Ionic Resorcinarenes as Drug Solubilization Agents in Water

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SUPPORTING INFORMATION

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GENERAL

We report the synthesis of novel macrocyclic receptor **R1** and that of **R2** which was synthesized according to reported procedures[1–3]. Drug compounds were purchased from Sigma Aldrich. The ^{1}H and ^{13}C NMR experiments were carried out in D_2O at 298 K on Bruker Avance 400 MHz spectrometers. HRMS was done using ESI in negative mode. ITC measurements were performed using the VP-ITC instrument made by Malvern Panalytical. DLS experiments were carried out in deionized water using a zetasizer nano from Malvern Panalytical. Cytotoxicity experiments were carried out on HEK-293 cells

SYNTHESIS

Synthesis of R1

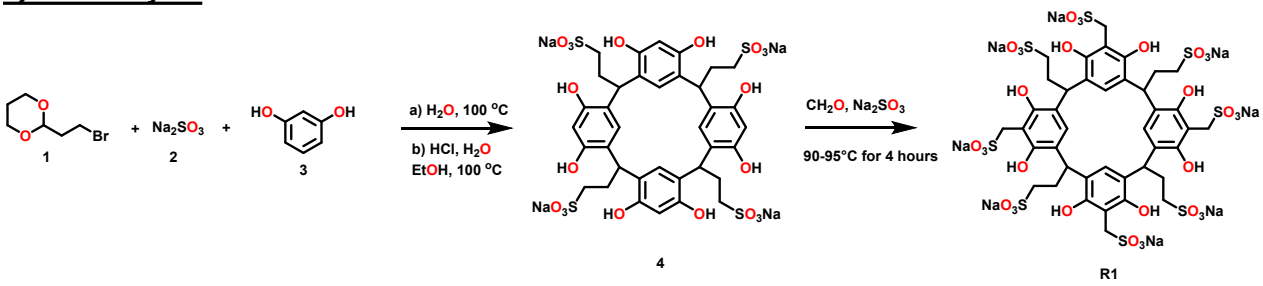


Figure S1: Synthesis of octa-sulfonated resorcinarenes **R1**.

A two-phase mixture of 2-(2-bromoethyl)-1,3-dioxane, **1** (4.0 g, 20 mmol) and an aqueous solution (20 mL) of Na_2SO_3, **2** (5.0 g, 40 mmol) was stirred at 100 °C for 24 hours. To the resulting homogeneous solution was added water (20 mL), and the mixture was washed with ether (40 mL x2) to get rid of unreacted **1**. To this were successively added ethanol (40 mL), resorcinol, **3** (4.0 g, 36 mmol), and concentrated HCl (6 mL). The mixture was stirred under nitrogen at 100 °C for 24 h. The solvent was evaporated, and the residue was taken in water (60 mL) and dialyzed three times against water (2 L) using a dialysis membrane having a transport critical molecular weight of 1000 (Spectra/Por membrane MWCO 1000) to remove inorganic salts. Most of the water was removed in vacuo, and the residue was triturated from methanol to give compound **4**. Compound **4** (0.01 mol), a solution of 37% formaldehyde (0.01mol) and sodium sulfite (0.01M) in H_2O (30 ml) was stirred and heated at 90–95°C for 4 h. Dilute hydrochloric acid was added after cooling until pH 7, then methanol (50 ml or more) was added to precipitate the product **R1.** The solid was filtered and dried. ESI_MS: $(C_{40}H_{45}O_{32}S_8)^{-3}$ calc. = 430.9899 found = 430.9892 (1.7238 ppm); $(C_{40}H_{44}O_{32}\ NaS_8)^{-3}$ calc. = 438.3172 found = 438.3165 (1.5637 ppm).

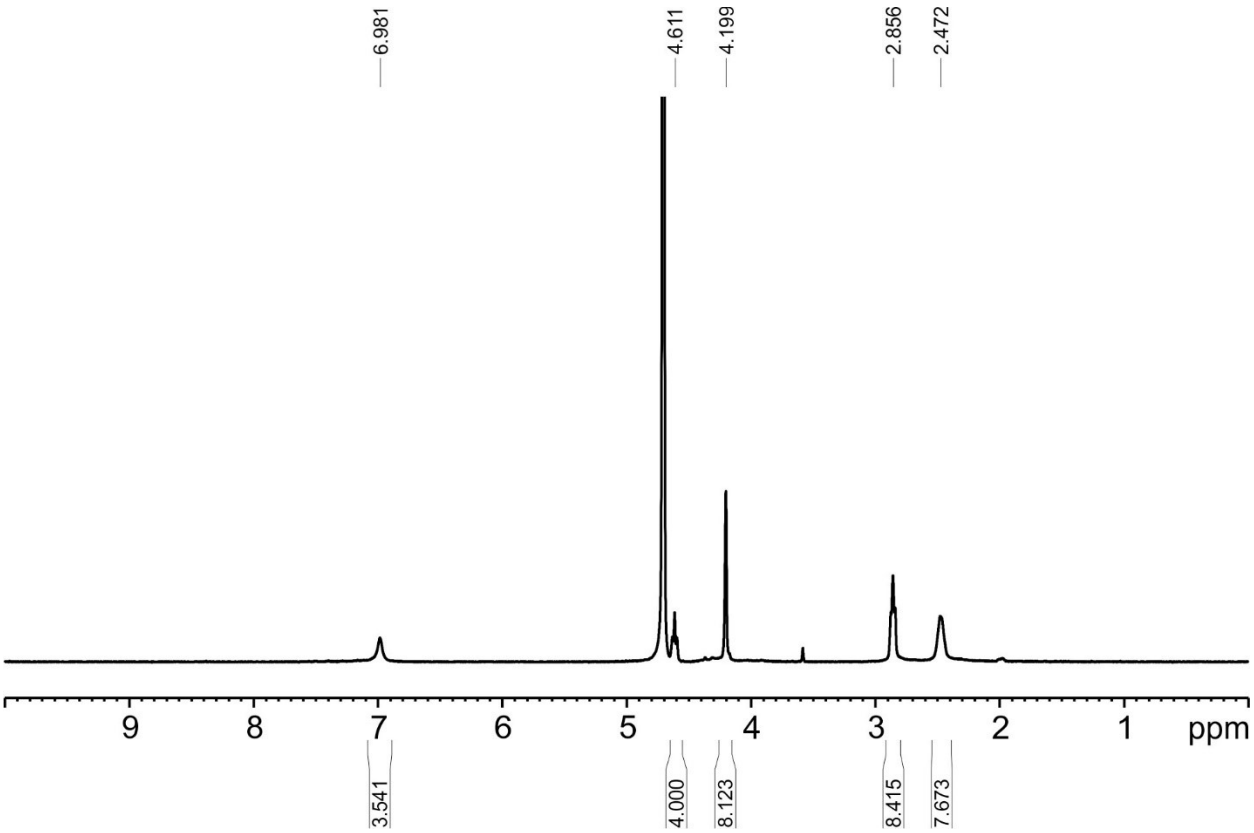


Figure S2: ^{1}H NMR spectra of octa sulfonated resorcinarene **R1** at 400 MHz in D_2O at 298 K.

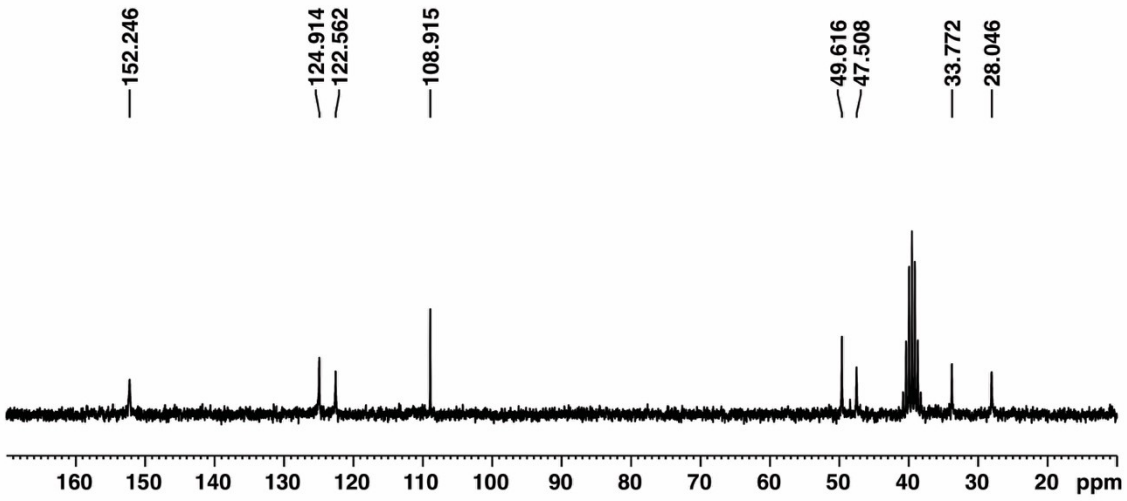


Figure S3: ^{13}C NMR spectra of octa sulfonated resorcinarene **R1** at 400 MHz in D_2O at 298 K.

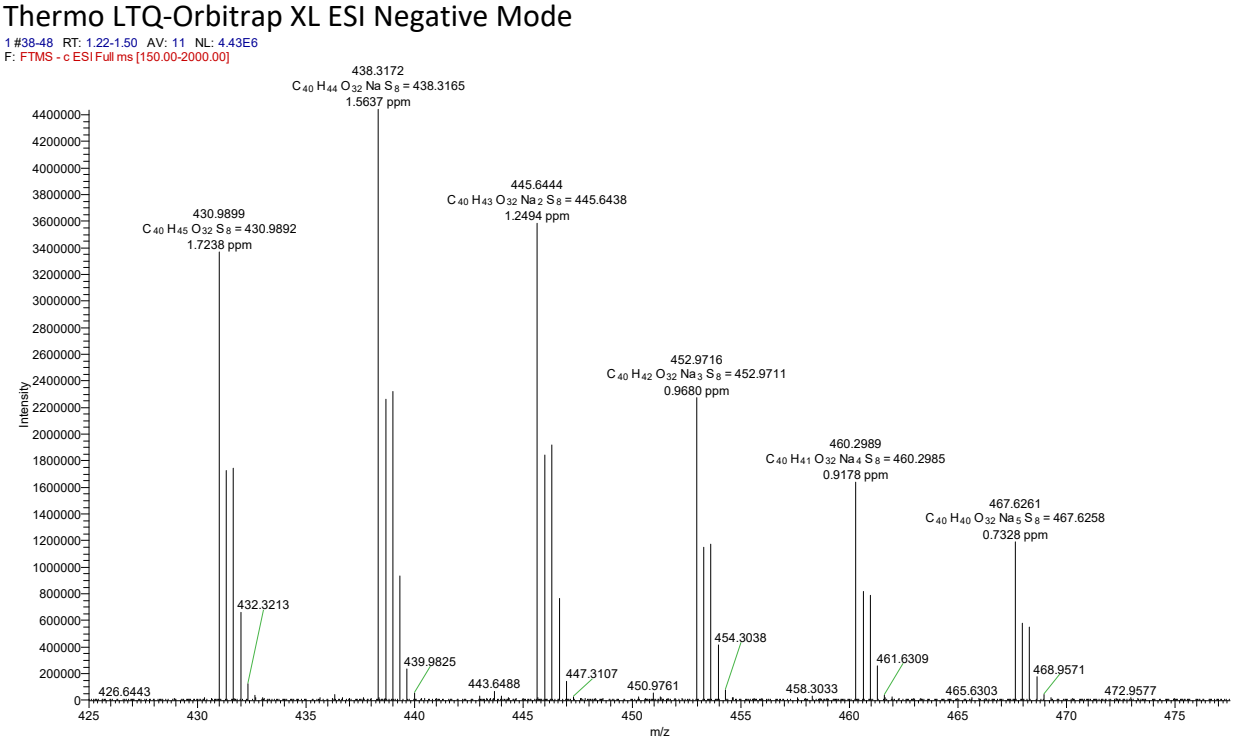


Figure S4. HRMS of octa sulfonated resorcinarene **R1**.

Synthesis of R2

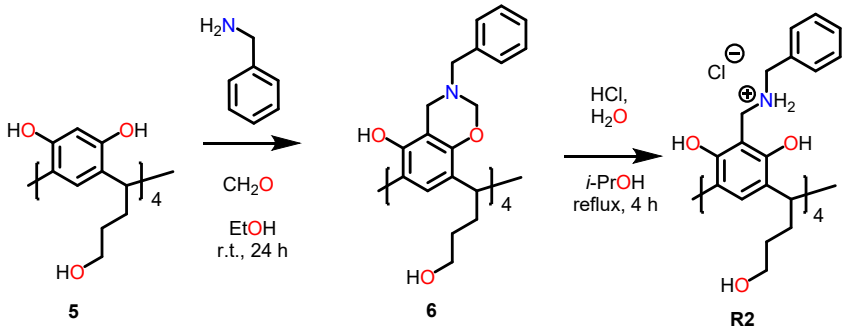


Figure S5: Synthetic scheme for N-benzyl-C-3 propanol resorcinarene **R2**.

Synthesis of this compound has been previously reported[4]. Briefly, resorcinarene, **5** (5.0 g, 6.9 mmol) and excess formaldehyde solution 36 % (28 mL), ethanol (60 ml), benzylamine (3.18 mL, 29.11 mmol) in ethanol (15 mL) were stirred together for 24 hours. The *N*-benzyl tetrabenzoxazine (**6**) precipitate was filtered and dried and used in the next step without further purification. **6** (1.0 g 0.92 mmol), isopropanol (40 ml), concentrated hydrochloric acid (3 ml), and H_2O (4 ml) were refluxed together for 4 hours. The precipitate was washed with ethyl acetate and then with diethyl ether to give the product **R2** (371.8 mg, 23.8 %).

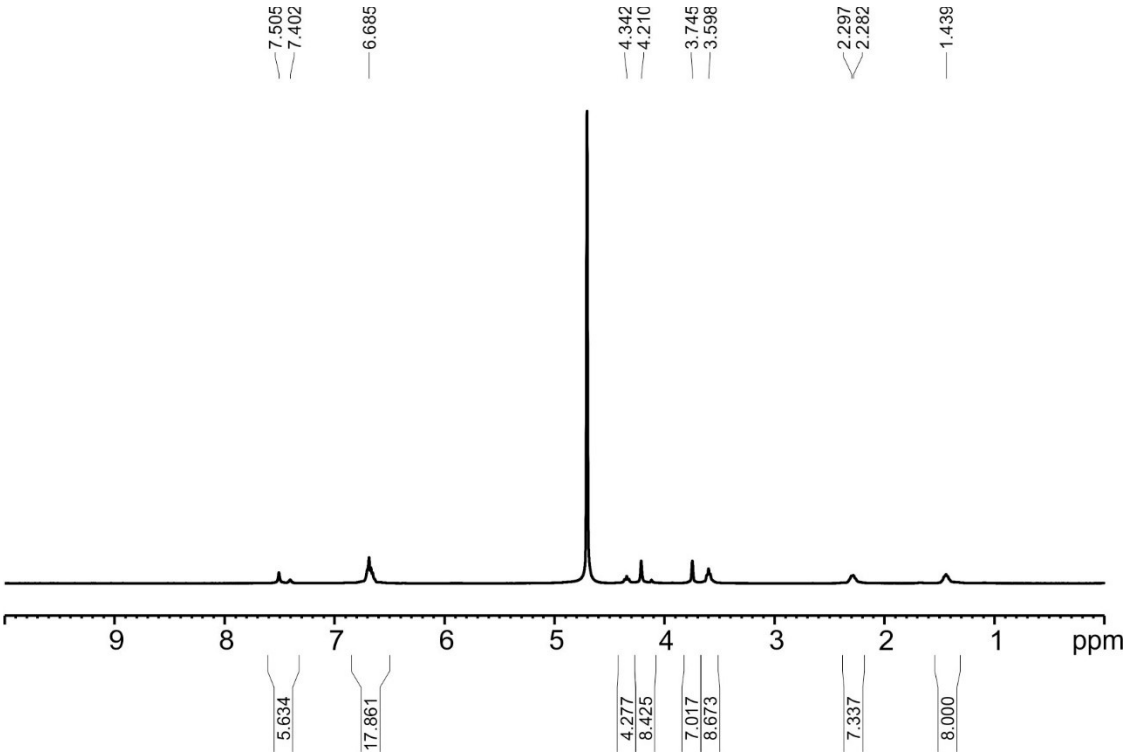


Figure S6: ^{1}H NMR spectra of N-benzyl-C-3 propanol resorcinarene **R2** at 400 MHz in D_2O at 298 K.

NMR SPECTROSCOPY

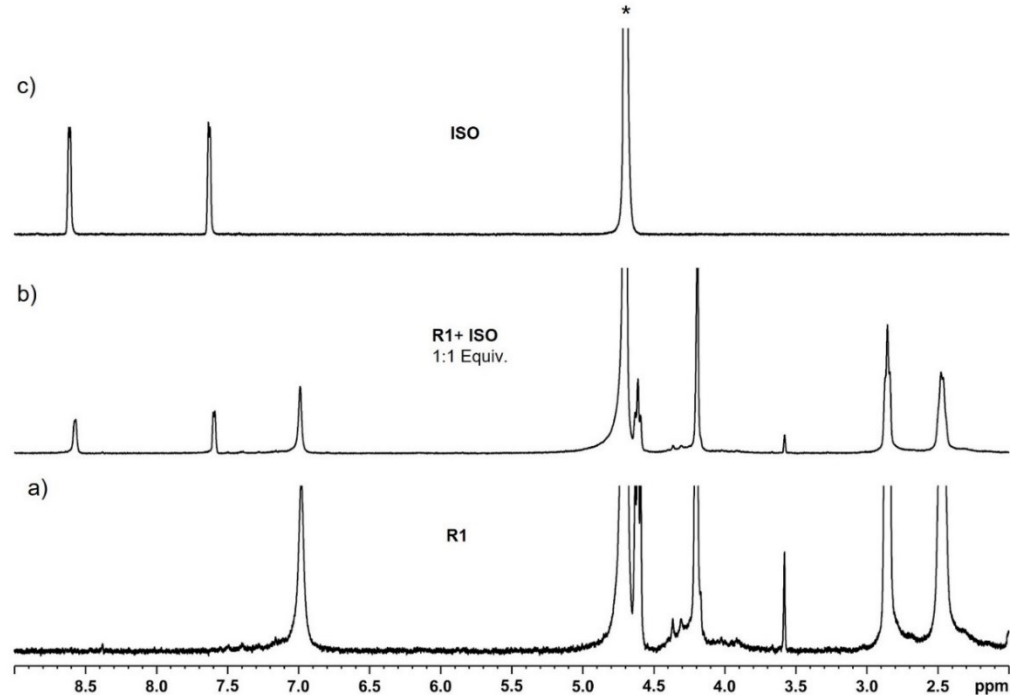


Figure S7: Sections of the ^{1}H NMR spectra at 400 MHz in D_2O at 298 K of pure (a) receptor **R1** and (c) drug **ISO**, and (b) an equimolar mixture of **R1** and **ISO**. The star indicates the D_2O solvent peak.

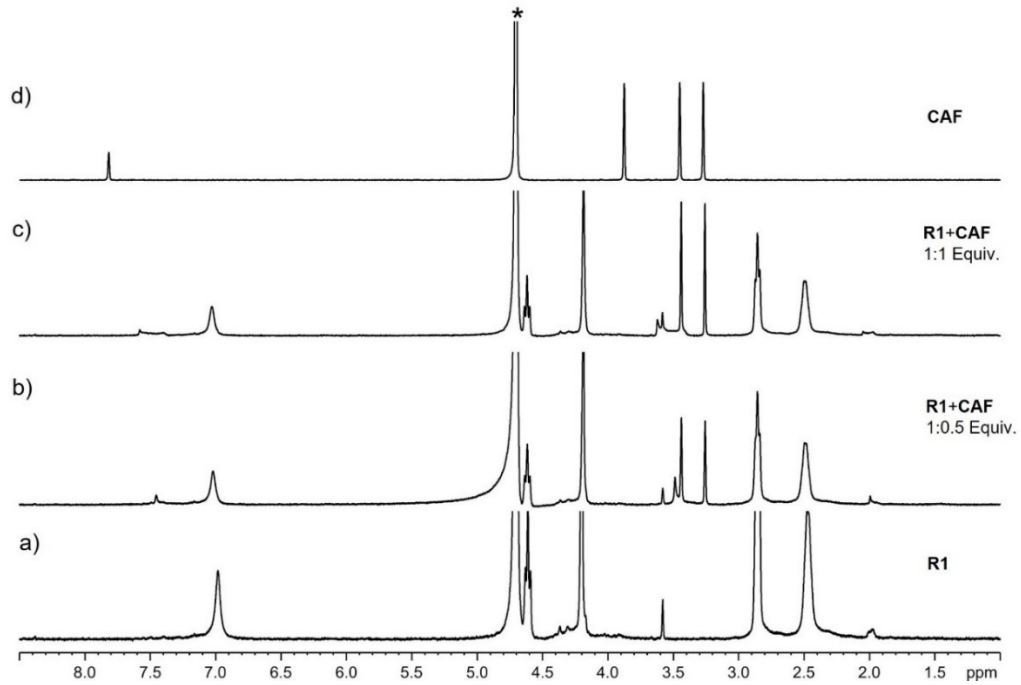


Figure S8: Sections of the ^{1}H NMR spectra at 400 MHz in D_2O at 298 K of pure (a) receptor **R1** and (d) drug **CAF**, and an equimolar mixture of **R1** and **CAF** (b)1:0.5 equivalent and (c) 1:1 equivalent. The star indicates the D_2O solvent peak.

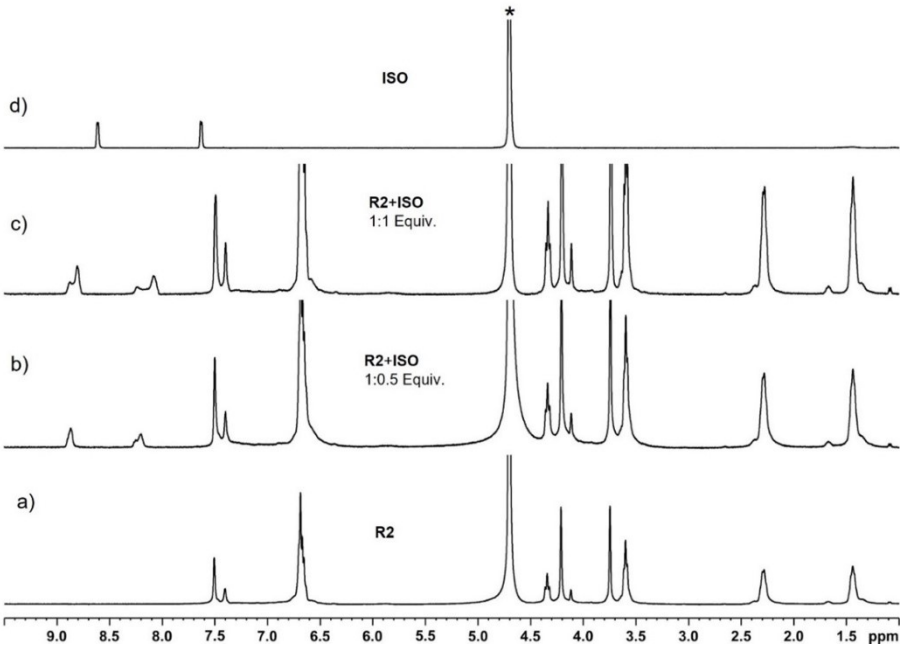


Figure S9: Sections of the ^{1}H NMR spectra at 400 MHz in D_2O at 298 K of pure (a) receptor **R2** and (d) drug **ISO**, and mixtures of **R2** and **ISO** (b)1:0.5 equivalent and (c) 1:1 equivalent. The star indicates the D_2O solvent peak.

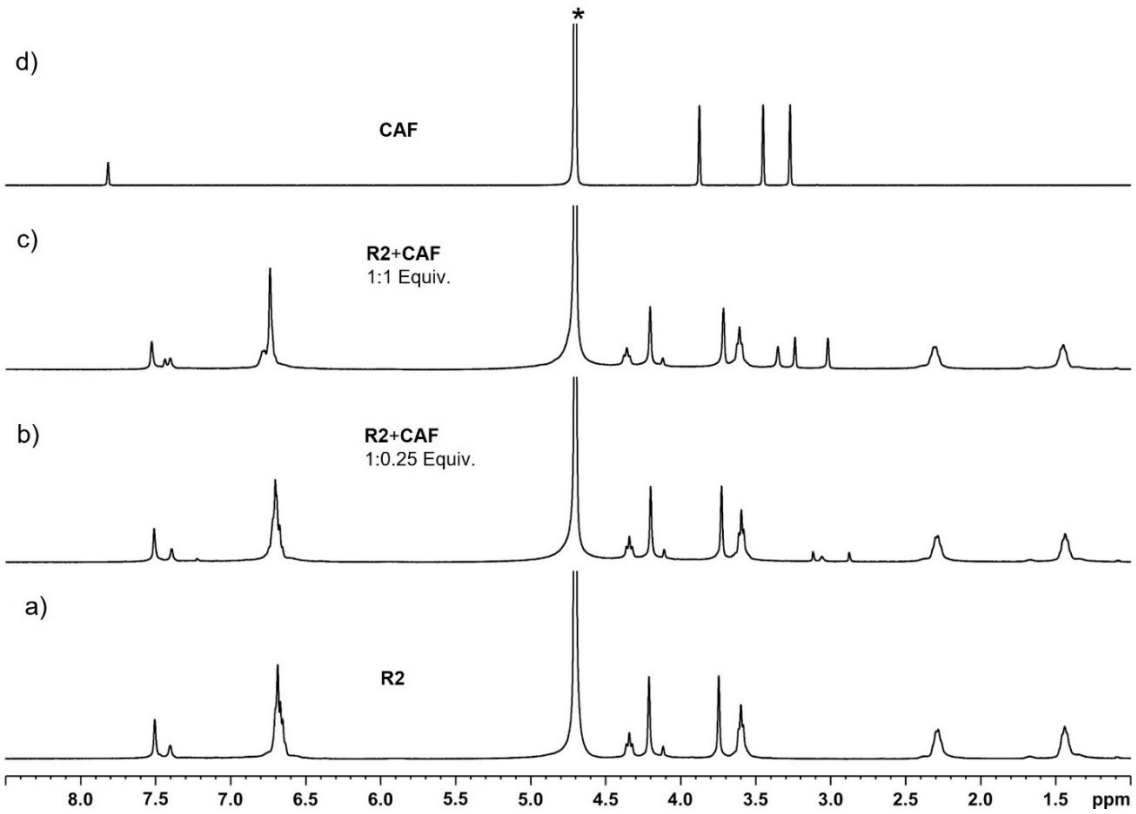


Figure S10: Sections of the ^{1}H NMR spectra at 400 MHz in D_2O at 298 K of pure (a) receptor **R2** and (d) drug **CAF**, and mixtures of **R2** and **CAF** (b)1:0.25 equivalent and (c) 1:1 equivalent. The star indicates the D_2O solvent peak.

ISOTHERMAL CALIROMETRIC TITRATIONS

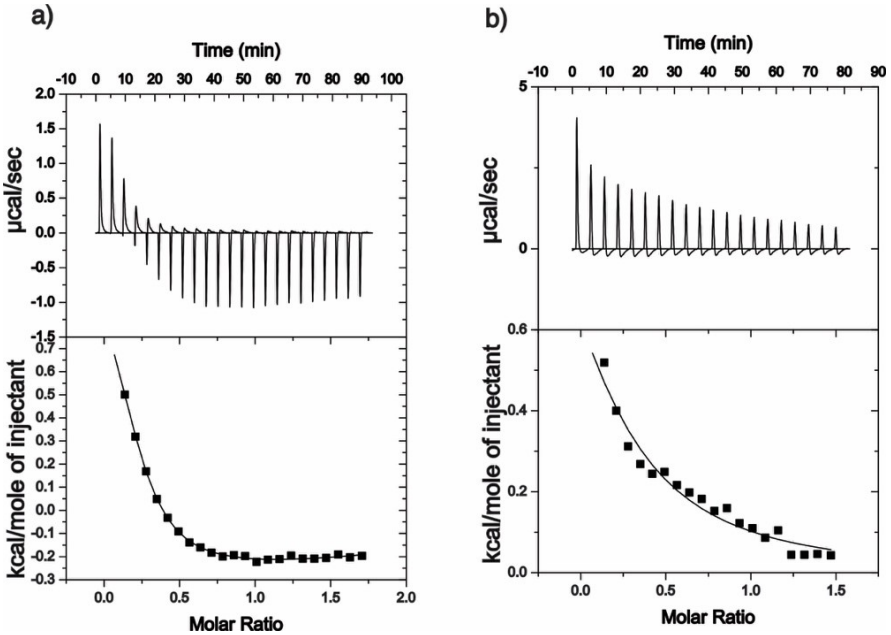


Figure S11: Isothermal calorimetric titration isotherms of **ISO** with: a) **R1** b) **R2** in deionized water at 298K

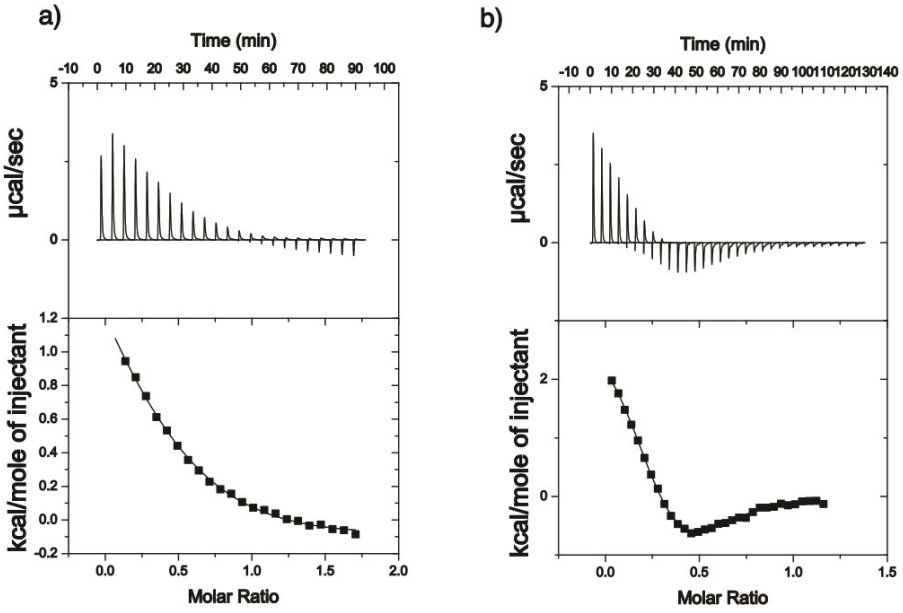


Figure S12: Isothermal calorimetric titration isotherms of **CAF** with: a) **R1** b) **R2** in deionized water at 298K

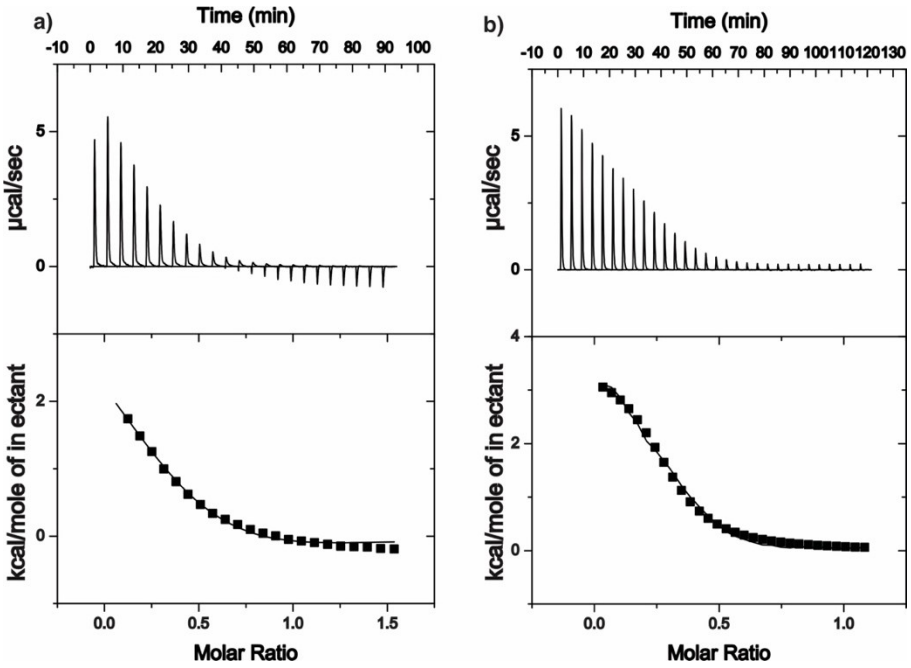


Figure S13: Isothermal calorimetric titration isotherms of **GRI** with: a) **R1** b) **R2** in deionized water at 298K

DYNAMIC LIGHT SCATTERING

3ml of drug concentration was prepared by measuring enough of a drug material directly into the sample cuvette to create 500mM, 100mM and 50mM of **ISO**, **CAF** and **GRI** respectively (**a**). DLS measurements were collected in triplicates and averaged. 30mg of **R1** and **R2** was also added directly into the same cuvette to create a 10mg/ml solution (**b**). DLS measurements are again collected in triplicates and averaged.

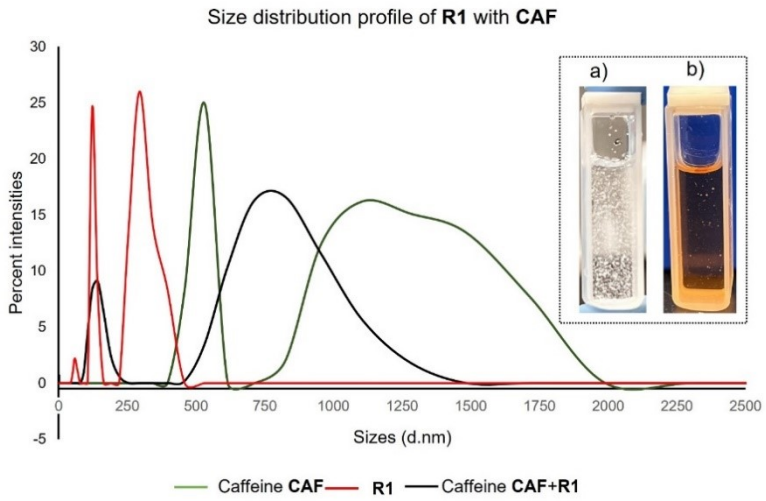


Figure S14: Illustration of the dynamic light scattering (DLS) experiment showing the size distribution profile of the pure drug **CAF**, pure receptor **R1**, and equimolar mixtures of the receptor and the drugs **CAF+R1**. Inset: Picture showing the pure drug (i) and the equimolar receptor-drug (ii) mixture, respectively.

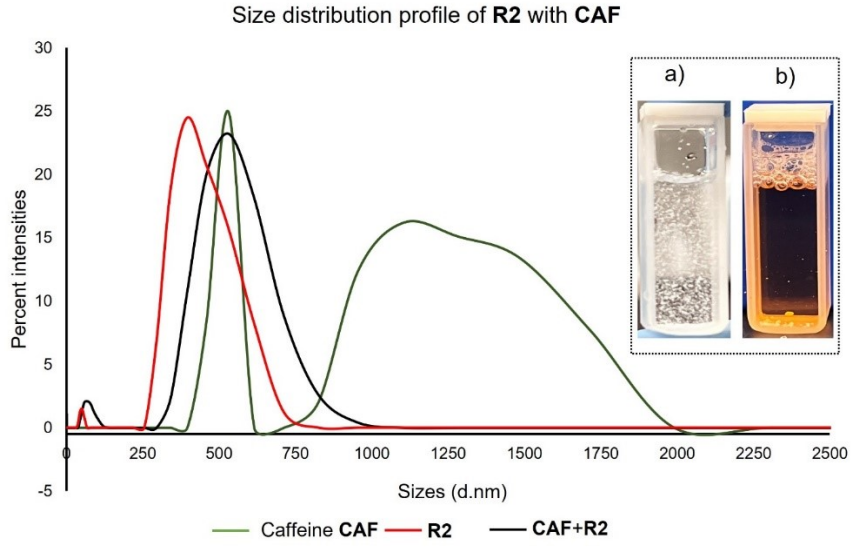
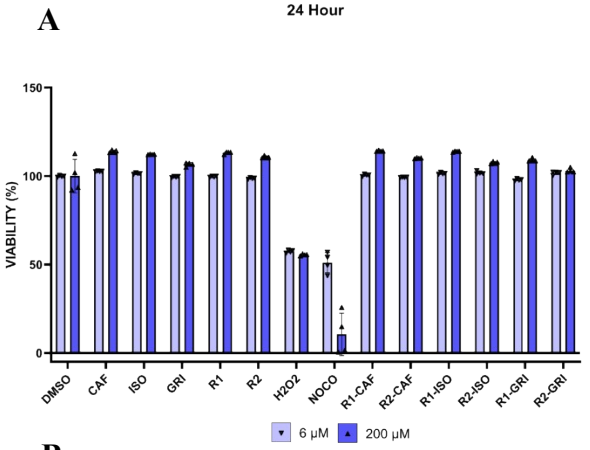
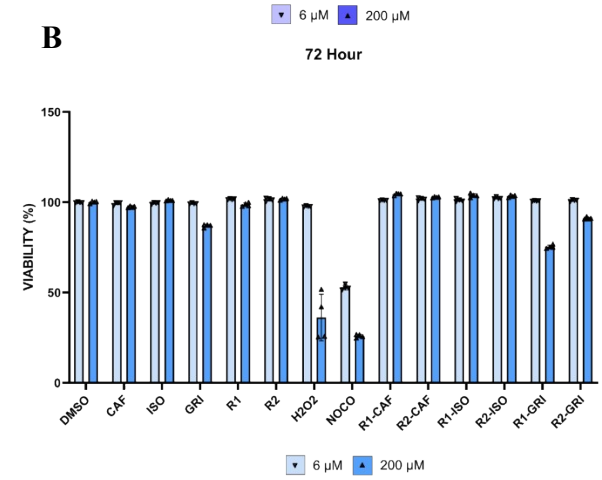


Figure S15: Illustration of the dynamic light scattering (DLS) experiment showing the size distribution profile of the pure drug **CAF**, pure receptor **R2**, and equimolar mixtures of the receptor and the drugs **CAF+R2**. Inset: Picture showing the pure drug (a) and the equimolar receptor-drug (b) mixture, respectively.

CYTOTOXICITY ASSESSMENTS





Tukey's multiple comparisons test (24 h)	Summary	Adjusted P Value
DMSO:6 µM vs. CAF:6 µM	**	0.0023
DMSO:6 µM vs. ISO:6 µM	ns	0.0989
DMSO:6 µM vs. GRI:6 µM	ns	0.9893
DMSO:6 µM vs. R1:6 µM	ns	0.8567
DMSO:6 µM vs. R2:6 µM	**	0.002
DMSO:6 µM vs. H2O2:6 µM	****	<0.0001
DMSO:6 µM vs. NOCO:6 µM	**	0.0062
DMSO:6 µM vs. R1-CAF:6 µM	ns	0.9205
DMSO:6 µM vs. R2-CAF:6 µM	ns	0.8179
DMSO:6 µM vs. R1-ISO:6 µM	***	0.0003
DMSO:6 µM vs. R2-ISO:6 µM	ns	0.4364
DMSO:6 µM vs. R1-GRI:6 µM	ns	0.0869
DMSO:6 µM vs. R2-GRI:6 µM	ns	0.4675
DMSO:200 µM vs. CAF:200 µM	ns	0.5665
DMSO:200 µM vs. ISO:200 µM	ns	0.6911
DMSO:200 µM vs. GRI:200 µM	ns	0.9926
DMSO:200 µM vs. R1:200 µM	ns	0.5888
DMSO:200 µM vs. R2:200 µM	ns	0.7892
DMSO:200 µM vs. H2O2:200 µM	*	0.0425
DMSO:200 µM vs. NOCO:200 µM	*	0.0267
DMSO:200 µM vs. R1-CAF:200 µM	ns	0.5636
DMSO:200 µM vs. R2-CAF:200 µM	ns	0.8428
DMSO:200 µM vs. R1-ISO:200 µM	ns	0.5792
DMSO:200 µM vs. R2-ISO:200 µM	ns	0.9594
DMSO:200 µM vs. R1-GRI:200 µM	ns	0.8397

Tukey's multiple comparisons test (24 h)	Summary	Adjusted P Value
DMSO:200 µM vs. R2-GRI:200 µM	ns	>0.9999
CAF:6 µM vs. R1-CAF:6 µM	ns	0.0714
CAF:6 µM vs. R2-CAF:6 µM	**	0.0025
CAF:200 µM vs. R1-CAF:200 µM	ns	0.995
CAF:200 µM vs. R2-CAF:200 µM	*	0.0314
ISO:6 µM vs. R1-ISO:6 µM	ns	>0.9999
ISO:6 µM vs. R2-ISO:6 µM	ns	0.9992
ISO:200 µM vs. R1-ISO:200 µM	*	0.0112
ISO:200 µM vs. R2-ISO:200 µM	**	0.0069
GRI:6 µM vs. R1-GRI:6 µM	ns	0.3071
GRI:6 µM vs. R2-GRI:6 µM	ns	0.3855
GRI:200 µM vs. R1-GRI:200 µM	ns	0.7305
GRI:200 µM vs. R2-GRI:200 µM	ns	0.1514
R1:6 µM vs. R1-CAF:6 µM	ns	0.8262
R1:6 µM vs. R1-ISO:6 µM	*	0.0111
R1:6 µM vs. R1-GRI:6 µM	ns	0.148
R1:200 µM vs. R1-CAF:200 µM	ns	0.4222
R1:200 µM vs. R1-ISO:200 µM	ns	0.9156
R1:200 µM vs. R1-GRI:200 µM	ns	0.0524
R2:6 µM vs. R2-CAF:6 µM	ns	0.7016
R2:6 µM vs. R2-ISO:6 µM	ns	0.1296
R2:6 µM vs. R2-GRI:6 µM	ns	0.1345
R2:200 µM vs. R2-CAF:200 µM	ns	0.9412
R2:200 µM vs. R2-ISO:200 µM	ns	0.1392
R2:200 µM vs. R2-GRI:200 µM	*	0.0228

Tukey's multiple comparisons test (72 h)	Summary	Adjusted P Value
DMSO:6 vs. CAF:6	ns	0.9633
DMSO:6 vs. ISO:6	ns	0.9132
DMSO:6 vs. GRI:6	ns	0.7413
DMSO:6 vs. R1:6	ns	0.0715
DMSO:6 vs. R2:6	ns	0.4728
DMSO:6 vs. H2O2:6	**	0.0015
DMSO:6 vs. NOCO:6	****	<0.0001
DMSO:6 vs. R1-CAF:6	**	0.0097
DMSO:6 vs. R2-CAF:6	ns	0.2528
DMSO:6 vs. R1-ISO:6	ns	0.6295
DMSO:6 vs. R2-ISO:6	ns	0.0898
DMSO:6 vs. R1-GRI:6	ns	0.2355
DMSO:6 vs. R2-GRI:6	ns	0.1383
DMSO:200 vs. CAF:200	ns	0.159
DMSO:200 vs. ISO:200	ns	0.6043
DMSO:200 vs. GRI:200	**	0.0021
DMSO:200 vs. R1:200	ns	0.9177
DMSO:200 vs. R2:200	ns	0.3686
DMSO:200 vs. H2O2:200	*	0.0311
DMSO:200 vs. NOCO:200	****	<0.0001
DMSO:200 vs. R1-CAF:200	*	0.0427
DMSO:200 vs. R2-CAF:200	ns	0.0593
DMSO:200 vs. R1-ISO:200	ns	0.1377
DMSO:200 vs. R2-ISO:200	ns	0.1336
DMSO:200 vs. R1-GRI:200	****	<0.0001

Tukey's multiple comparisons test (72 h)	Summary	Adjusted P Value
DMSO:200 vs. R2-GRI:200	**	0.0022
CAF:6 vs. R1-CAF:6	ns	0.2986
CAF:6 vs. R2-CAF:6	*	0.0129
CAF:200 vs. R1-CAF:200	**	0.0019
CAF:200 vs. R2-CAF:200	*	0.0114
ISO:6 vs. R1-ISO:6	ns	0.2135
ISO:6 vs. R2-ISO:6	ns	0.1838
ISO:200 vs. R1-ISO:200	ns	0.083
ISO:200 vs. R2-ISO:200	ns	0.1297
GRI:6 vs. R1-GRI:6	*	0.0156
GRI:6 vs. R2-GRI:6	ns	0.1519
GRI:200 vs. R1-GRI:200	*	0.0163
GRI:200 vs. R2-GRI:200	*	0.0199
R1:6 vs. R1-CAF:6	ns	0.5613
R1:6 vs. R1-ISO:6	ns	0.997
R1:6 vs. R1-GRI:6	ns	0.2268
R1:200 vs. R1-CAF:200	*	0.0146
R1:200 vs. R1-ISO:200	ns	0.1492
R1:200 vs. R1-GRI:200	**	0.002
R2:6 vs. R2-CAF:6	ns	>0.9999
R2:6 vs. R2-ISO:6	ns	0.6231
R2:6 vs. R2-GRI:6	ns	0.9998
R2:200 vs. R2-CAF:200	ns	0.6374
R2:200 vs. R2-ISO:200	ns	0.376
R2:200 vs. R2-GRI:200	**	0.0019

Figure S16: Compounds assayed for cytotoxicity (quantitative crystal violet assay). HEK-293 cells were incubated with a combination of carriers R1 or R2 and caffeine (CAF) or griseofulvin (GRIS) or isoniazid (ISO) to infer cytotoxicity via crystal violet assay. Cells are assayed at concentrations 6 or 200 µM for 24 (A) or 72 (B) hours. OD data are shown as viability percentages. Vehicle DMSO serves as negative control. Nocodazole or H2O2 serve as positive control. Data are representative of at least three biological replicates (mean ± SD), in technical triplicates. Two-way ANOVA Tukey's multiple comparison test results are shown in the summary tables.

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