | 0.25 | Fairly moderate | No |
|  | Ellman's reagent | 1.05 | 4 | Fairly moderate | No |
|  | Clopidogrel sulfate | 1.04 | 2 | Fairly moderate | Yes |
|  | Lovastatin | 1.04;1.01 | 0.5;0.25 | Fairly moderate | Yes |
|  | Harmine | 1.04 | 0.25 | Fairly moderate | Yes |
|  | Xanthurenic acid | 1.03 | 0.25 | Fairly moderate | Yes |
|  | Aclarubicin | 1.03 | 4 | Fairly moderate | No |
|  | Menadione | 1.03 | 1 | Fairly moderate | Yes |
|  | Methylphedrine ((1R,2S)-(-)-N-Methylephedrine) | 1.03 | 1 | Fairly moderate | No |
|  | Aluminum lactate | 1.03 | 2 | Fairly moderate | No |
|  | Hexamethonium Chloride | 1.03 | 0.25 | Fairly moderate | No |
|  | Vitamin B4 | 1.03 | 0.25 | Fairly moderate | Yes |
|  | Triethylene glycol | 1.03 | 0.5 | Fairly moderate | No |
|  | Deltaline | 1.02 | 0.25 | Fairly moderate | Yes |
|  | Sennoside A | 1.02 | 0.125 | Fairly moderate | Yes |
| ● | Chloroxine  | 1.01 | 4 | Fairly moderate | No |
|  | Tetrabromophenolphthalein ethyl ester, Potassium salt | 1.01 | 0.25 | Fairly moderate | Yes |
|  | Methyl ethyl ketone  | 1.01 | 0.125 | Fairly moderate | No |
|  | 3,3,5-Triiodo L-thyronine | 1.01 | 0.5 | Fairly moderate | No |
|  | Nalidixic acid | 1 | 2 | Fairly moderate | Yes |
|  | Putrescine dihydrochloride | 1 | 0.5 | Fairly moderate | No |
|  | Acetazolamide | 1 | 0.25 | Fairly moderate | No |
|  | Betaine | 1 | 4 | Fairly moderate | No |
|  | Maleic acid | 1 | 0.5 | Fairly moderate | No |
|  | Norfenefrine  | 1 | 0.5 | Fairly moderate | No |
|  | Azure A | 1 | 0.25 | Fairly moderate | Yes |
|  | Chlorpheniramine | 1 | 0.5 | Fairly moderate | No |
|  | Mustard oil | 1 | 4 | Fairly moderate | Yes |

**List of 113 hit compounds**

Hit compounds producing a SSMD score ≥1 in the primary screen ordered according to SSMD score. Drug names, concentrations producing SSMD ≥1, SSMD scores, SSMD effect types, and whether a dose-dependent trend was observed or not are shown. Yellow highlighted drugs were selected for confirmation testing. “●” denotes confirmed lead compounds (Supplementary File 2c-source data).

**Supplementary File 2d**

|  |  |  |
| --- | --- | --- |
| **MOA Category****(# cmpds)** | **Subcategory (# cmpds)** | **Compound Name** |
| **Neurotransmitter****modulator****(17)** | GABA signaling (4) | 3-Hydroxybenzylhydrazine dihydrochlorideChlordiazepoxideDiazepamNipecotic acid |
| Cholinergic signaling (3)  | Isopropamide IodideMethylatropine nitratePilocarpine hydrochloride |
| Nicotinic receptor modulator (3) | Hexamethonium chloridePentoliniumPyrantel |
| Dopamine release (2) | Lobeline sulfateAmantadine hydrochloride |
| Serotonin reuptake inhibitor (2) | ChlorpheniramineEscitalopram oxalate |
| Glycine/Acetylcholine receptor antagonist (1) | Strychnine |
| Glutamate signaling (1) | Xanthurenic acid |
| GHB receptor antagonist (1) | NCS-382 |
| **Ion transport****modulator****(9)** | Sodium channel blocker (2) | DeltalineDyclonine hydrochloride |
| Na+/K+ ATPase inhibitor (2) | DigoxinStrophanthin K |
| Calcium modulator (2) | Dantrolene sodiumEthaverine hydrochloride |
| Multiple ions carrier (2) | CalcimycinSuccimer |
| Proton (1) | Zinc pyrithione |
| **Adrenergic receptor modulator****(6)** | Beta-adrenergic modulator (3)  | Bamethan sulfateBopindolol maleateIsoxsuprine hydrochloride |
| α and β receptors modulator (3) | Methylphedrine ((1R,2S)-(-)-N-Methylephedrine)NorfenefrinePseudoephedrine, (1S,2S)-(+)- |
| **Antibacterial****agent****(6)** | Protein synthesis inhibitor (4) | Lincomycin hydrochlorideOxytetracycline hemicalcium saltStreptomycin sulfateThiostrepton |
| Peptidoglycan synthesis inhibitor (1) | Ampicillin sodium salt |
| Unknown (1) | Aluminum lactate |
| **Hormone****related****(5)** | Thyroid hormone (2) | 3,3,5-Triiodo L-thyronineRathyronine |
| Cortecosteroid (2) | CortexoloneDichlorisone acetate |
| Estrogen (1) | β-Estradiol 3-benzoate |
| **Chelating agent (5)** | CloxyquinHydroxyquinoline benzoateIodoquinolO-Phenanthroline Quinoline |
| **Therapeutic plant extract (5)** | Almond oil from prunus dulcisBelladonna tinctureMustard oilMyrrh oilRose oil |
| **NSAID (4)** | COX1 inhibitor (2) | IndomethacinSulindac |
| COX2 inhibitor (1) | Rofecoxib |
| Nonselective COX inhibitor (1) | Naproxen sodium |
| **Antioxidant (4)** | Flavonoid (3) | 6,7-DihydroxyflavoneAcacetinEupatorin |
| Glutathione S-transferase inducer (1) | 2-[N-(3-Phenylpropyl)thiocarbamoyl]-L-cysteine |
| **Vitamin (4)** | Vitamin B4PanthenolMenadioneLeucovorin calcium |
| **DNA synthesis inhibitor and cleavage (3)** | Nalidixic acid; 5-Fluoro-5'-Deoxyuridine; Aclarubicin |
| **Antifungal agent (3)** | Ergosterol inhibition(2) | Butoconazole nitrateMiconazole |
| Catalase and endoperoxide enzyme inhibitor (1) | Ciclopirox olamine |
| **Antimalarial (2)** | ArtemesininDihydroartemisinin |
| **Antimicrobial agent (2)** | ChloroxineChlorquinaldol |
| **Anticoagulant (2)** | Clopidogrel sulfateWarfarin |
| **HMG-CoA reductase inhibitor (2)** | CompactinLovastatin |
| **MAO-A inhibitor (2)** | HarmineSennoside A |
| **Others (19)** | 1,3 Diethyl 2 thiobarbituric acidAcetazolamideAluminum chloride hexahydrateAzure ABetaineCyclocreatineD-Galactosamine hydrochlorideDanthronDeoxycytidylic acidEllman's reagentEthohexadiolHexamethylene glycol (1,6 hexane diol)HydroquinoneHydroxypropyl celluloseLactuloseMagnesium gluconateMaleic acidMercaptamine HydrochlorideMethyl orange |
| **Unknown (13)** | 1,2-bis(trimethylsilyloxy)ethane2-Amino-5-(4-nitro-phenylsulfonyl)thiazole3-Amino-4-methoxybenzoic acid4-Hydroxy-6-Methylpyran-2-OneFrequentinMethyl ethyl ketone (2-Butanone)Neohesperidin dihydrochalconeOrthothymotinic acidPeruvian balsamPutrescine dihydrochlorideTerephthalic acidTetrabromophenolphthalein ethyl ester, Potassium saltTriethylene glycol |

**On-label MOA for 113 hit compounds**

Implicated MOA categories and subcategories are listed in order from most common to least common. The number of compounds per each category/subcategory are provided in the parentheses and compound names are listed.