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Transmembrane anion transport promoted by thioamides†

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Thioamide groups represent useful hydrogen-bonding motifs for the development of active transmembrane anion transporters. Using a 1,8-di(thioamido)carbazole scaffold the superior performance of thioamides compared with the parent amides has been demonstrated.

Introduction

Small molecules capable of performing protein functions represent attractive minimalistic models to study cellular processes and are appealing for potential biological applications.[1–3] In recent years, small molecules capable of facilitating the selective permeation of anions through lipid bilayers have emerged as promising candidates for the development of anticancer and antimicrobial agents as well as therapeutics for the treatment of channelopathies caused by faulty anion transport, such as cystic fibrosis, Dent's disease, and others.[4–6]

Transmembrane ion transport is a complex phenomenon influenced by a number of factors including binding energies and lipophilicity.[7–9] Subtle changes in the molecular structure of anionophores often result in dramatic differences in their performance.[10–12] Nevertheless, important insights into the design of this type of molecules have been obtained in recent years.[13] Hydrogen bonding is the most frequently employed interaction in the design of synthetic anion transporters. Several hydrogen bond donors arranged in a convergent manner ensure effective binding between the transporter and the anion, thus forming a supramolecular complex capable of permeating the membrane. Ureas and thioureas are among the most successful anion binding motifs employed in this type of molecules.[14,15] Thioureas usually outperform ureas as anion carriers because replacement of oxygen by sulfur improves the hydrogen bonding ability of the N–H groups, reduces tendency to self-assemble, and increases lipophilicity of the molecule. This strategy of using urea and thiourea derivatives has been

extensively used to produce anionophores with increased activity.[16] On the other hand, the potential of thioamides has remained largely unexplored despite a similar effect would be expected and amide groups are frequently employed in the construction of active anion receptors and transporters.[17] In this paper, we explore the potential of the thioamide moiety in the development of active anionophores.

Results and discussion

We have recently reported a family of 1,8-diamidocarbazoles as anion receptors and identified compound **4A** as a highly active anion carrier (Fig. 1).[18] We envisaged the potential of this motif to prepare their thioamide derivatives and to explore their anion transport properties.

Thioamides **1T–5T** as well as amido-thioamides **5AT** and **6AT** were synthesized from the corresponding 1,8-diamidocarbazoles by treatment with Lawesson's reagent and fully characterized (Fig. 1, see also ESI† for details).

^{1}H NMR titrations of the thioamides with TBACl in DMSO-d_6 + 0.5% H_2O resulted in large downfield shifts of the carbazole NH protons ($\Delta\delta_{max}$ from 1.83 to 2.55 ppm, except for the most weakly bonding **1T**, for which $\Delta\delta_{max} = 0.94$ ppm was observed) as well as in much smaller downfield shifts of the thioamide NHs ($0.42 < \Delta\delta_{max} < 0.65$ ppm, again with the exception of **1T**, where $\Delta\delta_{max} = 0.89$ ppm) (Fig. 2). In case of the amido-thioamides **5AT** and **6AT**, the CSNH protons shifted slightly upfield upon chloride binding (by 0.13–0.18 ppm), mirroring the behaviour of CONH protons of their parent diamide receptors.[19] All these observations suggest that the hydrogen bonds formed with the anion by the central carbazole NHs are much stronger than the bonds formed by the (thio)amide side arms.

This supposition was further corroborated by the X-ray crystal structure of the chloride complex of dithioamide **3T**, in which the carbazole NH–Cl distance is much shorter (2.248 Å) than the two thioamide NH–Cl distances (2.581 Å and 2.830 Å), see Fig. 1. The inability of the thioamide arms to form shorter bonds with chloride might be related to the steric bulk of the sulfur atom, which enforces significant torsion of the thioamide

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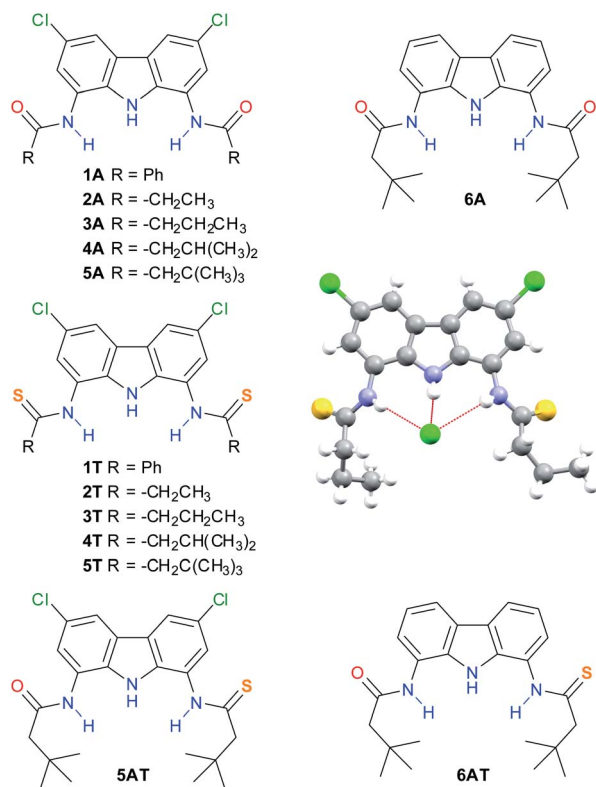
Fig. 1 Compounds included in this study and solid-state X-ray structure of compound **3T** complexing chloride (as TBACl; TBA cation omitted for clarity).

arms with respect to the carbazole plane (44–46°) as well as to geometric mismatch between the relatively wide binding cavity of the receptor and the relatively small chloride anion.

Quantitative analysis of the titration data revealed that the association constants with chloride turned out to be 2–3 times smaller for thioamides in comparison to their amide precursors (Table 1). This is in contrast to what might have been expected based on the much higher acidity of thioamides,[20] but in line with some literature precedents.[21,22] Very likely, the beneficial effect of enhanced hydrogen bond donating ability of the

thioamide moieties[23] is counterbalanced here by unfavourable conformational preferences of the thioamides.[24,25]

The anion transport properties of these compounds were investigated in POPC phospholipid vesicles using a chloride selective electrode.[26] In these assays, the facilitated chloride efflux induced by aliquots of the compounds was monitored over time. All receptors were first screened at 2% carrier to POPC molar ratio and compounds eliciting more than 50% chloride efflux were further analysed at different concentrations. For these latter derivatives, the concentration-dependent chloride efflux activity was then fitted using the Hill equation to obtain the EC_{50} parameters representing the amount of compound needed to induce 50% efflux of the encapsulated chloride. All the experiments were repeated at least three times using different batches of vesicles (Fig. 3).

The positive effect of replacing the amide groups by the thioamide was evident with all 1,8-di(thioamido)carbazoles outperforming their parent 1,8-diamidocarbazoles. The least active derivative was found to be the 1,8-dibenzothioamido derivative **1T**. For this compound, no EC_{50} could be calculated. The poor performance of transporters bearing aromatic substituents, such as **1T**, is likely related to their much lower chloride affinity.[18] Thioamides **2T–5T** were found to be highly active in the chloride efflux assay with nitrate in the external solution, with EC_{50} values in the submicromolar range. The most active derivative was **4T**, with a calculated EC_{50} of 67 nM. The positive effect of replacing even a single amide group by thioamide is demonstrated in the case of compounds **5AT** and **6AT**. Compound **5AT** displayed transport efficacy rivalling the best performers identified in this study. Compound **6AT** is an active transporter derived from a non-chlorinated carbazole core. None of the diamides derived from this core showed any chloride transport activity in our previous investigations.[18]

Experiments were also performed by suspending the vesicles in an external sulfate solution or an external mixture of sulfate and bicarbonate. These assays allow studying the impact of the anion composition of the external buffer on the observed chloride efflux. Bicarbonate is an anion of biological relevance and is significantly more hydrophilic than chloride and nitrate,

Table 1 Association constants K_a for 1 : 1 complexes of compounds **1T–5T**, **5AT** and **6AT** as well as their amide congeners (in brackets) with chloride (added as tetrabutylammonium salt) determined from ^{1}H NMR titration experiments in DMSO-d_6 + 0.5% H$_2$O at 293 K and transport activities expressed as EC_{50} (nM)

Compound	K_a (M^{-1})	EC_{50} (nM) NO$_3^-$/Cl$^-$	EC_{50} (% molar) NO$_3^-$/Cl$^-$	EC_{50} (nM) HCO$_3^-$/Cl$^-$	EC_{50} (% molar) HCO$_3^-$/Cl$^-$
1T	<10 (14)^a	—^{b}	—^{b}	—^{b}	—^{b}
2T	49 (104)^a	297	0.059	4290	0.858
3T	50 (109)^a	107	0.022	2910	0.582
4A	123^a	184	0.037	—^{b}	—^{b}
4T	46	66	0.013	385	0.077
5A	159^a	—^{b}	—^{b}	—^{b}	—^{b}
5T	52	93	0.019	465	0.093
5AT	67	61	0.012	1719	0.344
6A	48^a	—^{b}	—^{b}	—^{b}	—^{b}
6AT	23	353	0.071	—^{b}	—^{b}

^{a} The association constants determined for parent bisamides were taken from ref. 18. ^{b} The compound did not display enough activity to calculate an EC_{50} value.
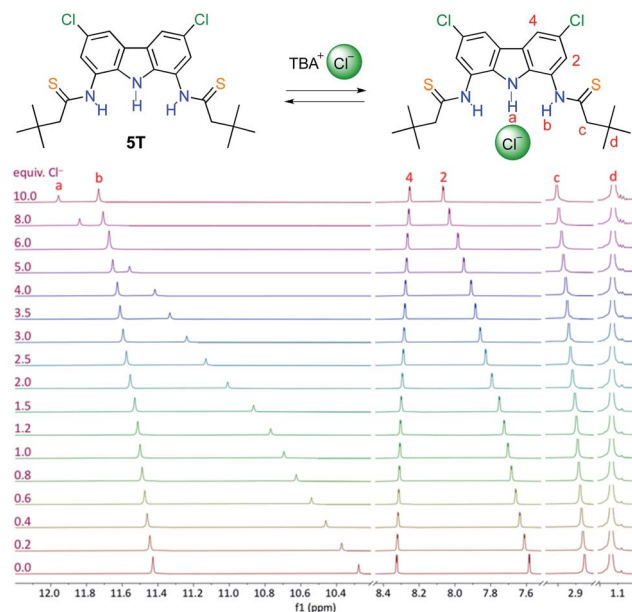
Fig. 2 ^{1}H NMR titration of **5T** (5 mM) with TBACl in DMSO-d_6 + 0.5% H_2O. T = 293 K.

thus more difficult to extract into the lipid bilayer. This was reflected in the higher calculated EC_{50} values. Only thioamides **4T** and **5T** displayed EC_{50} values in the submicromolar range under these conditions. No significant chloride efflux was detected in the presence of sulfate alone in the external milieu (see Fig. S56†). This result supports an exchange mechanism accounting for the transport activity displayed by these compounds, where the higher hydrophilicity of the doubly charged sulfate renders the compounds inactive as a result of their inability to facilitate the transport of this anion. This result also rules out any unspecific transport or membrane destabilization effect promoted by these compounds. The ability of some of these compounds to exchange chloride and bicarbonate bodes well for their potential biological applications.
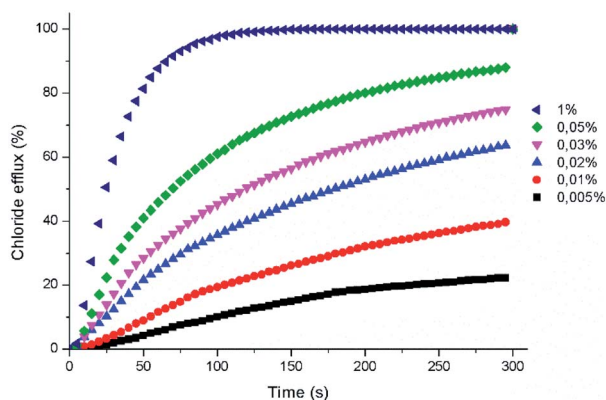

Fig. 3 Chloride efflux promoted by **4T** at different concentrations (1–0.005% carrier to POPC molar ratio, 5–0.025 μM) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM NaNO$_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

Conclusions

In conclusion, we have demonstrated the usefulness of thio-amides in the development of highly active transmembrane anion transporters.[27] These compounds display enhanced transmembrane transport activity compared to the parent amide derivatives. Taking into account the widespread use of amides as hydrogen-bonding motifs in successful anionophores, it is expected that this strategy will allow the identification of new candidates with improved anion transport efficiency.

Experimental

N,N'-(3,6-Dichloro-9H-carbazole-1,8-diyl)dibenzothioamide (1T)

A 50 ml, 2-neck, round-bottomed flask was dried for 10 min with a stream of hot air (*ca.* 500 °C) from heat-gun and cooled down to rt in a desiccator. Then the flask was charged with diamide **1A** (238 mg, 0.502 mmol), Lawesson's reagent (444 mg, 1.10 mmol) and a stir bar. Then the flask was equipped with a reflux condenser connected to a Schlenk line and its side neck was closed with a stopper. Air was removed by three pump/thaw cycles and then dry THF (30 ml) was added in a stream of argon through the side neck. The reaction mixture was intensively stirred and refluxed overnight. After this time the mixture was cooled down and 2.0 g of silica gel was added. Solvents were evaporated on a rotary evaporator and the solid residue was dried under high vacuum. Then the solid residue was suspended in hexane (40 ml) and put on top of a chromatographic column made of 110 g of silica suspended in CHCl$_3$. The column was eluted with CHCl$_3$ (*ca.* 500 ml), CHCl$_3$: CH$_3$CO$_2$Et = 40 : 1 (*ca.* 500 ml) and CHCl$_3$: CH$_3$CO$_2$Et = 30 : 1 until the desired product was completely washed out. Fractions containing pure product were combined and evaporated on a rotary evaporator yielding 293 mg (98.0%) of yellow solid. Mp: decomposition at 301 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.69 (s, 2H, thioamide NHs), 11.23 (s, 1H, carbazole NH), 8.37 (bs, 2H, carbazole CH-4/5), 8.03 (d, J = 7.6 Hz, 4H, phenyl CH-*ortho*), 7.77 (bs, 2H, carbazole CH-2/7), 7.56 (t, J = 7.3 Hz, 2H, phenyl CH-*para*), 7.48 (t, J = 7.5 Hz, 4H, phenyl CH-*meta*). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 199.19, 141.09, 133.99, 131.30, 127.98, 125.66, 124.62, 124.46, 122.98, 119.23. Elemental analysis: calcd for C$_{26}$H$_{17}$Cl$_2$N$_3$S$_2$: C, 61.66; H, 3.38; N, 8.30, found: C, 61.56; H, 3.52; N, 8.27. HR MS (TOF MS ES$^-$) m/z calcd for C$_{26}$H$_{16}$Cl$_2$N$_3$S$_2^-$: 504.0163 found: 504.0168.

Compounds **2T–5T**, **5AT** and **6AT** were prepared using similar procedures. Complete information is provided in the ESI.†

N,N'-(3,6-Dichloro-9H-carbazole-1,8-diyl)dipropanethioamide (2T)

Yellow solid, yield 79.7%. Mp: decomposition at 270 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.53 (s, 2H, thioamide NH); 10.84 (s, 1H, carbazole NH); 8.31 (d, J = 2.0 Hz, 2H, carbazole CH); 7.75 (d, J = 2.0 Hz, 2H, carbazole CH); 2.88 (q, J = 7.5 Hz, 4H, methylene CH$_2$); 1.35 (t, J = 7.5 Hz, 6H, methyl CH$_3$). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 208.02, 133.48, 124.93,

124.47, 123.81, 122.99, 119.04, 38.84, 13.92. Elemental analysis: calcd for $C_{18}H_{17}Cl_2N_3S_2$: C, 52.68; H, 4.18; N, 10.24, found: C, 52.64; H, 4.35; N, 10.09. HR MS (TOF MS ES$^-$) m/z calculated for $C_{18}H_{16}Cl_2N_3S_2^-$: 408.0163 found: 408.0161.

N,N′-(3,6-Dichloro-9H-carbazole-1,8-diyl)dibutanethioamide (3T)

Yellow solid, yield 80.2%. M.p. decomposition at 282 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.56 (s, 2H, thioamide NH), 10.64 (s, 1H, carbazole NH), 8.31 (d, J = 1.9 Hz, 2H, carbazole CH), 7.71 (d, J = 2.0 Hz, 2H, carbazole CH), 2.85 (t, 4H, J = 6.0 Hz, CH_2C=S), 1.93–1.81 (m, 4H, CH_2), 1.02 (t, J = 7.4 Hz, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 206.45, 133.45, 124.90, 124.54, 123.78, 123.06, 119.12, 47.69, 22.61, 13.27. Elemental analysis: calcd for $C_{20}H_{21}Cl_2N_3S_2$: C, 54.79; H, 4.83; N, 9.58, found: C, 54.88; H, 5.06; N, 9.47. HR MS (TOF MS ES$^-$) m/z calcd for $C_{20}H_{20}Cl_2N_3S_2^-$: 436.0476 found: 436.0472.

N,N′-(3,6-Dichloro-9H-carbazole-1,8-diyl)bis(3-methylbutanethioamide) (4T)

Yellow solid, yield 64.7%. ^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.57 (s, 2H, thioamide NH), 10.46 (s, 1H, carbazole NH), 8.33 (d, J = 1.5 Hz, 2H, carbazole CH-4), 7.66 (d, J = 1.5 Hz, 2H, carbazole CH-2), 2.75 (d, J = 7.2 Hz, 4H, CH_2), 2.40–2.25 (m, 2H, CH), 1.03 (d, J = 6.6 Hz, 12H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO} 205.56, 133.46, 124.89, 124.66, 123.79, 123.15, 119.22, 54.88, 39.50, 29.07, 21.96. Elemental analysis: calcd for $C_{22}H_{25}Cl_2N_3S_2$: C, 56.64; H, 5.40; N, 9.01, found: C, 56.75; H, 5.54; N, 8.94. HR MS (TOF MS ES$^-$) m/z calcd for $C_{22}H_{24}Cl_2N_3S_2^-$: 464.0789 found: 464.0786.

N,N′-(3,6-Dichloro-9H-carbazole-1,8-diyl)bis(3,3-dimethylbutanethioamide) (5T)

Yellow solid, yield 95.1%. M.p. decomposition at 278 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.43 (s; 2H; thioamide NH); 10.30 (s; 1H; carbazole NH); 8.33 (d; J = 2.0 Hz; 2H; carbazole CH-4/5); 7.59 (d; J = 2.0 Hz; 2H; carbazole CH-2/7); 2.85 (s; 4H; methylene CH_2); 1.14 (s; 18H; t-butyl CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 203.50, 133.43, 125.04, 124.75, 123.89, 123.17, 119.18, 59.12, 32.02, 29.77. Elemental analysis: calcd for $C_{24}H_{29}Cl_2N_3S_2$: C, 58.29; H, 5.91; N, 8.50, found: C, 58.37; H, 6.06; N, 8.25. HR MS (TOF MS ES$^-$) m/z calcd for $C_{24}H_{28}Cl_2N_3S_2^-$: 492.1102; found: 492.1113.

N-(3,6-Dichloro-8-(3,3-dimethylbutanethioamido)-9H-carbazol-1-yl)-3,3-dimethylbutanamide (5AT)

Yellow solid, yield 16.7%. ^{1}H NMR (DMSO-d_6) δ_{DMSO}: 11.55 (s, 1H, thioamides NH), 10.56 (s, 1H, carbazole NH), 10.00 (s, 1H, amide NH), 8.30 (d, J = 1.8 Hz, 1H, carbazole CH-5), 8.12 (d, J = 1.8 Hz, 1H, carbazole CH-4), 7.90 (d, J = 1.8 Hz, 1H, carbazole CH-2), 7.41 (d, J = 1.6 Hz, 1H, carbazole CH-7), 2.88 (s, 2H, CH_2C=S), 2.35 (s, 2H, CH_2C=O), 1.16 (s, 9H, t-Bu on the thioamide side), 1.08 (s, 9H, t-Bu on the amide side). ^{13}C NMR (DMSO-d_6) δ_{DMSO}: 203.85, 170.57, 133.40, 129.89, 125.06, 124.64, 124.59, 124.24, 124.00, 123.73, 122.96, 119.26, 117.74,

115.92, 58.90, 49.20, 32.07, 30.89, 29.78, 29.59. HR MS (TOF MS ES$^-$): m/z calcd for $C_{24}H_{28}Cl_2N_3OS^-$: 476.1330, found: 476.1331. Elemental analysis calcd for $C_{24}H_{29}Cl_2N_3OS$: C, 60.24; H, 6.11; N, 8.78; found: C, 60.61; H, 6.39; N, 8.39.

N-(8-(3,3-Dimethylbutanethioamido)-9H-carbazol-1-yl)-3,3-dimethylbutanamide (6AT)

Yellow solid, yield 40.3%, mp decomposition above 282 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.47 (s; 1H; thioamide NH); 10.06 (s; 1H; carbazole NH); 9.95 (s; 1H; amide NH); 8.08 (d, J = 7.5 Hz, 1H; CH-5 on the thioamide side); 7.94 (d, J = 7.7 Hz, 1H; CH-4 on the amide side); 7.61 (dd; J_1 = 7.8, J_2 = 1.0 Hz; 1H; CH-2 on the amide side); 7.34 (d, J = 7.7, 1H; CH-7 on the thioamide side); 7.22 (t; J = 7.7 Hz; 1H; CH-6 on the thioamide side); 7.17 (t; J = 7.8 Hz; 1H; CH-3 on the amide side); 2.87 (s; 2H; methylene CH_2 from the thioamide arm); 2.34 (s; 2H; methylene CH_2 from the amide arm); 1.17 (s; 9H; t-butyl CH_3 from the thioamide arm); 1.10 (s; 9H; t-butyl from the amide arm). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 202.76, 170.19, 134.29, 131.48, 124.60, 124.47, 124.10, 123.61, 123.40, 119.43, 119.07, 119.05, 118.28, 116.28, 58.89, 49.13, 31.91, 30.84, 29.85, 29.68. Elemental analysis: calcd for $C_{24}H_{31}N_3OS$: C, 70.38; H, 7.63; N, 10.26, found: C, 70.07; H, 7.61; N, 10.05. HR MS (TOF MS ES$^-$) m/z calcd for $C_{24}H_{30}N_3OS^-$: 408.2110 found: 408.2112.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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