Small molecule agonists of the RET receptor tyrosine kinase activate biased trophic signals that are influenced by the presence of GFRa1 co-receptors

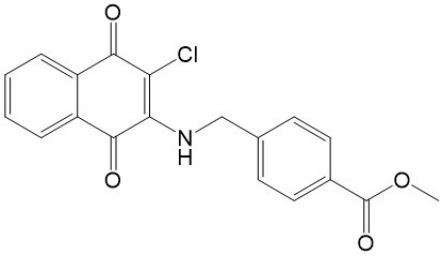
Sean Jmaeff, Yulia Sidorova, Hinyu Nedev, Mart Saarma, H. Uri Saragovi

List of supplemental data included. Synthesis of novel naphthoquinone derivatives, method, and NMR/MS characterization.

Abbreviations in Supplemental:

DBNQ - 2,3-dibromonaphthalene-1,4-dione
DCNQ - 2,3-dichloronaphthalene-1,4-dione (dichlone)
TEA – trimethylamine
MeOH – methanol
Et_2O – diethyl ether
DO – dioxane
EA – ethyl acetate
DCM – dichloromethane
DMF – dimethyl formamide
SP-HPLC – semi-preparative high pressure liquid chromatography
NCS – N-chlorosuccinimide
Chl - chloroform

Methyl 4-(((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)methyl) benzoate (Q101)

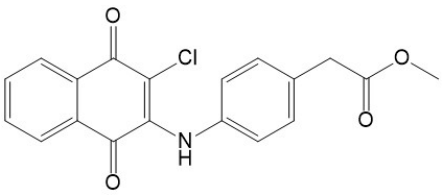


113 mg (0.5 mmol) of DCNQ, 101 mg (0.5 mmol) of methyl 4-(aminomethyl)-benzoate hydrochloride and 70 ul (0.5 mmol) of TEA were dissolved in 10 ml of MeOH and stirred for 18 h at RT. After precipitation with small amount of Et_2O, the red-orange solid was filtered and washed with cold Et_2O, yielding 47% of Q101.

^1H NMR ($CDCl_3$, 400 MHz) δ 8.21 (d, 2H), 8.18 (d, 2H), 8.07 (dd, 2H), 7.68 (d, 2H), 5.15 (d, 2H), 3.96 (s, 3H).

MS (ESI,+) *m/z* calcd for $C_{19}H_{15}ClNO_4$ $[M+H]^+$ calcd 356.06, found 356.3.

Methyl 2-(4-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)phenyl) acetate (Q105)

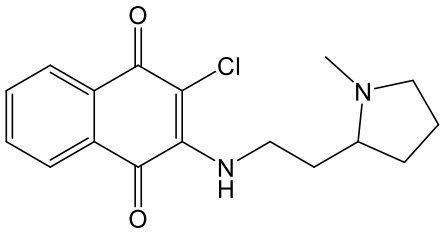


113 mg (0.5 mmol) of DCNQ, 83 mg (0.5 mmol) of methyl 2-(4-aminophenyl)acetate and 70 ul (0.5 mmol) of TEA were dissolved in 12 ml of MeOH and stirred 3 h at RT. After concentration of the solvent were isolated dark red needles with m.p.163-4° C.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.24 (d, 1H), 8.22 (d, 1H), 7.82 (m, 3H), 7.30 (d, 2H), 7.07 (d, 2H), 3.75 (s, 3H), 3.68 (s, 2H).

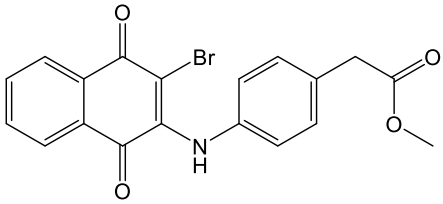
MS (ESI,+) *m/z* calcd for $C_{19}H_{15}ClNO_4$ $[M+H]^+$ 356.06, found 356.3.

2-Chloro-3-((2-(1-methylpyrrolidin-2-yl)ethyl)amino)naphthalene-1,4-dione (Q112)



57 mg (0.25 mmol) of DCNQ, 37.3 ul (0.25 mmol) of 2-(1-methyl-pyrrolidin-2-yl)ethan-1-amine and 35 ul (0.25 mmol) of TEA in 5 ml dry MeOH were stirred at RT for 3 hours and the solvent was concentrated *in vacuo*. The product yielded 33 mg (41%) as orange crystals. Chemical Formula: $C_{17}H_{19}ClN_2O_2$. Exact Mass: 318.11. MS (ESI,+) *m/z* calcd for $C_{17}H_{20}ClN_2O_2$ $[M+H]^+$ 319.11, found 319.1205

Methyl 2-(4-((3-bromo-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)phenyl) acetate (Q141)

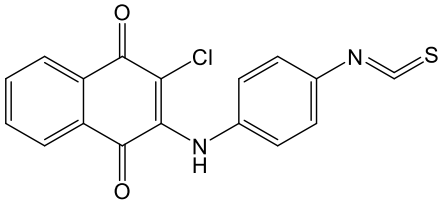


15.8 mg (0.05 mmol) of DBNQ and 8.3 mg (0.05 mmol) of methyl 2-(4-aminophenyl) acetate were dissolved in 1 ml 50% ethanol in water. The solution was stirred and heated at 60° C for 2 hrs. The dark red crystals were filtered and washed with small amount of cold ethanol. Yield 13 mg (64%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.24 (d, 1H), 8.22 (d, 1H), 7.78 (m, 3H), 7.32 (d, 1H), 7.09 (d, 2H), 3.75 (s, 3H), 3.68 (s, 2H).

MS (ESI,+) *m/z* calcd for $C_{19}H_{15}BrNO_4$ $[M+H]^+$ 400.01, found 400.1

2-Chloro-3-((4-isothiocyanatophenyl)amino)naphthalene-1,4-dione (Q143)

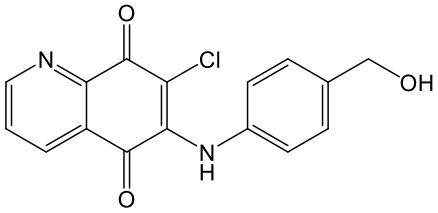


To a solution of 2-((4-aminophenyl)amino)-3-chloronaphthalene-1,4-dione (Ryan Inc) (23.8 mg, 0.08 mmol) in 20 ml of dry DCM was added37.1 mg (0.16 mmol) of 1,1'-thiocarbonylbis(pyridin-2(1H)-one). The mixture was stirred for 2 h at RT under nitrogen and concentrated under vacuum. The solvent was evaporated and the crude product was subjected to flash chromatography with chloroform as eluent. Fractions 2-6 were pooled and concentrated to afford 16 mg (88%) of Q143 as a dark red solid.

1H NMR (300 MHz, DMSO-d6) δ 9.46 (s, 1H), 8.11 – 8.02 (m, 2H), 7.96 – 7.78 (m, 2H), 7.46 – 7.35 (m, 2H), 7.23 – 7.08 (m, 2H).

MS (ESI,+) *m/z* calcd for $C_{17}H_{10}ClN_2O_2S$ $[M+H]^+$ 341.01, found 341.1.

7-Chloro-6-((4-(hydroxymethyl)phenyl)amino)quinoline-5,8-dione (Q525-1)

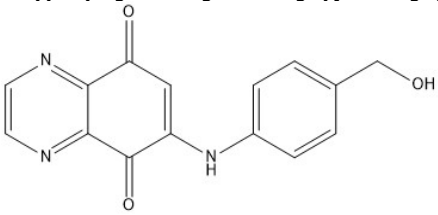


22.7 mg (0.1 mmol) of 6,7-dichloroquinoline-5,8-dione, 15.1 mg(0.1 mmol) of p-aminobenzyl alcohol were dissolved in 2 ml 50% ethanol and stirred at RT for 2 h until completion of the reaction (TLC). The solvent was concentrated and the two diastereomers were separated on semi-preparative HPLC. The H-NMR spectra of both compounds were run and analysed. The 6-substituted (7-Cl) isomer (structure shown above), was isolated in larger amount.

1H NMR (600 MHz, Methanol-d4) δ 8.84 (d, J = 4.7 Hz, 1H), 8.40 (dd, J = 7.9, 1.6 Hz, 1H), 7.69 (dd, J = 7.8, 4.8 Hz, 1H), 7.28 – 7.22 (m, 2H), 7.08 – 7.03 (m, 2H), 4.52 (s, 2H).

MS (+ESI) *m/z* calcd for $C_{16}H_{12}ClN_2O_3$ $[M+H]^+$ 315.05, found 315.07.

6-((4-(Hydroxymethyl)phenyl)amino)quinoxaline-5,8-dione (Q1041)



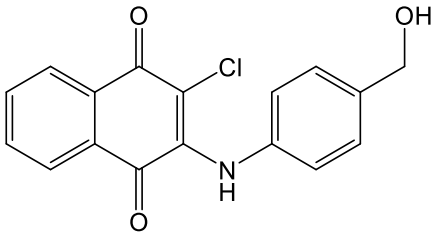
3 mg (0.019 mmol) of quinoxaline-5,8-dione, 2.54 mg (0.02 mmol) of p-aminobenzyl alcohol and 7.68 mg (0.02 mmol) of $CeCl_3.7H_2O$ are dissolved in 0.5 ml of absolute ethanol and stirred at RT overnight. After addition of 1 ml of water the mixture was extracted 3x with chloroform, the organic layer was dried with MgSO4, filtered and

evaporated. The SP-HPLC purification afforded two peaks, which were subjected to MS. The rose peak with RT=11' belonged to the expected product was lyophilized.

1H NMR (400 MHz, DMSO-d6) δ 9.49 (s, 1H), 9.02 (dd, J = 14.2, 2.3 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 8.5 Hz, 2H), 6.22 (s, 1H), 5.23 (s, 1H), 4.52 (d, J = 4.2 Hz, 2H).

MS (MALDI-TOF) *m/z* calcd for $C_{15}H_{12}N_3O_3$ $[M+H]^+$ 282.08, found 282.087.

2-Chloro-3-((4-(hydroxymethyl)phenyl)amino)naphthalene-1,4-dione (Q1047)



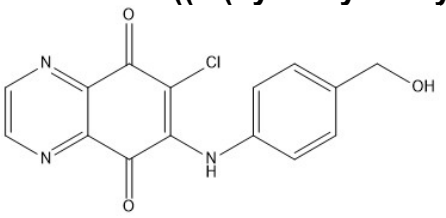
45.2 mg (0.2 mmol) of 2-chloronaphthalene-1,4-dione and 25 mg (0.2mmol) of (4-aminophenyl)methanol were dissolved in 3 ml of dry methanol and stirred overnight at room temperature. The mixture was left for 10 min at 4° C, the unreacted products were filtered and after the evaporation of the solvent, the residue was dissolved in a minimal amount of 1.5% DMF in chloroform and subjected on a flash chromatography. Fractions 11-19 were collected.

R_f=0.2 (chloroform/methanol=98/2), R_f=0.78 (ethyl acetate).

1H NMR (400 MHz, DMSO-d6) δ 9.30 (s, 1NH), 8.04 (d, 2H), 7.84 (dtd, J = 7.5, 1.4 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 7.09 (d, J = 8.2 Hz, 2H), 5.17 (s, 1OH), 4.48 (s, 2H).

MS: (TOF, positive) *m/z* calcd for $C_{17}H_{13}ClNO_3$ $[M+H]^+$ 314.05, found 314.0589

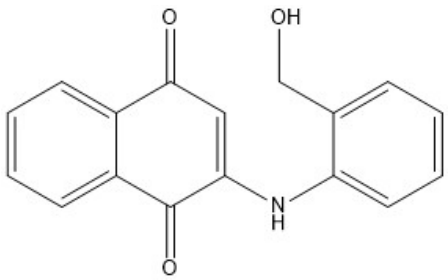
6-Chloro-7-((4-(hydroxymethyl)phenyl)amino)quinoxaline-5,8-dione (Q1048)



A solution of 2-chloro-3-((4-(hydroxymethyl)phenyl)amino)naphthalene-1,4-dione (Q1047) (1.9 mg, 6.76 umol) in 0.75 ml methanol was treated with NCS (0.9 mg, 6.75 umol). The mixture was stirred at RT overnight, the solvent was evaporated and the residue was purified by flash chromatography on Silica gel 60A in mini column with ethyl acetate as an eluent. Fractions 2-6 were collected, pooled and the solvent was evaporated to afford 0.5 mg of Q-1048 as a red solid.

MS (+ESI) *m/z* calcd for $C_{15}H_{11}ClN_3O_3$ $[M+H]^+$ 316.05, found 316.049.

2-Chloro-3-((4-(hydroxymethyl)phenyl)amino)naphthalene-1,4-dione (Q2003)

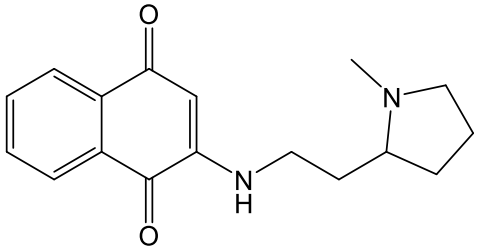


189.6 mg (1.2 mmol) of naphthalene-1,4-dione and 123 mg (1 mmol) of o-aminobenzyl alcohol are dissolved in 8 ml of methanol and stirred overnight at 45° C. The solvent was evaporated and the residue was subjected to flash chromatography with chloroform, C/MeOH=99/1 and C/MeOH=98/2. Fractions 11-15 were pooled and the solvent was evaporated to afford the Q2003 as a dark red solid.

1H NMR (400 MHz, Methanol-d4) δ 8.14 (d, J = 7.6 Hz, 1H), 8.04 (d, J = 7.7 Hz, 1H), 7.81 (t, J = 7.5 Hz, 1H), 7.75 (dd, J = 8.3, 6.7 Hz, 1H), 7.49 (d, J = 7.6 Hz, 1H), 7.46 – 7.38 (m, 2H), 7.28 (t, J = 7.1 Hz, 1H), 6.03 (d, J = 1.5 Hz, 1H), 4.64 (s, 2H), 4.58 (s, 0H).

MS (+ESI) *m/z* calcd for $C_{17}H_{14}NO_3$ $[M+H]^+$ 280.09, found 280.097.

2-((2-(1-Methylpyrrolidin-2-yl)ethyl)amino)naphthalene-1,4-dione (Q2004)



87 mg (0.55 mmol) of naphthalene-1,4-dione and 72 ul (0.5 mmol) of 2-(1-methyl-pyrrolidin-2-yl)ethan-1-amine were dissolved in 2 ml of MeOH and stirred for 2 hrs at RT. The solvent was evaporated, the crude product was dissolved in minimal amount of chloroform and purified by flash chromatography. As eluent was used chloroform with increasing concentration of MeOH up to 25%. The product was collected from fractions 12-20. After evaporating of the organic solvents, Q2004 yielded 60 mg (42%) as a dark orange solid.

1H NMR (400 MHz, Chloroform-d) δ 8.11 (dt, J = 7.8, 0.8 Hz, 1H), 8.04 (ddd, J = 7.7, 1.4, 0.5 Hz, 1H), 7.72 (td, J = 7.6, 1.4 Hz, 1H), 7.61 (td, J = 7.6, 1.3 Hz, 1H), 5.70 (s, 1H), 3.26 (ddq, J = 20.0, 13.4, 6.7 Hz, 2H), 2.40 (s, 4H), 2.01 (s, 1H), 1.77 (s, 2H), 1.58 (s, 6H).

MS (+ESI) *m/z* calcd for $C_{17}H_{21}N_2O_2$ $[M+H]^+$ 285.15, found 285.07.