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Supplementary information

Probing the free-state solution behavior of drugs and their tendencies to self-aggregate into nano-entities

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Supplementary Information

Protocol for probing the free-state solution behavior of drugs and their tendencies to self-aggregate into nano-entities

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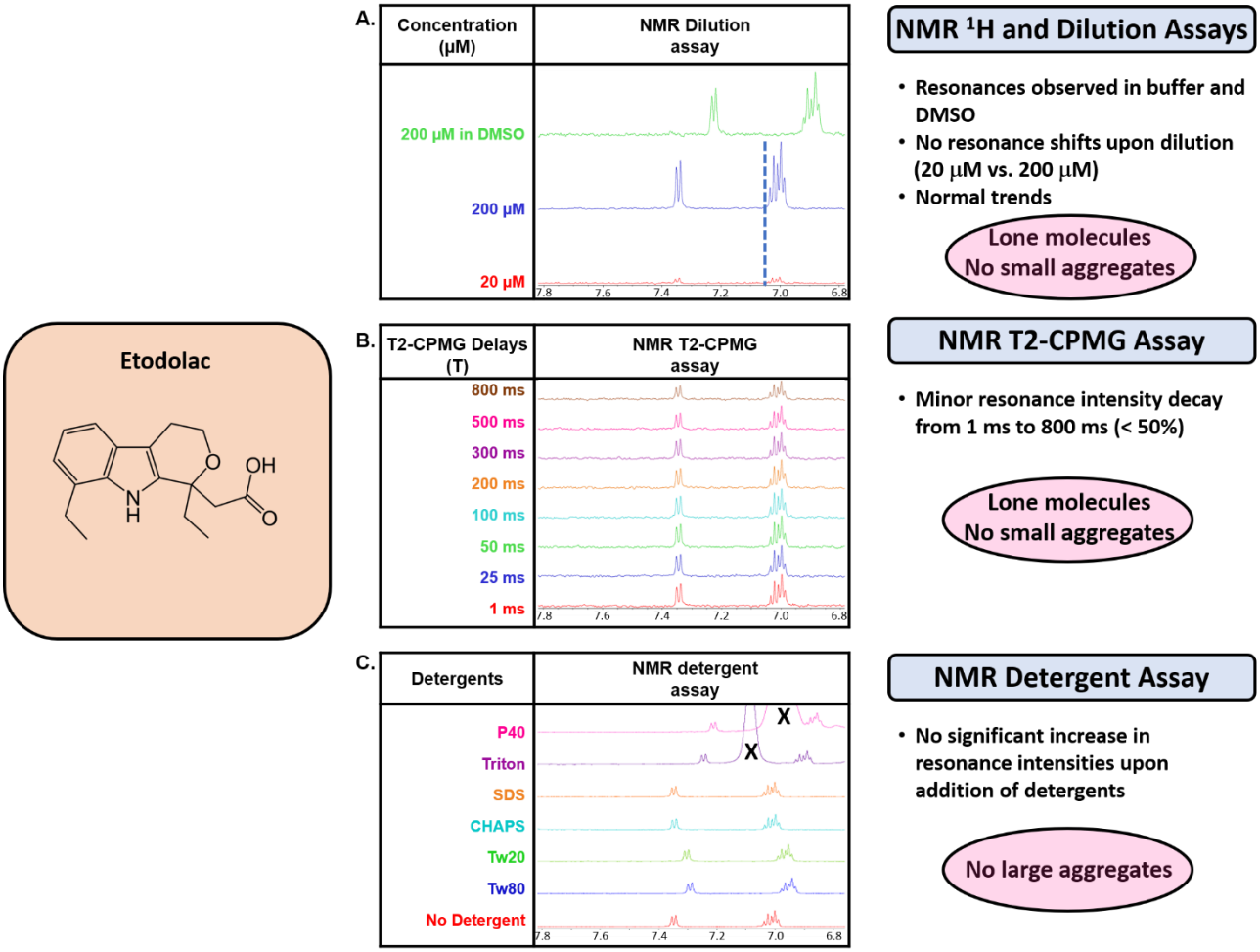
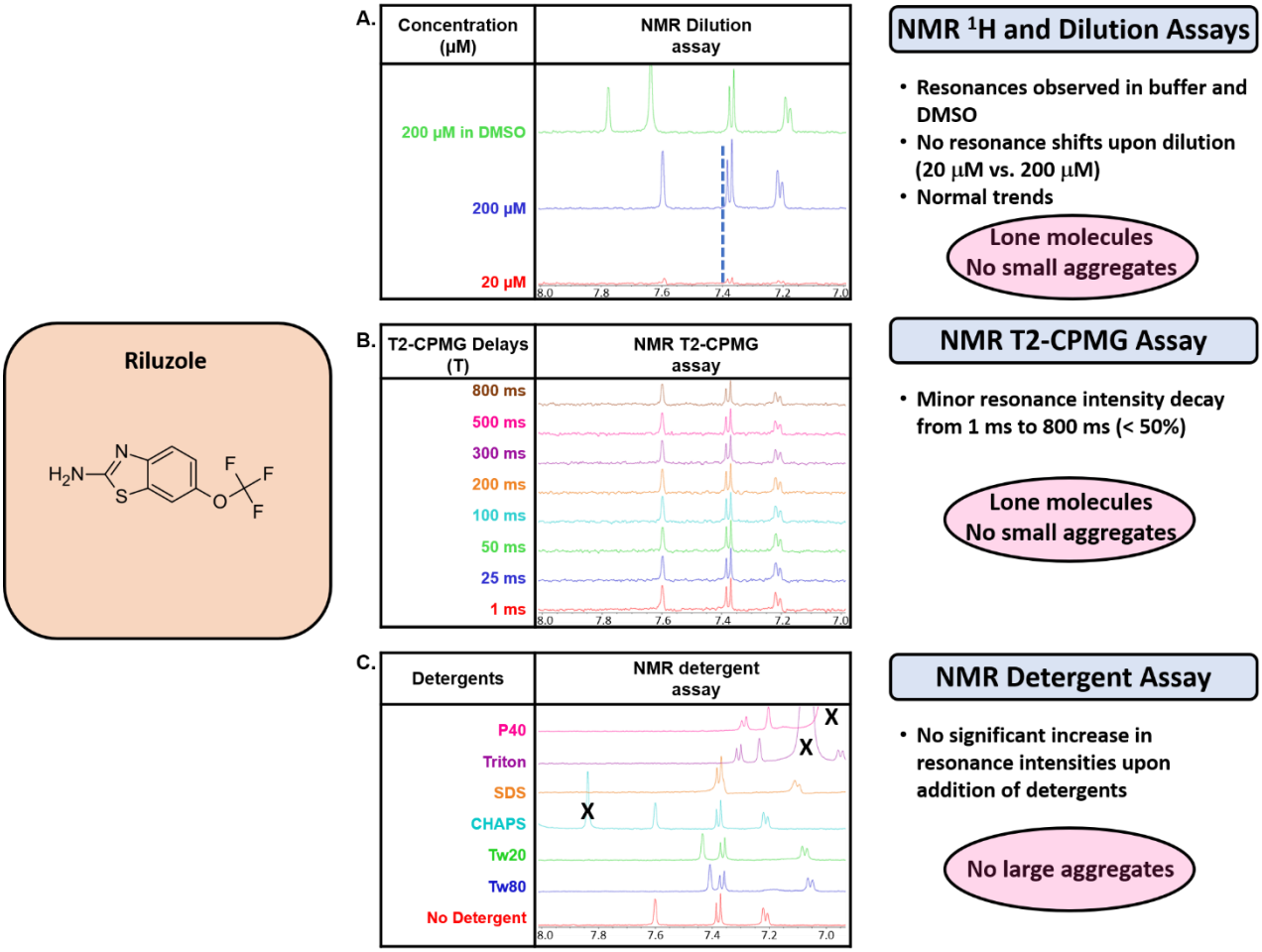
Probing the solution behavior of etodolac



Supplementary Figure 1 | Probing the solution behavior of etodolac. Shown are NMR data from, (A) the NMR ^{1}H and Dilution Assays, (B) the T2-CPMG Assay, and (C) The NMR Detergent Assay. The chemical structure is shown on the left. "x" in (C) denote resonances that arise from the detergent and not from the compound.

The NMR ^{1}H Assay for etodolac in Supplementary Figure 1A resulted in observable resonances in both buffer and DMSO-d_6 at 200 μM nominal concentration. Also, the samples were clear with no precipitate upon visual inspection, confirming that etodolac was soluble in both solvents. Furthermore, upon dilution in buffer from 200 μM to 20 μM, normal trends were notable (i.e. Supplementary Figure 1A - decreases in signal intensity with no change in chemical shifts). These observations were consistent with a behavior of lone tumbling molecules with no self-association tendencies. These conclusions were corroborated by data from the NMR T2-CPMG Assay (Supplementary Figure 1B) where the resonance intensities were minimally affected upon comparison of the resonances of the spectra employing delay times of 1 ms versus 800 ms (minor resonance intensity decay < 50%). Additionally, no significant increases in resonance intensities were noted upon addition of detergents in the NMR Detergent Assay (Supplementary Figure 1C) reporting that no large aggregates exist. The same finding can be observed with riluzole (Supplementary Figure 2). None of these two drugs exhibit evidence of aggregation at these concentrations.

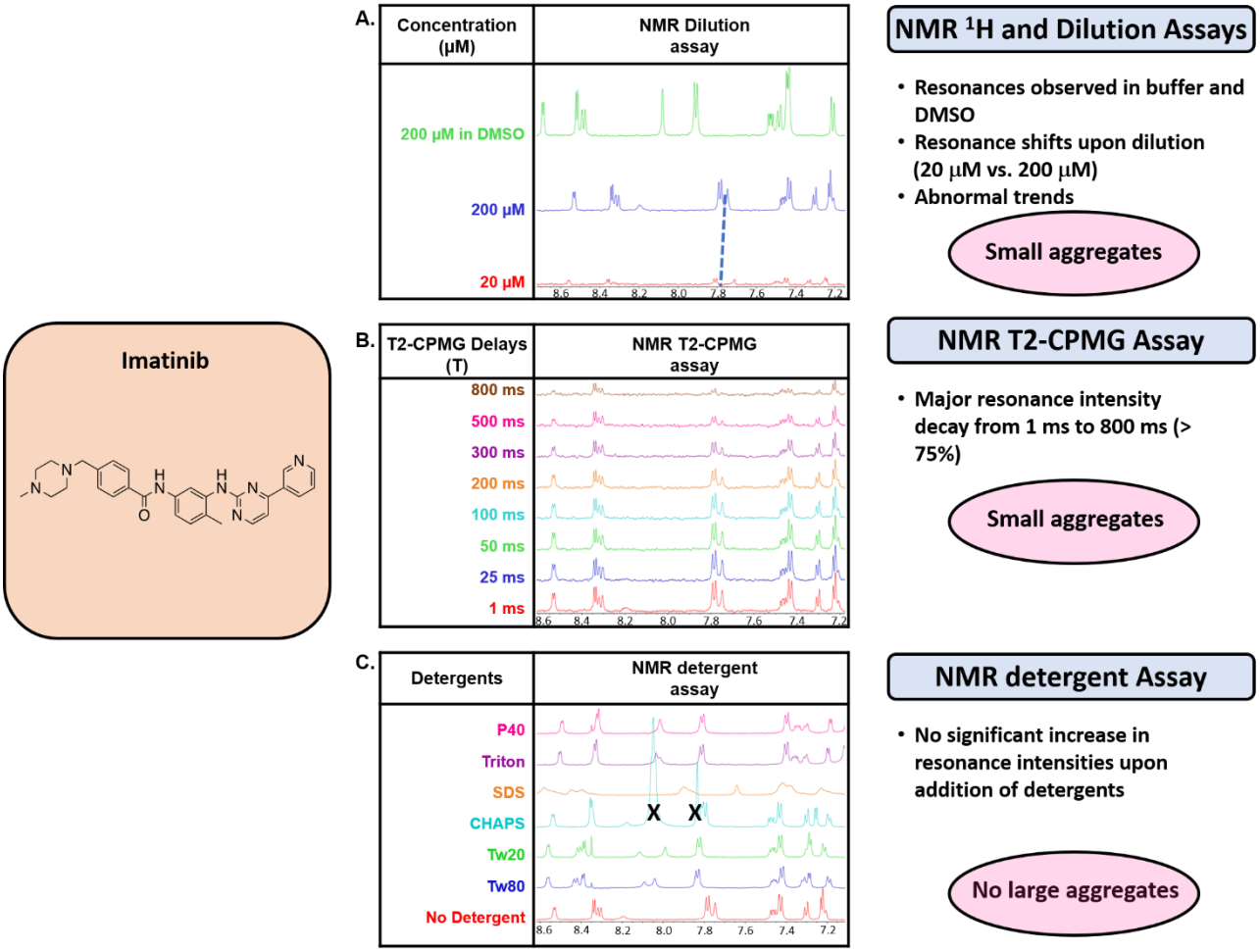
Probing the solution behavior of riluzole



Supplementary Figure 2 | Probing the solution behavior of riluzole. Shown are NMR data from, (A) the NMR 1H and Dilution Assays, (B) the T2-CPMG Assay, and (C) The NMR Detergent Assay. The chemical structure is shown on the left. "x" in (C) denote resonances that arise from the detergent and not from the compound.

Probing the solution behavior of imatinib

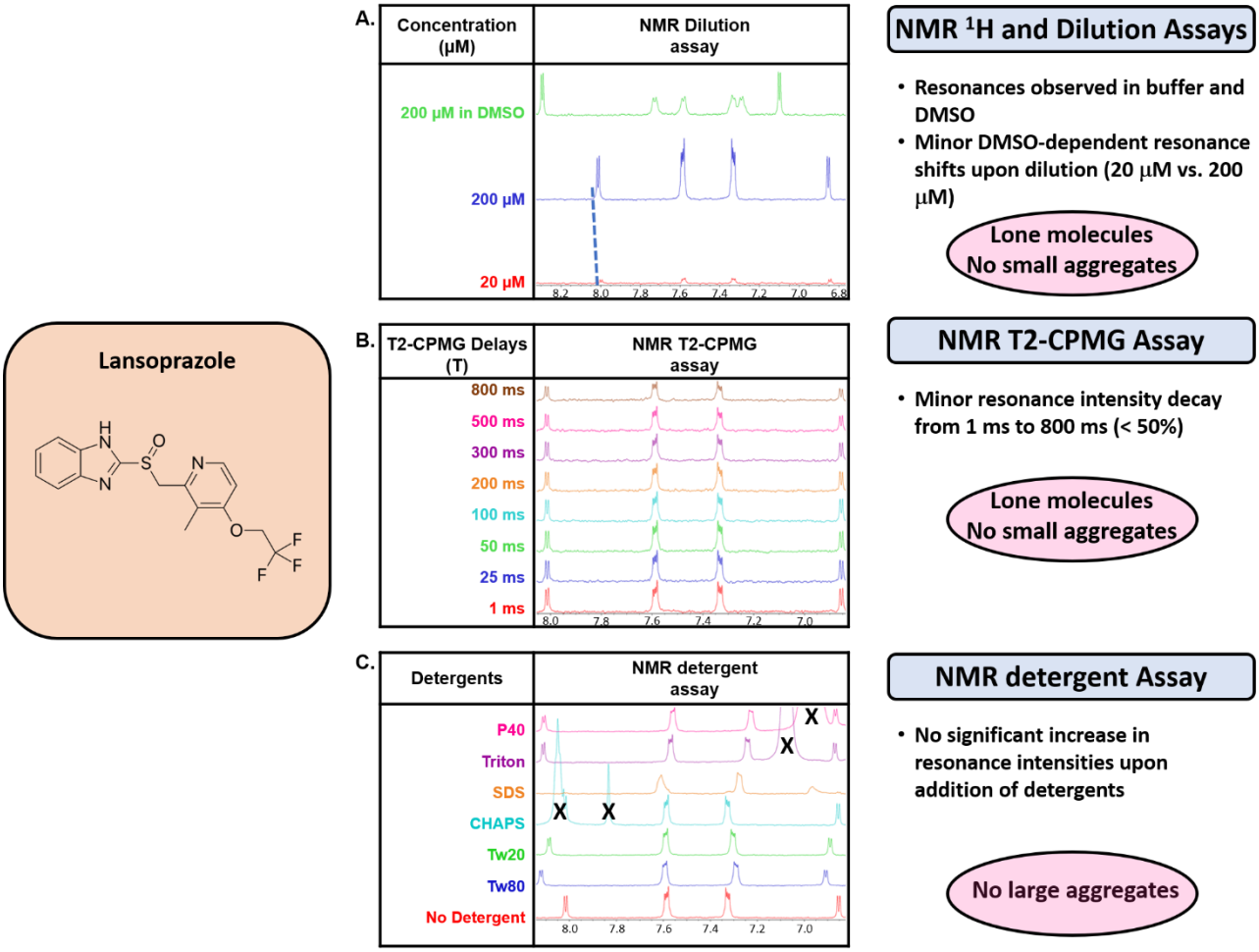
NMR resonances were observed for imatinib in the NMR ^{1}H Assay acquired in buffer and DMSO-d_6 at 200 μM nominal concentration (Supplementary Figure 3A). Again, the samples were clear with no precipitate upon visual inspection. Dilution in buffer from 200 μM to 20 μM results in observation of abnormal trends (i.e. Supplementary Figure 3A - decreases in intensity along with changes in chemical shifts). This would be consistent with a behavior of self-association into small aggregates. The NMR T2-CPMG Assay (Supplementary Figure 3B) supports these conclusions as resonance intensities were significantly reduced after 800 ms (intensity decay > 75%). Interestingly, no significant increases in resonance intensities were noted upon addition of detergents in the NMR Detergent Assay (Supplementary Figure 3C) reporting that no large aggregates exist. Thus, the data suggests that imatinib self-associates into small aggregates and not into large aggregates.



Supplementary Figure 3 | Probing the solution behavior of imatinib. Shown are NMR data from, (A) the NMR ^{1}H and Dilution Assays, (B) the T2-CPMG Assay, and (C) The NMR Detergent Assay. The chemical structure is shown on the left. "x" in (C) denote resonances that arise from the detergent and not from the compound.

Probing the solution behavior of lansoprazole

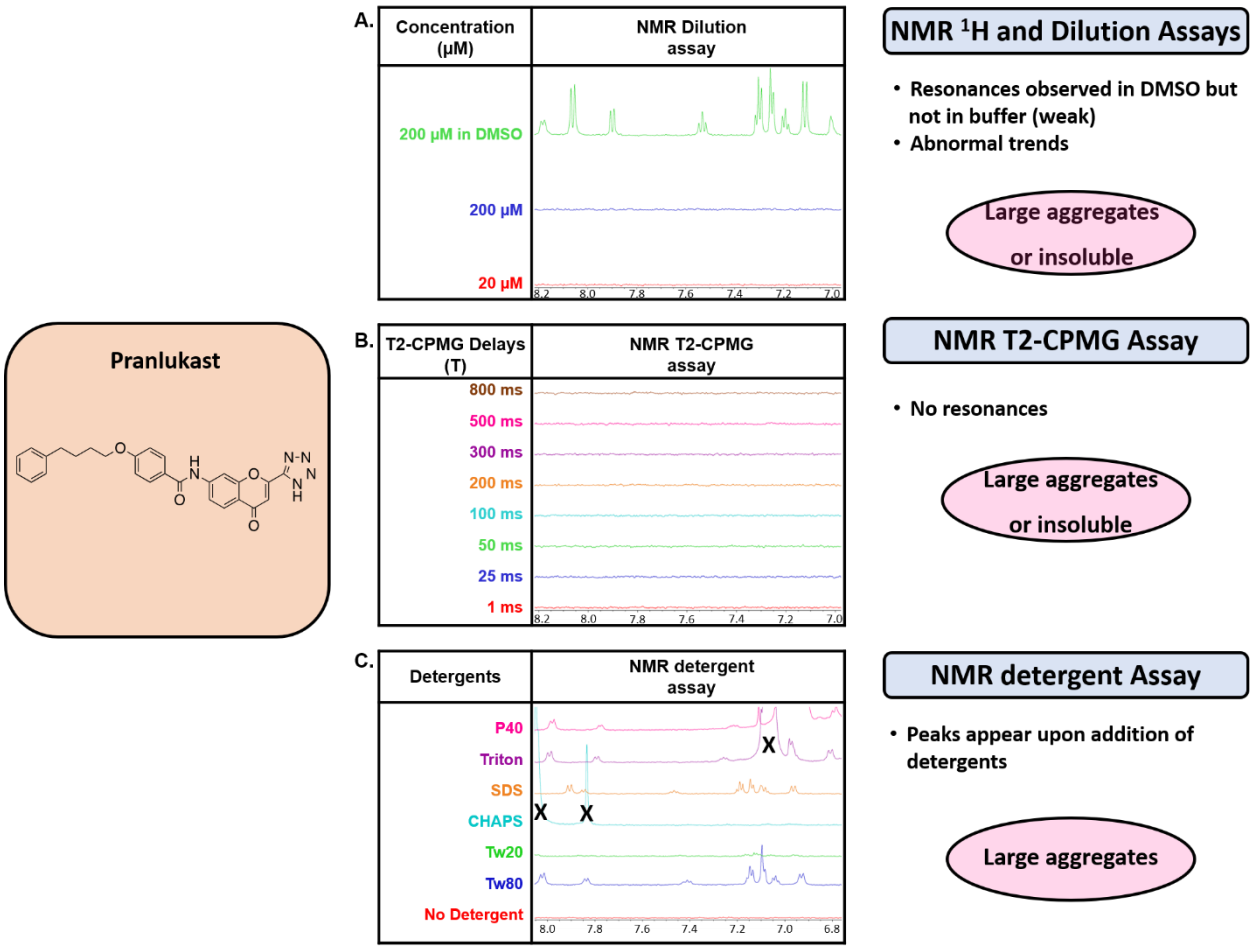
Here is a particular example where caution must be taken upon interpretation of the data. T2-CPMG and detergent assays of lansoprazole do not suggest any aggregation phenomenon. However, upon looking at the dilution assay, small changes in chemical shifts can be observed for some resonances upon dilution from 200 to 20 µM. For the sake of simplicity and speed, the dilution assay involves diluting the samples while also diluting the amount of DMSO at the same time. Therefore, some cases as this one may arise where observed shift can be attributed to such variation in DMSO concentration. Retesting of lansoprazole dilutions with constant amount of DMSO results in no observable changes in chemical shifts (data not shown). The fact that only some lansoprazole resonances would shift upon dilution can be an indication that care must be taken with interpretation. It is likely that some hydrogens are more sensitive to the changes in chemical environment caused by this variation in DMSO. Although, it is conceivable that some aggregates could also preferentially induce changes in chemical shifts for specific resonances. This highlights the importance of looking at the data as a whole.



Supplementary Figure 4 | Probing the solution behavior of lansoprazole. Shown are NMR data from, (A) the NMR 1H and Dilution Assays, (B) the T2-CPMG Assay, and (C) The NMR Detergent Assay. The chemical structure is shown on the left. "x" in (C) denote resonances that arise from the detergent and not from the compound.

Probing the solution behavior of pranlukast

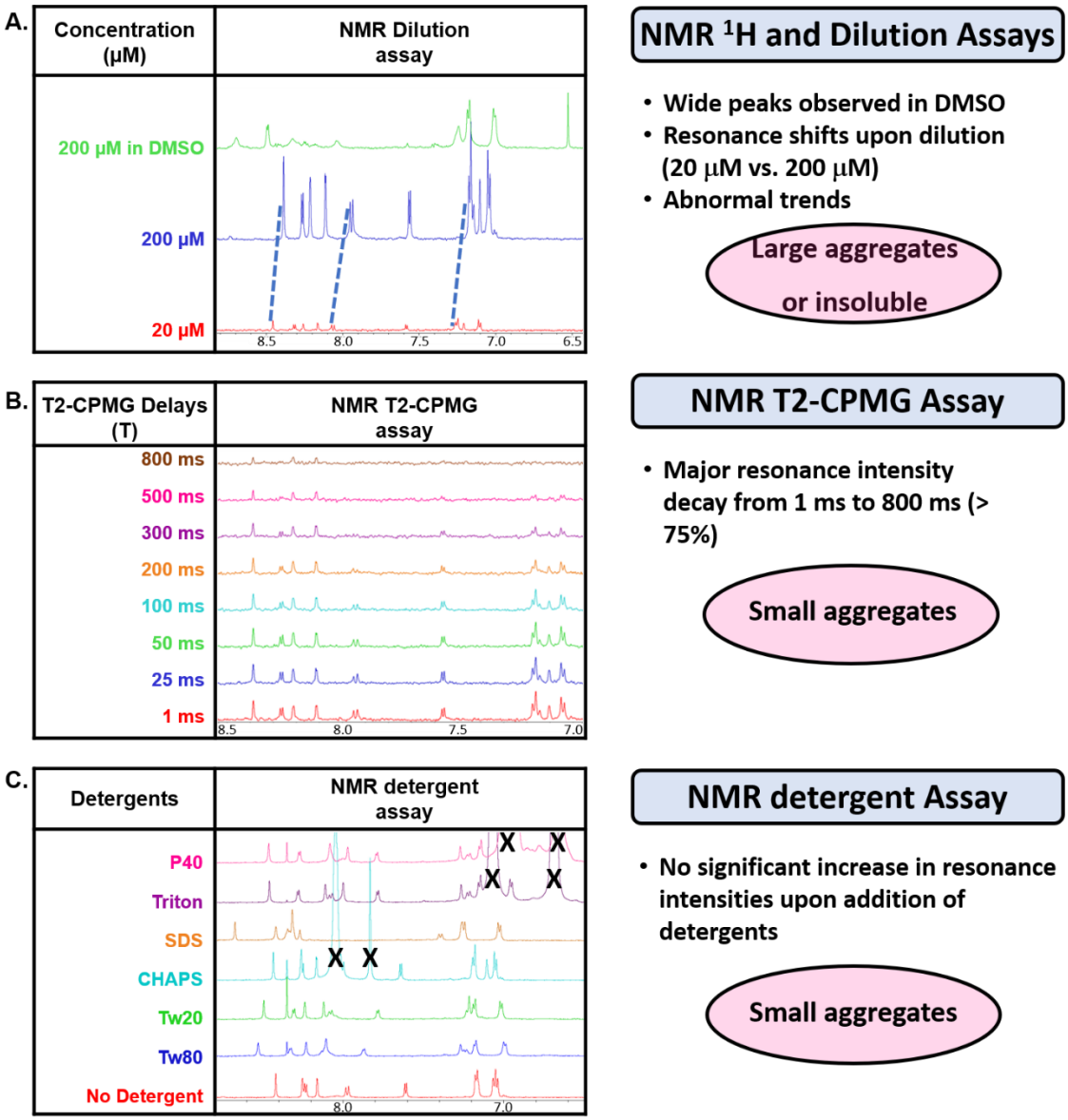
NMR resonance for pranlukast were observed in DMSO-d_6, but not in buffer at 200 µM nominal concentration in the NMR ^{1}H Assay (Supplementary Figure 5A). However, samples in buffer had cloudiness upon inspection but no precipitate, confirming that the compound was still in solution. Dilution in buffer from 200 µM to 20 µM did not result in appearance of signal but resulted in significant decrease in sample cloudiness without any observable precipitate. The NMR T2-CPMG Assay therefore did not provide any information due to the lack of resonances. The NMR Detergent Assay confirms the presence of large aggregates since the addition of several detergents results in appearance of resonances (Supplementary Figure 5C).



Supplementary Figure 5 | Probing the solution behavior of pranlukast. Shown are NMR data from, (A) the NMR ^{1}H and Dilution Assays, (B) the T2-CPMG Assay, and (C) The NMR Detergent Assay. The chemical structure is shown on the left. "x" in (C) denote resonances that arise from the detergent and not from the compound.

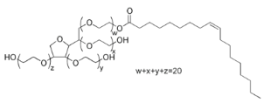
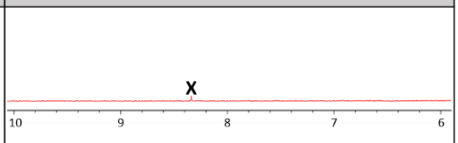
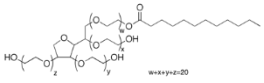
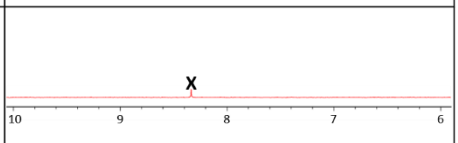
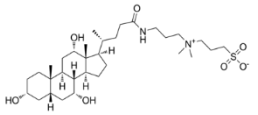
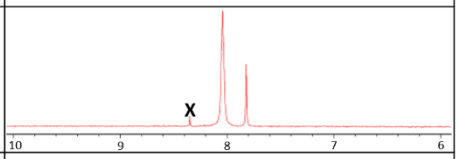
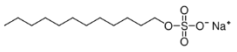
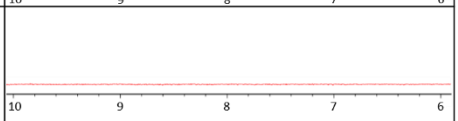
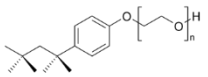
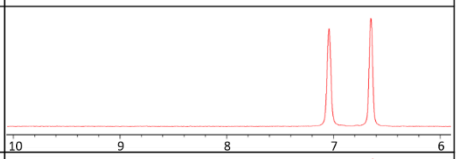
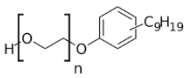
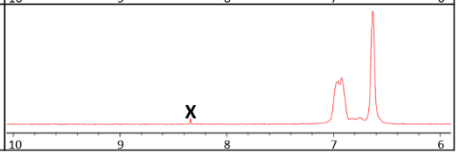
Example of a possible DMSO aggregator

NMR resonances for an undisclosed compound were observed in both DMSO-d_6 and buffer. However, the NMR profile in DMSO would suggests that the compounds is aggregating (broad peaks, low signal intensity) in this solvent (Supplementary Figure 6A). Although the NMR peaks appear much sharper in buffer, the dilution from 200 μM to 20 μM results in changes in chemical shifts for all the observed aromatic resonances. No precipitate or cloudiness could be visible in the samples. The NMR T2-CPMG Assay supports the dilution results as the signals exhibit a significant decay after 800 ms (Supplementary Figure 6B). Finally, no significant increase in resonance intensities could be observed in the NMR Detergent Assay (Supplementary Figure 6C). The overall data suggests the presence of small aggregates in buffer, with apparent aggregation of the molecule in DMSO as well.



Supplementary Figure 6| Example of a compound that possibly aggregates in both buffer and DMSO. Shown are NMR data from, (A) the NMR ^{1}H and Dilution Assays, (B) the T2-CPMG Assay, and (C) The NMR Detergent Assay. The chemical structure is shown on the left. "x" in (C) denote resonances that arise from the detergent and not from the compound.

Detergents properties

Detergent	Chemical structure	Class	CMC	Spectrum (aromatic region)
Tween 80		Non-ionic	0.028 mM	
Tween 20		Non-ionic	0.042 mM	
CHAPS		Zwitterionic	8-10 mM	
SDS		Ionic	6-8 mM	
Triton X-100		Non-ionic	0.2 mM	
Nonidet P-40		Non-ionic	0.29 mM	

Supplementary Table 1 | Properties of detergents used in this report. “x” in NMR spectra denote resonances that arise from the impurities and not from detergents.

Experimental guidelines for various sample set sizes

Number of compounds	Time per compound	Methods

1 - 20	• **Preparation time**: 30-40 min
	• **NMR time**: For one sample
	  • T2 CPMG : 5.5 min (4 scans)
	  • ^{1}H NMR : 4 min (16 scans), 7 min (32 scans), 13.3 min (64 scans) and 26 min (128 scans)
	• **Analysis time**: 40-50 min
		• **NMR dilution assay**: All concentrations
		• **NMR T2-CPMG assay**: All delay times
		• **NMR detergent assay**: 3 detergents or more (e.g. Tween 80, NP40 and CHAPS)
		• **Note:** The number of scans should be same for all dilution experiments. However they can be 16, 32, 64, 128 or more (for dilution and detergent assays)

20 - 50	• **Preparation time**: 15-20 min
	• **NMR time**: For one sample
	  • T2 CPMG : 5.5 min (4 scans)
	  • ^{1}H NMR : 4 min (16 scans) or 7 min (32 scans)
	• **Analysis time**: 30 min
		• **NMR dilution assay**: Fast track method
		• **NMR T2-CPMG assay**: All delay times
		• **NMR detergent assay**: 1 or 2 detergents only (e.g. Tween 80 and NP40)
		• **Number of scans** (dilution and detergent assays): 16 or 32
		• **Note:** The number of scans should be same for all dilution experiments. However they can be 16 or 32 (for dilution and detergent assays)

> 50	• **Preparation time**: 15-20 min
	• **NMR time**: For one sample
	  • T2 CPMG : 5.5 min (4 scans)
	  • ^{1}H NMR : 4 min (16 scans)
	• **Analysis time**: 25 min
		• **NMR dilution assay**: Fast track method
		• **NMR T2-CPMG assay**: 1 ms and 800 ms delays only
		• **NMR detergent assay**: 1 or 2 detergents only (e.g. Tween 80 and NP40)
		• **Note:** The number of scans should be same for all dilution experiments. 16 scans often result in satisfactory signal-to-noise (for dilution and detergent assays)

Supplementary Table 2 | Experimental guidelines according to various sample set sizes. Presented here are suggestions in order to mitigate increases in NMR acquisition time with larger sample sets.

Compounds information

Compounds	SMILES	Supplier	Catalog No.	CAS	Drug class	References in the manuscript
Valsartan	CCCCC(=O)N(CC1=CC=C(C=C1)C2=CC=CC=C2C3=NNN=N3)C(C(C)C)C(=O)O	BETAPHARMA	56-02004	137862-53-4	Antihypertensive	Figures 6
Etodolac	CCC1=C2C(=CC=C1)C3=C(N2)C(OCC3)(CC)CC(=O)O	SIGMA	E0516-50MG	41340-25-4	NSAID	Figure S1
Riluzole	C1=CC2=C(C=C1OC(F)(F)F)SC(=N2)N	SIGMA	R116-250MG	1744-22-5	Amyotrophic lateral sclerosis treatment	Figure S2
Methylene blue	CN(C)C1=CC2=C(C=C1)N=C3C=CC(=[N+](C)C)C=C3S2.[Cl-]	SIGMAALDRICH	M9140-25G	7220-79-3	Antidote for methemoglobiniziant poisoning	Figure 7
Imatinib	CC1=C(C=C(C=C1)NC(=O)C2=CC=C(C=C2)CN3CCN(CC3)C)NC4=NC=CC(=N4)C5=CN=CC=C5	BETAPHARMA	86-33437	152459-95-5	Chemotherapy	Figure S3
Lansoprazole	CC1=C(C=CN=C1CS(=O)C2=NC3=CC=CC=C3N2)OCC(F)(F)F	SIGMA	L8533-250MG	103577-45-3	Proton pump inhibitor	Figure S4
Pranlukast	C1=CC=C(C=C1)CCCCOC2=CC=C(C=C2)C(=O)NC3=CC=CC4=C3OC(=CC4=O)C5=NNN=N5	BETAPHARMA	56-05418	103177-37-3	Antiasthmatic agent (anti-inflammatory)	Figure S5
Candesartan Cilexetil	CCOC2=NC1=CC=CC(=C1[N]2CC3=CC=C(C=C3)C4=CC=CC=C4C5=N[N]N=N5)C(=O)OC(C)OC(=O)OC6CCCCC6	BETAPHARMA	14-31650	145040-37-5	Antihypertensive	Figure 8

Lapatinib	CS(=O)(=O)CCNCC1=CC=C(O1)C2=CC3=C(C=C2)N=CN=C3NC4=CC(=C(C=C4)OCC5=CC(=CC=C5)F)Cl	Ontario Chemicals Inc	L1034	231277-92-2	Chemotherapy	2, 10
Clofazimine	CC(C)N=C1C=C2C(=NC3=CC=CC=C3N2C4=CC=C(C=C4)Cl)C=C1NC5=CC=C(C=C5)Cl	Sigma	C8895	2030-63-9	Anti-leprosy	2
Sorafenib	CNC(=O)C1=NC=CC(=C1)OC2=CC=C(C=C2)NC(=O)NC3=CC(=C(C=C3)Cl)C(F)(F)F	SynChem	BAM66401	475207-59-1	Chemotherapy	2
Curcumin	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O	Sigma	C1386	458-37-7	NA	2

Supplementary Table 3 | Compounds information.