**Supplemental Material**

Identification of Estrogen-Related Receptor Alpha Agonists in the Tox21 Compound Library

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**Supplementary Table S1.** qHTS primary and follow-up screen data

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Chemical Name** |  | **ERR** |  | **PGC/ERR** |  |
| **(CASRN, Supplier)** |  | **Primary** | **Follow-up** | **Primary** | **Follow-up** |
| **NCGC#** | **Structure** | **EC50 (µM)** | **EC50 (µM)** | **EC50 (µM)** | **EC50 (µM)** |
| **[Purity Rating]** |  | **[Efficacy**§ **(%)]** | **[Efficacy**§ **(%)]** | **[Efficacy**§ **(%)]** | **[Efficacy**§ **(%)]** |
|  |  | **Cluster 1.10** |  |  |  |
| Adriamycin hydrochloride(25316-40-9, Toronto Research)NCGC00260032-011[A](25316-40-9, Tocris)NCGC00024415-342[A] | 25316-40-9 | Inactive\*1 | Inactive\*2 | 0.235 ± 0.0324[57.5 ± 15.0]1 | 0.419 ± 0.0482[75.7 ± 7.71]2 |
| Daunomycin Hydrochloride(23541-50-6, LKT Labs)NCGC00258711-01[A] | 23541-50-6 | Inactive\* | Inactive\* | 0.109 ± 0.0304[39.3 ± 14.5] | 0.344 ± 0.0395[107 ± 9.17] |
| Pirarubicin(72496-41-4, Sigma)NCGC00344543-01[B] | Chemical Structure | Inactive\* | Inactive\* | 0.406 ± 0.0265[50.9 ± 10.0] | 0.692 ± 0.0882[87.7 ± 4.21] |
|  |  | **Cluster 1.13** |  |  |  |
| Biochanin A(491-80-5, Sigma)NCGC00256458-01[A] | Chemical Structure | 3.47 ± 1.96[169 ± 7.73] | 11.3 ± 2.03[172 ± 7.54] | 7.92 ± 2.21[168 ± 25.7] | 13.1 ± 1.81[123 ± 4.87] |
| Genistein(446-72-0, Light Biologicals)NCGC00254275-01[A] | Chemical Structure | 4.34 ± 1.43[101 ± 9.53] | 12.1 ± 0.00[125 ± 3.97] | 8.15 ± 1.84[115 ± 18.2] | 11.2 ± 0.758[88.1 ± 1.97] |
|  |  | **Cluster 1.15** |  |  |  |
| 5,6-Benzoflavone(6051-87-2, Acros)NCGC00255199-01[A] | Chemical Structure | 0.102 ± 0.0183[216 ± 28.0] | 0.360 ± 0.0813[239 ± 8.62] | 0.203 ± 0.0701[109 ± 2.52] | 0.337 ± 0.0938[127 ± 21.9] |
| Flavone(525-82-6, Sigma)NCGC00260532-011[A](525-82-6, Sigma)NCGC00090962-052[A] | Chemical Structure | 5.99 ± 3.28[70.7 ± 9.22]1 | 11.7 ± 1.99[74.2 ± 7.19]2 | 8.50 ± 2.06[34.0 ± 5.90]1 | 9.54 ± 0.00[31.8 ± 5.45]2 |
|  |  | **Cluster 2.12** |  |  |  |
| Apigenin(520-36-5, Light Biologicals)NCGC00256419-01[A] | Chemical Structure | 5.72 ± 1.03[119 ± 19.6] | 7.73 ± 1.62[102 ± 12.1] | 4.87 ± 2.48[80.4 ± 32.9] | 12.8 ± 2.88[80.6 ± 5.13] |
| Chrysin(480-40-0, Sigma)NCGC00255307-01[A] | Chemical Structure | 7.42 ± 0.483[190 ± 46.1] | 7.48 ± 2.81[145 ± 28.8] | 2.63 ± 0.171[53.7 ± 5.79] | 11.4 ± 4.25[95.3 ± 9.57] |
|  |  | **Cluster 2.13** |  |  |  |
| Daidzein(486-66-8, Sigma)NCGC00258995-011[A](486-66-8, Light Biologicals)NCGC00257367-012[A] | Chemical Structure | 16.6 ± 2.82[154 ± 38.8]1 | 30.3 ± 0.00[135 ± 6.41]2 | 24.2 ± 2.78[124 ± 18.7]1 | 34.5 ± 6.44[111 ± 16.5]2 |
| Formononetin(485-72-3, Light Biologicals)NCGC00255167-01[A] | Chemical Structure | 4.26 ± 1.03[162 ± 15.3] | 7.73 ± 1.62[161 ± 11.0] | 12.5 ± 8.16[155 ± 66.9] | 13.1 ± 0.851[118 ± 13.0] |
| Ipriflavone(35212-22-7, Sequoia)NCGC00018139-05[A] | Chemical Structure | 1.68 ± 0.599[143 ± 25.9] | 2.03 ± 0.279[95.3 ± 9.78] | 1.91 ± 0.736[78.8 ± 11.8] | 2.87 ± 2.08[74.8 ± 17.5] |
|  |  | **Cluster 6.20** |  |  |  |
| 2-Amino-6-methoxybenzothiazole(1747-60-0, Sigma)NCGC00256938-01[A] | Chemical Structure | 22.4 ± 9.37[37.4 ± 7.25] | 14.2 ± 1.81[47.2 ± 3.86] | Inactive\* | Inactive\* |
| Riluzole(1744-22-5, Vitas)NCGC00015882-15[A] | Chemical Structure | 10.2 ± 0.665[67.8 ± 13.3] | 10.7 ± 2.44[44.8 ± 8.78] | 10.6 ± 0.00[40.0 ± 4.54] | 18.0 ± 1.17[42.3 ± 7.00] |
|  |  | **Cluster 7.9** |  |  |  |
| Albendazole(54965-21-8, Sigma)NCGC00255250-01[A] | Chemical Structure | 3.87 ± 1.27[35.2 ± 9.15] | Inactive\* | 12.0 ± 10.7[75.0 ± 24.7] | 2.53 ± 0.747[56.1 ± 12.2] |
| Carbendazim(10605-21-7, Light Biologicals)NCGC00254328-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
| Parbendazole(14255-87-9, Prestwick)NCGC00016706-07[A] | Chemical Structure | 1.02 ± 0.0665[35.8 ± 13.9] | Inactive\* | 6.47 ± 4.69[94.2 ± 14.9] | 3.65 ± 2.00[69.6 ± 16.6] |
| Thiophanate(23564-06-9, Light Biologicals)NCGC00255347-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
|  |  | **Cluster 7.10** |  |  |  |
| Cyclobendazole(31431-43-3, GVK)NCGC00262953-01[A] | Chemical Structure | Inactive\* | Inactive\* | 6.44 ± 0.420[38.8 ± 2.95] | Inactive\* |
| Fenbendazole(43210-67-9, Vitas)NCGC00016855-10[A] | Chemical Structure | 3.26 ± 0.555[34.1 ± 14.9] | Inactive\* | 16.5 ± 9.00[138 ± 48.9] | 5.71 ± 0.786[49.5 ± 1.06] |
| Mebendazole(31431-39-7, Microsource)NCGC00016806-13[A] | Chemical Structure | Inactive\* | Inactive\* | 4.06 ± 0.265[65.2 ± 5.05] | 3.45 ± 0.234[48.7 ± 7.77] |
| Nocodazole(31430-18-9, Prestwick Chemical, Inc.)NCGC00015647-18[A] | Chemical Structure | Inactive\* | Inactive\* | 4.81 ± 3.14[82.4 ± 18.3] | 6.50 ± 1.62[53.4 ± 4.83] |
| Oxfendazole(53716-50-0, Vitas)NCGC00095157-05[ND] | Chemical Structure | Inactive\* | Inactive\* | 15.4 ± 7.23[111 ± 25.8] | 6.28 ± 3.65[51.8 ± 7.24] |
|  |  | **Cluster 8.14** |  |  |  |
| Benzyl salicylate(118-58-1, Sigma)NCGC00256928-01[A] | Chemical Structure | 28.5 ± 3.64[35.0 ± 7.44] | 22.7 ± 5.13[52.7 ± 6.67] | Inactive\* | 23.1 ± 15.5[33.6 ± 14.4] |
| Phenyl 1-hydroxy-2-naphthoate(132-54-7, Sigma)NCGC00255458-01[A] | Chemical Structure | 22.3 ± 8.72[167 ± 43.8] | 12.1 ± 0.00[137 ± 7.73] | 10.9 ± 1.25[74.6 ± 7.93] | 10.8 ± 1.24[86.8 ± 4.83] |
| Salicylic acid p-tolyl ester(607-88-5, TCI)NCGC00256803-01[A] | Chemical Structure | Inactive\* | 27.0 ± 0.00[42.6 ± 1.96] | Inactive\* | 21.8 ± 4.56[34.9 ± 0.752] |
|  |  | **Cluster 8.15** |  |  |  |
| Benzylparaben(94-18-8, Sigma)NCGC00255352-01[A] | Chemical Structure | 12.5 ± 2.24[47.7 ± 8.50] | 28.1 ± 9.57[66.6 ± 9.42] | 12.4 ± 0.837[47.1 ± 4.80] | 24.9 ± 10.4[45.5 ± 11.7] |
| Butylparaben(94-26-8, Enamine)NCGC00254294-01[A] | Chemical Structure | 15.4 ± 5.07[34.8 ± 4.52] | Inactive\* | 20.4 ± 7.64[30.0 ± 8.84] | 31.6 ± 2.14[35.9 ± 4.61] |
| Heptylparaben(1085-12-7, Alfa Aesar)NCGC00257279-01[A] | Chemical Structure | Inactive\* | Inactive\* | 21.3 ± 13.3[31.6 ± 5.06] | Inactive\* |
| Hexylparaben(1083-27-8, Light Biologicals)NCGC00257292-01[A] | Chemical Structure | 6.42 ± 1.15[85.2 ± 7.20] | 11.6 ± 0.758[75.3 ± 4.44] | 14.9 ± 10.8[85.6 ± 21.0] | 14.2 ± 8.75[57.7 ± 8.34] |
| Methylparaben(99-76-3, Enamine)NCGC00253939-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
| Phenylparaben(17696-62-7, TCI)NCGC00257341-01[A] | Chemical Structure | 25.8 ± 5.81[41.0 ± 5.80] | 29.2 ± 1.90[68.3 ± 6.85] | 39.7 ± 18.6[71.1 ± 21.1] | 35.4 ± 2.40[87.0 ± 10.0] |
| sec-Butylparaben(17696-61-6, Light Biologicals)NCGC00257425-01[A] | Chemical Structure | Inactive\* | 7.82 ± 2.50[31.5 ± 3.48] | Inactive\* | Inactive\* |
| tert-Butyl 4-hydroxybenzoate(25804-49-3, Sigma)NCGC00256248-01[A] | Chemical Structure | Inactive\* | 12.1 ± 0.00[37.2 ± 7.08] | Inactive\* | Inactive\* |
|  |  | **Cluster 8.18** |  |  |  |
| 4-Ethoxyphenol(622-62-8, Sigma)NCGC00256790-01[A] | Chemical Structure | Inactive\* | Inactive\* | 59.2 ± 8.14[35.2 ± 3.39] | Inactive\* |
| 4-(Hexyloxy)phenol(18979-55-0, Sigma)NCGC00257244-01[A] | Chemical Structure | Inactive\* | Inactive\* | 16.5 ± 9.66[68.8 ± 13.1] | 29.8 ± 11.2[47.6 ± 7.25] |
|  |  | **Cluster 10.14** |  |  |  |
| Naphthalen-2-yl 2-aminobenzoate(63449-68-3, Oakwood)NCGC00256876-01[A] | Chemical Structure | 17.3 ± 1.99[192 ± 15.9] | 20.3 ± 8.74[156 ± 16.9] | 15.8 ± 4.41[110 ± 14.7] | 17.6 ± 5.67[106 ± 7.21] |
| Phenethyl anthranilate(133-18-6, Light Biologicals)NCGC00257318-01[A] | Chemical Structure | 2.15 ± 0.598[187 ± 33.0] | 4.63 ± 0.302[156 ± 16.2] | 4.06 ± 3.98[131 ± 64.6] | 3.54 ± 0.240[106 ± 6.28] |
|  |  | **Cluster 15.23** |  |  |  |
| Atorvastatin(134523-00-5, Waterstone)NCGC00255181-01[A] | Chemical Structure | 6.04 ± 1.36[71.2 ± 12.6] | 9.69 ± 0.632[70.6 ± 4.86] | Inactive\* | Inactive\* |
| Atorvastatin Calcium(134523-03-8, Light Biologicals)NCGC00255845-01[A] | Chemical Structure | 1.79 ± 0.752[62.2 ± 2.13] | 5.22 ± 0.600[50.5 ± 4.09] | Inactive\* | Inactive\* |
| Cerivastatin sodium(143201-11-0, Sequoia)NCGC00164625-04[A] | Chemical Structure | 0.0986 ± 0.0126[63.1 ± 15.8] | 0.173 ± 0.0117[64.5 ± 7.04] | Inactive\* | Inactive\* |
| Fluvastatin(93957-54-1, Enamine)NCGC00256490-01[A] | Chemical Structure | 1.54 ± 0.177[69.4 ± 14.4] | 2.63 ± 0.448[54.4 ± 5.56] | Inactive\* | Inactive\* |
|  |  | **Cluster 17.8** |  |  |  |
| 2,7-Acetylaminofluorene(304-28-9, ChemService)NCGC00259068-011[A](304-28-9, Light Biologicals)NCGC00256587-012[A] | Chemical Structure | Inactive\*1 | Inactive\*2 | Inactive\*1 | Inactive\*2 |
| N-(2-Fluorenyl)-2,2,2-trifluoroacetamide(363-17-7, Light Biologicals)NCGC00255119-01[A] | Chemical Structure | 8.70 ± 5.54[37.7 ± 7.42] | Inactive\* | 17.7 ± 14.2[34.7 ± 10.4] | Inactive\* |
|  |  | **Cluster 18.9** |  |  |  |
| Isoproturon(34123-59-6, Sigma)NCGC00255738-01[A] | Chemical Structure | 27.3 ± 0.00[30.6 ± 5.61] | Inactive\* | Inactive\* | Inactive\* |
|  |  | **Cluster 18.15** |  |  |  |
| Bromindione(1146-98-1, Microsource)NCGC00160590-03[C] | Chemical Structure | 9.52 ± 6.61[80.9 ± 31.5] | Inactive\* | 16.8 ± 5.99[44.0 ± 7.95] | Inactive\* |
|  |  | **Cluster 19.17** |  |  |  |
| 2-Ethylanthracene-9,10-dione(84-51-5, Sigma)NCGC00256285-01[A] | Chemical Structure | 5.45 ± 0.00[130 ± 20.3] | 8.24 ± 0.537[105 ± 9.70] | 9.28 ± 2.75[65.4 ± 2.63] | 10.1 ± 3.79[73.1 ± 5.54] |
| 2-Methylanthraquinone(84-54-8, Acros)NCGC00255621-01[A] | Chemical Structure | 25.2 ± 1.71[115 ± 20.2] | 9.49 ± 2.64[55.9 ± 3.72] | 15.4 ± 5.59[45.5 ± 5.31] | 18.8 ± 10.3[53.7 ± 6.54] |
| 2-(Propan-2-yl)-9H-thioxanthen-9-one(5495-84-1, Light Biologicals)NCGC00256279-01[A] | Chemical Structure | 5.39 ± 1.50[201 ± 44.0] | 6.82 ± 0.784[134 ± 13.0] | 3.59 ± 0.458[90.5 ± 5.56] | 8.19 ± 4.46[97.8 ± 19.3] |
|  |  | **Cluster 20.8** |  |  |  |
| Nitazoxanide(55981-09-4, Toronto Research)NCGC00258778-011[C](55981-09-4, NIEHS)NCGC00090774-012[A] | Chemical Structure | 1.45 ± 0.895[123 ± 11.1]1 | 2.22 ± 1.69[99.7 ± 18.7]2 | 1.41 ± 1.00[63.5 ± 16.3]1 | 1.11 ± 0.512[74.1 ± 13.9]2 |
| Tenonitrozole(3810-35-3, Vitas)NCGC00160655-03[A] | Chemical Structure | 0.729 ± 0.124[77.0 ± 17.8] | 1.03 ± 0.257[71.2 ± 7.67] | 0.688 ± 0.192[32.8 ± 5.46] | 1.60 ± 0.104[57.3 ± 3.69] |
|  |  | **Cluster 21.10** |  |  |  |
| Resveratrol(501-36-0, ChromaDex)NCGC00258925-011[A](501-36-0, Prestwick)NCGC00015894-022[A] | Chemical Structure | 4.43 ± 0.927[199 ± 11.6]1 | 3.74 ± 0.672[229 ± 12.7]2 | 5.30 ± 0.345[165 ± 7.28]1 | 5.87 ± 0.397[270 ± 15.0]2 |
|  |  | **Cluster 22.12** |  |  |  |
| 2-(2H-Benzotriazol-2-yl)-4-methylphenol(2440-22-4, Sigma)NCGC00257391-01[A] | Chemical Structure | 21.8 ± 2.50[194 ± 25.2] | 12.6 ± 0.851[157 ± 5.58] | 14.2 ± 6.75[78.6 ± 22.5] | 16.1 ± 6.94[110 ± 14.0] |
|  |  | **Cluster 22.22** |  |  |  |
| 13-cis Retinoic acid(4759-48-2, Sigma)NCGC00257647-01[I] | Chemical Structure | 6.61 ± 3.86[43.7 ± 5.32] | 20.9 ± 0.00[38.1 ± 6.00] | Inactive\* | Inactive\* |
| Sorbic acid(110-44-1, Enamine)NCGC00253957-01[A] | Chemical Structure | Inactive\* | 42.6 ± 0.00[47.2 ± 4.29] | Inactive\* | 40.3 ± 3.28[34.9 ± 5.80] |
| trans-Retinoic acid(302-79-4, Sigma)NCGC00259879-011[B](302-79-4, Labotest)NCGC00017280-102[I] | Chemical Structure | 14.5 ± 6.25[51.5 ± 14.8]1 | Inactive\*2 | 22.0 ± 8.49[31.1 ± 8.46]1 | Inactive\*2 |
|  |  | **Cluster 24.11** |  |  |  |
| 4,4'-Sulfonyldiphenol(80-09-1, Sigma)NCGC00256437-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
| 4-Phenylphenol(92-69-3, Sigma)NCGC00256447-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
| Hydroquinone(123-31-9, Enamine)NCGC00254037-01[A] | Chemical Structure | 17.0 ± 4.75[45.6 ± 13.7] | 22.2 ± 10.6[44.3 ± 7.09] | Inactive\* | 22.4 ± 1.51[81.7 ± 9.08] |
| p-Phenylazophenol(1689-82-3, Sigma)NCGC00260514-01[A] | Chemical Structure | 9.11 ± 0.616[95.1 ± 9.31] | 10.3 ± 2.32[91.4 ± 16.9] | 27.8 ± 3.19[97.5 ± 5.98] | 33.2 ± 2.16[125 ± 9.78] |
|  |  | **Cluster 24.23** |  |  |  |
| 2-Carboxyethyl acrylate(24615-84-7, Sigma)NCGC00257334-01[Z] | Chemical Structure | Inactive\* | 28.1 ± 1.90[45.0 ± 8.28] | Inactive\* | Inactive\* |
|  |  | **Cluster 25.22** |  |  |  |
| 4-Hydroxybutyl prop-2-enoate(2478-10-6, Light Biologicals)NCGC00256652-01[Z] | Chemical Structure | 56.7 ± 3.84[103 ± 27.1] | 42.9 ± 0.00[109 ± 3.09] | Inactive\* | 44.6 ± 3.02[68.6 ± 6.84] |
| 2-Hydroxyethyl acrylate(818-61-1, Light Biologicals)NCGC00256462-01[ND] | Chemical Structure | 56.7 ± 3.84[96.5 ± 19.7] | 38.4 ± 0.00[142 ± 4.31] | 55.2 ± 10.3[39.7 ± 5.03] | 40.2 ± 5.12[79.2 ± 13.0] |
|  |  | **Cluster 26.8** |  |  |  |
| 1-Amino-2-methylanthraquinone(82-28-0, Enamine)NCGC00256386-01[A] | Chemical Structure | 6.57 ± 1.94[107 ± 17.3] | 8.24 ± 2.70[92.1 ± 11.1] | 5.86 ± 3.64[52.0 ± 6.73] | 6.08 ± 2.21[59.6 ± 8.86] |
| 2-Aminoanthraquinone(117-79-3, Sigma)NCGC00253972-01[A] | Chemical Structure | 4.46 ± 1.24[140 ± 13.5] | 4.95 ± 1.38[133 ± 18.6] | 2.18 ± 0.354[77.8 ± 3.23]^ | 6.35 ± 2.19[85.9 ± 11.3] |
|  |  | **Cluster 26.23** |  |  |  |
| 2-Phenylethyl 3-phenylprop-2-enoate(103-53-7, Sigma)NCGC00256862-01[A] | Chemical Structure | 18.6 ± 7.81[123 ± 13.3] | 22.1 ± 6.16[111 ± 18.8] | 7.39 ± 3.11[48.0 ± 8.54] | 10.6 ± 2.57[59.3 ± 3.46] |
| Benzyl cinnamate(103-41-3, Acros)NCGC00254607-01[A] | Chemical Structure | 33.4 ± 13.2[52.5 ± 13.9] | 32.6 ± 7.89[30.6 ± 10.5] | 25.3 ± 1.71[42.2 ± 11.9] | 35.7 ± 6.43[32.6 ± 2.45] |
|  |  | **Cluster 27.22** |  |  |  |
| 2-(2-Ethoxyethoxy)ethyl prop-2-enoate(7328-17-8, Sigma)NCGC00256257-01[Z] | Chemical Structure | Inactive\* | 43.0 ± 4.95[54.7 ± 5.71] | Inactive\* | Inactive\* |
| 2-(Dihydrocyclopentadienyloxy)ethyl methacrylate(68169-03-9, Sigma)NCGC00254736-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
| 2-Phenoxyethyl acrylate(48145-04-6, Sigma)NCGC00255604-01[Z] | Chemical Structure | 63.6 ± 4.30[57.3 ± 8.84] | 28.1 ± 1.90[31.5 ± 1.32] | 61.3 ± 7.05[39.9 ± 13.1] | 41.3 ± 2.69[39.9 ± 7.64] |
| Tripropylene glycol diacrylate(42978-66-5, Sigma)NCGC00255375-01[Z] | Chemical Structure | 19.4 ± 2.23[96.5 ± 25.4] | 10.5 ± 1.21[101 ± 19.6] | 22.6 ± 1.53[70.4 ± 10.5] | 7.16 ± 0.466[60.8 ± 8.63] |
|  |  | **Cluster 29.9** |  |  |  |
| 1,4-Benzenediamine(106-50-3, Enamine)NCGC00256482-01[ND] | Chemical Structure | Inactive\* | Inactive\* | 32.1 ± 15.7[33.4 ± 10.8] | Inactive\* |
| 3,4,4'-Triaminodiphenyl ether(6264-66-0, Light Biologicals)NCGC00255251-01[C] | Chemical Structure | 4.86 ± 0.00[103 ± 30.0] | 7.65 ± 0.880[83.6 ± 6.08] | 8.99 ± 0.608[87.9 ± 9.09] | 17.1 ± 6.09[86.0 ± 8.78] |
| 4-Aminoazobenzene(60-09-3, Sigma)NCGC00164077-02[A] | Chemical Structure | 10.2 ± 2.99[91.9 ± 15.6]3 | 14.4 ± 4.08[81.3 ± 7.60] | 8.26 ± 5.17[46.6 ± 6.59]3 | 20.5 ± 6.03[69.8 ± 6.35] |
| 4-Biphenylamine hydrochloride(2113-61-3, Enamine)NCGC00257524-01[A] | Chemical Structure | 14.3 ± 9.75[40.0 ± 5.82] | 18.5 ± 2.55[32.5 ± 2.72] | Inactive\* | 40.5 ± 3.30[31.9 ± 3.26] |
| 4'-Fluoro-4-aminodiphenyl(324-93-6, Light Biologicals)NCGC00255397-01[A] | Chemical Structure | Inactive\* | 22.3 ± 7.60[34.4 ± 1.39] | Inactive\* | 30.5 ± 4.95[30.6 ± 4.59] |
| Benzidine(92-87-5, Enamine)NCGC00254474-01[A] | Chemical Structure | Inactive\* | 23.4 ± 11.0[38.2 ± 5.22] | Inactive\* | Inactive\* |
|  |  | **Cluster 29.10** |  |  |  |
| 2,3-Diaminotoluene(2687-25-4, Sigma)NCGC00254106-01[C] | Chemical Structure | 14.4 ± 12.6[106 ± 28.8] | 21.2 ± 5.92[52.4 ± 10.7] | 16.6 ± 13.4[44.6 ± 10.9] | 26.5 ± 9.98[56.2 ± 6.79] |
| 3,4-Diaminotoluene(496-72-0, Light Biologicals)NCGC00254324-01[A] | Chemical Structure | 3.84 ± 2.84[202 ± 40.4] | 8.40 ± 3.16[55.2 ± 16.6] | 3.71 ± 2.11[80.6 ± 5.22] | 15.2 ± 5.43[99.0 ± 10.7] |
| 3-Methylaniline(108-44-1, Light Biologicals)NCGC00254415-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
|  |  | **Cluster 30.6** |  |  |  |
| Phenazopyridine hydrochloride(136-40-3, Sigma)NCGC00256528-01[A] | Chemical Structure | 9.73 ± 1.12[108 ± 14.2] | 9.72 ± 1.82[84.9 ± 18.1] | 8.75 ± 1.63[67.5 ± 20.2] | 20.1 ± 6.94[82.8 ± 13.6] |
|  |  | **Cluster 30.9** |  |  |  |
| 2-Aminoanthracene(613-13-8, Sigma)NCGC00257001-01[B] | Chemical Structure | 22.1 ± 5.05[61.9 ± 6.39] | 10.8 ± 1.24[51.6 ± 11.4] | 22.4 ± 13.0[36.9 ± 8.97] | 14.3 ± 9.38[52.7 ± 17.0] |
| 3,3'-Diaminobenzidine(91-95-2, Acros)NCGC00255443-01[Z] | Chemical Structure | Inactive\* | Inactive\* | 23.3 ± 12.7[30.5 ± 8.21] | Inactive\* |
|  |  | **Cluster 31.9** |  |  |  |
| 2-Amino-4-phenylthiazole hydrobromide hydrate(52253-69-7, Sigma)NCGC00257725-011[A](52253-69-7, NIEHS)NCGC00091948-012[A] | Chemical Structure | 38.8 ± 22.0[48.0 ± 5.99]1 | 14.6 ± 3.54[41.2 ± 4.85]2 | Inactive\*1 | Inactive\*2 |
| 2-Amino-5,6-dimethylbenzothiazole(29927-08-0, Sigma)NCGC00258632-011[A](29927-08-0, NIEHS)NCGC00091571-012[A] | Chemical Structure | 14.5 ± 5.01[145 ± 29.7] 1 | 11.0 ± 1.98[132 ± 12.2] 2 | 23.0 ± 7.94[99.7 ± 11.0] 1 | 12.7 ± 5.33[85.7 ± 7.23] 2 |
| 2-Aminobenzothiazole(136-95-8, Sigma)NCGC00258372-011[A](136-95-8, NIEHS)NCGC00091895-012[A] | Chemical Structure | 47.6 ± 15.6[38.1 ± 9.67]1 | Inactive\*2 | Inactive\*1 | Inactive\*2 |
|  |  | **Cluster 32.12** |  |  |  |
| Dihydralazine(484-23-1, Labotest)NCGC00159421-03[ND] | Chemical Structure | 30.0 ± 3.45[78.4 ± 8.18] | 0.391 ± 0.115[35.7 ± 4.91] | 21.2 ± 10.8[36.2 ± 2.27] | Inactive\* |
| Hydralazine hydrochloride(304-20-1, Light Biologicals)NCGC00256719-01[A] | Chemical Structure | 2.84 ± 0.967[129 ± 4.64] | Inactive\* | 4.75 ± 3.93[110 ± 42.6] | 0.543 ± 0.0880[31.1 ± 2.81] |
|  |  | **Cluster 33.9** |  |  |  |
| 4-(2-Phenylpropan-2-yl)-N-[4-(2-phenylpropan-2-yl)phenyl]aniline(10081-67-1, TCI)NCGC00257239-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
| 10,11-Dihydro-5H-dibenz[b,f]azepine(494-19-9, GVK)NCGC00253630-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
| N-Phenyl-1-naphthylamine(90-30-2, Sigma)NCGC00259622-011[A](90-30-2, Sigma)NCGC00256930-012[A] | Chemical Structure | 14.3 ± 5.67[44.4 ± 6.71]1 | 27.1 ± 9.89[74.3 ± 8.58]2 | 19.6 ± 4.21[41.3 ± 11.3]1 | 34.0 ± 0.00[67.6 ± 11.7]2 |
| N-Phenyl-2-naphthylamine(135-88-6, Sigma)NCGC00256440-01[A] | Chemical Structure | 13.7 ± 4.52[77.3 ± 16.6] | 35.4 ± 2.40[81.1 ± 6.54] | 27.7 ± 5.17[62.0 ± 12.9] | 38.4 ± 4.41[60.0 ± 0.834] |
|  |  | **Cluster 33.14** |  |  |  |
| Anthracene(120-12-7, Enamine)NCGC00254204-01[B] | Chemical Structure | Inactive\* | 34.3 ± 5.56[32.9 ± 8.05] | Inactive\* | Inactive\* |
| Benz(a)anthracene(56-55-3, Light Biologicals)NCGC00254419-01[A] | Chemical Structure | 13.3 ± 5.26[129 ± 39.7] | 26.2 ± 3.60[133 ± 8.64] | 21.1 ± 3.59[68.5 ± 18.0] | 26.5 ± 9.98[83.4 ± 17.5] |
| Benzo(b)fluoranthene(205-99-2, Sigma)NCGC00254223-01[A] | Chemical Structure | 15.5 ± 13.2[35.6 ± 6.09] | 11.0 ± 5.95[44.1 ± 6.42] | 56.7 ± 3.84[43.6 ± 3.52] | Inactive\* |
| Dibenz(a,h)anthracene(53-70-3, Sigma)NCGC00257287-01[A] | Chemical Structure | 0.598 ± 0.167[122 ± 14.7] | 3.01 ± 0.346[125 ± 5.38] | 3.10 ± 3.92[76.9 ± 49.3] | 2.87 ± 0.851[68.9 ± 6.73] |
| Fluoranthene(206-44-0, Light Biologicals)NCGC00254498-01[A] | Chemical Structure | Inactive\* | Inactive\* | Inactive\* | Inactive\* |
|  |  | **Cluster 37.19** |  |  |  |
| Fuberidazole(3878-19-1, Light Biologicals)NCGC00255946-01[A] | Chemical Structure | 21.1 ± 3.59[79.2 ± 6.34] | 8.17 ± 3.67[50.2 ± 5.92] | 19.6 ± 7.56[41.4 ± 6.37] | 33.0 ± 4.54[66.0 ± 8.73] |
| Thiabendazole(148-79-8, Sigma)NCGC00259946-011[A](148-79-8, NIEHS)NCGC00016410-062[A] | Chemical Structure | 27.6 ± 3.17[98.4 ± 24.3]1 | 8.44 ± 5.52[71.4 ± 12.5]2 | 28.8 ± 5.19[46.8 ± 5.08]1 | 8.90 ± 5.17[41.1 ± 4.56]2 |
|  |  | **Cluster 40.23** |  |  |  |
| Lovastatin(75330-75-5, Vitas)NCGC00023509-131[A](75330-75-5, NIEHS)NCGC00023509-032[A] | Chemical Structure | 2.78 ± 0.354[64.4 ± 10.9]1 | 8.75 ± 1.57[49.5 ± 8.39]2 | Inactive\*1 | Inactive\*2 |
| Simvastatin(79902-63-9, Toronto Research)NCGC00254418-01[B] | Chemical Structure | 13.5 ± 3.27[64.8 ± 25.6] | Inactive\* | Inactive\* | Inactive\* |
|  |  | **Cluster 42.9** |  |  |  |
| 3'-Methyl-4-dimethylaminoazobenzene(55-80-1, Sigma DiscoveryCPR)NCGC00256428-01[A] | Chemical Structure | 3.07 ± 0.751[231 ± 22.9] | 5.40 ± 1.93[205 ± 33.9] | 5.56 ± 4.61[177 ± 89.6] | 4.89 ± 1.12[131 ± 13.0] |
| Methyl Red(493-52-7, Sigma)NCGC00255799-01[A] | Chemical Structure | 5.98 ± 3.19[55.6 ± 16.0] | 3.68 ± 1.21[54.0 ± 6.86] | 8.12 ± 3.04[41.5 ± 4.35] | 9.04 ± 4.17[67.7 ± 9.33] |
| Michler's ketone(90-94-8, Sigma)NCGC00254018-01[A] | Chemical Structure | Inactive\* | Inactive\* | 20.0 ± 6.43[61.9 ± 3.43] | 11.1 ± 3.09[62.4 ± 7.73] |
| N,N-Dimethyl-4-nitrosoaniline(138-89-6, Sigma)NCGC00257004-01[A] | Chemical Structure | 6.89 ± 0.792[71.5 ± 12.8] | Inactive\* | 8.99 ± 0.608[127 ± 89.0] | 12.1 ± 1.39[112 ± 33.9] |

§: The % efficacy is based on the maximal efficacy produced by Genistein.

\*: Compound was identified as inactive if the efficacy was below 30% of Genistein’s activity.

#: At our lowest concentration, the efficacy is already higher than 100% and therefore we generated an estimate based on the lowest test concentration.

Purity Ratings: A = MW Confirmed, Purity > 90%; B = MW Confirmed, Purity 75 – 90%; C = MW Confirmed, Purity 50 – 75%; I = MW Confirmed, Two or more isomers detected; Z = MW Confirmed, No Purity Info; Ac = Purity > 90%, Low concentration of sample; ND = Not determined yet

1: This compound was used in the primary screen and picked for the follow-up screen; however, we ran out of the sample and therefore, had to create a new stock.

2: This is the lot we created to replace 1 for the follow-up screens.

3: n=48, for this sample.

^: n=2, the third sample was an outlier.

**Supplementary Table S2.** qHTS primary and follow-up screen data for singletons

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Chemical Name** |  | **ERR** |  | **PGC/ERR** |  |
| **(CASRN, Supplier)** | **Structure** | **Primary** | **Follow-up** | **Primary** | **Follow-up** |
| **NCGC#** |  | **EC50 (µM)** | **EC50 (µM)** | **EC50 (µM)** | **EC50 (µM)** |
| **[Purity Rating]** |  | **[Efficacy**§ **(%)]** | **[Efficacy**§ **(%)]** | **[Efficacy**§ **(%)]** | **[Efficacy**§ **(%)]** |
|  |  | **Single Actives** |  |  |  |
| 4-Chloro-1,2-diaminobenzene(95-83-0, Sigma)NCGC00091663-03[C] | Chemical Structure | 4.91 ± 4.42[159 ± 50.7]3 | 9.72 ± 1.82[78.1 ± 6.28] | 3.10 ± 3.92[76.9 ± 49.3]3 | 19.5 ± 4.46[83.3 ± 4.96] |
| Amlenanox(68302-57-8, Bosche)NCGC00167472-03[A] | Chemical Structure | 1.50 ± 0.173[178 ± 43.0] | 2.97 ± 0.341[172 ± 13.7] | 1.52 ± 0.318[73.8 ± 5.62] | 1.94 ± 0.660[117 ± 7.31] |
| Axitinib(319460-85-0, Sequoia)NCGC00241108-04[I] | Chemical Structure | 0.948 ± 0.312[128 ± 17.5] | 0.756 ± 0.173[86.3 ± 8.76] | 1.16 ± 0.197[80.4 ± 5.85] | 1.39 ± 0.249[71.8 ± 3.07] |
| C.I. Disperse Yellow 3(2832-40-8, Sigma Chemical Company)NCGC00357249-011[ND](2832-40-8, Sigma)NCGC00164349-012[A] | Chemical Structure | 4.50 ± 0.305[219 ± 27.8]1 | 3.82 ± 0.00[183 ± 3.94]2 | 3.37 ± 1.27[90.1 ± 5.90]1 | 6.30 ± 0.426[142 ± 10.5]2 |
| Fanetizole(79069-94-6, Enamine)NCGC00160438-02[B] | Chemical Structure | 1.36 ± 0.729[86.3 ± 20.1] | 5.47 ± 0.370[139 ± 3.66] | 2.58 ± 0.354[75.6 ± 4.59] | 14.3 ± 0.930[155 ± 6.13] |
| Febuxostat(144060-53-7, Tocris)NCGC00182059-031[A](144060-53-7, APAC)NCGC00182059-022[A] | Chemical Structure | 0.562 ± 0.127[173 ± 41.7]1 | 1.71 ± 0.477[203 ± 22.8]2 | 0.738 ± 0.564[83.8 ± 16.1]1 | 1.53 ± 0.493[129 ± 4.08]2 |
| Forskolin(66575-29-9, Sigma)NCGC00255526-01[A] | Chemical Structure | Inactive\* | Inactive\* | 1.73 ± 0.199[277 ± 27.3] | 0.964 ± 0.111[172 ± 10.4] |
| Frentizole(26130-02-9, Vitas)NCGC00160657-03[A] | Chemical Structure | 1.19 ± 0.00[249 ± 39.0] | 2.02 ± 0.131[181 ± 16.7] | 0.690 ± 0.222[96.7 ± 8.43] | 2.27 ± 0.313[122 ± 3.29] |
| HMR1426(262376-75-0, Pharma)NCGC00254151-01[C] | Chemical Structure | 3.45 ± 1.26[108 ± 10.6] | 4.30 ± 1.57[45.4 ± 7.25] | 7.04 ± 2.76[58.0 ± 12.7] | 5.94 ± 3.25[41.8 ± 16.5] |
| Para-Azoxyanisole(1562-94-3, Sigma)NCGC00258714-011[A](1562-94-3, Sigma)NCGC00091863-042[B] | Chemical Structure | 0.654 ± 0.0426[166 ± 17.0]1 | 1.61 ± 0.222[177 ± 13.7]2 | 0.660 ± 0.112[82.2 ± 6.32]1 | 1.91 ± 1.25[113 ± 12.4]2 |
| Suberoylanilide hydroxamic acid (SAHA)(149647-78-9, Prestwick)NCGC00168085-05[ND] | Chemical Structure | Inactive\* | Inactive\* | 1.96 ± 0.133[112 ± 4.69] | 3.33 ± 0.383[191 ± 4.26] |

§: The % efficacy is based on the maximal efficacy produced by Genistein.

\*: Compound was identified as inactive if the efficacy was below 30% of Genistein’s activity.

#: At our lowest concentration, the efficacy is already higher than 100% and therefore we generated an estimate based on the lowest test concentration.

Purity Ratings: A = MW Confirmed, Purity > 90%; B = MW Confirmed, Purity 75 – 90%; C = MW Confirmed, Purity 50 – 75%; I = MW Confirmed, Two or more isomers detected; ND = Not determined yet

1: This compound was used in the primary screen and picked for the follow-up screen; however, we ran out of the sample and therefore, had to create a new stock.

2: This is the lot we created to replace 1 for the follow-up screens.