Supplemental Data

Small-molecule ligands that bind the RET receptor activate neuroprotective signals independent of but modulated by co-receptor GFRα1

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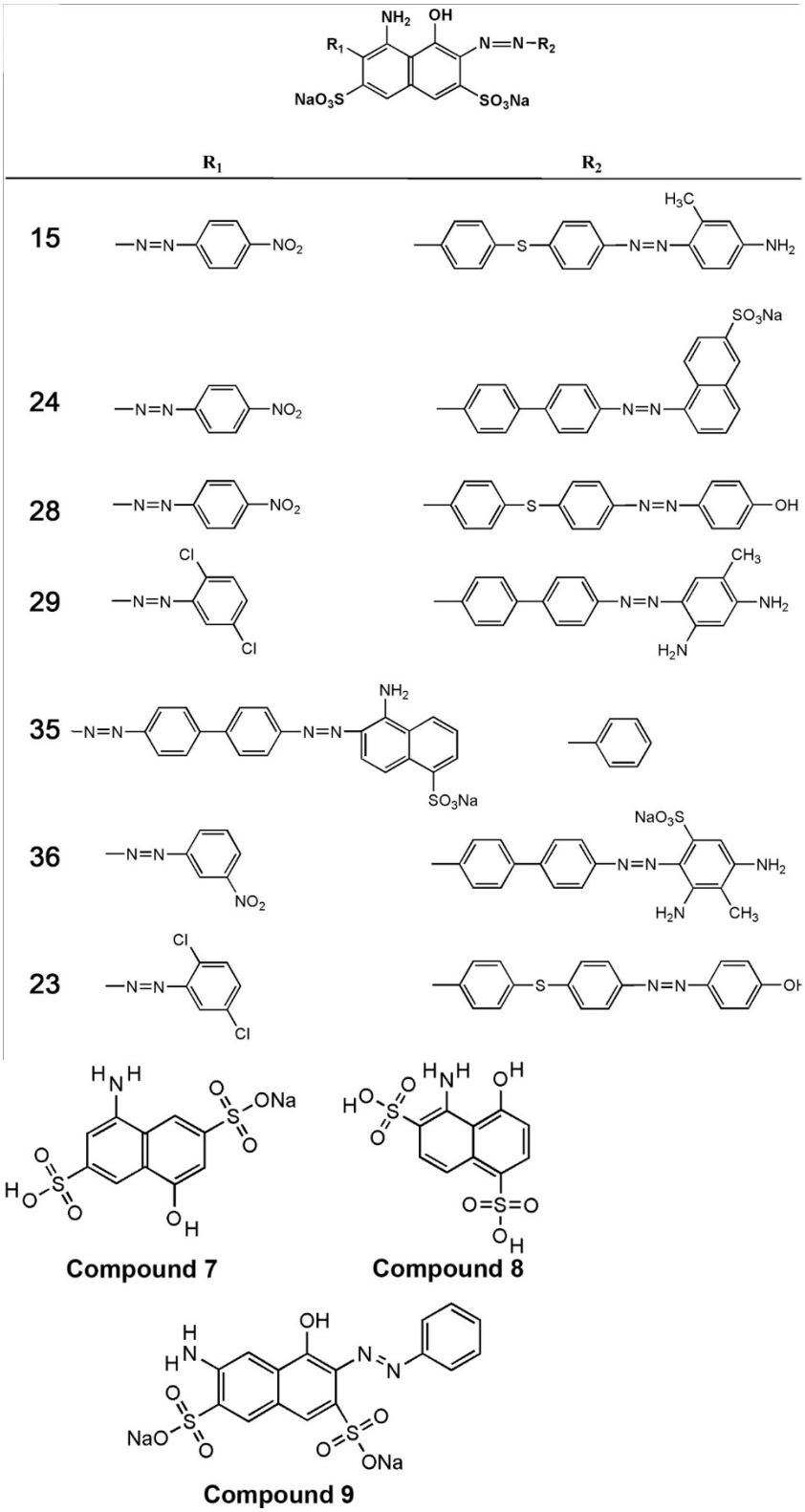
	MG87 RET/GFRa1		MG87 RET	
Compound	**5 µM**	**20 µM**	**5 µM**	**20 µM**
116	102.3124	134.8646	99.44444	117.8862
206	162.1520	122.5338	121.6667	102.0325
210	268.2869	265.0870	133.3333	121.1382
302	121.7084	180.3675	102.7778	105.2846
226	262.9070	227.1760	182.7778	163.4146
224	131.9018	247.6306	99.44444	111.7886
65	162.0104	230.2708	130.5556	174.3902
47	136.6210	166.2476	140.0000	180.4878
29	258.9429	330.0290	185.0000	252.0325

Supplemental Table 1. Initial Compound Screening. In initial screens, ~200 compounds were tested in MG87 cells with and without GFRα1 expression at 5 and 20 µM. Data from a select panel are shown as the average relative increase in luminescence versus vehicle from 4 experiments**. Compound 29** was selected as a candidate hit as it generated significant increases in both cell types, and subsequent analogues were then tested. In these assays, GDNF as positive control induces higher increases in luciferase activity, ranging from 60-80 fold over vehicle, consistent with previous reports (Sidorova et al, 2010).

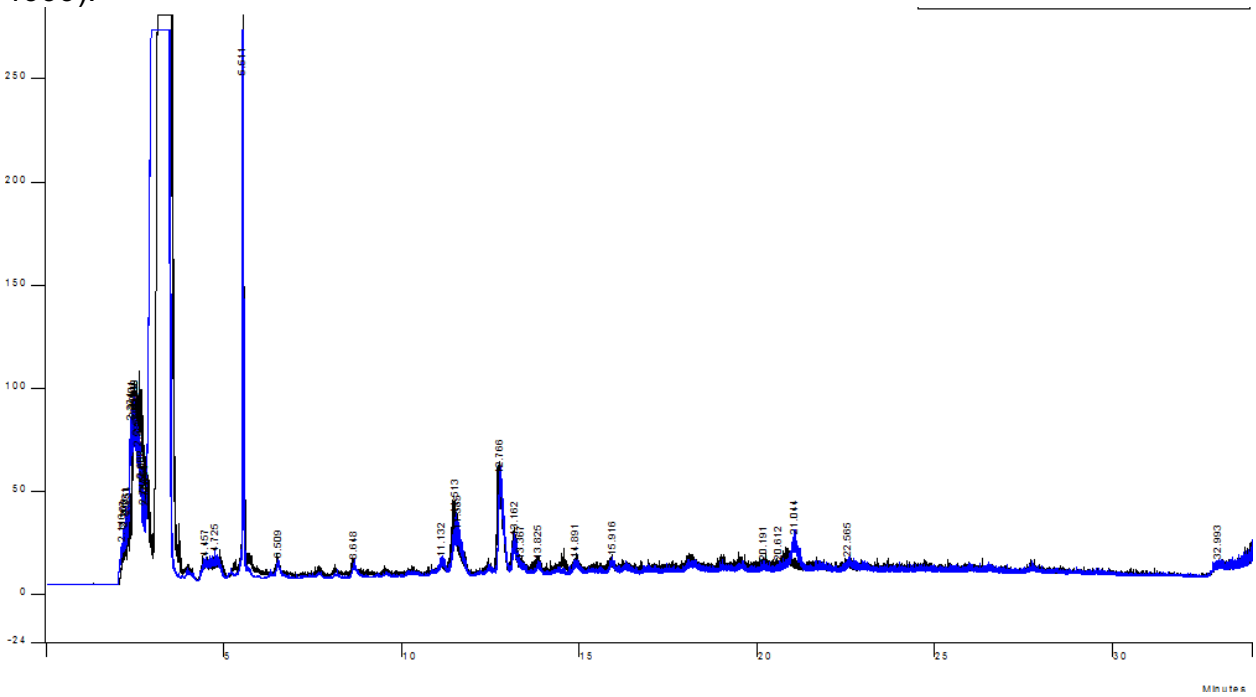
Supplemental Figure 1.

Compound Structures. Overview of the structures as they are described by the NCI.



Structural Properties of NCI Database Compounds and 4-amino-5-hydroxynaphthalene-1,3-disulfonic Acid

4-amino-5-hydroxynaphthalene-1,3-disulfonic acid was obtained commercially from TCI America (Product Code: A0363) as a dark powder with reported purity of >85.0%. Both Compound 8 and this product were purified on a 250 mm x 10 mm Kromasil 100-5-C18 semi-preparative column. A solvent gradient of 5-30%B was used for 30 minutes, where solvent A was HPLC grade H_2O with 0.1% TFA, and solvent B was a 70/30 mix of Acetonitrile/H_2O with 0.1% TFA. Samples of each were lyophilized yielding an off-white powder and dissolved in D_2O for subsequent 1H-NMR at 500 MHz. ESI mass spectra were obtained in negative mode with a Bruker Maxis Impact. Compounds 15, 23, 29, and 35 were dissolved in DMSO for mass spec analysis. Sulfonic acids ionize preferentially in negative mode, and commonly yield di-charged ions by ESI (Holcapek 1999).



Supplemental Figure 2. HPLC chromatogram of the 5-30%B gradient showing 2 mg injections of 4-amino-5-hydroxynaphthalene-1,3-disulfonic Acid (Blue) and Compound 8 (Black). Both were relatively pure with the major fraction eluting ~3min. The overlay of the chromatograms suggest that the impurities in the mixture are likely to be similar.

Supplemental Figure 3.
H-NMR resonances and the detected mass of 4-amino-5-hydroxynaphthalene-1,3-disulfonic Acid and of Compound 8 show that they are likely the same molecule. Therefore compound 8 is incorrectly identified in the NCI database. Other agents (compounds 15,23,29,35) are also shown, and they correspond to the NCI-reports.
Compound 8 (NSC37052). 1H-NMR (D_2O, 500 MHz): δ 8.17 (s, 1H), 7.90 (dd, J = 8.6, 0.9 Hz, 1H), 7.44 – 7.37 (t, 1H), 6.84 (dd, J = 7.8, 0.9 Hz, 1H).
MS (ESI,-) *m/z* calcd for $C_{10}H_7NO_7S_2$ $[M-2H]^{2-}$ calcd: 158.4837, found: 158.4841
4-amino-5-hydroxynaphthalene-1,3-disulfonic acid. 1H-NMR (D_2O, 500 MHz): δ 8.16 (s, 1H), 7.89 (dd, J = 8.5, 1.0 Hz, 1H), 7.44 – 7.37 (t, 1H), 6.84 (dd, J = 7.8, 1.1 Hz, 1H). MS (ESI,-) *m/z* calcd for $C_{10}H_7NO_7S_2$ $[M-2H]^{2-}$ calcd: 158.4837, found: 158.4840
Compound 15 (NSC65571). MS (ESI,-) *m/z* calcd for $C_{35}H_{25}N_9O_9S_3$ $[M-2H]^{2-}$ calcd: 405.5474, found: 405.5460
Compound 23 (NSC75661). MS (ESI,-) *m/z* calcd for $C_{34}H_{21}Cl_2N_7O_8S_3$ $[M-2H]^{2-}$ calcd: 410.5001, found: 410.5009
Compound 29 (NSC79730). MS (ESI,-) *m/z* calcd for $C_{35}H_{25}Cl_2N_9O_7S_2$ $[M-2H]^{2-}$ calcd: 408.5353, found: 408.5356
Compound 35 (NSC79745). MS (ESI,-) *m/z* calcd for $C_{38}H_{25}N_8NaO_{10}S_3$ $[M-2H]^{2-}$ calcd: 436.0382, found: 436.0397