Does lipophilicity affect the effectiveness of a transmembrane anion transporter? Insight from squaramido-functionalized bis(choloyl) conjugates

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Supporting Information

Figures S1~18. ^{1}H NMR, ^{13}C NMR and HR-ESI-MS of compounds **1-6**

Figures S19~25. Ionophoric activity of compounds **1-6**

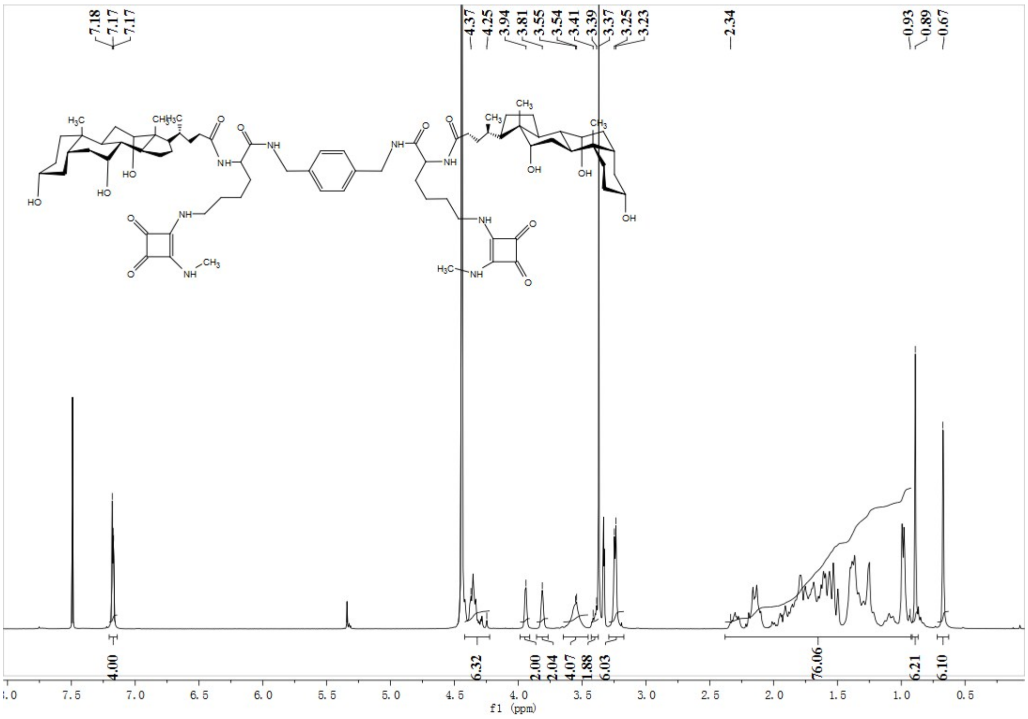


Fig. S1. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ = 2/1) of compound **1**.

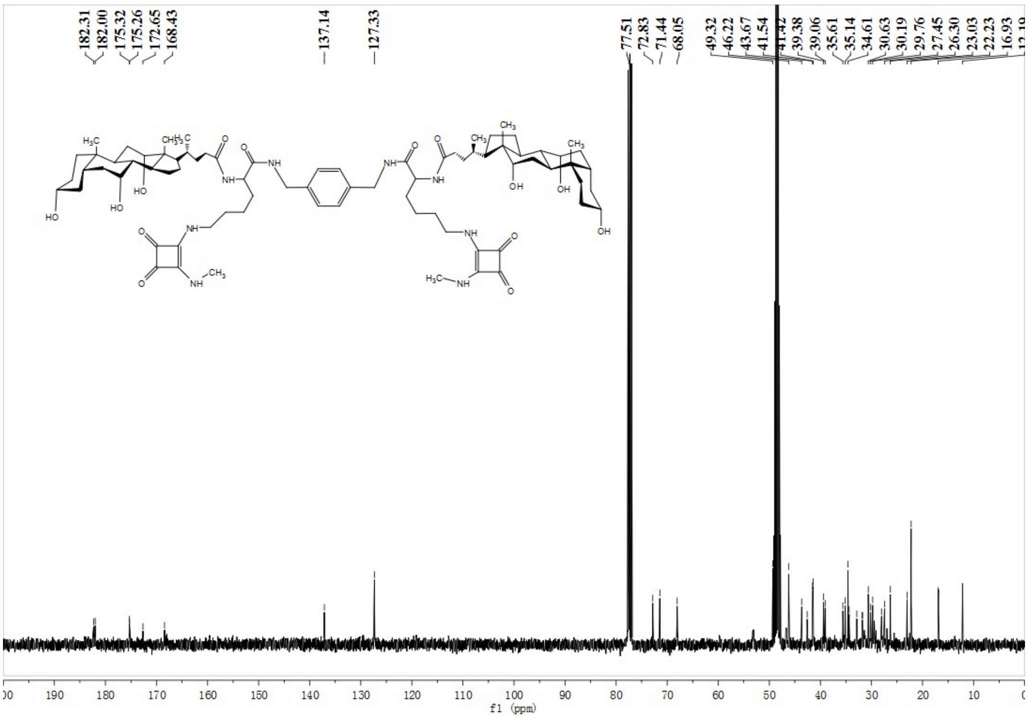
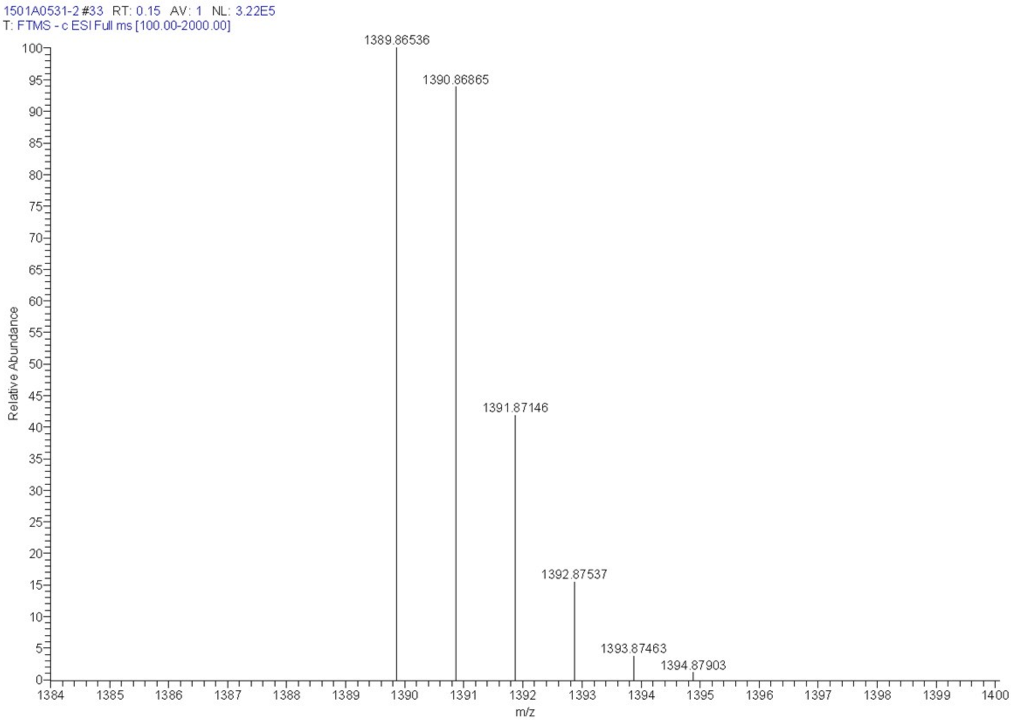


Fig. S2. ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$, 2/1) of compound **1**.



SPECTRUM - simulation:				
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1389.86536	1389.86947	-2.96	24.5	$C_{78}H_{117}O_{14}N_8$

Fig. S3. HR-ESI-MS of compound **1**.

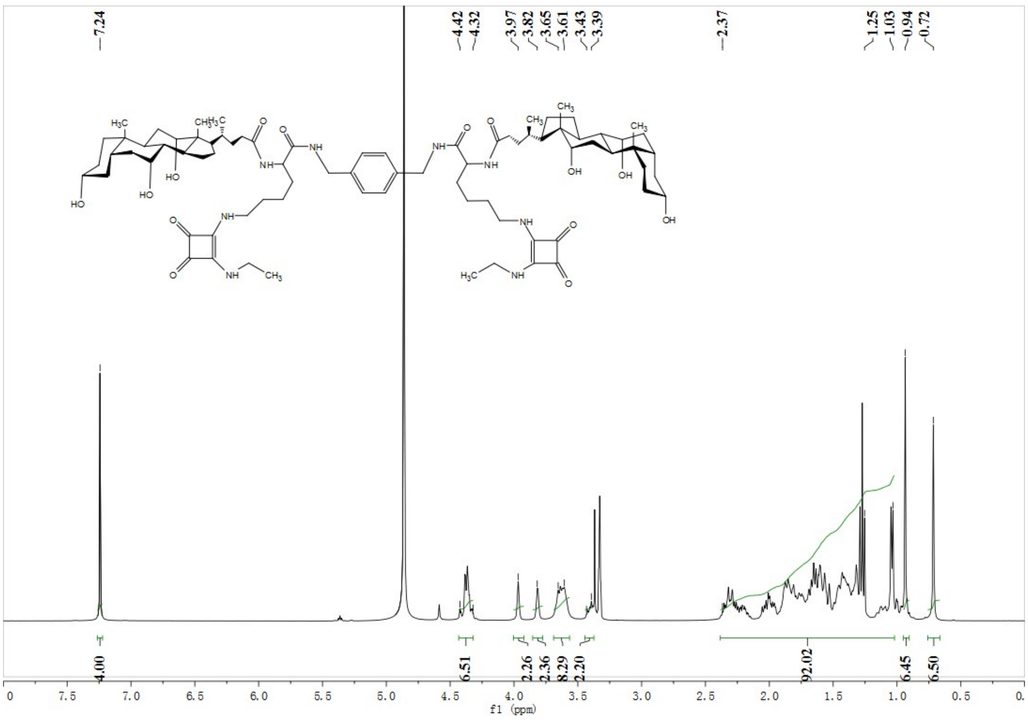


Fig. S4. ^{1}H-NMR (400 MHz, CD_3OD) of compound **2**.

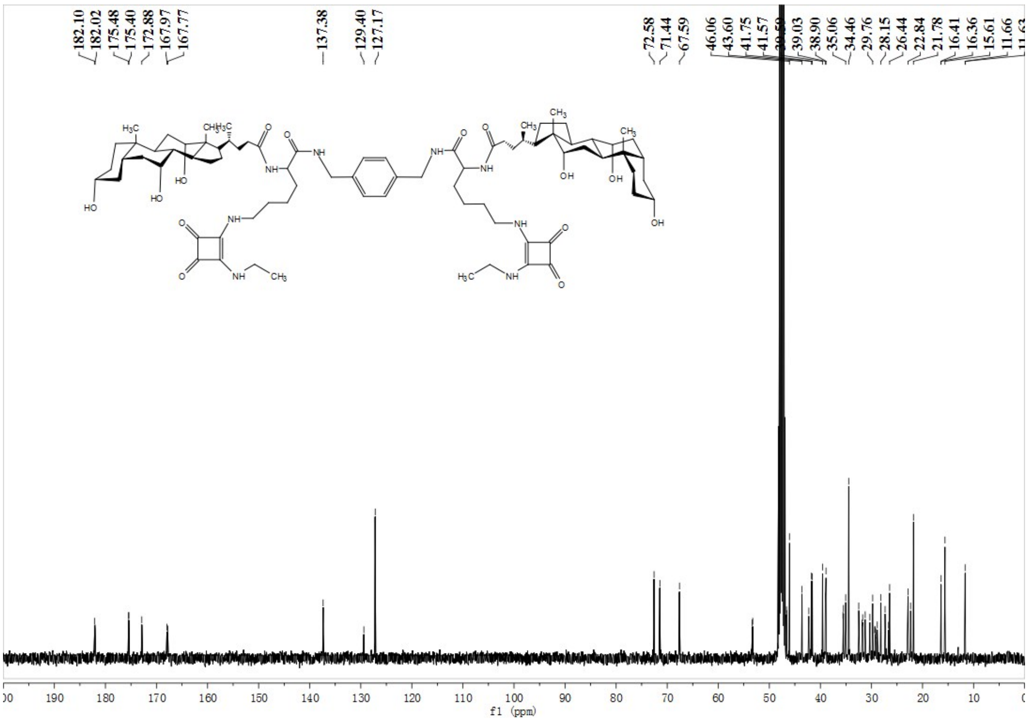
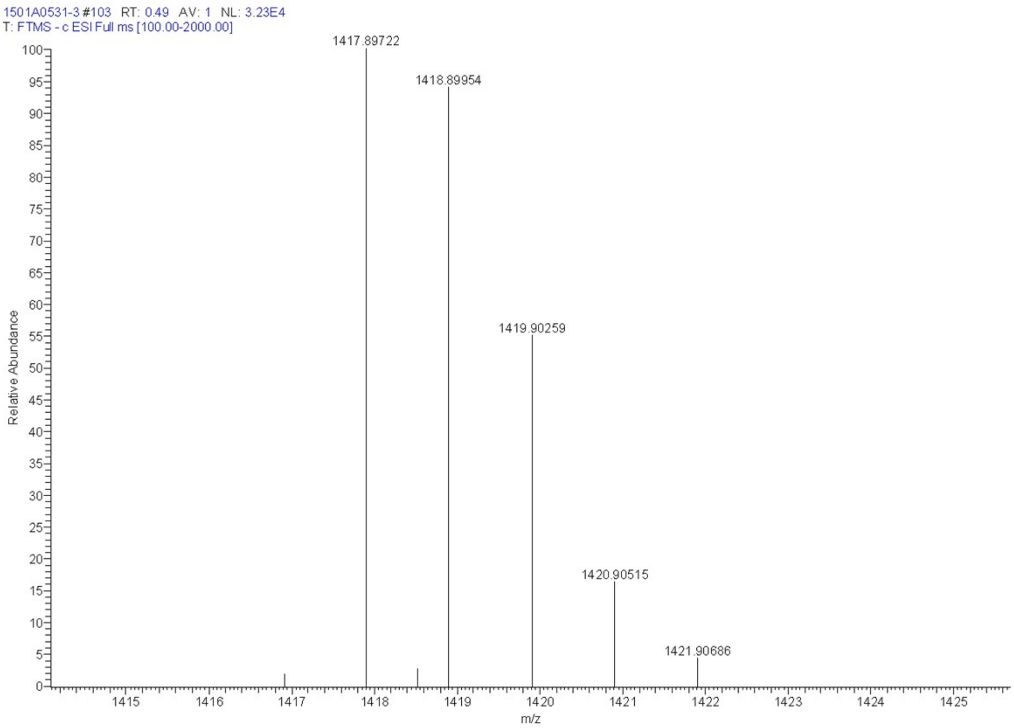


Fig. S5. ^{13}C-NMR (100 MHz, CD_3OD) of compound **2**.



SPECTRUM - simulation:				
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1417.89722	1417.90077	-2.51	24.5	$C_{80}H_{121}O_{14}N_8$

Fig. S6. HR-ESI-MS of compound **2**.

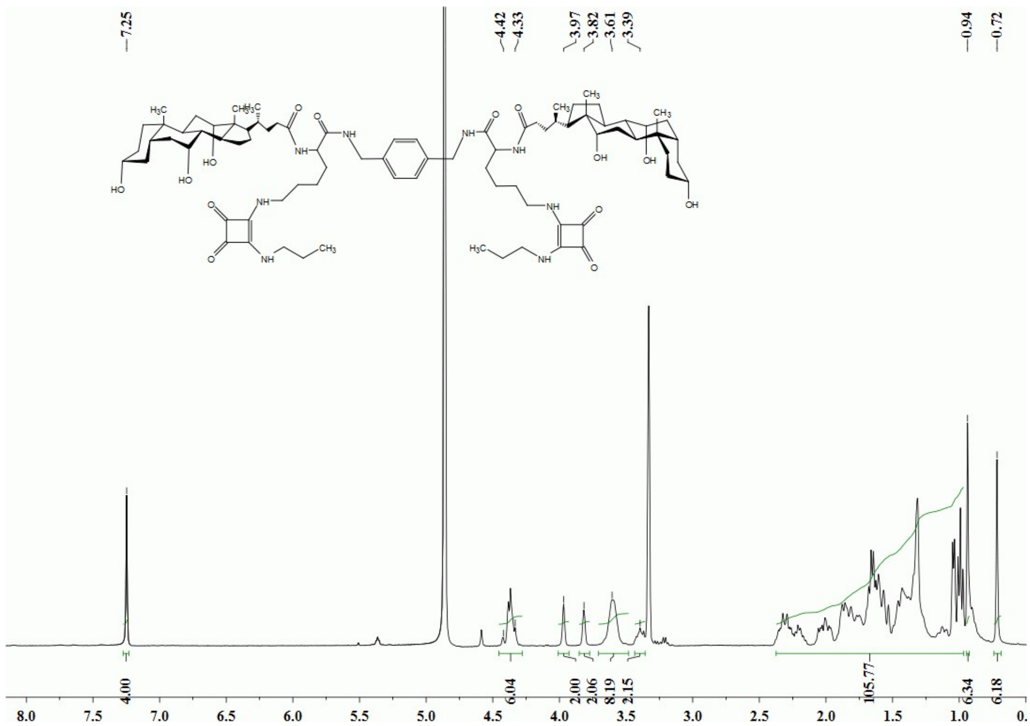


Fig. S7. ^{1}H-NMR (400 MHz, CD_3OD) of compound **3**.

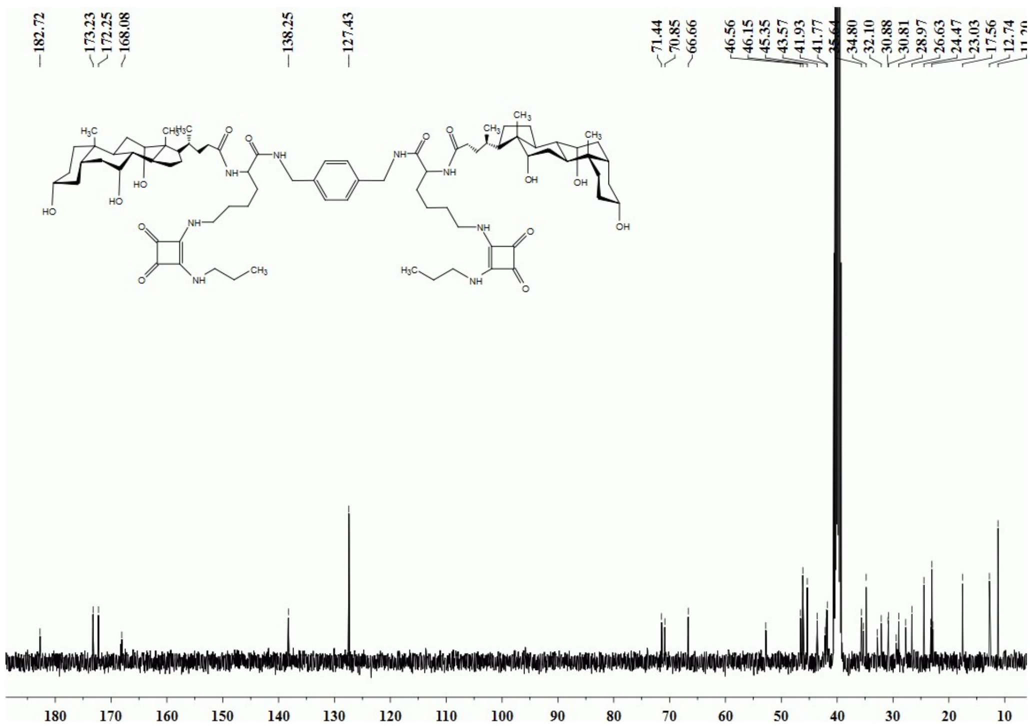
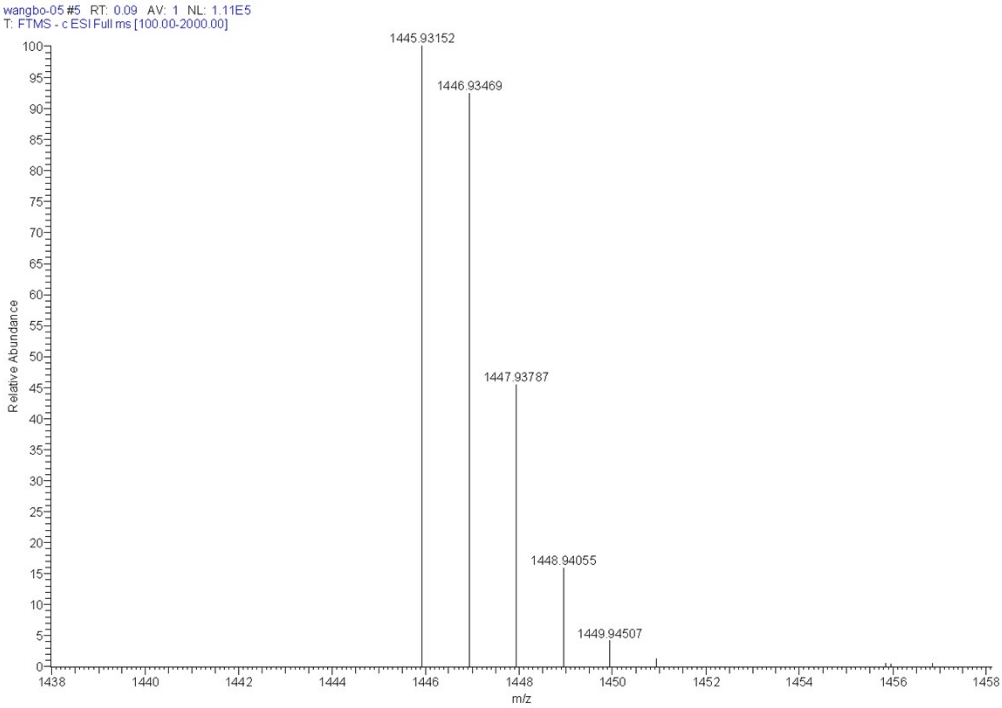


Fig. S8. ^{13}C-NMR (100 MHz, d_6-DMSO) of compound **3**.



SPECTRUM - simulation:				
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1445.93152	1445.93207	-0.38	24.5	$C_{82}H_{125}O_{14}N_8$

Fig. S9. HR-ESI-MS of compound **3**.

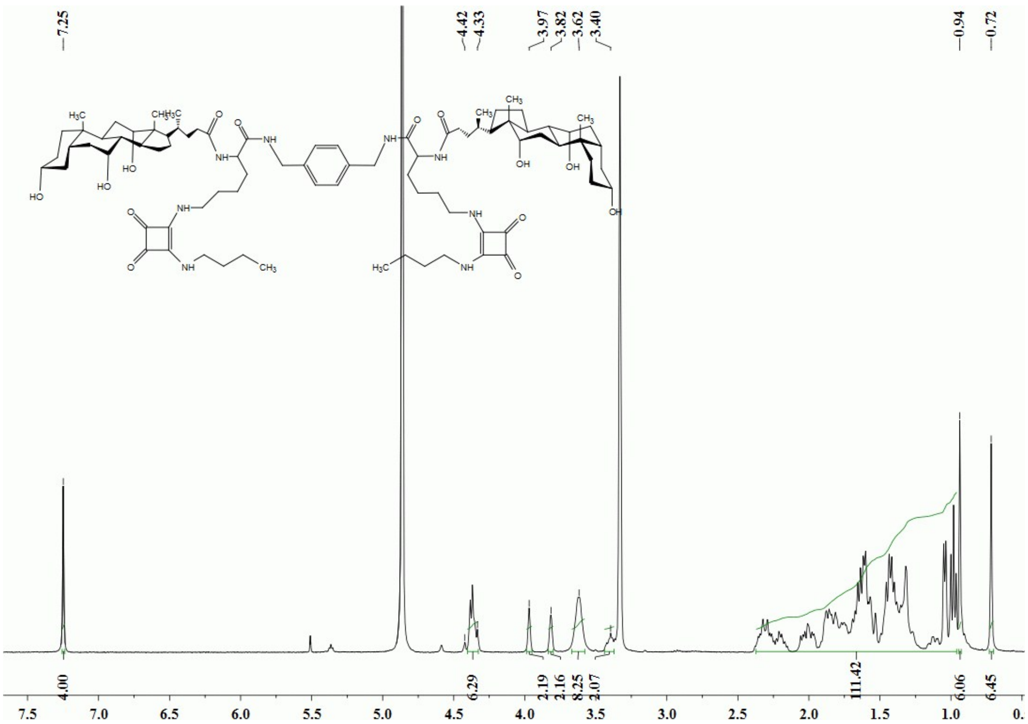


Fig. S10. ^{1}H-NMR (400 MHz, CD_3OD) of compound **4**.

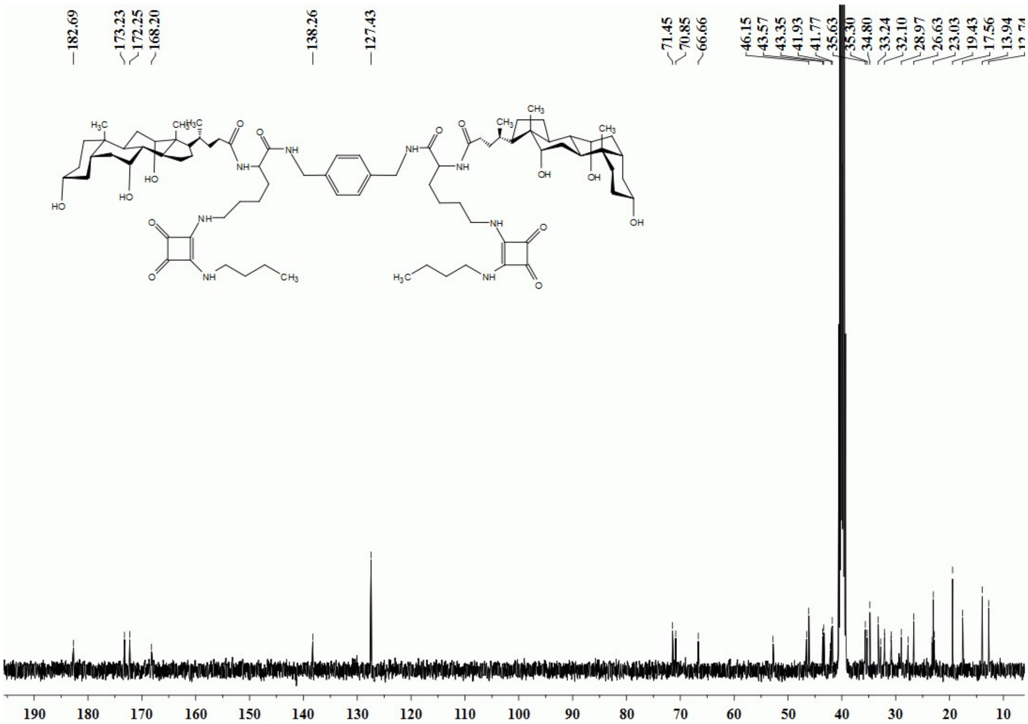
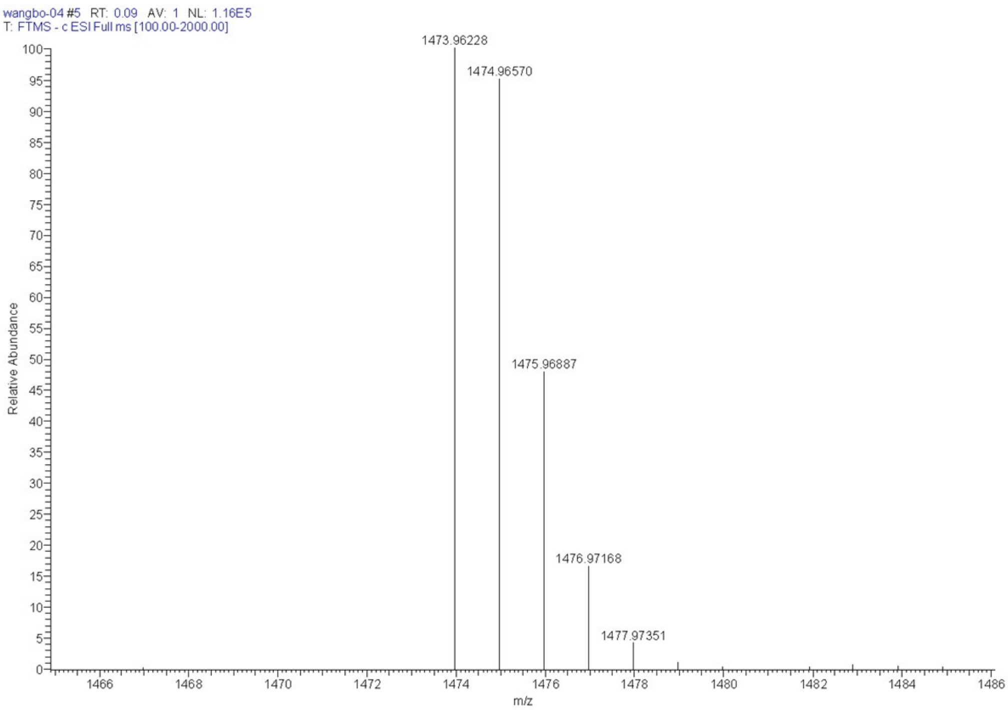


Fig. S11. [13]C-NMR (100 MHz, d_6-DMSO) of compound **4**.



SPECTRUM - simulation:

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1473.96228	1473.96337	-0.74	24.5	$C_{84}H_{129}O_{14}N_8$

Fig. S12. HR-ESI-MS of compound **4**.

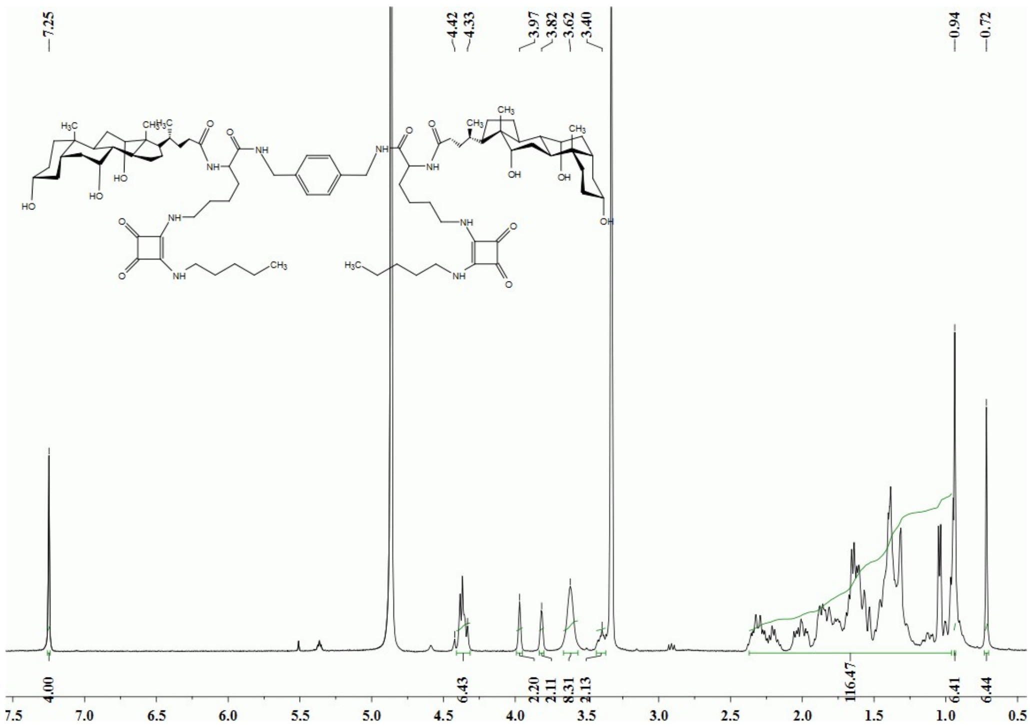


Fig. S13. ^{1}H-NMR (400 MHz, CD_3OD) of compound **5**.

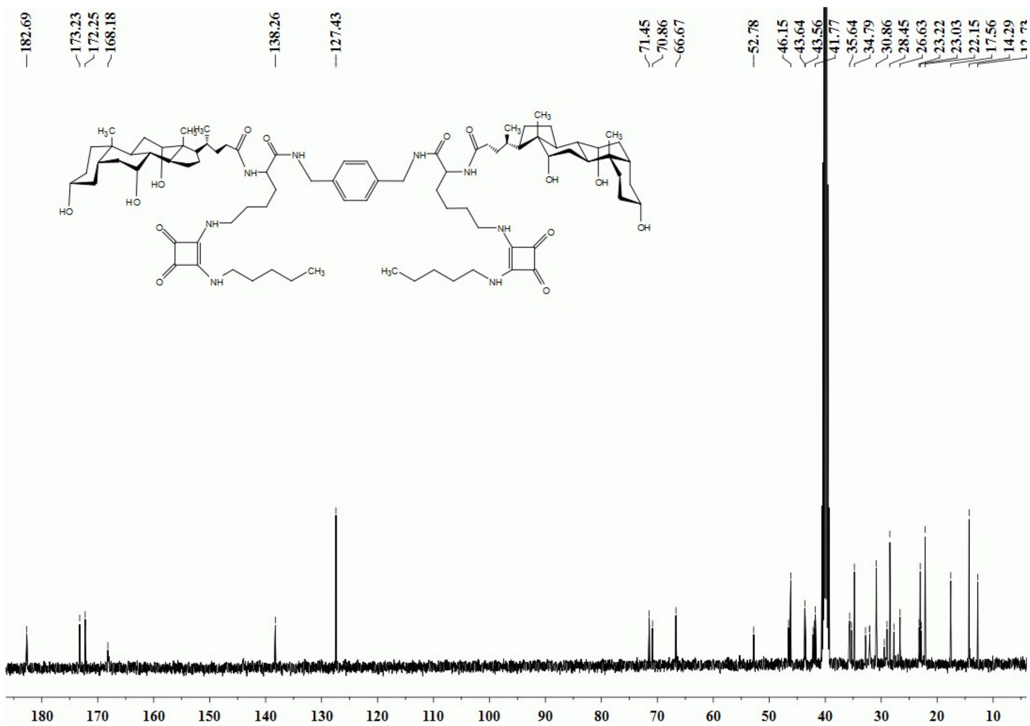
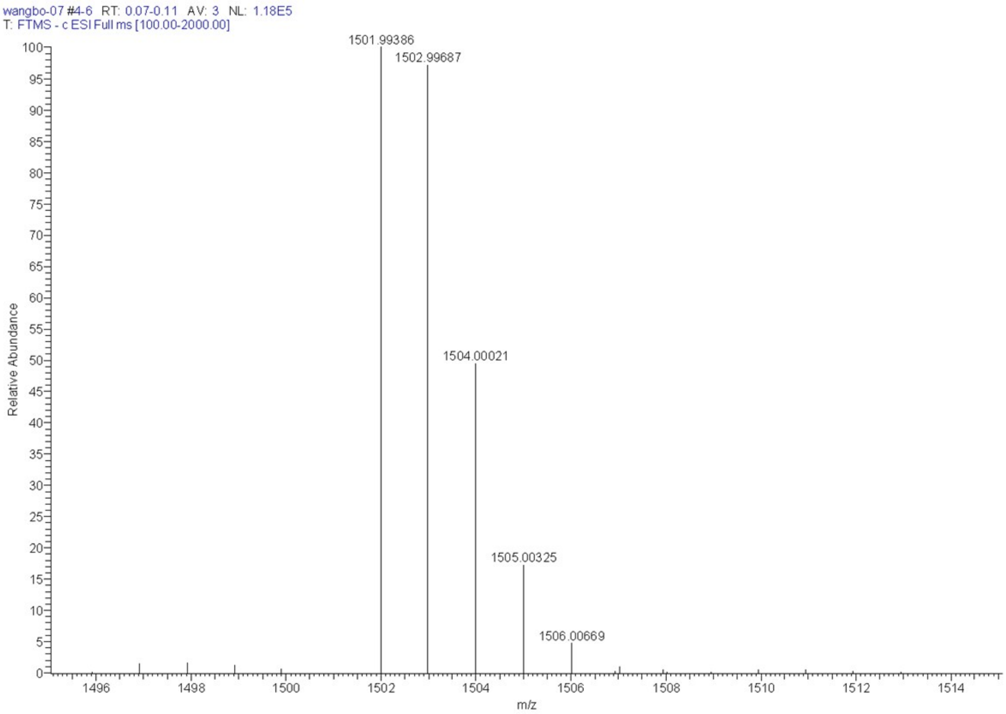


Fig. S14. ^{13}C-NMR (100 MHz, d_6-DMSO) of compound **5**.



SPECTRUM - simulation:				
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1501.99386	1501.99467	-0.54	24.5	$C_{86}H_{133}O_{14}N_8$

Fig. S15. HR-ESI-MS of compound **5**.

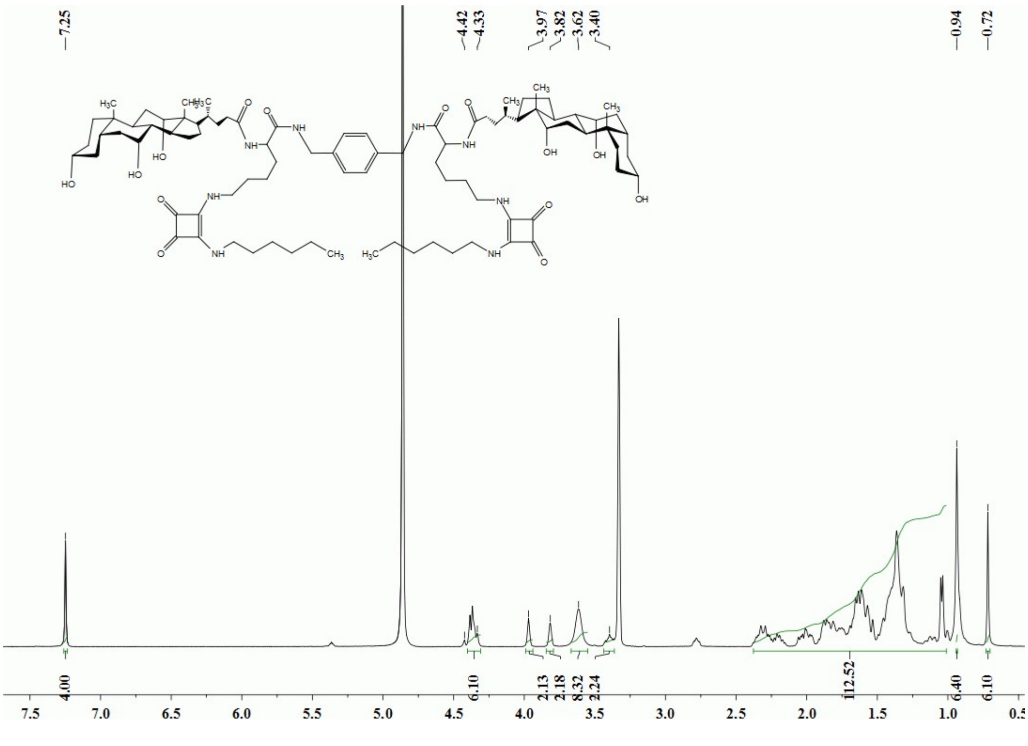


Figure S16. ^{1}H-NMR (400 MHz, CD_3OD) of compound **6**.

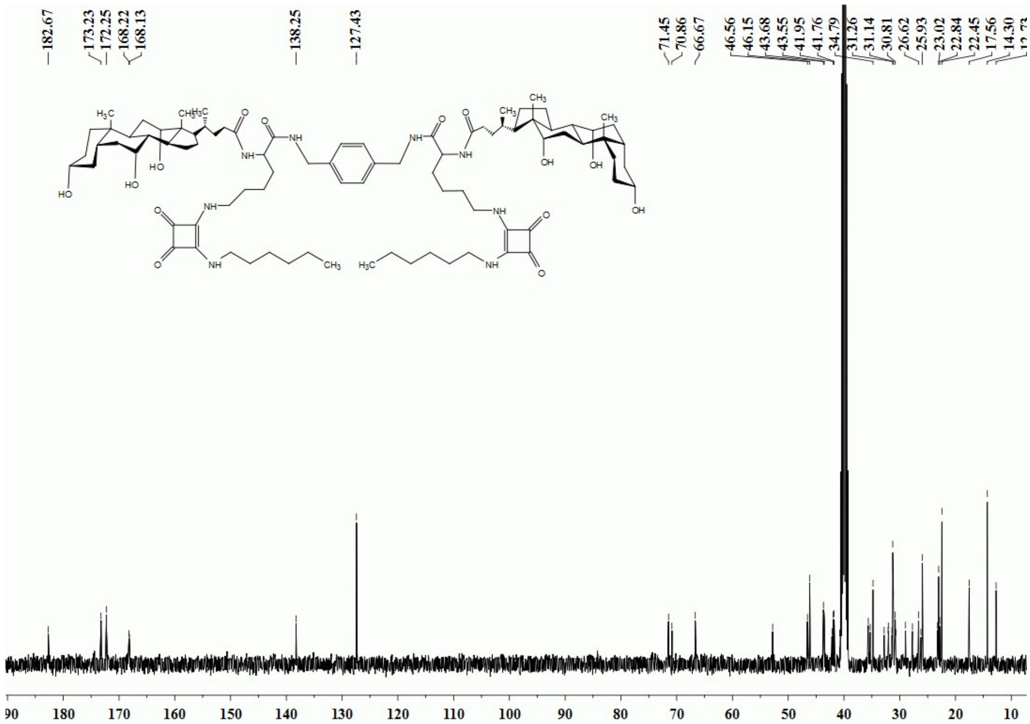
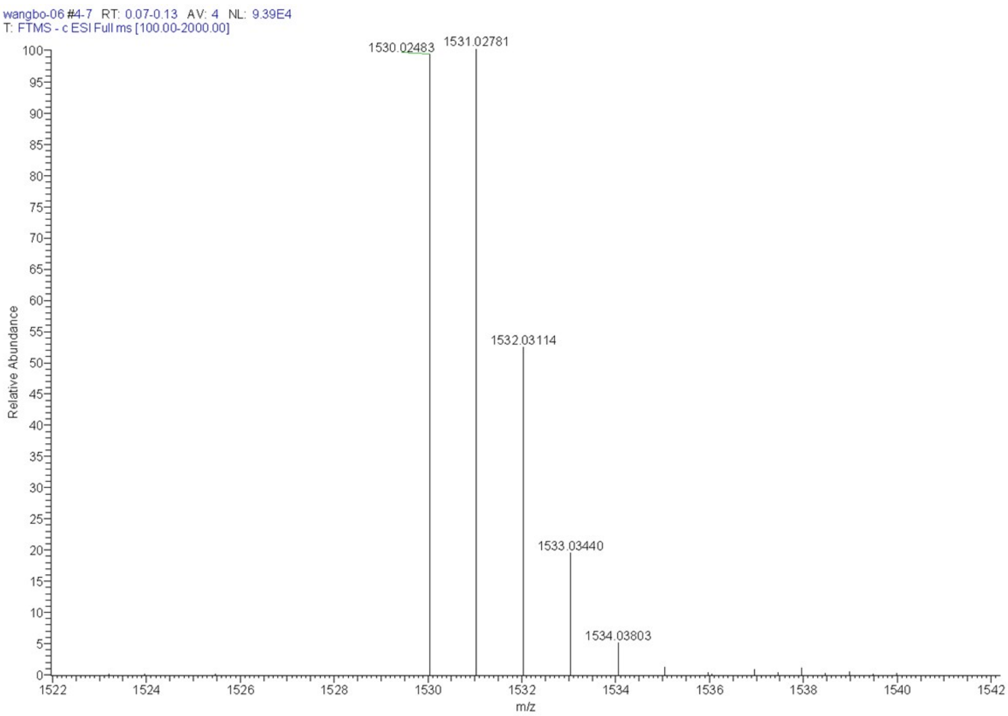


Figure S17. ^{13}C-NMR (100 MHz, d_6-DMSO) of compound **6**.



SPECTRUM - simulation:				
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1530.02483	1530.02597	-0.75	24.5	$C_{88}H_{137}O_{14}N_8$

Fig. S18. HR-ESI-MS of compound **6**.

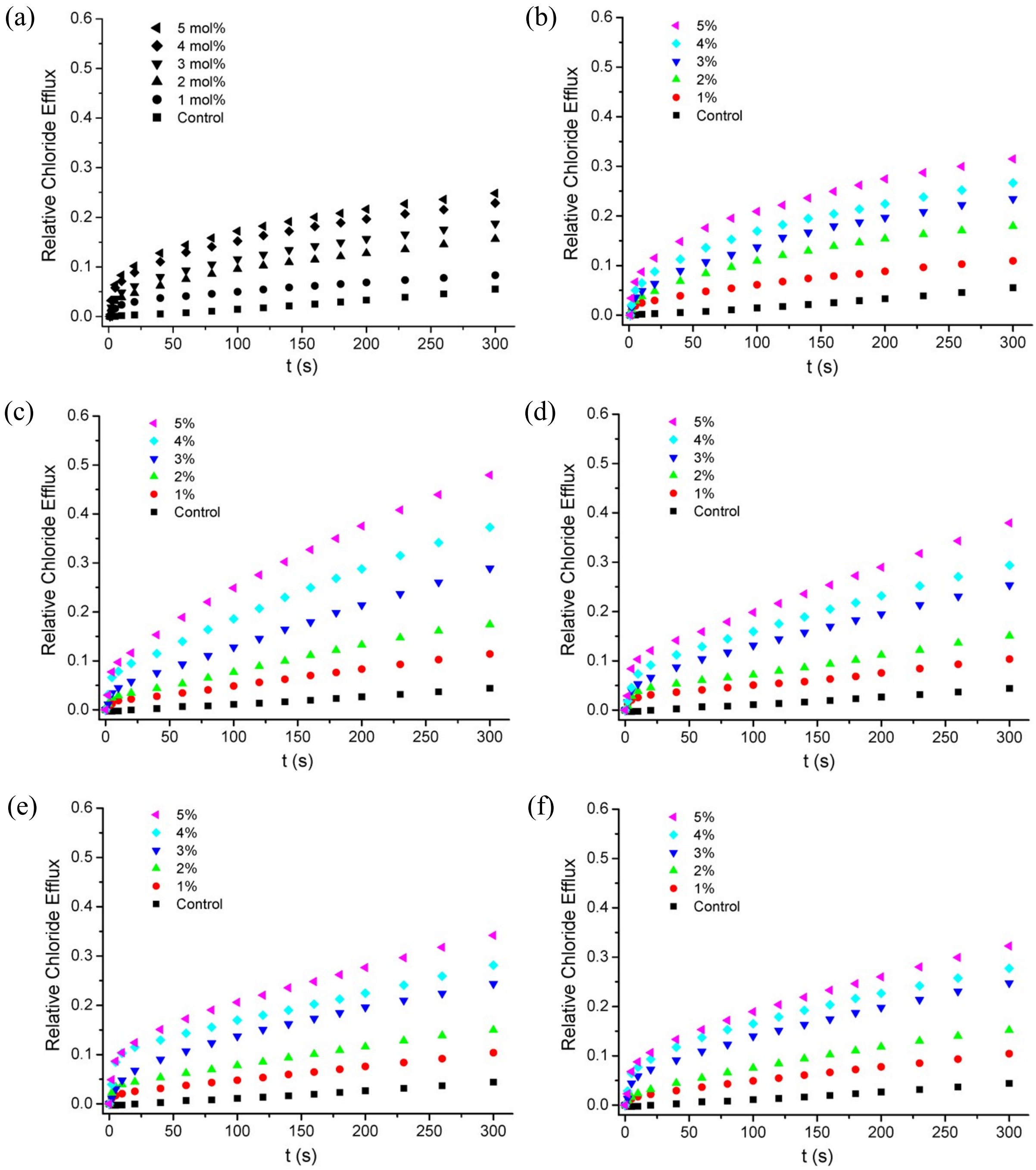


Fig. S19. Relative chloride efflux promoted by compounds **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f) of varying concentrations in EYPC vesicles loaded with 500 mM NaCl buffered to pH 7.0 with 25 mM HEPES. The vesicles were dispersed in 500 mM $NaNO_3$ buffered to pH 7.0 with 25 mM HEPES.

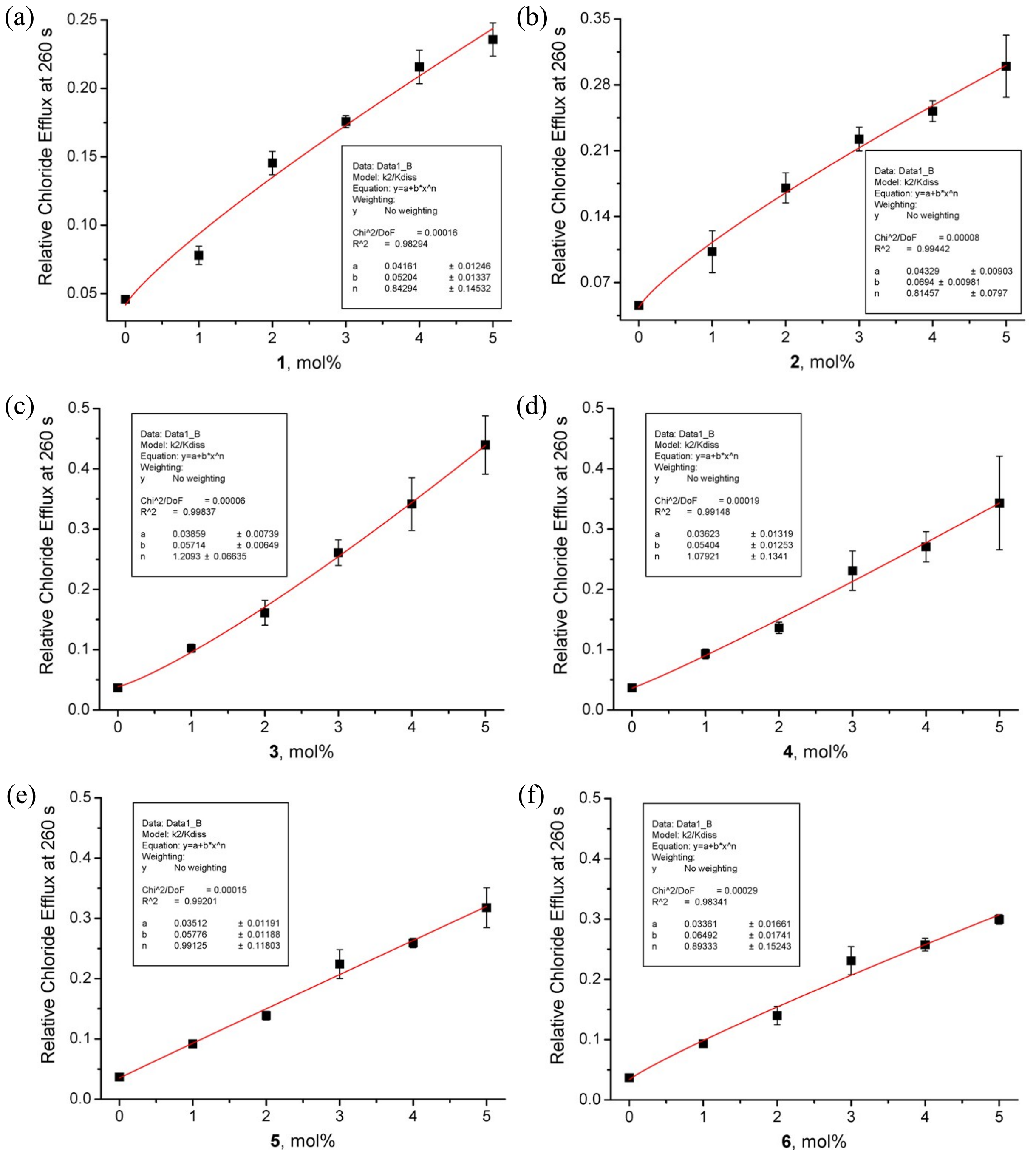


Fig. S20. Plots of the relative chloride efflux at 260 s against the mol% concentrations of compounds **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f), according to the Eq. $k_{obs} = k_0 + k_2 \times [\text{compound}]^n / K_{diss}$.

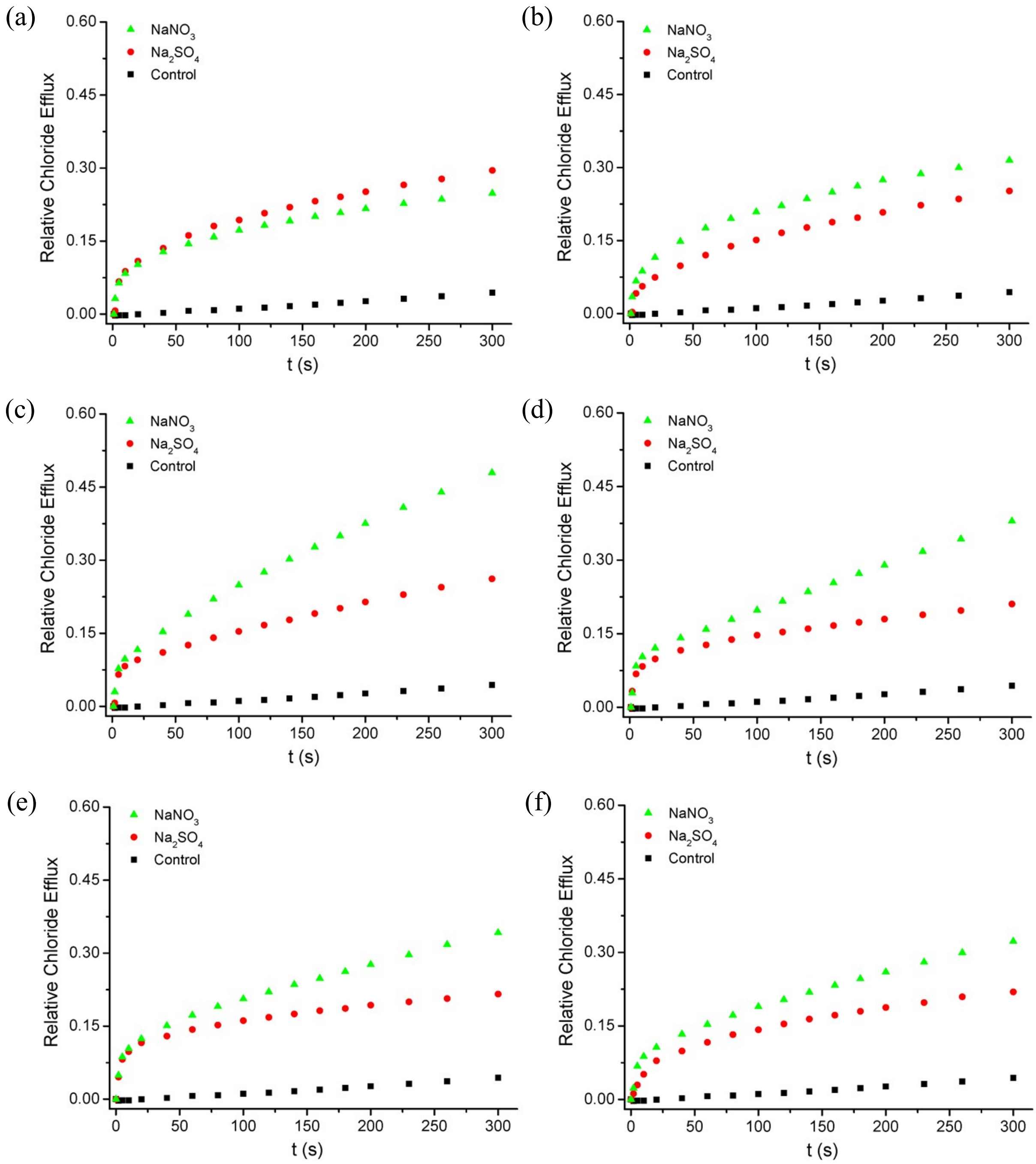


Fig. S21. (b) Relative chloride efflux, promoted by 5 mol% of compounds **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f) in EYPC vesicles loaded with 500 mM NaCl buffered to pH 7.0 with 25 mM HEPES. The vesicles were dispersed in 25 mM HEPES buffer (pH 7.0) containing 500 mM $NaNO_3$ and 250 mM Na_2SO_4, respectively. The experiment that was conducted in $NaNO_3$ media with DMSO was used as a control.

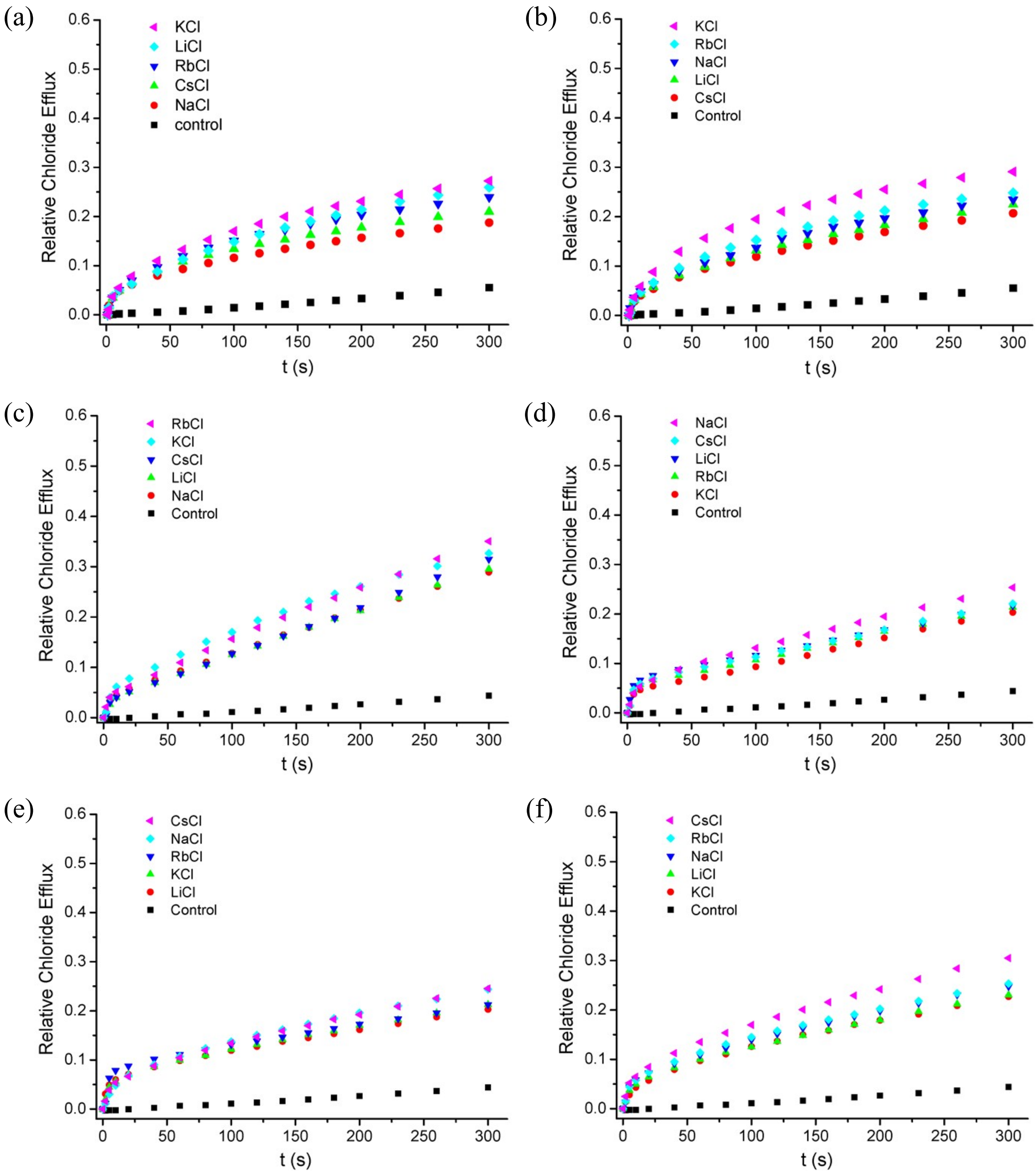


Fig. S22. Relative chloride efflux promoted by 3 mol% of compounds **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f) in EYPC vesicles loaded with 500 mM MCl (M = Li, Na, K, Rb and Cs) buffered to pH 7.0 with 25 mM HEPES. The vesicles were dispersed in 500 mM $NaNO_3$ buffered to pH 7.0 with 25 mM HEPES.

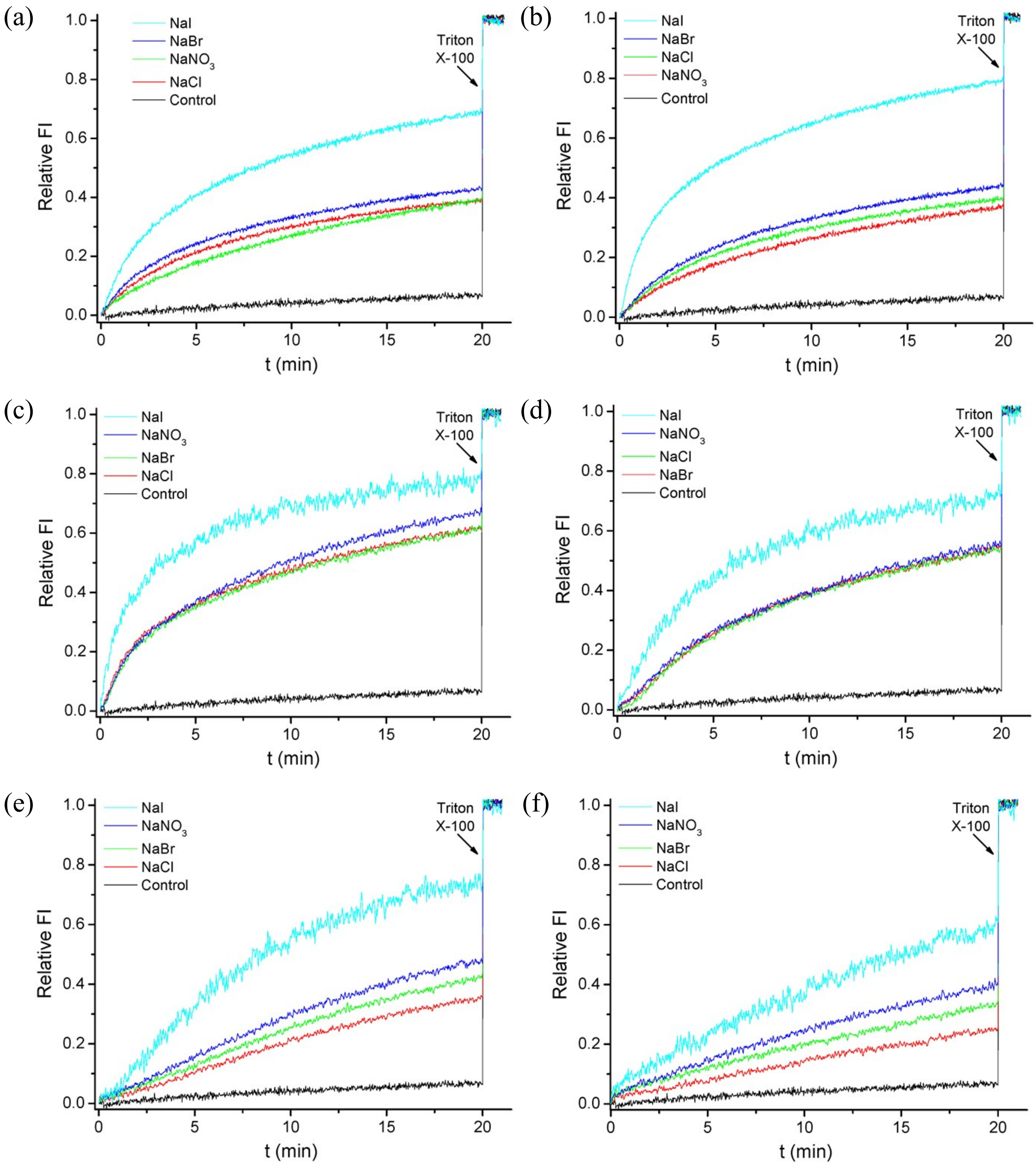


Fig. S23. Discharge of a pH gradient by 3 mol% of compounds **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f) across EYPC-based liposomal membranes, under the measuring conditions of internal vesicles: 0.1 mM pyranine in 25 mM HEPES (50 mM NaX, pH 7.0) and external vesicles: 25 mM HEPES (50 mM NaX, pH 8.0) (X = NO_3, Cl, Br and I). Ex 460 nm; em 510 nm. The experiment that was conducted in NaCl media with DMSO was used as a control.

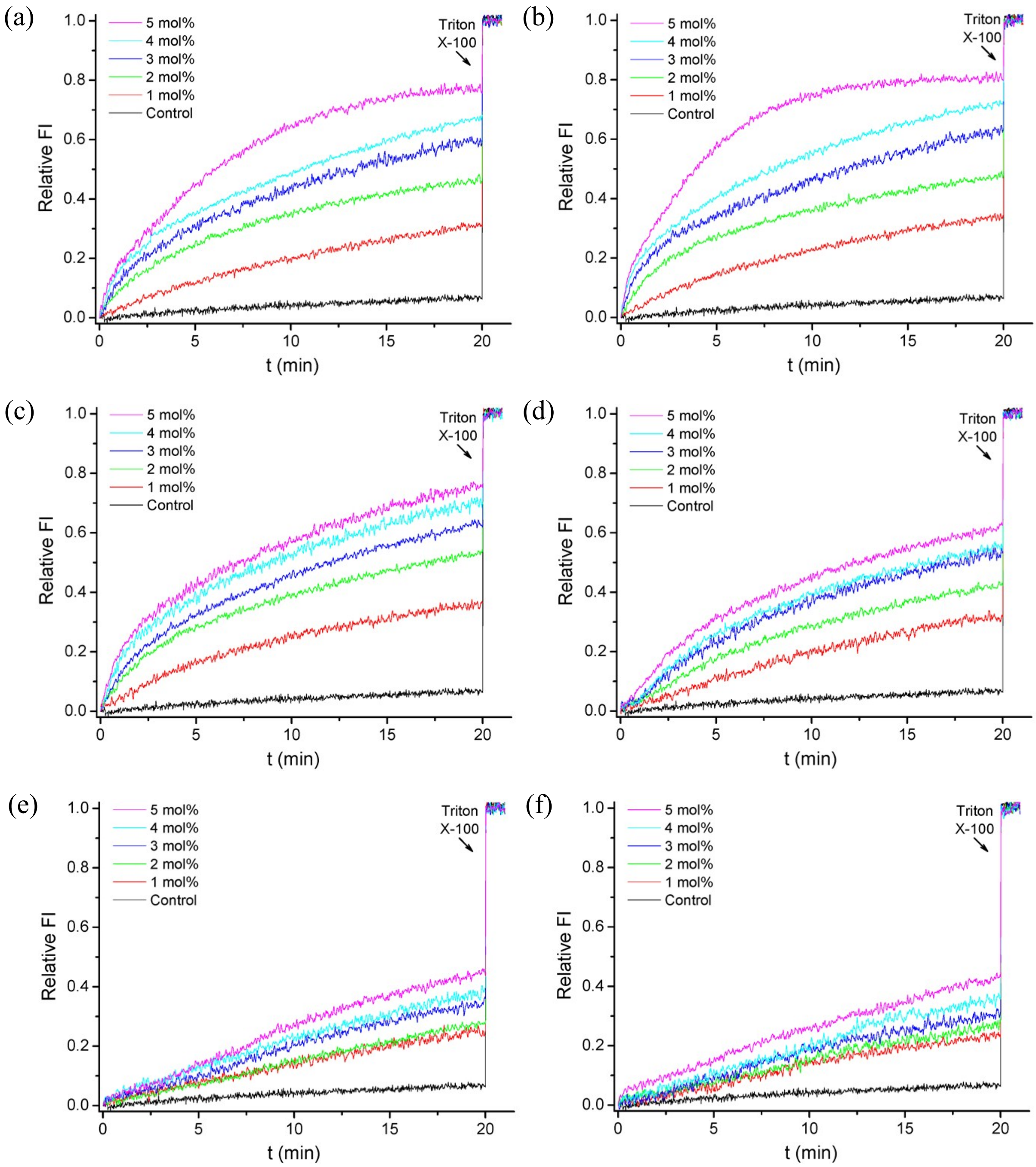


Fig. S24. Discharge of a pH gradient by compounds **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f) of varying concentrations across EYPC-based liposomal membranes, under the measuring conditions of internal vesicles: 0.1 mM pyranine in 25 mM HEPES (50 mM NaCl, pH 7.0) and external vesicles: 25 mM HEPES (50 mM NaCl, pH 8.0). Ex 460 nm; em 510 nm. DMSO was used as a control.

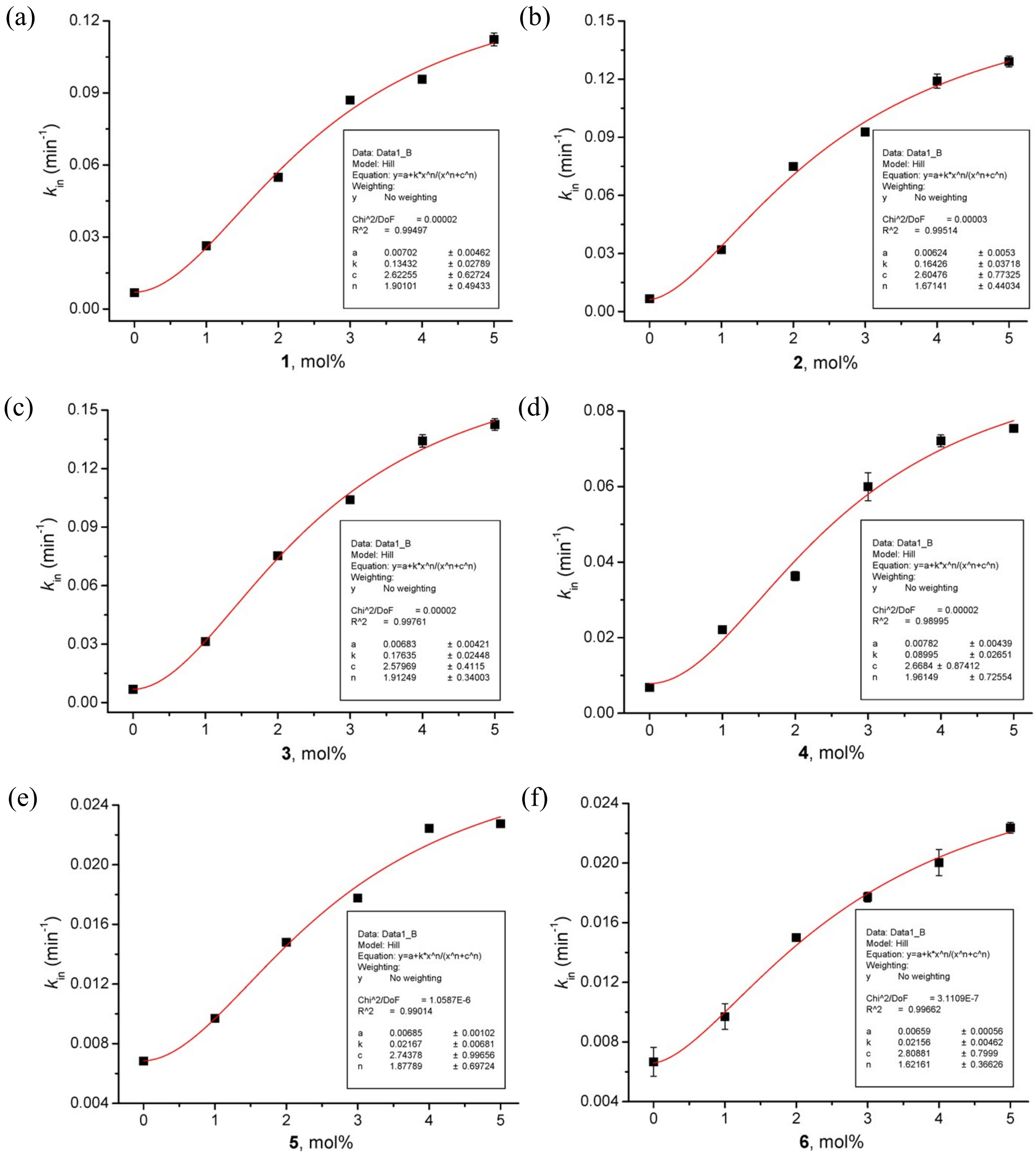


Fig. S25. Hill plots of the initial rate constants (k_{in}'s) against the mol% concentrations of compounds **1** (a), **2** (b), **3** (c), **4** (d), **5** (e) and **6** (f), according to the Eq. $k_{in} = k_0 + k_{max} \times [\text{compound}]^n / ([\text{compound}]^n + [EC_{50}]^n)$.