Ametantrone-based compounds as potential regulators of Tau pre-mRNA alternative splicing

Gerard Artigas,[a] Paula López-Senín,[a] Carlos González,[b] Núria Escaja,[a] and Vicente Marchán*[,a]

[a] Departament de Química Orgànica and IBUB

Universitat de Barcelona

Martí i Franquès 1-11, E-08028 Barcelona (Spain)

Fax: (+) (34) 93 339 7878

E-mail: vmarchan@ub.edu

[b] Instituto de Química Física Rocasolano

CSIC

Serrano 119, E-28006 Madrid (Spain)

SUPPLEMENTARY INFORMATION

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1. Synthesis of the ligands

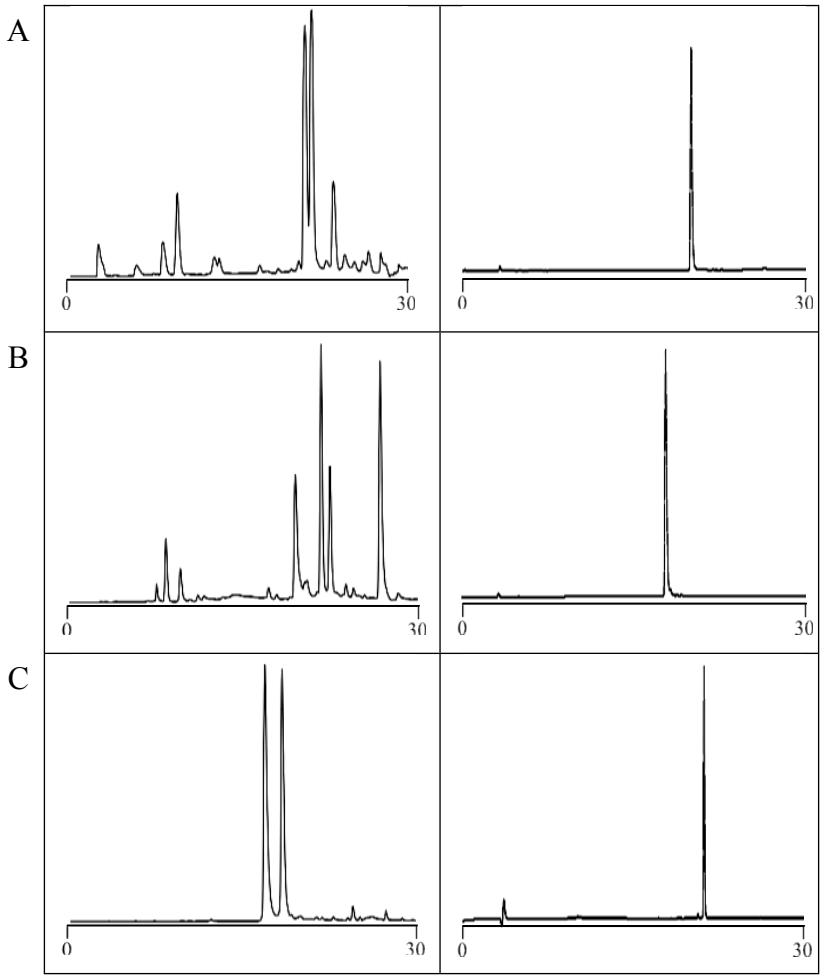
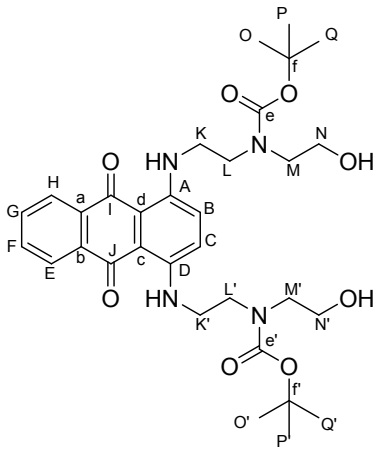
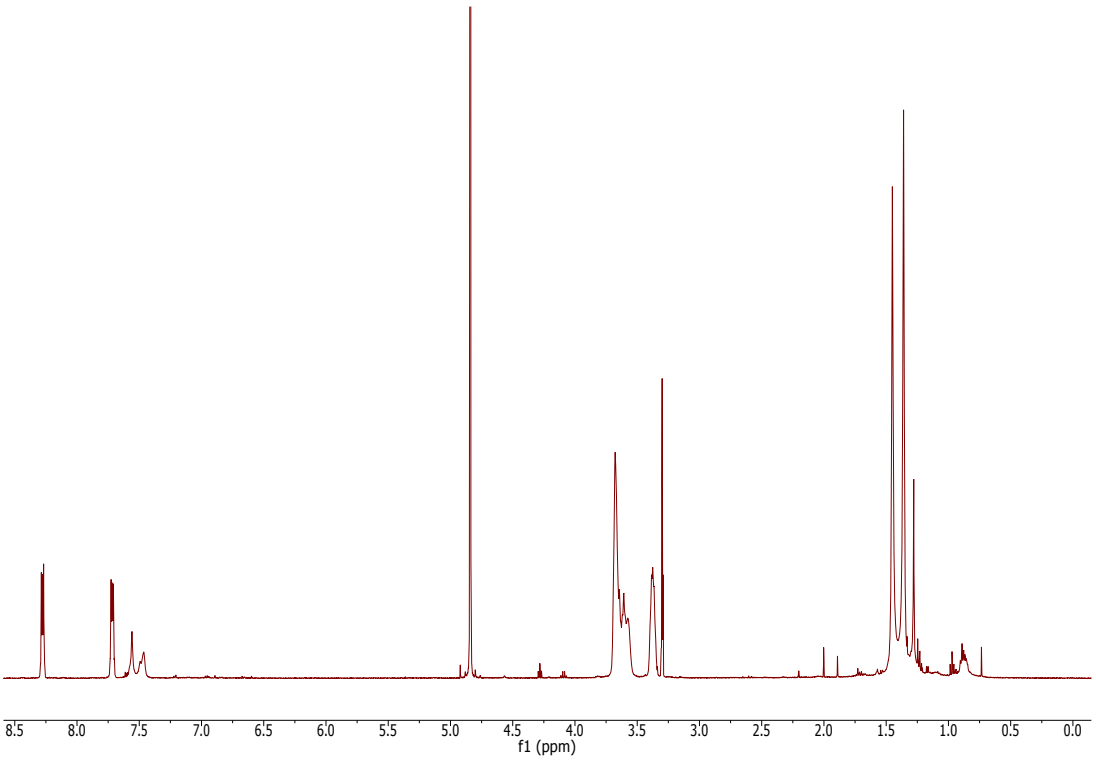


Figure S1. Reversed-phase HPLC traces for the ligands Amt-Nea (A), Amt-Nea2 (B) and Amt-Azq (C): reaction crude (left) and purified (right).

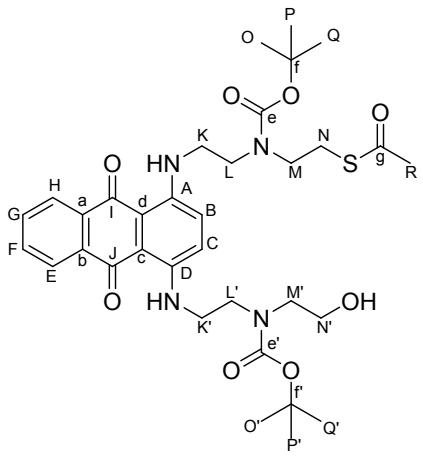
2. ^{1}H NMR spectra of the compounds

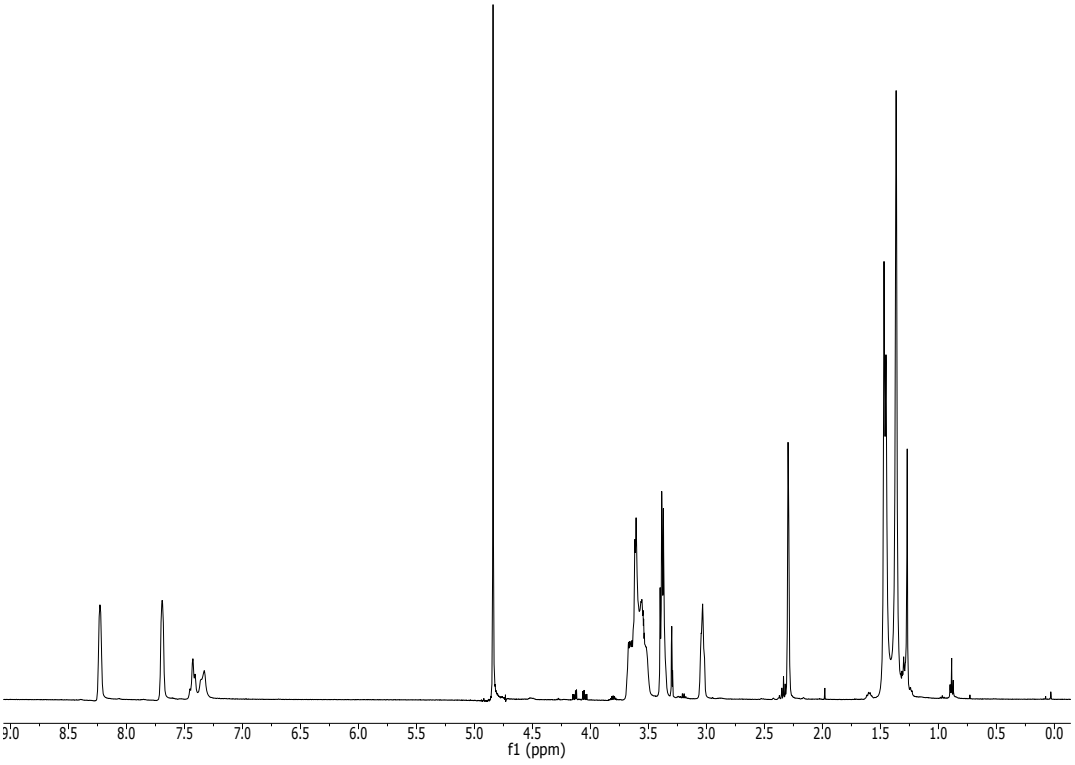
Amt(Boc)$_2$ (1)





Amt(Boc)$_2$SAc (2)





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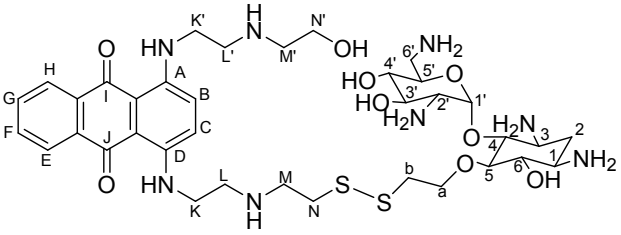
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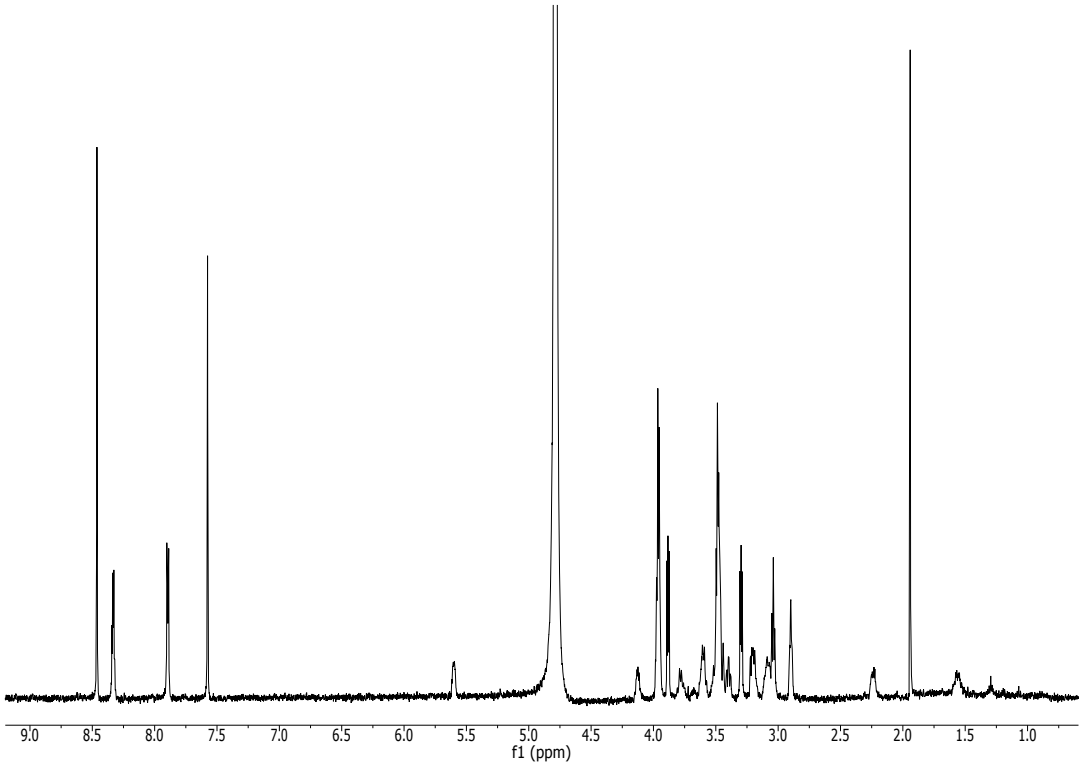
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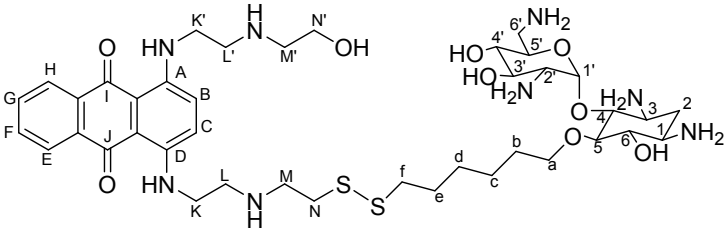
f1 (ppm)

Amt-Nea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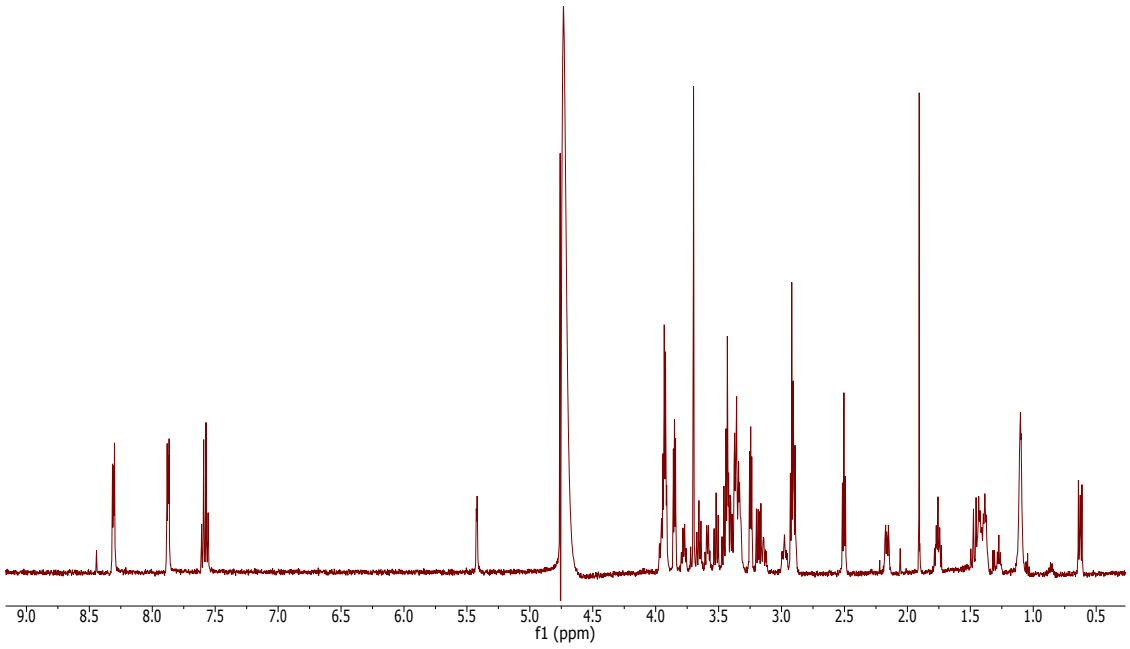
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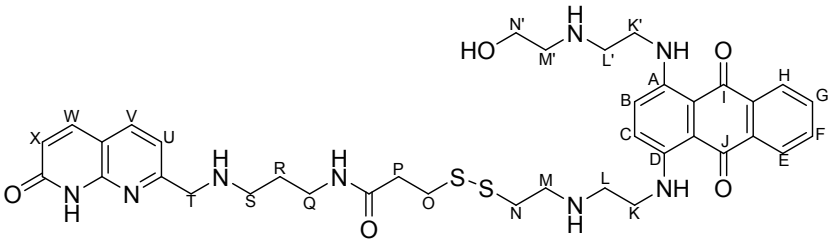
0.5
f1 (ppm)

Amt-Azq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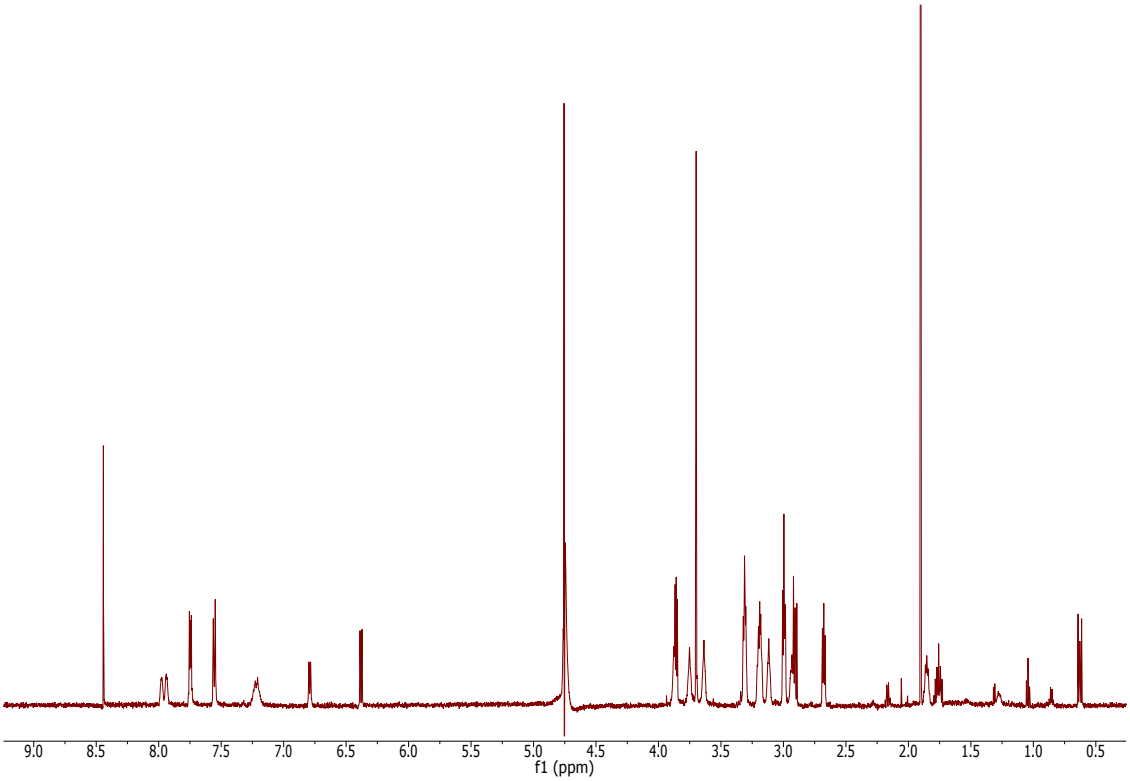
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0.5
f1 (ppm)

3. UV-VIS TITRATION EXPERIMENTS

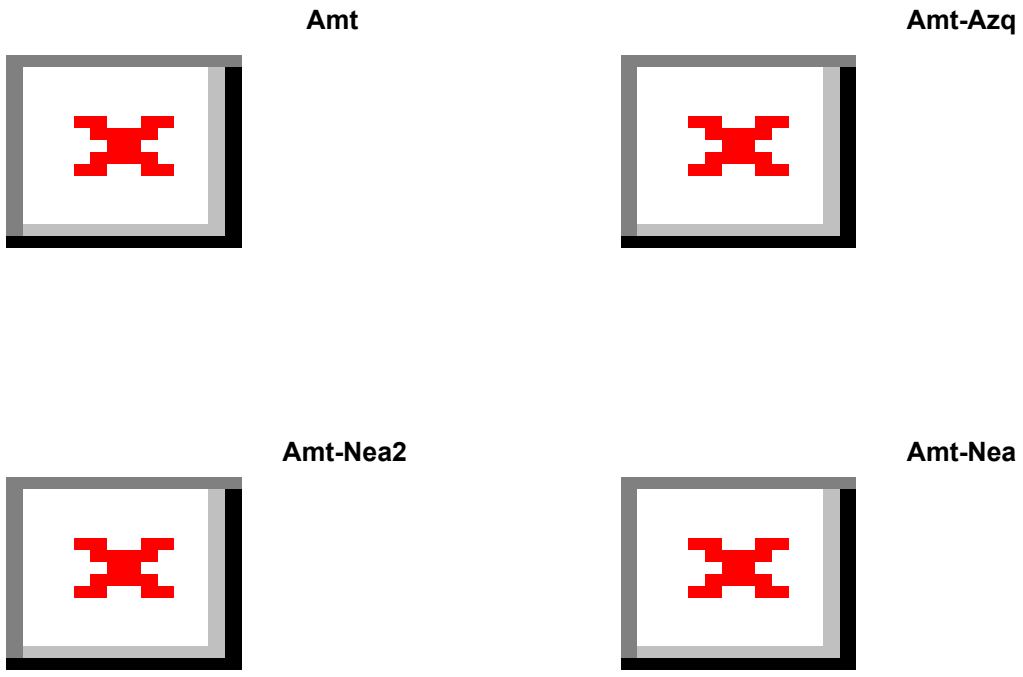

Figure S2. UV-Vis spectra of the ametantrone-containing ligands in the absence and in the presence of increasing amounts of wt RNA in a 10 mM sodium phosphate buffer, pH 6.8, containing 100 mM NaCl and 0.1 mM Na_2EDTA. Ligand concentrations: 10 μM **(**Amt) and 8.4 μM (Amt-Azq, Amt-Nea and Amt-Nea2).

4. FLUORESCENCE TITRATION EXPERIMENTS

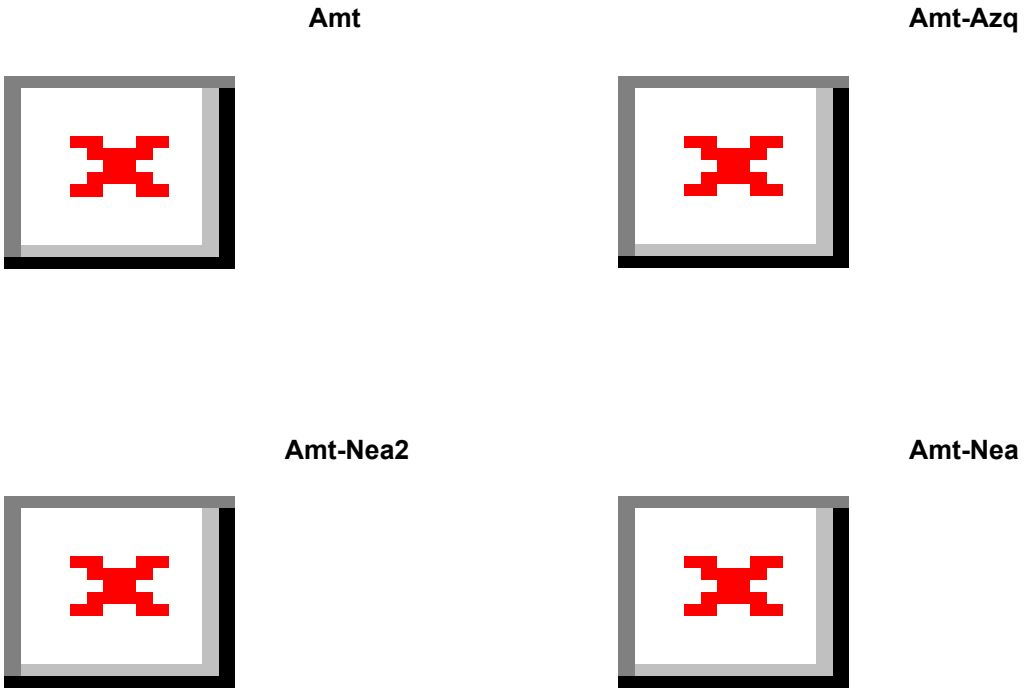

Figure S3. Fluorescence emission spectra of the ametantrone-containing ligands in the absence and in the presence of increasing amounts of wt RNA in a 10 mM sodium phosphate buffer, pH 6.8, containing 100 mM NaCl and 0.1 mM Na_2EDTA. Ligand concentrations: 2 μM. The emission spectra were recorded from 600 ~ 850 nm with λ_{ex} = 547 nm.

5. NMR TITRATION EXPERIMENTS

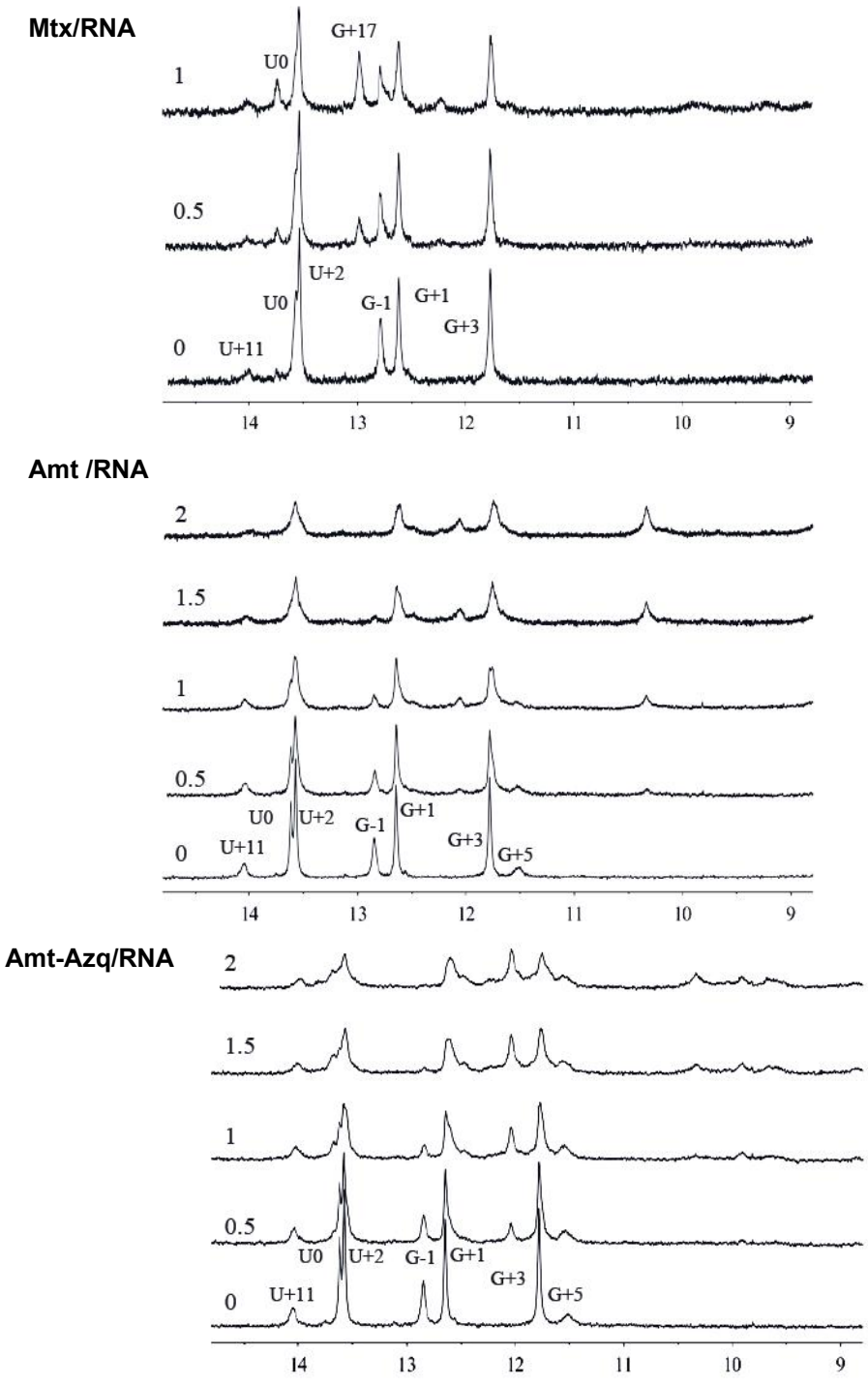

Figure S4. Imino region of the NMR spectra of wt RNA alone and in the presence of increasing amounts of the ligands (**Mtx**, **Amt** or **Amt-Azq**; the ratio ligand/RNA is indicated in each series of spectra from bottom to top. Imino proton signals are labelled according to the numbering scheme shown in Scheme 1. The RNA concentration was 100 μM in a 10 mM sodium phosphate buffer, pH 6.8, in a 90%/10% H_2O/D_2O mixture (T = 5ºC).

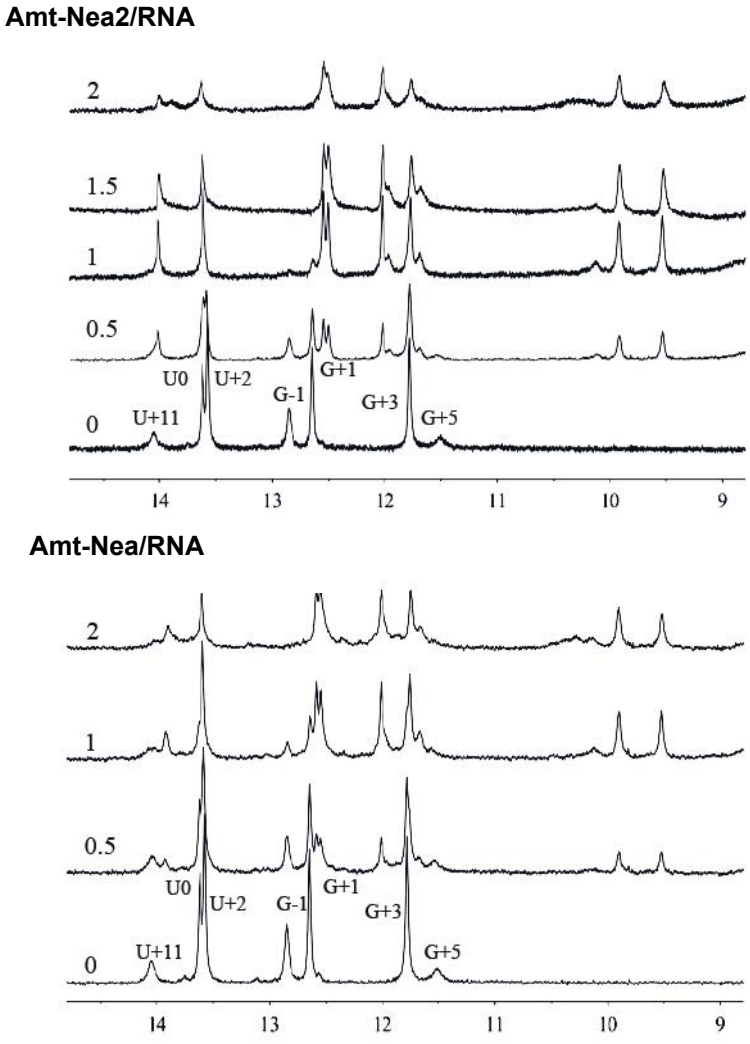

Figure S5. Imino region of the NMR spectra of wt RNA alone and in the presence of increasing amounts of the ligands (**Amt-Nea2** or **Amt-Nea**; the ratio ligand/RNA is indicated in each series of spectra from bottom to top. Imino proton signals are labelled according to the numbering scheme shown in Scheme 1. The RNA concentration was 100 μM in a 10 mM sodium phosphate buffer, pH 6.8, in a 90%/10% H_2O/D_2O mixture (T = 5ºC).

6. 2D NMR SPECTRA

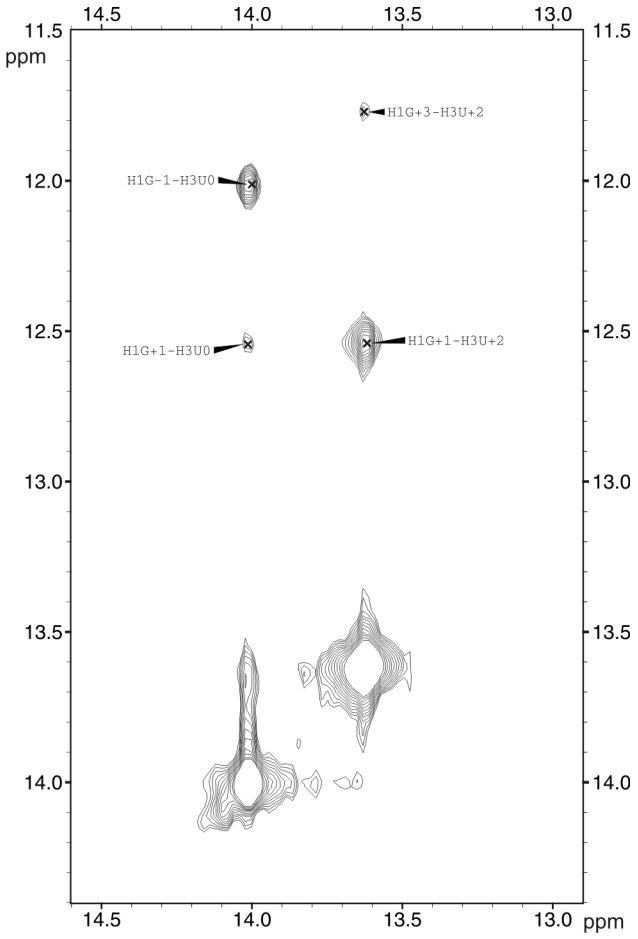

Figure S6. Imino-imino region of the NOESY spectrum (t_m=250ms) of the 1:1 complex Amt-Nea2:wt Tau RNA in H_2O/D_2O 9:1 in phosphate buffer pH=6.8, T=5ºC. [RNA]= 280 μM.

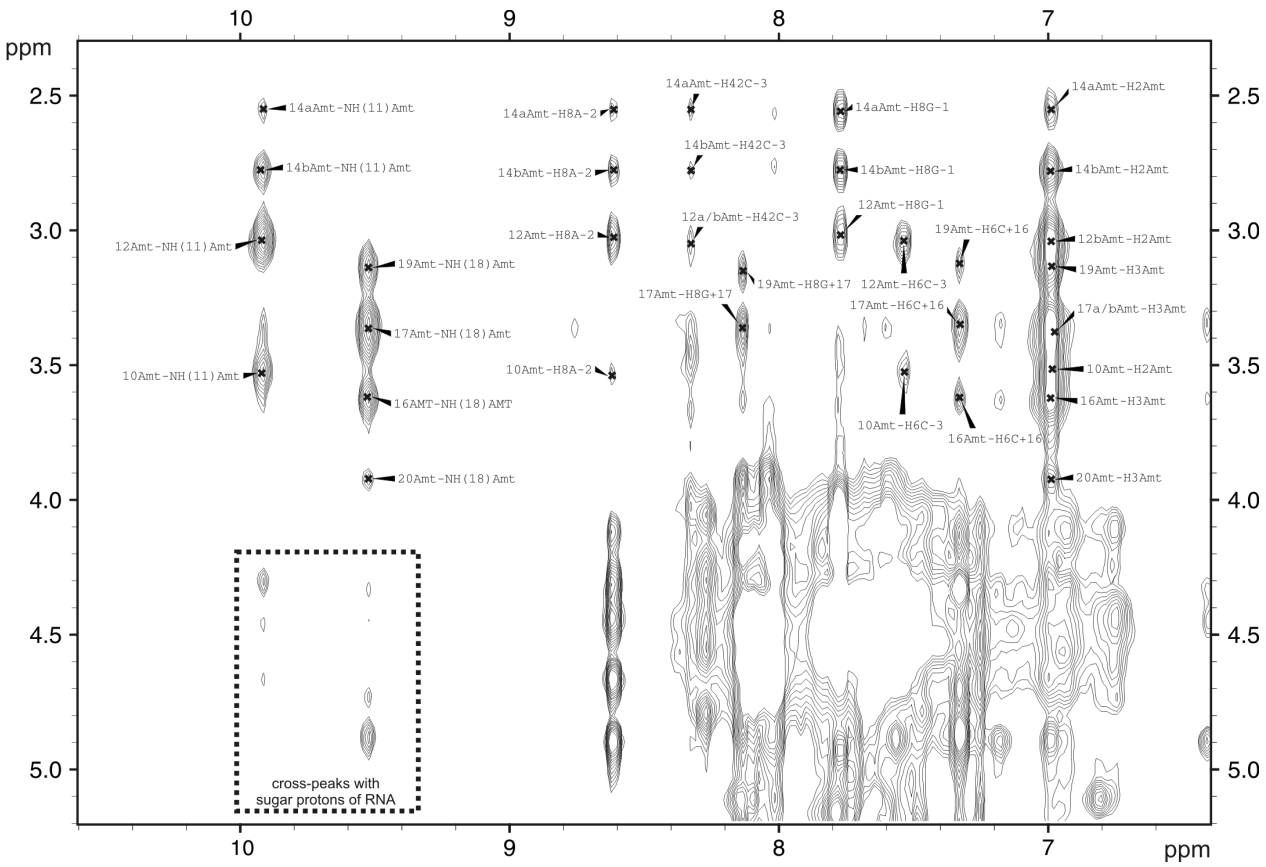

Figure S7. Amino/aromatic-methylene protons region of the NOESY spectrum (t_m=250ms) of the 1:1 complex Amt-Nea2:wt Tau RNA in H_2O/D_2O 9:1 in phosphate buffer pH=6.8, T=5ºC. [RNA]= 280 μM. Labelled cross-peaks correspond to contacts between methylene protons of the side chains of ametantrone with aromatic/amino protons of RNA and with the amino protons of the side chain of ametantrone. Proton signals are labelled according to the number schemes shown in Figure 1 and Figure 2D in the manuscript.

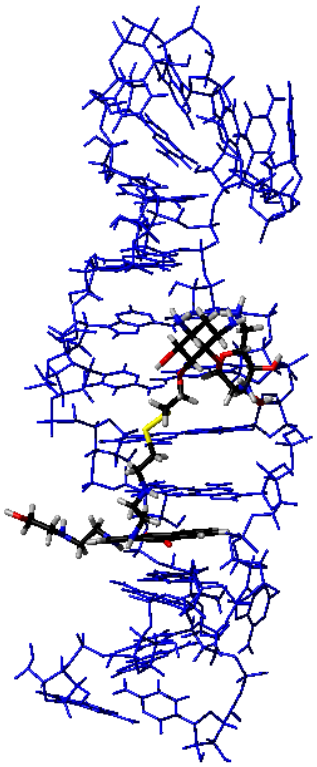

Figure S8. Model of the 1:1 complex Tau RNA:AmtNea2. Coordinates for the Tau RNA:Mtx complex determined by Varani *et al*.[30] were used to build the model, as described in Methods in the manuscript.

7. UV MELTING CURVES OF RNA-LIGAND COMPLEXES

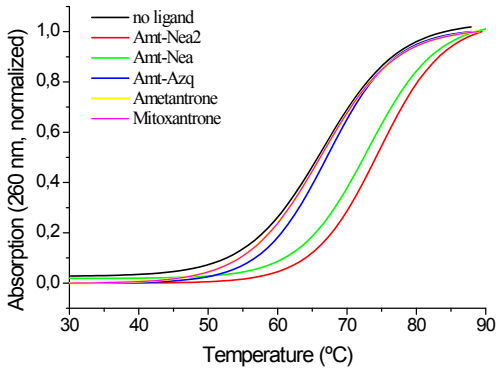

Figure S9. UV melting profiles for the wt RNA oligonucleotide and its ligand complexes at a [ligand]/RNA ratio of 1.0.

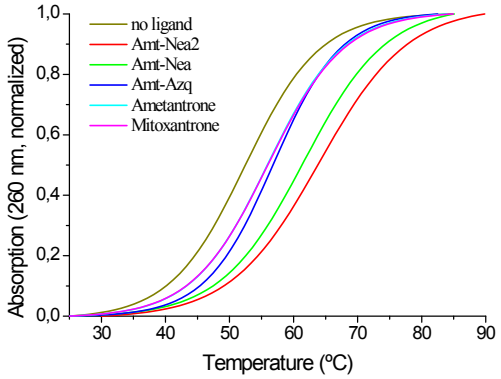

Figure S10. UV melting profiles for the +3 mutated RNA oligonucleotide and its ligand complexes at a [ligand]/RNA ratio of 1.0.

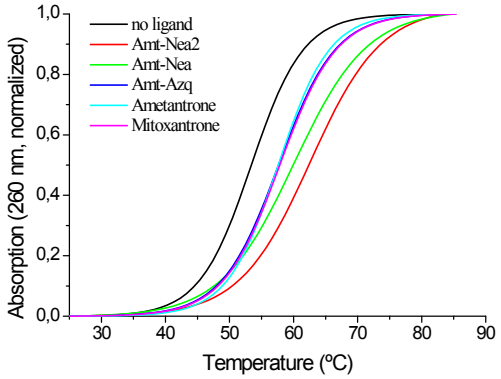

Figure S11. UV melting profiles for the +14 mutated RNA oligonucleotide and its ligand complexes at a [ligand]/RNA ratio of 1.0.

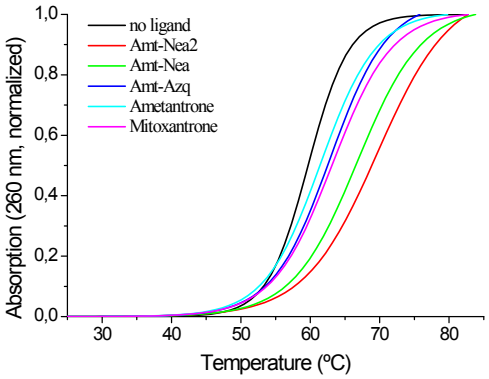

Figure S12. UV melting profiles for the +16 mutated RNA oligonucleotide and its ligand complexes at a [ligand]/RNA ratio of 1.0.

8. NMR TITRATION EXPERIMENTS WITH A-SITE RNA COMPETITORS

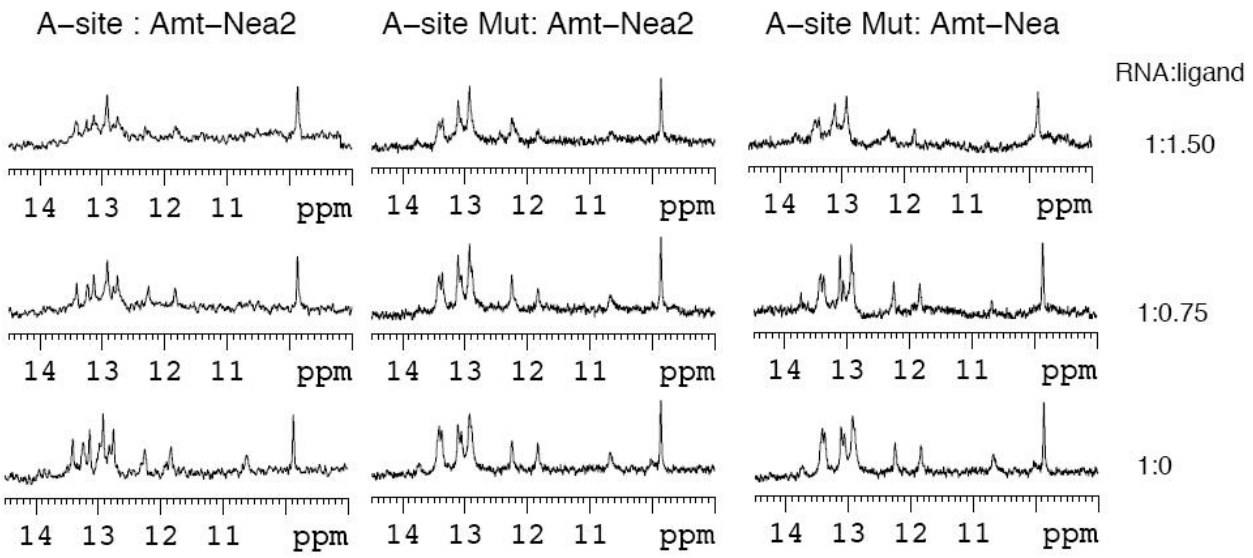

Figure S13. Imino region of the NMR spectra of the E. coli A-site fragment RNA ($^{5'}$rGGCGUCACACCUUCGGGUGAAGUCGCC) and its related mutant sequence ($^{5'}$rGGCGUCGCACCUUCGGGUGCGUCGCC), alone and in the presence of increasing amounts of Amt-Nea ligands. From bottom to top: ligand/RNA ratio = 0.0, 0.75, and 1.5. Experimental conditions: 40 μM RNA concentration, 10 mM sodium phosphate buffer, pH 6.8, in a 90%/10% H_2O/D_2O mixture (T = 5ºC).