Alteration of RNA splicing by small molecule inhibitors of the interaction between NHP2L1 and U4

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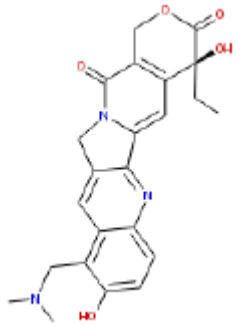
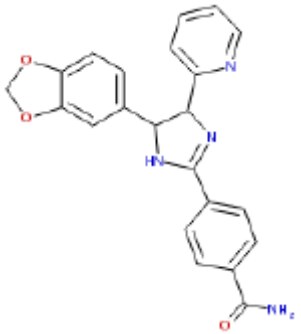
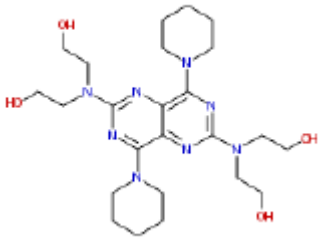
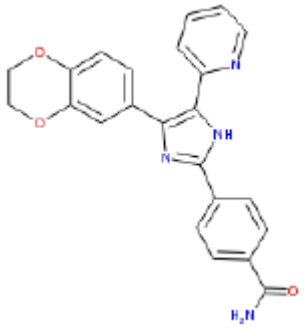
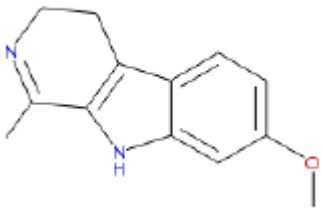
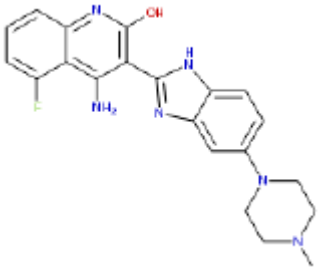
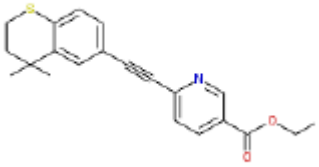
Supplementary Material.

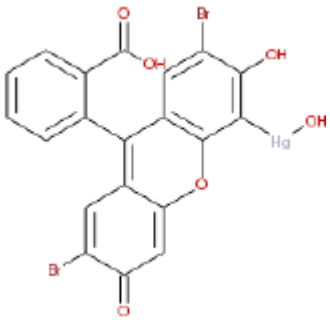
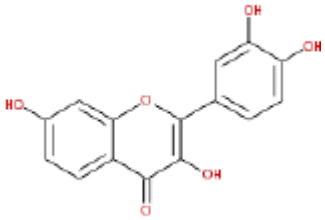
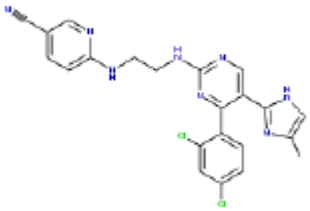
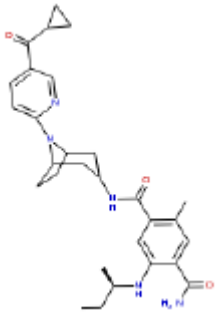
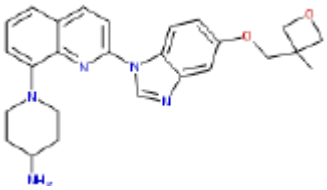
-Tables S1, 2, 3

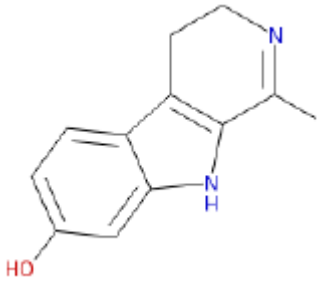
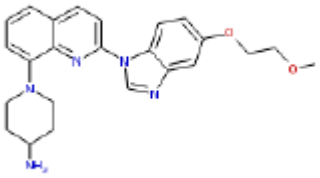
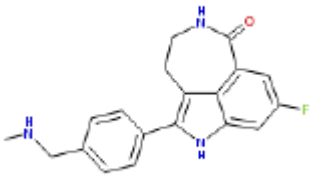
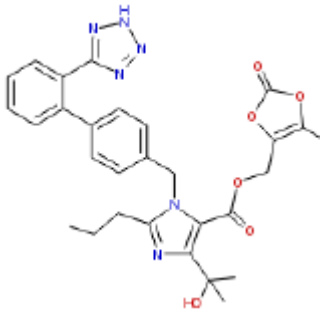
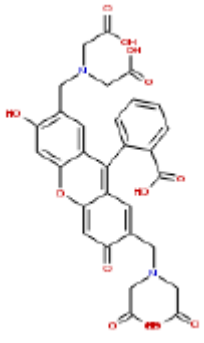
-Figures S1-6

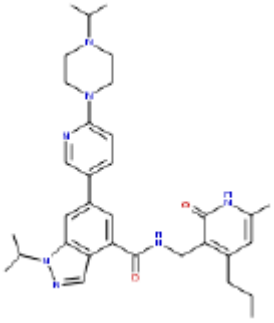
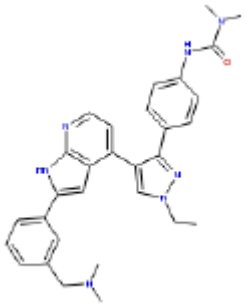
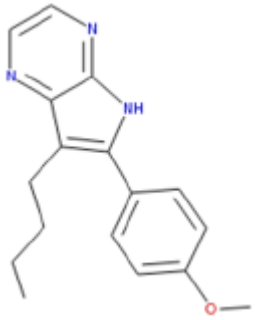
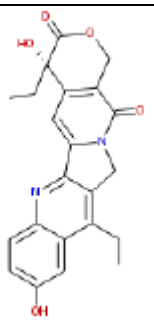
Table S1. 53 unique compounds with % Inhibition ≥ 40% were selected from the primary screening (at 15 µM) for dose response analysis to determine the IC_{50} values where applicable.

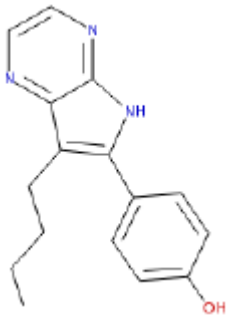
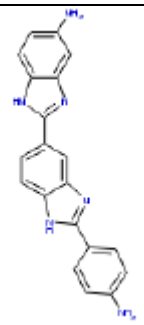
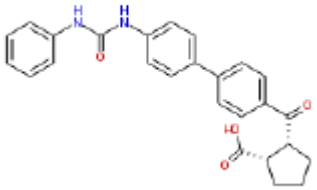
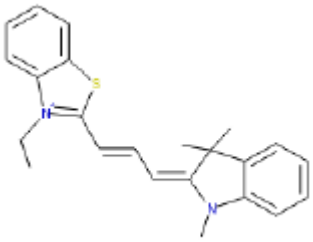
No.	SJ Number	Compound name	Structure	%Inhibition (at 15 µM)	IC_{50}
1	SJ000287149-13	Topotecan		81.6%	2.1 µM
2	SJ000574283-2	SB-431542		70.9%	9.2 µM
3	SJ000285745-10	Dipyridamole		69.8%	ND*

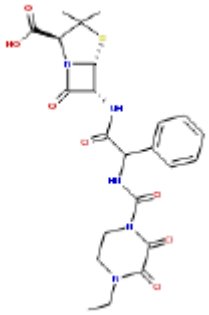
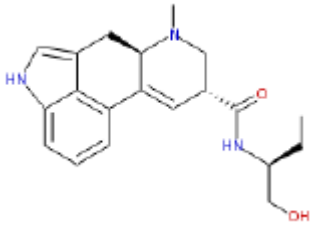
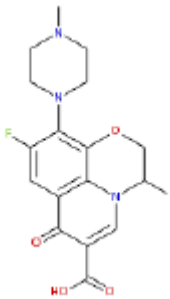
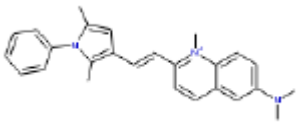
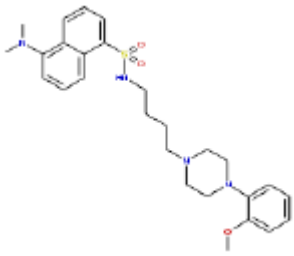
4	SJ000773191-2	D 4476		69.7%	34.7 μM
5	SJ000285455-2	Harmaline		67.5%	33.7 μM
6	SJ000574286-4	Dovitinib		67.2%	ND
7	SJ000561072-6	Tazarotene		65.5%	21.1 μM

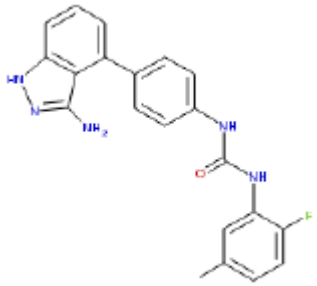
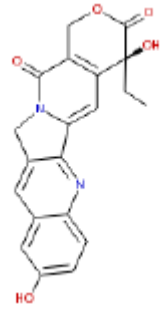
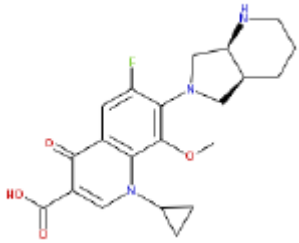
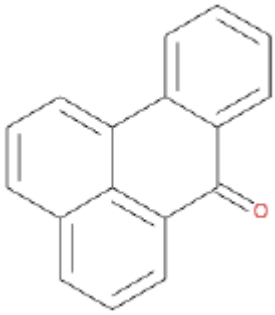
8	SJ000285323-3	Merbromin		64.9%	29.6 μM
9	SJ000286937-3	Fisetin		64.2%	51.4 μM
10	SJ000855356-1	CHIR-99021		62.7%	ND
11	SJ000855455-1	XL888		62.6%	30.2 μM
12	SJ000791567-6	Crenolanib		62.5%	9.3 μM

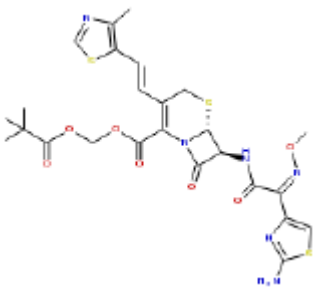
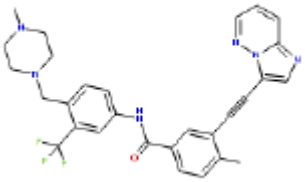
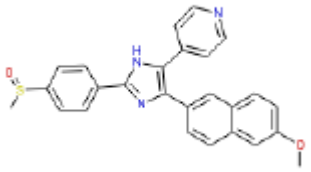
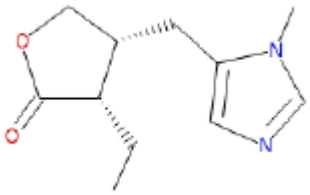
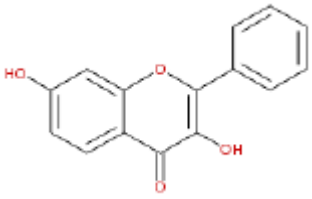
13	SJ000285345-2	Harmalol		61.7%	49.9 μM
14	SJ000855162-1	CP-673451		61.4%	ND
15	SJ000784257-6	Rucaparib		57.8%	ND
16	SJ000286080-2	Olmesartan medoxomil		55.7%	ND
17	SJ000285441-1	Calcein		55.3%	ND

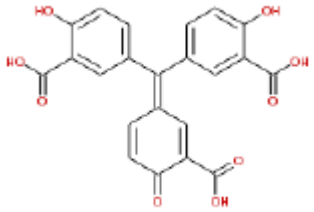
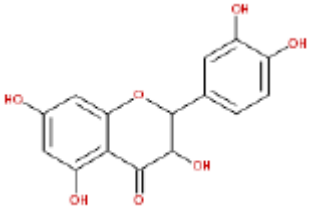
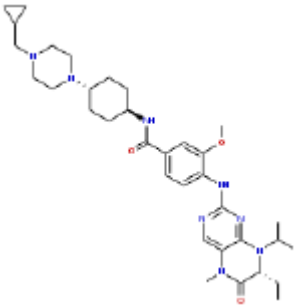
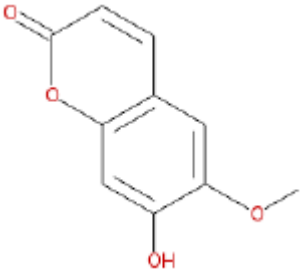
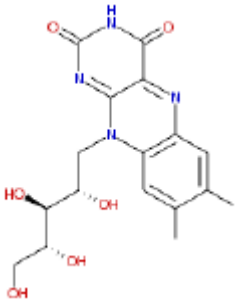
18	SJ000833872-2	UNC1999		55.2%	53.5 μM
19	SJ000852778-2	GSK1070916		55.0%	ND
20	SJ000312173-2	Aloisine		54.7%	3.4 μM
21	SJ000311679-7	SN-38		53.5%	1.6 μM

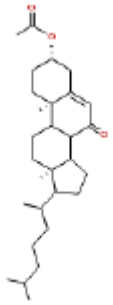
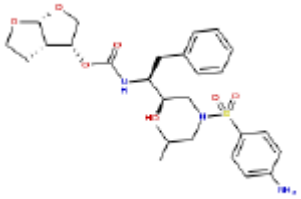
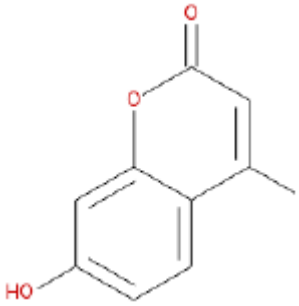
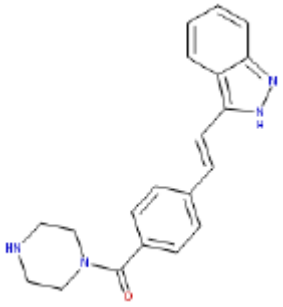
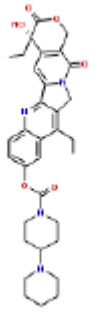
22	SJ000312172-5	Aloisine A		52.8%	ND
23	SJ000288308-1	RO 90-7501		52.8%	16.5 µM
24	SJ000785921-2	A 922500		52.6%	31.3 µM
25	SJ000288306-1	AC-93253 iodide		52.5%	11.4 µM

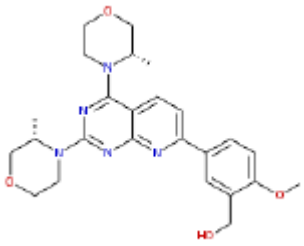
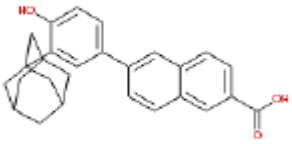
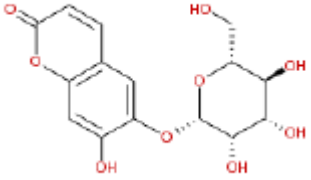
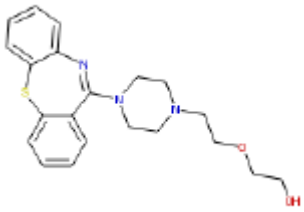
26	SJ000285538-5	Piperacillin		51.4%	ND
27	SJ000285656-8	Methyl-Ergonovine		50.6%	ND
28	SJ000285911-12	Levofloxacin		50.6%	45.4 µM
29	SJ000286160-5	Pyrvinium pamoate		50.5%	ND
30	SJ000287706-2	ST-148		50.4%	23.3 µM

31	SJ000560618-6	Linifanib		50.3%	24.1 μM
32	SJ000286965-1	(S)-10-Hydroxy-camptothecin		50.0%	2.4 μM
33	SJ000562694-2	Moxifloxacin		49.5%	ND
34	SJ000286099-2	Benzanthrone		49.4%	ND

35	SJ000285553-3	Cefditorin pivoxil		47.0%	ND
36	SJ000791566-7	Ponatinib		46.7%	ND
37	SJ000852750-2	Tie2 kinase inhibitor		46.5%	56.4 μM
38	SJ000285536-14	Pilocarpine		45.7%	ND
39	SJ000286967-1	3,7-Dihydroxyflavone		45.6%	ND

40	SJ000287594-2	Aurintricarboxylic acid		45.1%	ND
41	SJ000288237-5	Taxifolin		45.0%	ND
42	SJ000780471-10	Volasertib		44.7%	ND
43	SJ000285342-1	Scopoletin		44.5%	66.9 μM
44	SJ000286086-4	Riboflavine		44.4%	59.8 μM

45	SJ000287024-1	7-Oxocholesteryl acetate		44.1%	ND
46	SJ000312367-5	Darunavir		43.9%	ND
47	SJ000287434-5	Hymecromone		43.9%	ND
48	SJ000855214-1	KW-2449		42.9%	33.9 μM
49	SJ000312345-11	Irinotecan		42.6%	50.1 μM

50	SJ000571306-5	AZD8055		42.2%	40.6 μM
51	SJ000546519-2	AGN-192837		41.4%	2.3 μM
52	SJ000285590-4	Aesculin		41.3%	ND
53	SJ000312300-3	Seroquel		40.1%	ND

SJ corresponds to St Jude Children's Research Hospital

*ND: IC_{50} value is not determined.

Table S2. IC_{50} from Dose Response curve of Topotecan and camptothecin derivatives

Drug name	IC_{50}[1]	IC_{50}[2]
Topotecan	2.1 ± 0.3 μM	3.1 ± 0.2 μM
(*S*)-10-HO-camptothecin	2.4 ± 0.2 μM	2.4 ± 0.3 μM
SN-38	1.6 ± 0.1 μM	2.1 ± 0.2 μM
Irinotecan	50.1 ± 2.8 μM	ND[5]
(*S*)-camptothecin[3]	NT[4]	ND
9-NH_2-(*S*)-camptothecin[3]	NT	80.5 ± 3.6 μM
9-NO_2-10-HO-(*S*)-camptothecin[3]	NT	ND
N-Desmethyl topotecan[3]	NT	9.9 ± 0.8 μM
7,11-diethyl-10-HO-(*S*)-camptothecin[3]	NT	2.0 ± 0.2 μM

[1]The screening IC_{50} values (using RISE, an in house data analysis software) were reported.

[2]The IC_{50} values confirmed with re-purchased powders.

[3]Additonal topotecan analogs were obtained for analog activity exploration. These analogs were not presented in the screening library.

[4]NT: IC_{50} value was not tested.

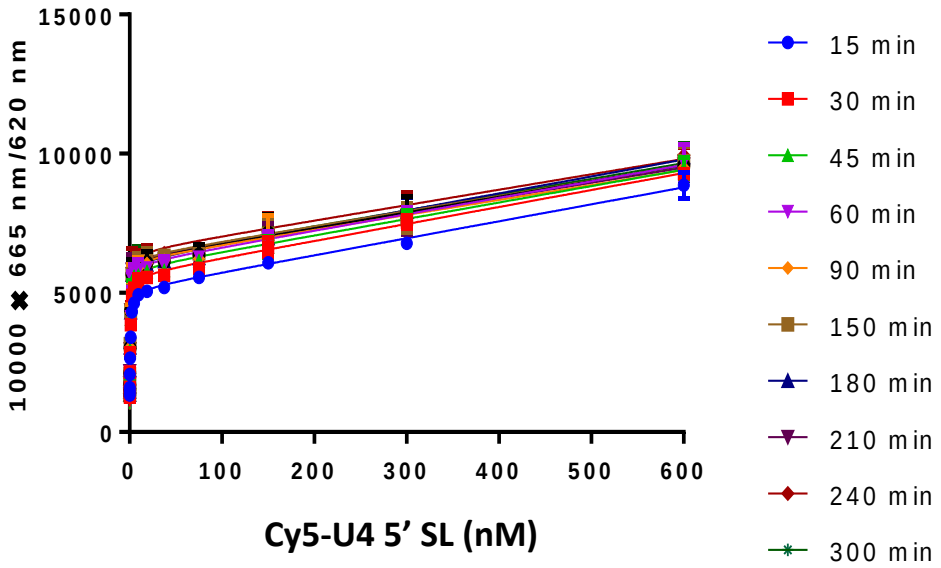
[5]ND: not determined

Table S3. Binding of topotecan to U4 by surface plasma resonance.

Interaction	**K_D (μM)**	**Rmax (RU)**
Topotecan + U4	91 (± 7)	27 (±1)
Topotecan + NHP2L1	X	X

X = minimal binding observed

A



B

	Kd
15 min	0.8355
30 min	0.7290
45 min	0.6805
60 min	0.7133
90 min	0.6620
120 min	0.6853
150 min	0.6874
180 min	0.6447
210 min	0.6511
240 min	0.7200
300 min	0.6814

Figure S1. TR-FRET signal stability. Increasing concentrations of Cy5-U4 5' SL were incubated with 2nM Terbium-anti-His and 2nM His-NHP2l1. (A)The TR-FRET signals are depicted as a function of the concentration of Cy5-U4 5' SL after 15min to 300 min incubation times . (B) K_d (nM) of the interaction His-NHP2l1 and Cy5-U4 5' SL at the different incubation times.

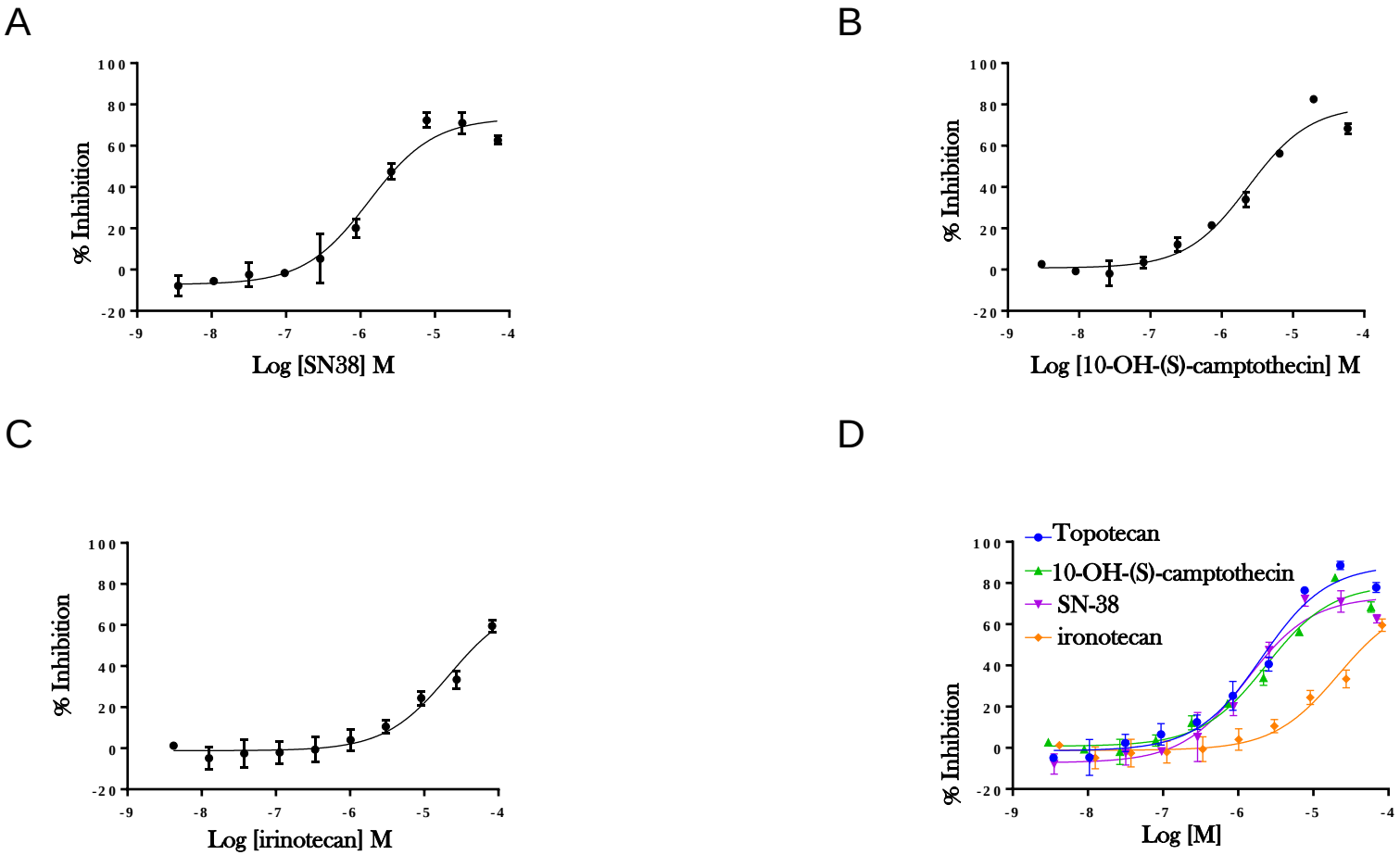


Figure S2. Dose response curve of Camptothecin derivatives in the library. Increasing concentration of SN-38 (A), 10-OH-camptothecin (B), or irinotecan (C) were incubated for 45min with 2nM Tb-anti-His, 2nM His-NHP2L1, 2nM Cy5-U4 5' SL. The TR-FRET signals were determined and converted into percentage of inhibition by reference to controls. Sigmoidal curve was fitted to the resulting data using GraphPad Prism 6.07. (D) Dose response curves of the four compounds relative to % inhibition of NHP2L1-U4 binding. IC_{50} values were determined from log[concentration]-percent inhibition curves constructed for each small molecule.

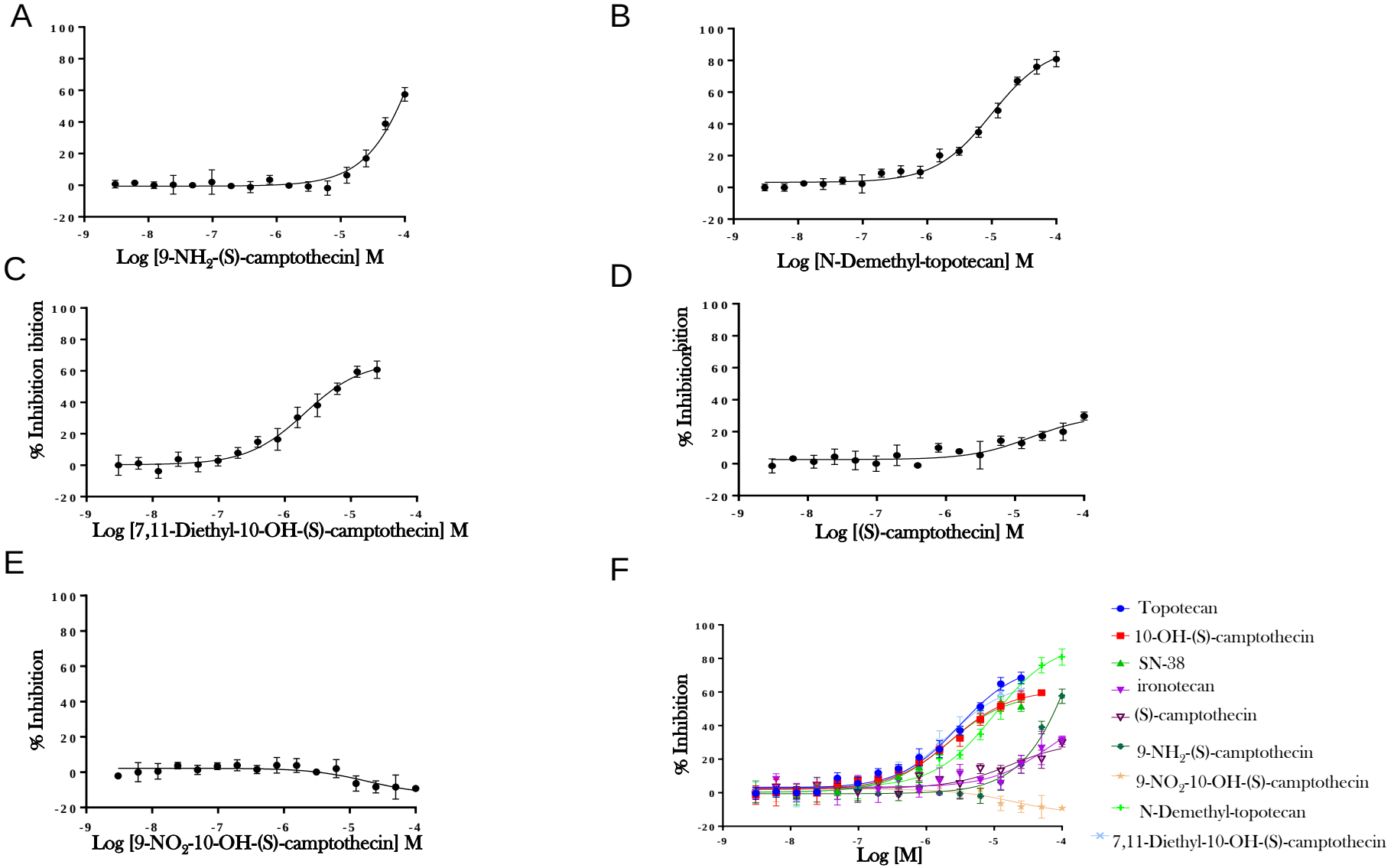


Figure S3. Dose response curves of additional camptothecin derivatives. Increasing concentration of 9-NH_2-(*S*)-camptothecin (A), N-Demethyl-topotecan (B), 7,11-Diethyl-10-OH-(S)-camptothecin (C), (S)-camptothecin (D), 9-NO_2-10-OH-(*S*)-camptothecin (E) were incubated for 45min with 2nM Tb-anti-His, 2nM His-NHP2L1, 2nM Cy5-U4 5' SL. The TR-FRET signals were determined and converted into percentage inhibition by reference to controls. Sigmoidal curve was fitted to the resulting data using GraphPad Prism 6.07. (F) Dose response curves of the nine compounds.

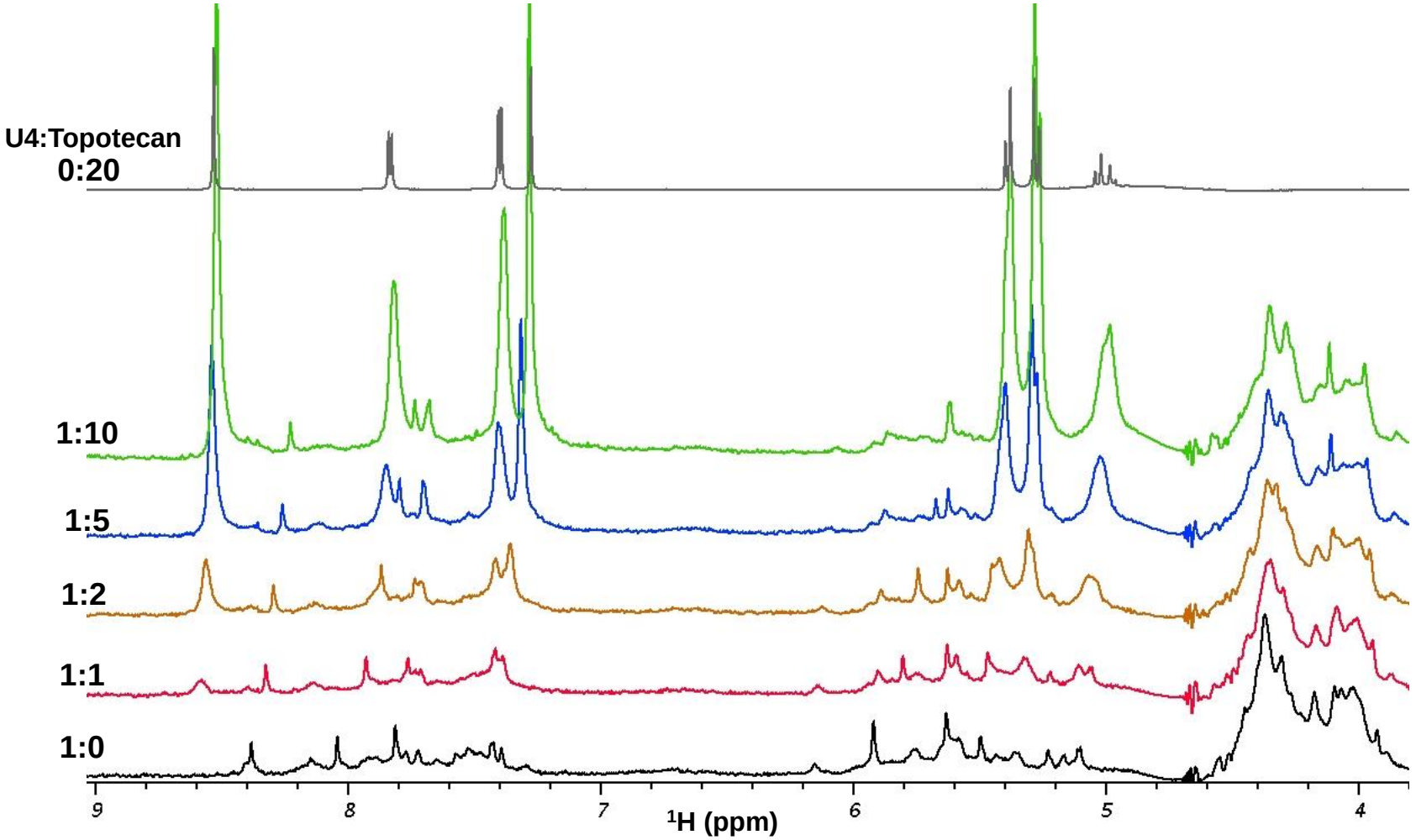
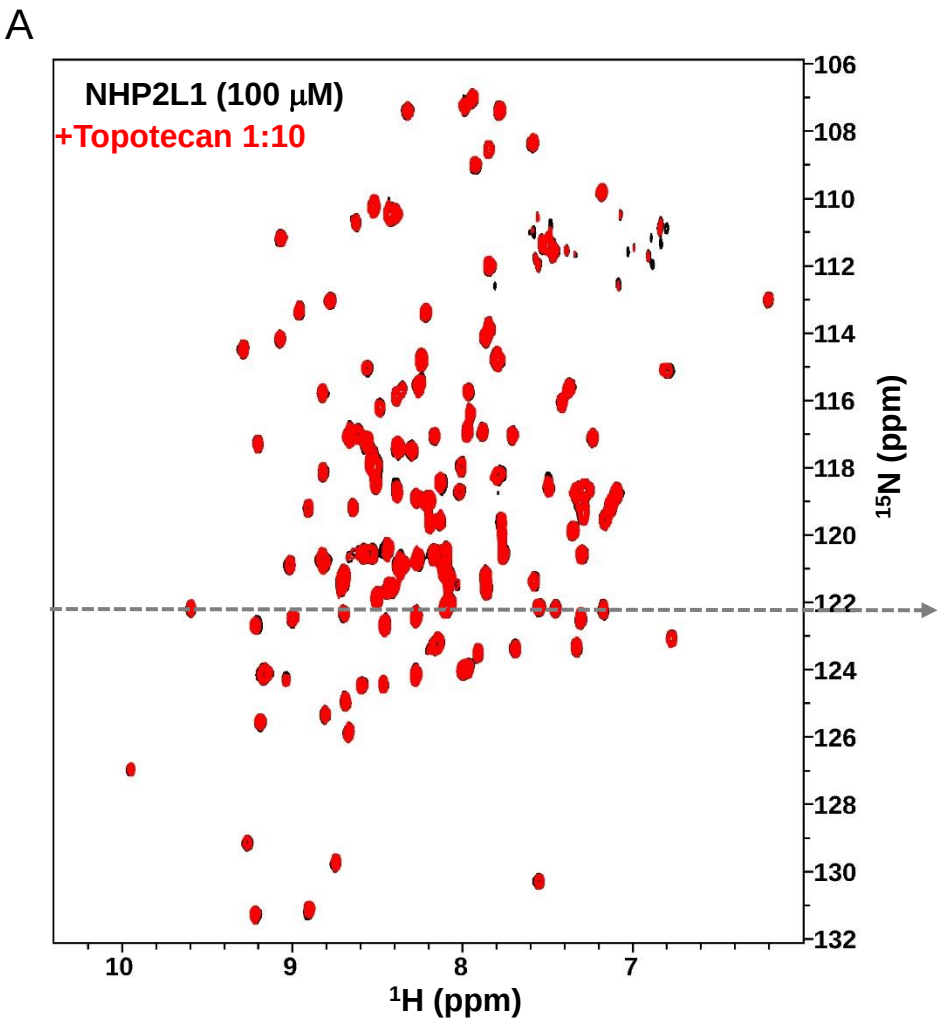


Figure S4. Topotecan binds to U4. 1D ^{1}H NMR spectra of free U4 5' SL (100μM) or after addition of increasing concentrations of topotecan (1:0; 1:1; 1:2;1:5; 1:10 molar ratios) indicate small molecule binding as shown by the chemical shift. The spectrum of free topotecan is shown on top.



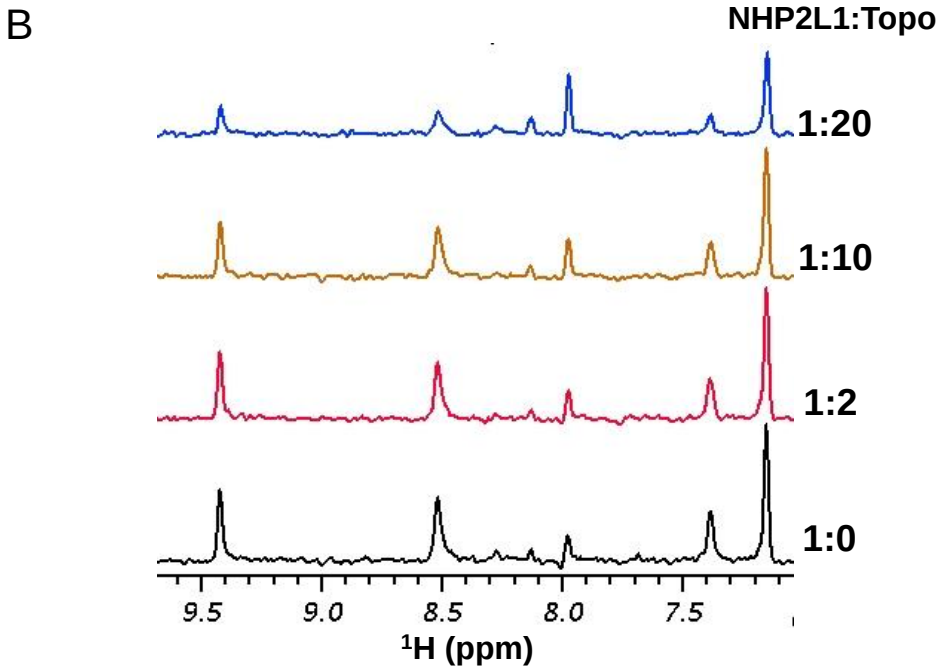


Figure S5. Topotecan does not bind to free NHP2L1. (A) Overlay of 2D ^{1}H-^{15}N TROSY spectra of 100μM ^{15}N-labelled NHP2L1 in the absence (black) and presence (red) of 1mM Topotecan. (B) 1D slices are cross-section of "A" taken at the grey dashed line with free NHP2L1 or after addition of increasing concentrations of topotecan (1:0; 1:2; 1:10;1:20 molar ratios) indicate no binding of the small molecule to NHP2L1.

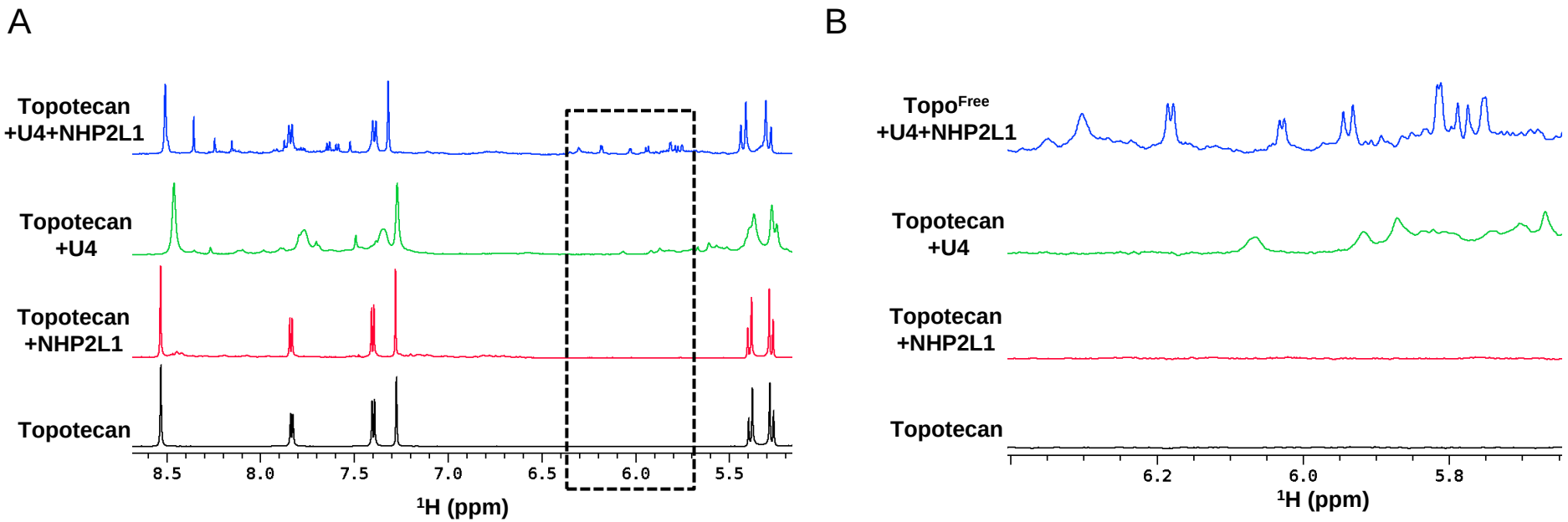


Figure S6. Topotecan binds to U4-NHP2L1 complex. (A) The 1D spectra of topotecan alone or with U4, or with NHP2L1, or with U4 and NHP2L1 showed additional resonances (in dashed box) only in the spectrum of topotecan with U4 and NHP2L1 complex. These small populations of topotecan resonances (blue) at 1:1:20 (NHP2L1:U4:topotecan), could possibly represent topotecan bound to U4 in complex with NHP2L1. These conformers are not represent when topotecan interacts with free U4 (Green). (B) represents expanded region inside the dashed box.