Supplementary Information

For

"Small Molecule-Mediated Inhibition of Translation by Targeting a Native RNA G-Quadruplex"

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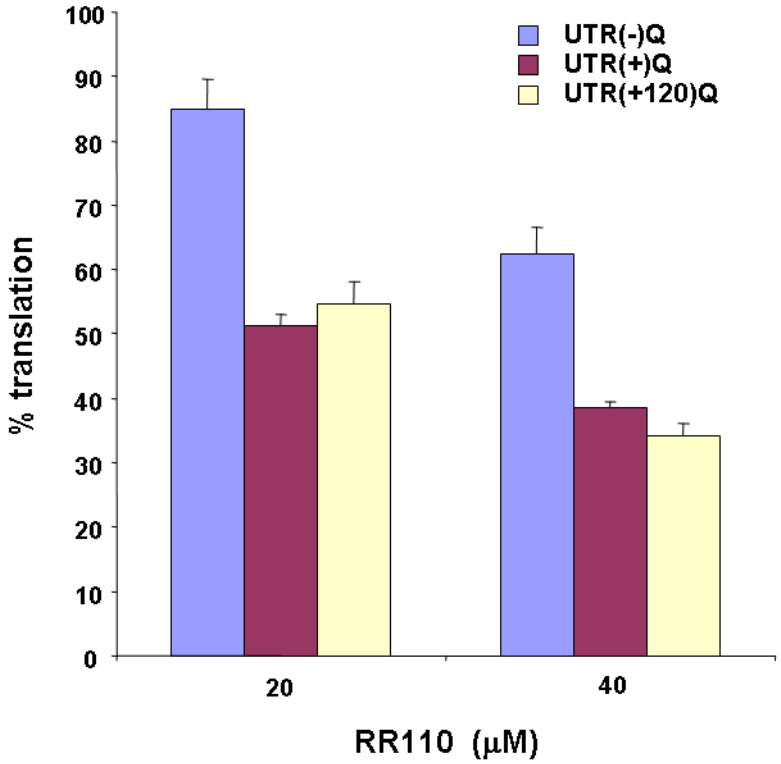


Fig. S1 Relative *in vitro* translation efficiencies of NRAS UTR(–)Q, NRAS UTR(+)Q and NRAS UTR(+120)Q mRNAs in the presence of the 20 and 40 μM of RR110, as measured by luciferase activities. For each construct, the measured luciferase activity was normalized to the activity obtained in the absence of the ligand, which was set as 100%. Experiments were performed at least three times using at least two separate batches of RNA. The average values are presented along with the standard error on the mean.

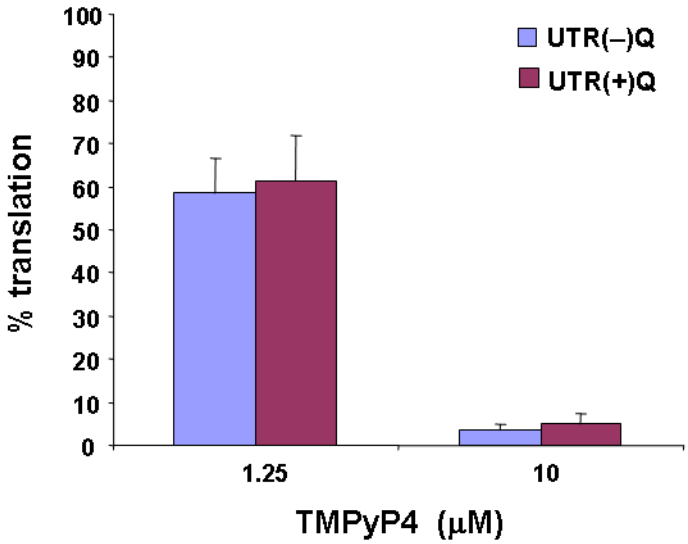


Fig. S2 Relative translation efficiencies of NRAS UTR(–)Q and NRAS UTR(+)Q mRNAs in the presence of the indicated amounts of TMPyP4, as measured by luciferase activities. For each construct, the measured luciferase activity was normalized to the activity obtained in the absence of the ligand, which was set as 100%. Experiments were performed three times. The average values are presented along with the standard error on the mean.

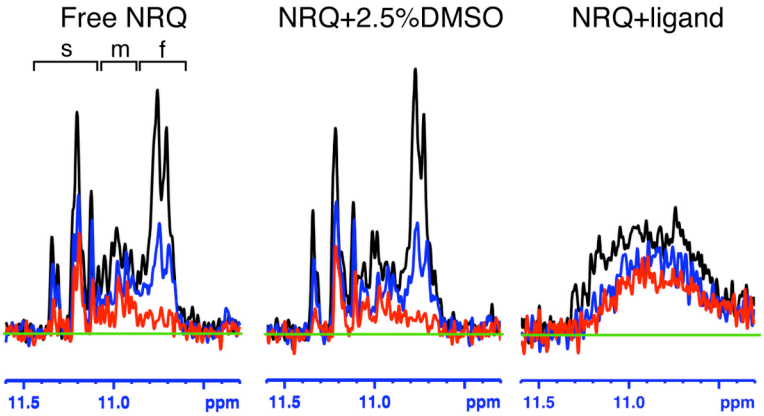


Fig. S3 HDX analysis of the RNA G-quadruplex (NRQ) imino protons. Overlay of imino proton spectra of the NRQ recorded at different time points during the course of the HDX. The black, blue and red spectra correspond to 0, 0.5 and 12 hours after HDX, respectively. The states of the NRQ are indicated on the top panel and the baselines of individual spectra are shown in green. Regions of the slow (s), medium (m) and fast (f) exchanging imino protons are indicated on the left panel.

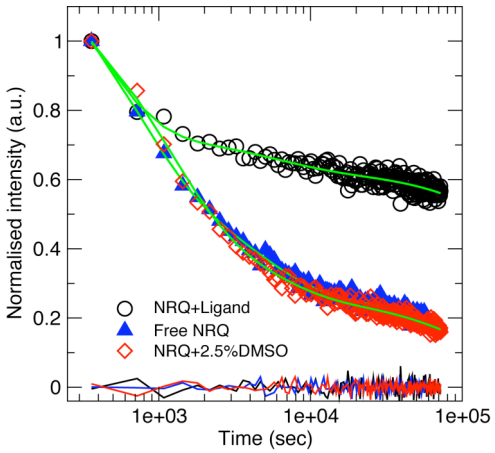


Fig. S2 Rate analysis of the imino proton HDX of the RNA G-quadruplex (NRQ). The overall imino proton signals are integrated and normalized with respect to the first time point. The imino proton resonance intensities are plotted as a function of HDX time and are fitted to three exponentially decaying components with the fitting residuals shown below. The addition of DMSO alone has little effect to the HDX kinetics of the NRQ (filled blue triangle vs. open red diamond), while the binding of RR110 shows a significantly larger population of the exchangeable imino groups (open black circle), suggesting a stabilizing effect upon ligand binding.

Table S1 Analysis of the NMR imino proton hydrogen-deuterium exchange of the NRQ RNA G-quadruplex

	Slow phase		Medium phase		Fast phase	
Sample	A_s (%)[a]	k_s (s^{-1})	A_m (%)[a]	k_m (s^{-1})	A_f (%)[a]	k_f (s^{-1})
Free NRQ	21	6.04e-6	27	2.47e-4	52	1.61e-3
NRQ+2.5% DMSO	21	6.26e-6	19	2.11e-4	60	1.00e-3
NRQ+RR110	43	1.61e-6	8	1.88e-4	49	2.78e-3

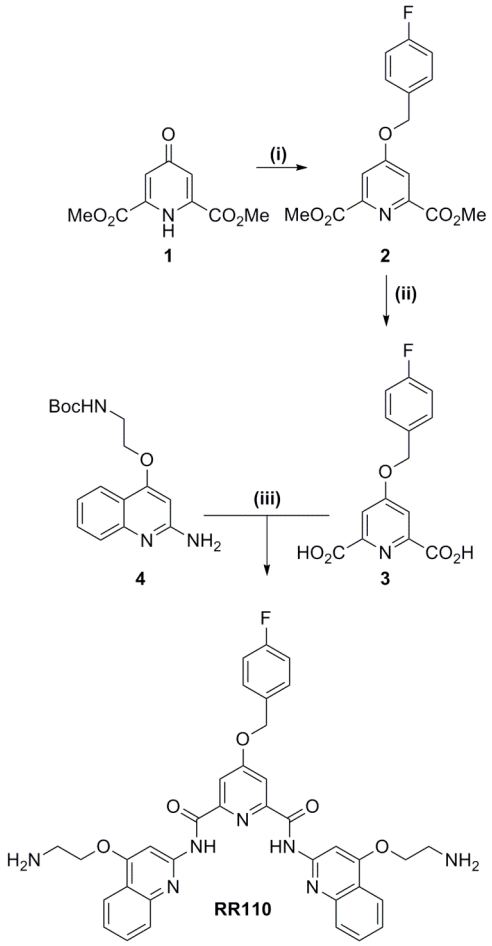
a. The initial amplitudes are expressed in percentage of the total population after normalization by the sum of the initial amplitudes of the three phases.

Synthesis of RR110

General experimental

All solvents and reagents were purified by standard techniques reported in Perrin, D. D.; Armarego, W. L. F., *Purification of Laboratory Chemicals*, 3rd edition, Pergamon Press, Oxford, 1988 or used as supplied from commercial sources, as appropriate. NMR spectra were acquired on Bruker DRX-400, DPX-400 and DRX-500 instruments using deuterated solvents as detailed and at ambient probe temperature (300 K). Notation for the ^{1}H NMR spectral splitting patterns includes: singlet (*s*), doublet (*d*), triplet (*t*), broad (*br*) and multiplet/overlapping peaks (*m*). Signals are quoted as δ values in ppm, coupling constants (*J*) are quoted in Hertz. Mass spectra were recorded on a Micromass Q-Tof (ESI) spectrometer. TLC was performed on Merck Kieselgel 60 F254 plates, and spots were visualised under UV light. Flash chromatography was performed using Merck Kieselgel 60 at rt under a positive pressure of nitrogen using distilled solvents. HPLC purifications were performed by using a Varian Pursuit C18, 5μ column (250 × 21.2 mm) at flow rate of 12.0 mL.min^{-1}.

Synthesis



Scheme S1 General scheme for the synthesis of RR110. (i) 4-Fluorobenzyl alcohol, triphenylphosphine, THF, DIAD, 0 °C to rt. (ii) aq. NaOH, MeOH, rt. (iii) **3**, **4**, pyridine, triphenylphospite, 50 °C to 100 °C; then TFA/DCM, rt.

Dimethyl-4-(4-fluoro-benzyloxy)-pyridine-2,6-dicarboxylate (2)

The known[1] chelidamic acid dimethyl ester **(1)** (200 mg, 0.9 mmol)**,** 4-fluorobenzyl alcohol (154 µl, 1.4 mmol) and triphenylphosphine (373 mg, 1.4 mmol) were dissolved in freshly distilled THF (5 ml) then cooled to 0 °C. DIAD (325 µl, 1.7 mmol) was added dropwise under argon. The mixture was allowed to warm to rt and stirred for 3 days. The solvent was removed *in vacuo* and the product purified by

[1] R. Rodriguez, S. Müller, J. A. Yeoman, C. Trentesaux, J.-F Riou and S. Balasubramanian *J. Am. Chem. Soc.,* 2008, **130**, 15758-15759.

column chromatography (50% EtOAc, 50% petroleum ether) to obtain the title compound as a white powder (200 mg, 0.6 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.88 (2H, *s*), 7.45-7.40 (2H, *m*), 7.14-7.07 (2H, *m*), 5.20 (2H, *s*), 4.01 (6H, *s*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.5, 165.8, 162.9 (*d*, *J* 246 Hz), 149.9, 130.5, 122.7 (*d*, *J* 8 Hz), 115.8 (*d*, *J* 22 Hz), 114.7, 70.1, 53.2; HRMS (ES) calculated for $C_{16}H_{14}FNNaO_5$ ($[M + Na]^+$) m/z: 342.0748, found 342.0751.

4-(4-fluoro-benzyloxy)-pyridine-2,6-dicarboxylate (3)

An aqueous sodium hydroxide solution (2 M*,* 0.6 ml) was added dropwise to a solution of **2** (200 mg, 0.63 mmol) in MeOH (10.0 ml) at rt and the solution was stirred for 1 h. MeOH was removed *in vacuo*. A 5% formic acid solution was added dropwise to lower the pH to 3.0-4.0. The compound was then extracted with EtOAc. The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo* to obtain compound **3** as a white powder (180 mg, 0.63 mmol, quantitative yield), which was used without further purification.

4-(4-Fluorobenzyloxy)pyridine-2,6-dicarboxylic acid *bis*-{[4-(2-aminoethoxy)quinolin-2-yl]amide}, trifluoroacetic acid salt (RR110)

Compound **3** (43 mg, 0.15 mmol) and the known[1] compound **4** (94 mg, 0.31 mmol) were dissolved in pyridine (1.0 ml) at 50 °C, stirred for 30 min, followed by the dropwise addition of triphenylphosphite (85 µl, 0.32 mmol). The reaction mixture was warmed to 100 °C and stirred for 5 h under argon. After removal of the solvent *in vacuo*, The crude product was purified by column chromatography (50% EtOAc, 50% petroleum ether) to obtain a white powder which was dissolved in DCM (1.5 ml) and TFA (0.5 ml) and stirred for 4 h at rt. The solvent was removed *in vacuo* to give a

yellow solid which was purified by HPLC (gradient: 10 to 100% MeCN, 0.1% TFA over 20 min, R_t=15.0-16.0 min) to yield the title compound as a white powder (12 mg, 0.03 mmol, 8% yield over two steps). ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.49 (2H, *d*, *J* 8.5 Hz), 8.19 (2H, *s*), 8.18 (2H, *s*), 8.07 (2H, *d*, *J* 8.5 Hz), 7.94 (2H, *dd*, *J* 8.5, 8.0 Hz), 7.68 (2H, *dd*, *J* 8.5, 8.0 Hz), 7.61-7.57 (2H, *m*), 7.20-7.15 (2H, *m*), 5.45 (2H, *s*), 4.76 (4H, *t*, *J* 4.0 Hz), 3.68 (4H, *t*, *J* 4.0 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ_C 169.8, 166.7, 164.3 (*d*, *J* 245 Hz), 164.2, 152.5, 151.0, 143.7, 134.1, 132.8, 131.3 (*d*, *J* 8 Hz), 127.6, 124.5, 124.1, 120.2, 116.6 (*d*, *J* 22 Hz), 114.5, 95.3, 71.7, 67.6, 39.8; HRMS (ES) calculated for $C_{36}H_{33}FN_7O_5$ ($[M + H]^+$) m/z: 662.2521, found 662.2501.