Electronic Supplementary Material (ESI) for Chemical Communications
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Thiourea isosteres as anion receptors and transmembrane transporters†

Electronic supplementary information

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General remarks

^{1}H NMR (300 MHz) and ^{13}C{^{1}H} NMR (75 MHz) were determined on a Bruker AV300 spectrometer. Chemical shifts for ^{1}H NMR are reported in parts per million (ppm), calibrated to the solvent peak set. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet. Chemical shifts for ^{13}C{^{1}H} NMR are reported in ppm, relative to the central line of a septet at δ = 39.52 ppm for DMSO-d_6. HR-MS(ES) spectra were recorded using a Bruker Apex III spectrometer and reported as *m/z* (relative intensity).
All solvents and starting materials were purchased from commercial sources and used without further purification unless otherwise stated. Dry dichloromethane was obtained by distillation over CaH_2 prior to use. POPC was supplied by Genzyme. NMR titrations were performed by addition of aliquots of the putative anionic guest as the tetrabutylammonium (TBA) salt (0.15 M), in a solution of the receptor (0.01 M) in DMSO-d_6 to 0.01 M solution of the receptor.
Chloride concentrations during transport experiments were determined using an Accumet chloride selective electrode.

Synthesis:

The thiourea intermediates **4** and **5** were prepared according to a literature method[1] by adding 1-hexylamine (5 mM, 0.7 mL, in 10 mL dry DCM) dropwise over an hour to one equivalent of the related isothiocycante (phenyl-isothiocyante) 0.6 mL, 5 mM and pentafluorophenyl isothiocyanate (0.7 mL, 0.5 mM), respectively) in dry 15 ml dry DCM at room temperature. The reation mixture was stirred over night, quenched with 10 ml water and washed with 1 M HCl (2 x 10 mL) and 1 M NaOH (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and crude product was isolated after evaporation of the solvent under reduce pressure.

The cyanoguanidine receptors **1** and **2** were prepared following the method described by Novak *et al.*[2] by reacting the thiourea with an excess of MeI in acetone at room temperature under N_2-atmosphere over night. The excess of MeI was removed under reduced pressure. The crude product was treated without purification with cyanoamine (3 time excess) in the precence of 15% 1,4-diazabicyclo[2.2.2]octane (DABCO) in 15 mL buthanol. The reation mixture was refluxed over night under N_2. The solvent was removed under reduced perssue. The residue was taken up in 15 mL water, extracted with dichloromethane (3 x 7 mL), dried over $MgSO_4$ and the solvent was removed to give the crude product.

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Compound 1

Recrystallised from DCM/Et_2O, plattlet crystals filtered off, washed with Et_2O and dired on air; yield: 85.2 %, HR-MS m/z 267.1570 $[M+Na]^+$, calc. $C_{14}H_{20}N_4Na$ = 267.1586; ^1H NMR in $CDCl_3$ δ [ppm], 7.70 (1, s, NH), 7.45 (2, t, C_6H_5), 7.34 (1, t, C_6H_5), 7.26 (2, d, C_6H_5), 4.94 (1, s, NH), 3.27 (2, m, CH_2), 1.51 (2, t, CH_2), 1.28 (6, s, $(CH_2)_3$) and 0.89 (3, t, CH_3); ^{13}C 158.8, 135.5, 130.1, 127.4, 125.5, 118.1, 42.0, 31.3, 29.2, 26.3, 22.5, 13.9.

Compound 2

Recrystallised from Et_2O/hexane, washed with hexane and dired on air, yield 13.8 %, HR-MS m/z 335.1292 $[M+H]^+$, calc. $C_{14}H_{16}N_4F_5$ = 335.1295, 357.1111 $[M+Na]^+$, calc. $C_{14}H_{15}N_4F_5Na$ = 357.1115; ^1H NMR in DMSO-d_6 δ [ppm], 9.12 (1, s, NH), 7.49 (1, s, NH), 3.17 (2, d, CH_2), 1.46 (2, s, CH_2), 1.26 (6, s, $(CH_2)_3$) and 0.86 (3, t, CH_3); ^{13}C 158.0, 145.4, 142.2, 139.0 136.6 116.5, 41.5, 30.8, 28.6, 25.6, 22.0, 13.8. Block cyrstalls grown by slow diffusion of hexane into Et_2O solution.

Compound 4

Yield: 99%; HR-MS m/z 259.1240 $[M+Na]^+$, calc. $C_{13}H_{20}N_2SNa$ = 259.1251; ^1H NMR in DMSO-d_6 δ [ppm], 9.42 (1, s, NH), 7.71 (1, s, NH), 7.40 (2, d, C_6H_5), 7.30 (2, t, C_6H_5), 7.09 (1, t, C_6H_5), 3.45 (2, d, CH_2), 1.53 (2, t, CH_2), 1.28 (6, m, $(CH_2)_3$) and 0.87 (3, t, CH_3); ^{13}C 180.2, 139.3, 128.5, 123.9, 122.9, 43.8, 31.9, 28.4, 26.1, 22.1, 13.9.

Compound 5

Recrystallised from dichloroethane/hexane, crystals filtered off, washed with Et_2O and dired on air; yield: 95.8 %, HR-MS m/z 349.0766 $[M+Na]^+$, calc. $C_{13}H_{15}F_5N_2SNa$ = 349.0780; ^1H NMR in DMSO-d_6 δ [ppm], 9.13 (1, s, NH), 8.16 (1, s, NH), 3.43 (2, q, CH_2), 1.52 (2, t, CH_2), 1.27 (6, s, $(CH_2)_3$) and 0.87 (3, t, CH_3); ^{13}C 182.1, 145.2, 142.8, 138.4, 115.0, 44.7, 30.9, 28.2, 25.9, 22.0, 13.8.

Compound 3

Compound **3** was prepared after the method described by Tullio et al.[3, 4] The analytical data of the intermediate *3-(1 H-imidazol-1-yl)-4H-1,2,4-benzothiadiazine 1,1-dioxide* (**6**) are in agreement to reported data:[4] yield 81.9 %, ESI-MS (negative) m/z 247.1 $[M-H]^-$, ^1H NMR in DMSO-d_6 δ [ppm] 9.30 (1, s, 2-H imidazole), 8.01 (1, s, 5-H imidazole), 7.74 (1, d, H phenol), 7.56 (2, m, H phenol), 7.36 (2, M, H phenol + H imidazole).

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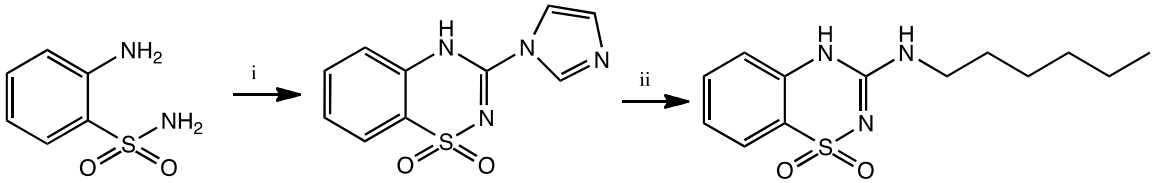
Compound **6** (0.5 g, 2.0 mmol) and a 70 % w/v aqueous solution of 1-hexylamine (5 mL) was heated in a sealed vessel over night at 150 °C. After the mixture was cooled, the excess amine was eliminated by distillation under reduced pressure. The residue was taken up in EtOH and water was added. The white precipitate was collected by filtration, washed with water and Et_2O, and dried in vacuum. Yield: 21.8 %. HR-MS m/z 282.1272 $[M+H]^+$, calc. $C_{13}H_{20}N_3O_2S$ = 282.1286, 304.1087 $[M+Na]^+$, calc. $C_{13}H_{20}N_3O_2SNa$ = 304.1102; ^1H NMR in DMSO-d_6 δ [ppm] 10.49 (1, s, NH), 7.64 (1, d, C_6H_4), 7.54 (1, t, C_6H_4), 7.24 (1, t, C_6H_4), 7.20 (1, d, C_6H_4), 7.07 (1, s, NH), 3.21 (2, q, CH_2), 1.51 (2, t, CH_2), 1.28 (6, s, $(CH_2)_3$) and 0.88 (3, t, CH_3); ^{13}C 151.1, 135.7, 132.3, 123.6, 122.8, 122.6, 116.4, 40.4, 30.9, 28.7, 25.9, 22.0, 13.9.



i: Dioxane, 1,1'-Thiocarbonyldiimidazole, reflux, 6 h;
ii: 1-hexaneamine/H_20 70% v/v, 150°, 12 h, sealed vessel

Crystallography

Compound 4

Cystalline needles of **4** were grown by slow evapouration form DCM, Data were collected on a Bruker Nonius APEXII mounted at the window of an FR591 rotating Mo anode with confocal optics. Cystal data for $C_{13}H_{20}N_2S$, M = 236.37, orthorhombic, a = 8.5794(2) Å, b = 10.2955(2) Å, c = 15.2141(3) Å, V = 1343.85(5) $Å^3$, T = 120 K, $P2_12_12_1$ (no. 19), Z = 4, 10 129 reflections measured, 3059 unique (R_{int} = 0.0379) of which 3059 were used in the calculations, R_1 = 0.0345 (2871 with F > 2s(F)). Absolute structure was determined by anomalous dispersion effects, the Flack parameter refined to 0.00(6).

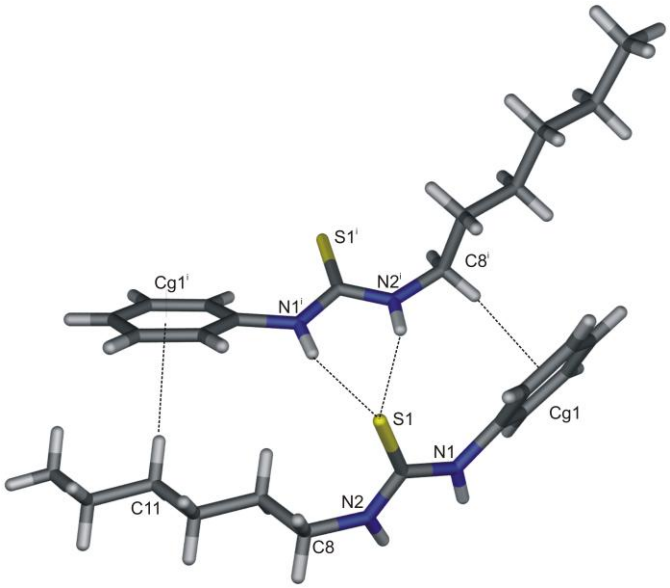


Fig. S1 Schematic draw of the crystal structure of **4** with selected atoms labelled, dotted lines represent hydrogen bonds, Symmetry code: i = 1/2+x,1/2-y,2-z.

Table S1 Hydrogen bonds in **1**, **2** and **4**.

D-H	A	D-H [Å]	H···A [Å]	D···A [Å]	D-H···A [°]
1					
N1-H1	$N8^{i}$	0.86	2.16	2.932(4)	149
N2-H7	$N8^{ii}$	0.86	2.35	3.122(3)	150
N5-H15	$N4^{iii}$	0.86	2.17	2.950(4)	150
N6-H21	$N4^{iv}$	0.86	2.42	3.127(3)	140
2					
N1-H1	$N4^{v}$	0.86	2.06	2.857(3)	153
N2-H2	$N4^{vi}$	0.86	2.26	3.013(3)	147
4					
N1-H1	$S1^{vii}$	0.86	2.54	3.3639(15)	162
N2-H2	$S1^{vii}$	0.86	2.62	3.4376(15)	160
C8-H8B	$Cg1^{vii}$	0.97	2.63	3.5078(18)	151
C11-H11B	$Cg1^{viii}$	0.97	2.92	3.8595(18)	163

Symmetry codes: i = 1+x,y,z; ii = 1/2+x,3/2-y,-z; iii = -1+x,y,z; iv = -1/2+x,1/2-y,-z; v = 2-x,1-y,1-z; vi = 2-x,-1/2+y,1/2-z; vii = 1/2+x,1/2-y,2-z; viii = -1/2+x,1/2-y,2-z.

Anion Transport Studies

Preparation of Vesicles

A lipid film of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and cholesterol (0% or 30%) was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing

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with a metal chloride (MCl) salt solution (489 mM MCl, 5 mM phosphate buffer at pH 7.2). The lipid suspension was then subjected to nine freeze-thaw cycles and allowed to age for 30 min at room temperature before extruding 25 times through a 200 nm polycarbonate membrane. The resulting unilamellar vesicles were dialyzed against the external medium to remove unencapsulated MCl salts.

Chloride Transport Assays

Unilamellar POPC vesicles containing NaCl, prepared as described above, were suspended in 489 mM $NaNO_3$ solution buffered to pH 7.2 with sodium phosphate salts. The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule (10 mM) was added to start the experiment and the chloride efflux was monitored using a chloride sensitive electrode. At 5 min, the vesicles were lysed with 50 µl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO *v/v*) and a total chloride reading was taken at 7 min.

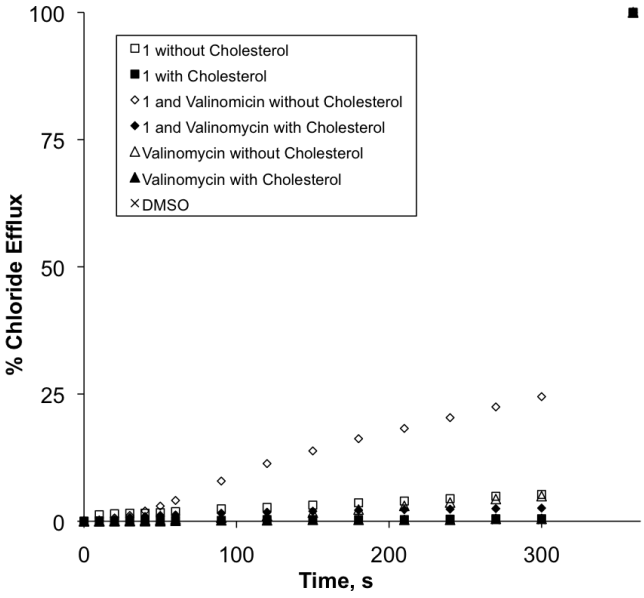

Fig. S2 Chloride efflux promoted by 0.02 molar equiv. of receptor **1** in the presence and absence of valinomycin (0.02 molar equiv.) from unilamellar POPC vesicles and unilamellar POPC/cholesterol (7:3) vesicles, loaded with 489 mM KCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were dispersed in 489 mM KNO_3 buffered to pH 7.2 with 5 mM sodium phosphate salts. Each point represents the average of three trials.

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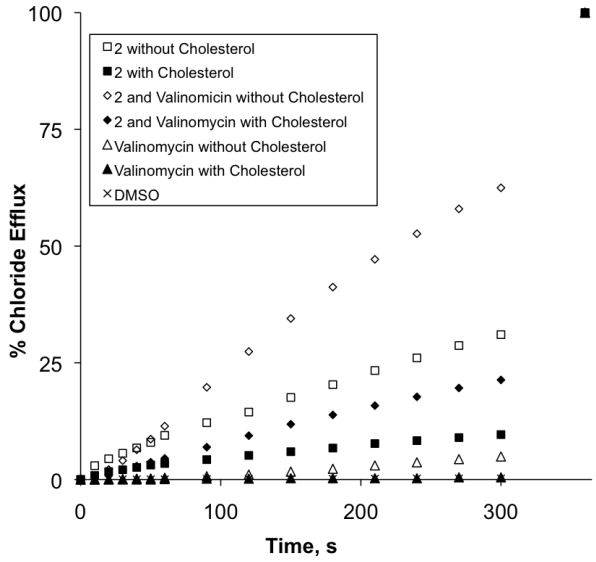

Fig. S3 Chloride efflux promoted by 0.02 molar equiv. of receptor **2** in the presence and absence of valinomycin (0.02 molar equiv.) from unilamellar POPC vesicles and unilamellar POPC/cholesterol (7:3) vesicles, loaded with 489 mM KCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were dispersed in 489 mM KNO_3 buffered to pH 7.2 with 5 mM sodium phosphate salts. Each point represents the average of three trials.

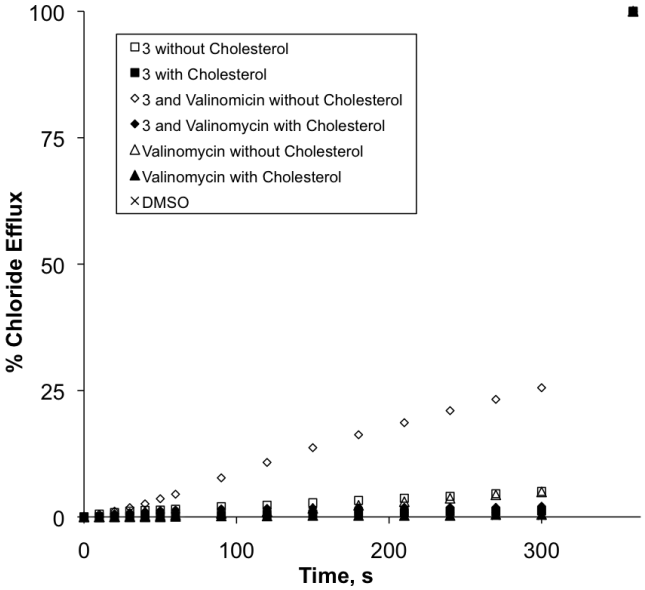

Fig. S4 Chloride efflux promoted by 0.02 molar equiv. of receptor **3** in the presence and absence of valinomycin (0.02 molar equiv.) from unilamellar POPC vesicles and unilamellar POPC/cholesterol (7:3) vesicles, loaded with 489 mM KCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were dispersed in 489 mM KNO_3 buffered to pH 7.2 with 5 mM sodium phosphate salts. Each point represents the average of three trials.

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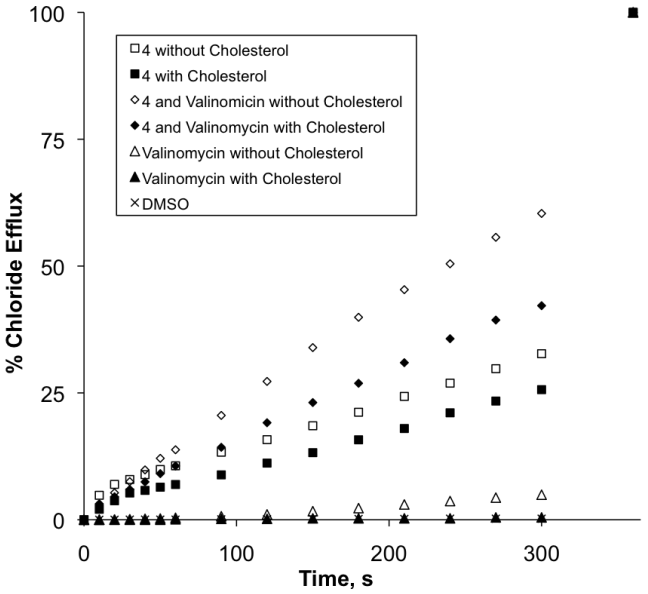

Fig. S5 Chloride efflux promoted by 0.02 molar equiv. of receptor **4** in the presence and absence of valinomycin (0.02 molar equiv.) from unilamellar POPC vesicles and unilamellar POPC/cholesterol (7:3) vesicles, loaded with 489 mM KCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were dispersed in 489 mM KNO_3 buffered to pH 7.2 with 5 mM sodium phosphate salts. Each point represents the average of three trials.

Proton NMR studies

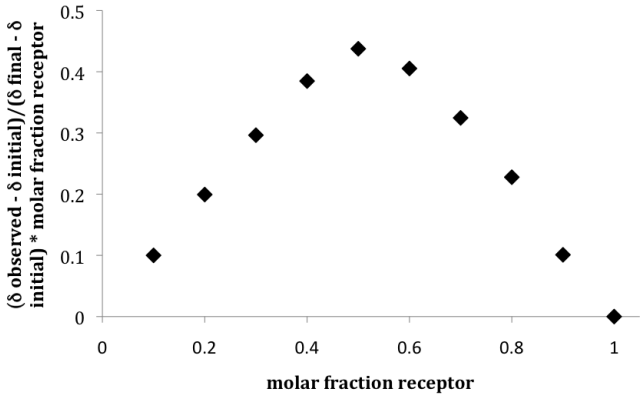

Fig. S6 Job plots in DMSO-d_6 /0.5% H_2O for compound **1** with $TBA_2{\cdot}SO_4$, NH at 8.9 ppm.

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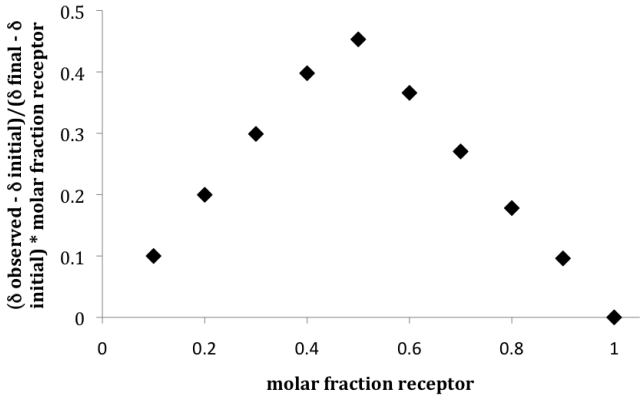

Fig. S7 Job plots in DMSO-d_6 /0.5% H_2O for compound **2** with $TBA_2{\cdot}SO_4$, NH at 7.5 ppm.

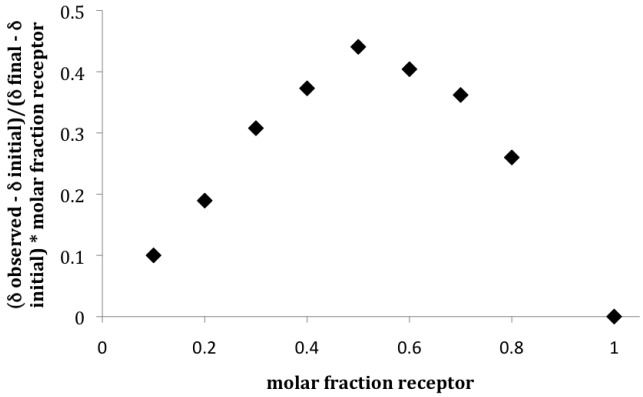

Fig. S8 Job plots in DMSO-d_6 /0.5% H_2O for compound **3** with $TBA_2{\cdot}SO_4$, NH at 7.1 ppm.

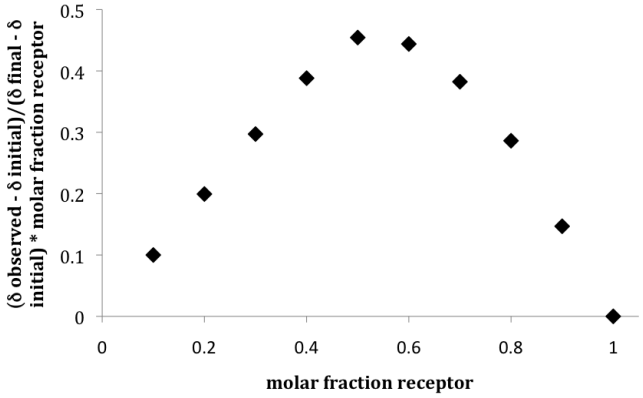

Fig. S9 Job plots in DMSO-d_6 /0.5% H_2O for compound **4** with $TBA_2{\cdot}SO_4$, NH at 7.7 ppm.

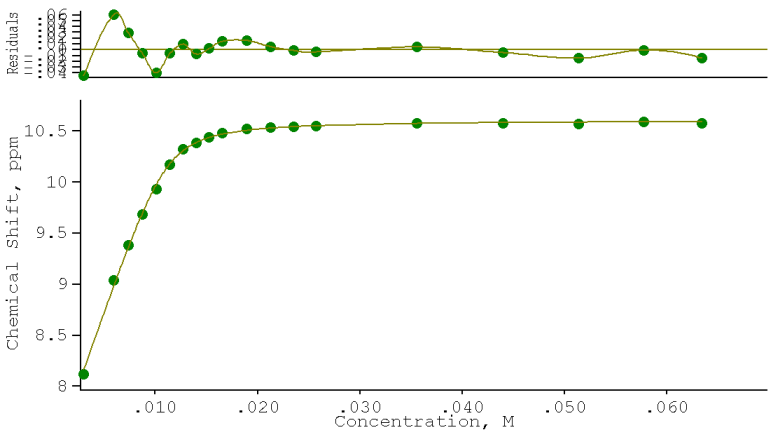

Fig. S10 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **1** with $TBA_2 \cdot SO_4$, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.34 ppm, K_a = 3890 M^{-1} error = 8 %.

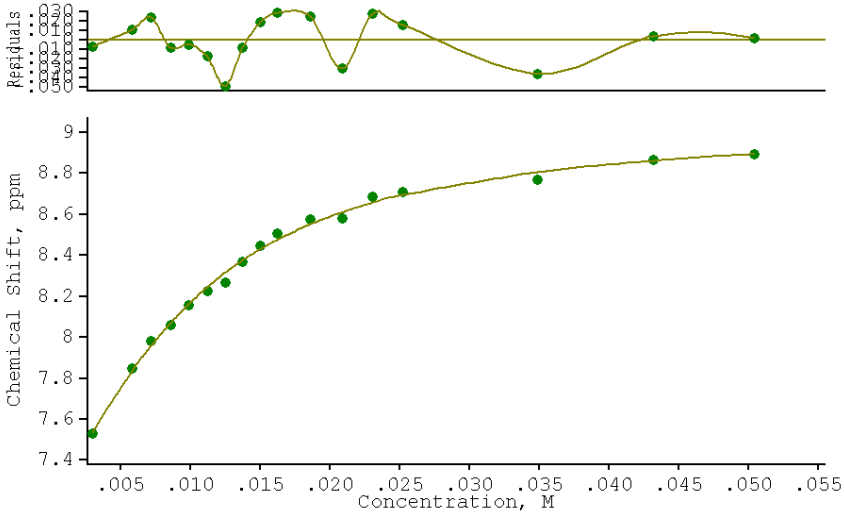

Fig. S11 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **1** with $TBA \cdot H_2PO4$, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.34 ppm, K_a = 236 M^{-1} error = 10 %.

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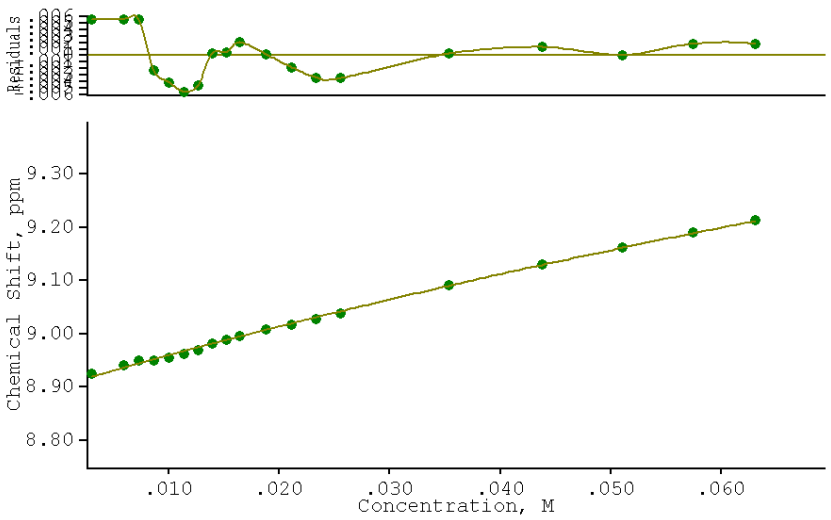

Fig. S12 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **1** with TBA·Cl, Changes in chemical shift are fitted to a 1:1 binding model, NH at 8.90 ppm, K_a = < 10 M^{-1}.

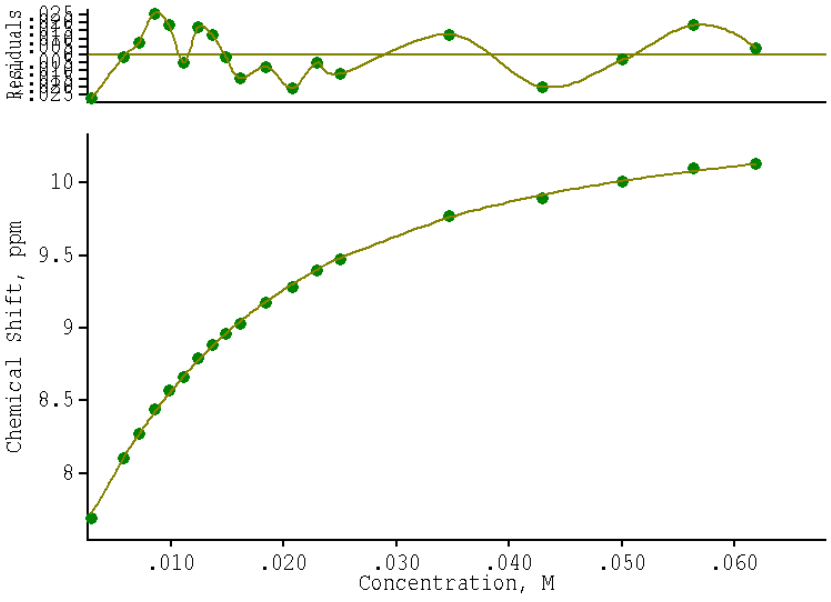

Fig. S13 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **1** with TBA·PhCOO, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.18 ppm, K_a = 106 M^{-1} error = 3 %.

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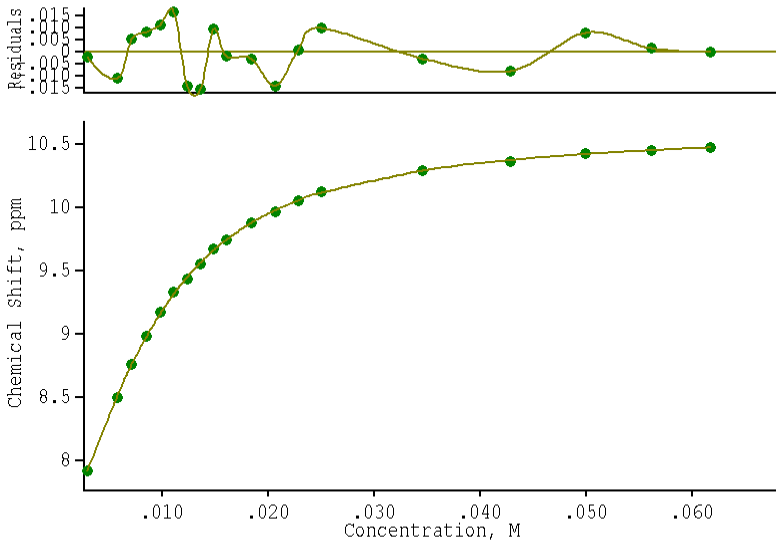

Fig. S14 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **1** with TBA·CH_3COO, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.18 ppm , K_a = 324 M^{-1} error = 2 %.

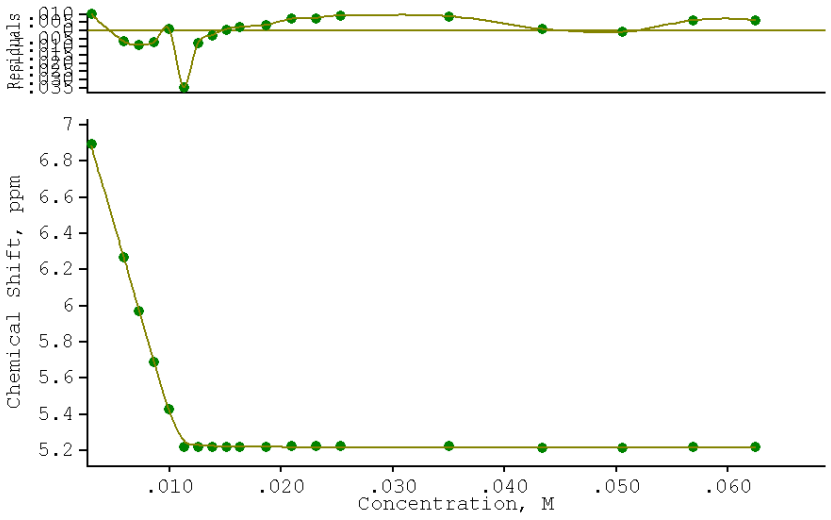

Fig. S15 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **2** with TBA_2·SO_4, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.50 ppm, K_a = > 10000 M^{-1}.

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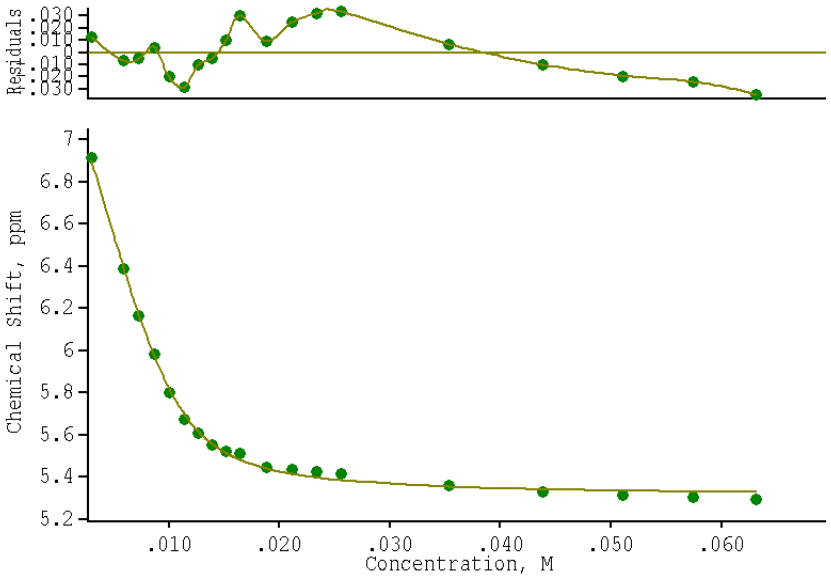

Fig. S16 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **2** with TBA· CH_3COO, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.50 ppm, K_a = 1713 M^{-1} error = 8 %.

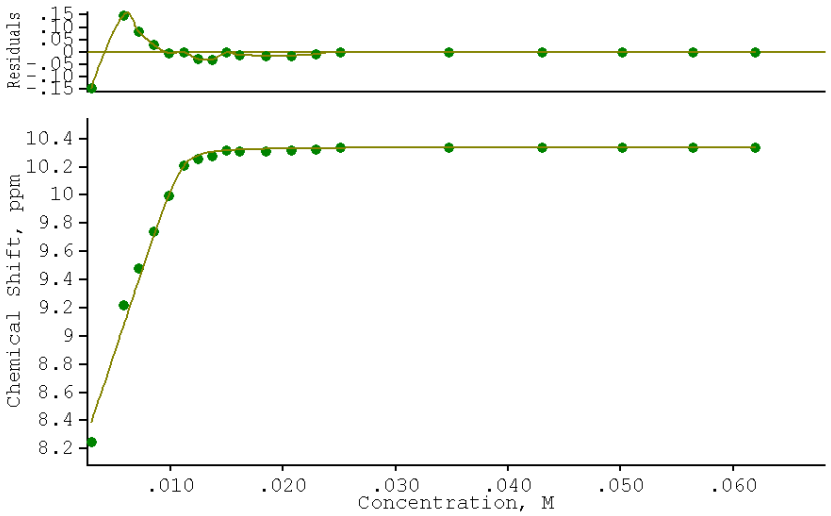

Fig. S17 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **3** with TBA_2·SO_4, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.07 ppm, K_a = > 10000 M^{-1}.

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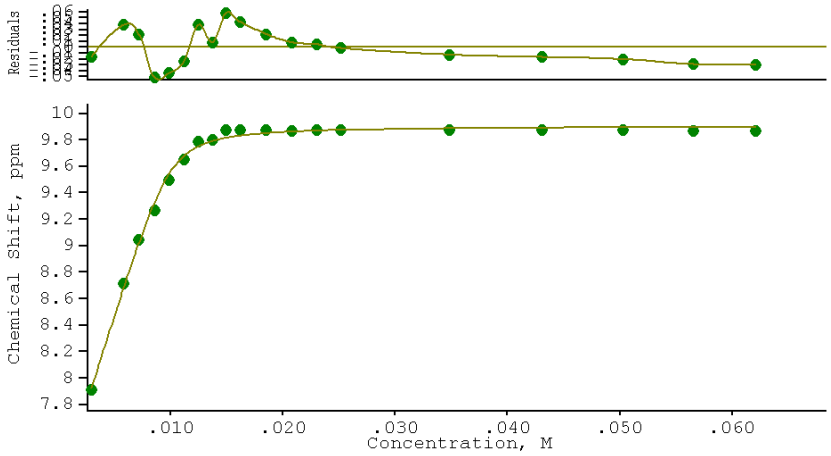

Fig. S18 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **3** with TBA·H_2PO_4, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.07 ppm, K_a = 5548 M^{-1} error = 15 %.

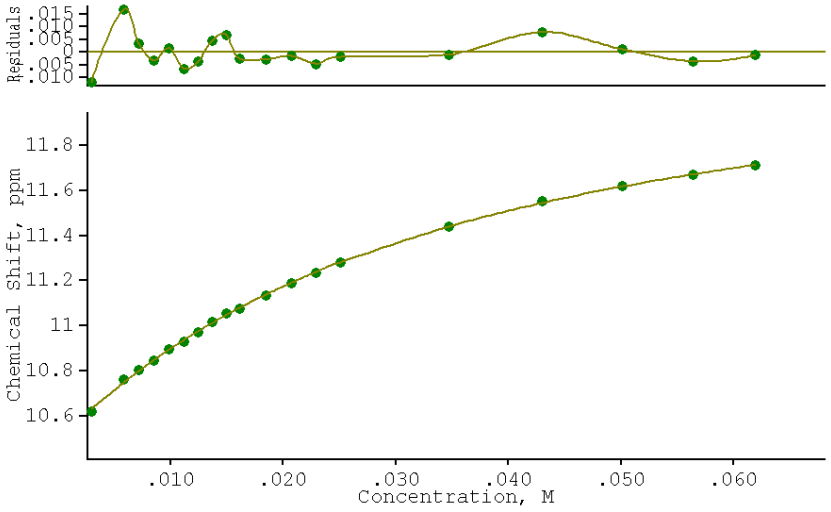

Fig. S19 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **3** with TBA·Cl, Changes in chemical shift are fitted to a 1:1 binding model, NH at 10.49 ppm, K_a = 36 M^{-1} error = 4 %.

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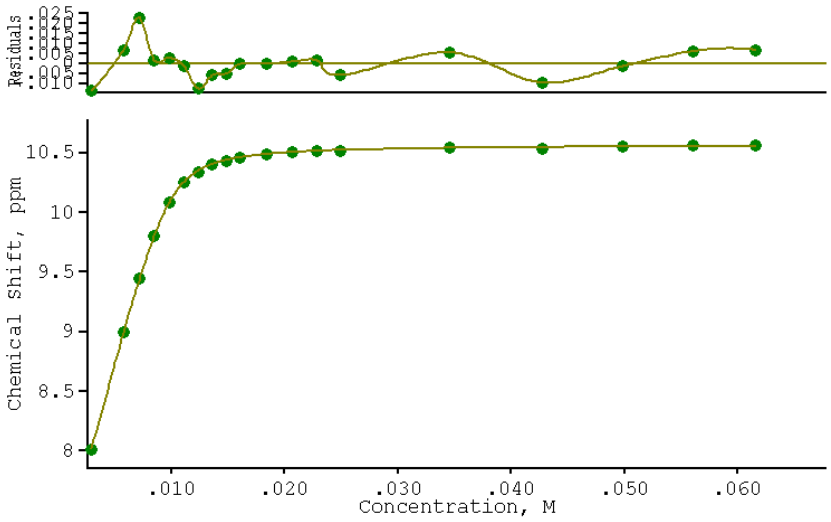

Fig. S20 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **3** with TBA·PhCOO, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.07 ppm, K_a = 5034 M^{-1} error = 3 %.

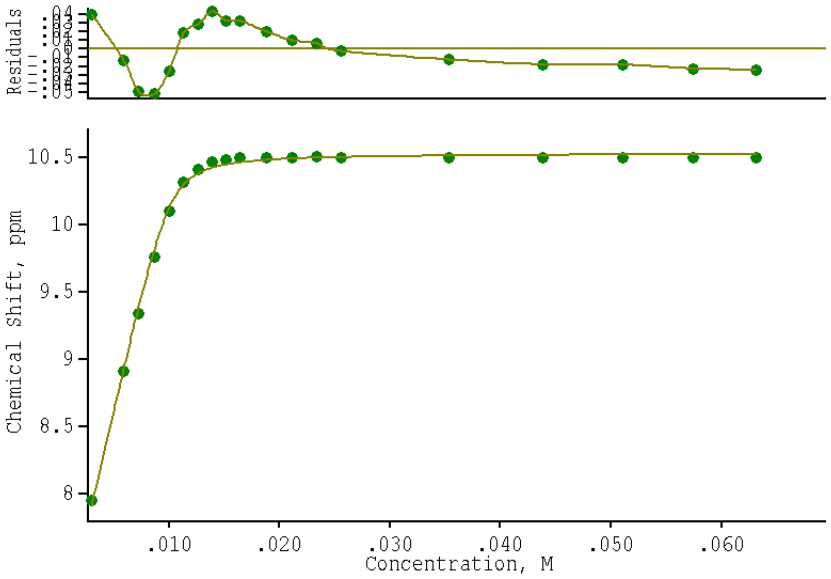

Fig. S21 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **3** with TBA·CH_3COO, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.08 ppm, K_a = 8146 M^{-1} error = 13 %.

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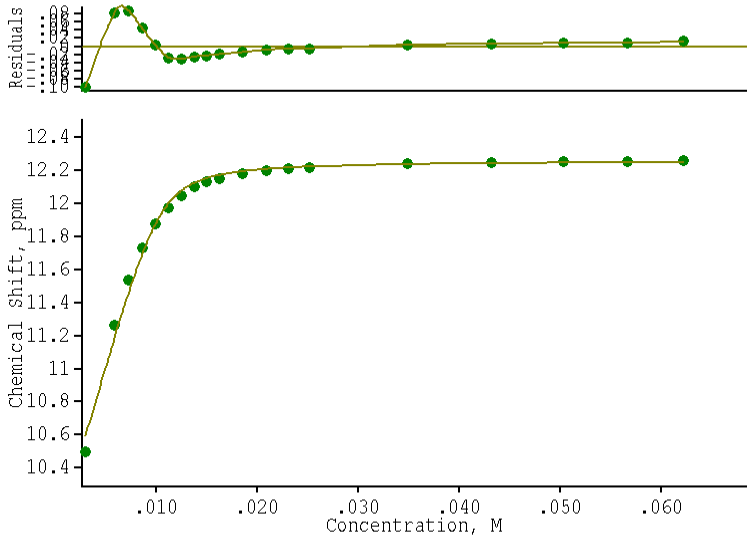

Fig. S22 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **4** with $TBA_2 \cdot SO_4$, Changes in chemical shift are fitted to a 1:1 binding model, NH at 9.44 ppm, K_a = 4071 M^{-1} error = 20 %.

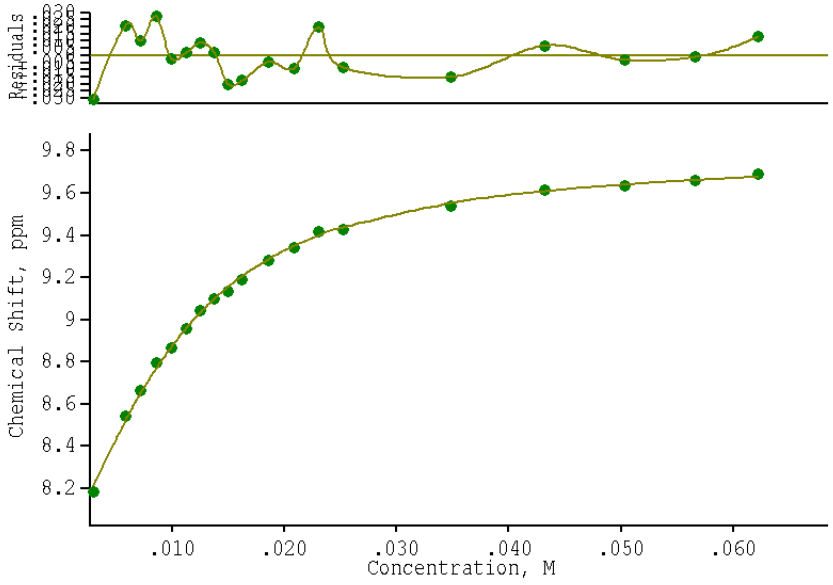

Fig. S23 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **4** with $TBA \cdot H_2PO_4$, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.73 ppm, K_a = 264 M^{-1} error = 5 %.

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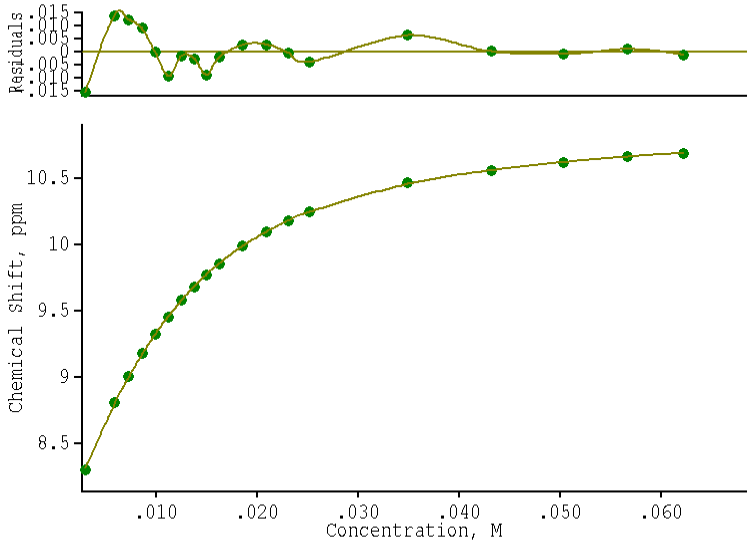

Fig. S24 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **4** with TBA·PhCOO, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.73 ppm, K_a = 209 M^{-1} error = 1 %.

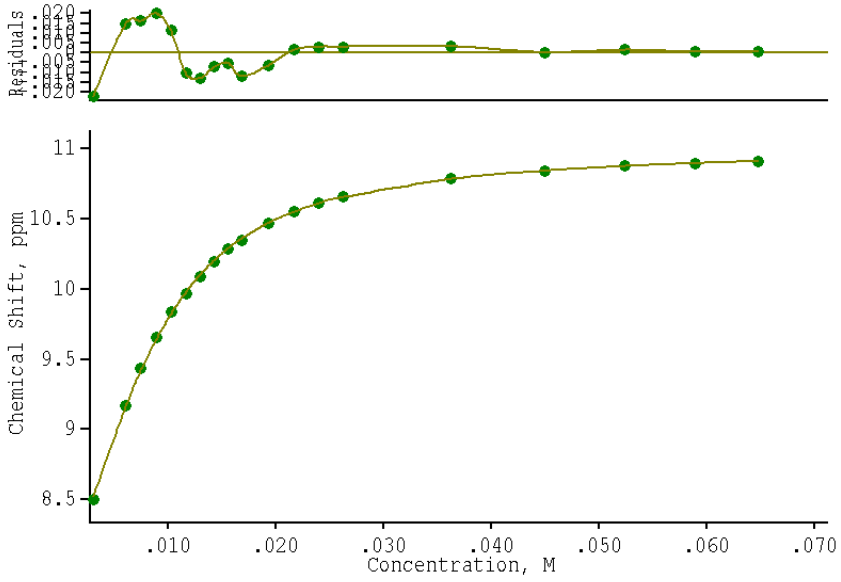

Fig. S25 ^{1}H NMR titration in DMSO-d_6/H_2O 0.5% for compound **4** with TBA·CH_3COO, Changes in chemical shift are fitted to a 1:1 binding model, NH at 7.73 ppm, K_a = 435 M^{-1} error = 2 %.

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