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The role of lipophilicity in transmembrane anion transport†

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The transmembrane anion transport activity of a series of synthetic molecules inspired by the structure of tambjamine alkaloids can be tuned by varying the lipophilicity of the receptor, with carriers within a certain log *P* range performing best.

Development of small molecules capable of facilitating anion transport through lipid bilayers is an important goal.[1] These molecules could have potential applications in channel replacement therapies, as novel chemotherapeutics or biomembrane research tools.[2] Despite this, little is known regarding the requirements to produce efficient anion transporters and in particular the molecular design of anion transporters. It is clear that ion translocation through a lipid membrane is a complex event involving many parameters. Anion binding affinity is the most commonly studied parameter in anion transporters. Steroid based "cholapods" developed by A. P. Davis and colleagues are excellent examples of very efficient anion transporters displaying extremely high anion affinities.[3] On the other hand, an important number of efficient nitrate transmembrane carriers exhibit negligible nitrate binding affinity.[4] Moreover, systems displaying very similar anion binding affinities can present dramatic differences in anion transport efficiency.

Lipophilicity is a parameter of paramount importance in the pharmaceutical industry.[5] However, it has received little attention in the context of transmembrane anion transport, despite its widespread use in pharmaceutics, which also involves membrane translocation steps.[6] P. A. Gale and colleagues have recently introduced lipophilicity calculations, along with total and polar surface areas assessment for several urea and thiourea based anion transporters.[7] Nevertheless typical modifications introduced in anion receptors, such as replacing urea by thiourea groups or introducing electron-withdrawing substituents (such as fluorinated groups), modify both lipophilicity and anion binding strength, making it difficult to assess the contribution of each factor in the final observed transport efficiency.

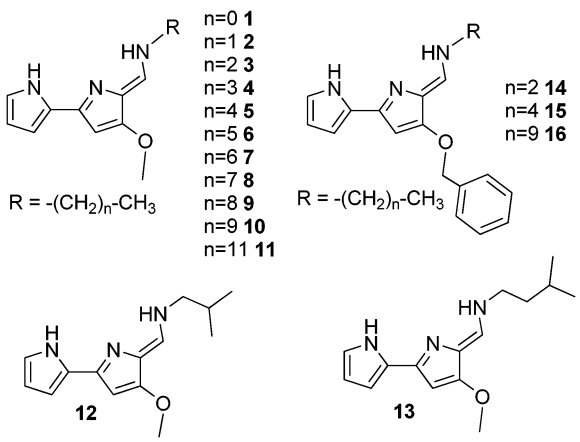

Fig. 1 Tambjamine derivatives **1–16**.

We have recently reported the anion transport properties of some tambjamine alkaloids and related analogs.[8] We envisaged that these easy-to-make compounds offered a unique opportunity to investigate the impact of lipophilicity on the overall efficiency of an anion transporter. We first prepared compounds **1–13** by systematically varying the substitution of the enamine alkyl chain (Fig. 1). A number of these compounds have been identified as naturally occurring bioactive derivatives (*e.g.*, **11–13**).[9] Displaying the same bipyrrole–enamine moiety as anion binding motif, it is expected that these compounds would have essentially similar anion binding affinity and polar surface areas, determined by the nitrogen and oxygen atoms. This was supported by computational studies and the estimation of chloride binding constants in DMSO solution for representative derivatives (see ESI† for details).

Log *P*, the logarithm of the octanol/water partition coefficient, is the most widely used measure of the lipophilicity. The log *P* values for these derivatives were calculated using the VCCLab software and consensus values for log *P* were used.[10] These values represent the average of the log *P* values calculated through different structure-based and property-based methods (see ESI† for details). The consensus log *P* value has been shown to provide accurate results in the prediction of log *P* values.[11] These calculations were performed for two different tautomeric forms of the neutral and N-protonated forms of compounds **1–16** (Table S1, ESI†). There is a linear

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Table 1 Transport activities ($\%s^{-1}$) and calculated log *P* values of compounds **1–16**. Carrier concentration 1 μM, 0.2 mol% carrier to lipid

Compound	Transport activity/$\%s^{-1}$	Log *P*
1	0.034	0.93 ± 0.46
2	0.070	1.34 ± 0.51
3	0.090	1.78 ± 0.52
4	0.193	2.22 ± 0.57
5	0.394	2.69 ± 0.63
6	0.467	3.16 ± 0.71
7	0.682	3.63 ± 0.79
8	0.680	4.26 ± 0.69
9	0.685	4.70 ± 0.74
10	0.530	5.13 ± 0.81
11	0.499	6.09 ± 0.92
12	0.205	2.11 ± 0.56
13	0.319	2.59 ± 0.64
14	0.299	3.44 ± 0.69
15	0.698	4.20 ± 0.78
16	0.248	6.64 ± 0.96

relationship between the calculated set of log *P* values for all tautomers. At physiological pH values it is expected that the tambjamine derivatives are protonated and therefore this is likely the active form of these molecules. We also checked the reliability of the calculated values using experimental data. To obtain a measure of the lipophilicity of the compounds we used reverse phase HPLC.[12] Retention times are directly proportional to the lipophilicity of the compounds. These results correlated well with the predicted log *P* values (see Fig. S9, ESI†).

The anion transport properties of these molecules were assayed in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) vesicles (see ESI† for details). Chloride efflux from chloride loaded vesicles in the presence of external bicarbonate was monitored using a chloride selective electrode.[13] Direct evidence of bicarbonate transport was obtained by ^{13}C NMR (see Fig. S16, ESI†). We chose this assay due to the biological relevance of the chloride/bicarbonate exchange process. For comparative purposes the relative transport activity was expressed as the initial rate of chloride efflux ($\%s^{-1}$).[14] This value results from the fitting of the traces representing the chloride efflux (see ESI† for details). Hill analysis suggested a discrete carrier mechanism, with EC_{50} values (concentration needed for inducing 50% of chloride efflux after five minutes) in the submicromolar range for the most active derivatives.[15]

There are substantial differences in the transmembrane anion transport activity of these compounds, ranging from limited to excellent at the concentration screened (Table 1). A plot of the transport activity *vs.* log *P* showed a maximum activity for compounds having log *P* values close to 4.2 ± 0.5 (Fig. 2). The transport activity diminished for compounds having log *P* values above or below this value. Compounds with log *P* values lower than 2 displayed very limited activity. It should be noted that diminished activity as a result of an increase in the lipophilicity probably also reflected the poorer deliverability from the aqueous phase to the lipid bilayer and/or the lower mobility of these compounds within the membranes. These results showed that carrier activity is influenced by ionophore lipophilicity with an optimal value of this parameter giving a maximum in transmembrane transport activity.

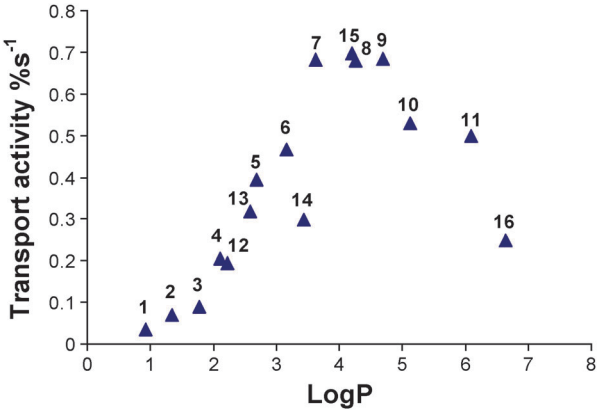

Fig. 2 Representation of transport activity, measured as initial chloride efflux ($\%s^{-1}$) *vs.* calculated average log *P* for compounds **1–16**.

Compounds **1–13** feature aliphatic hydrophobic side groups. In order to assess whether the relationship between hydrophobicity and transport activity also holds for aromatic side groups we prepared compounds **14** and **16** in which the O–Me fragment was replaced by an O–Bn substituent (Fig. 1). This modification impacts significantly on the overall lipophilicity of the molecule without modifying the anion binding cleft. The transport activity of **14** and **16** matched fairly well with the trend shown by **1–13**. The *n*-propyl substituted derivative **3** induced very limited chloride efflux, being too hydrophilic. Replacing the O–Me fragment by a O–Bn substituent as in **14** increases the log *P* value of the molecule significantly, resulting in an improved transport activity (Table 1, Fig. 3). Likewise, modifying the lipophilicity of compound **10** (R = *n*-decyl) as in compound **16** had a detrimental effect as a result of the increase in the already higher than optimal log *P* value. Since the maximum transport activity was found for compounds having log *P* values around 4.2 we decided to prepare an analog displaying this log *P* value. Theoretical calculations indicated an optimal value for the R = *n*-pentyl O–Bn derivative **15**. This compound was synthesized and was found to be among the most active of the series, confirming that log *P* values may be used to predict transport activity.

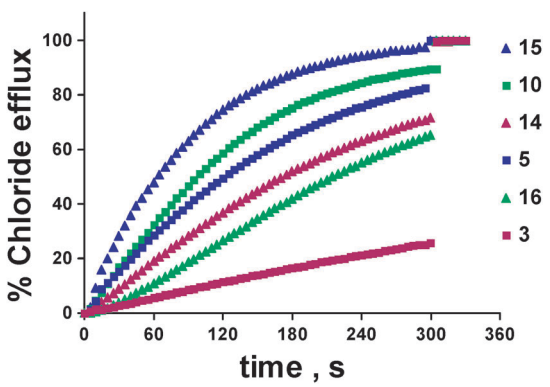

Fig. 3 Comparison of chloride efflux mediated by –OMe substituted compounds **3**, **5**, **10** (square symbols) and the parent –OBn substituted derivatives **14**, **15**, **16** (triangle symbols) in phospholipid vesicles (1 μM, 0.2 mol% carrier to lipid concentration). Each trace represents the average of three trials.

In conclusion, we have studied the transmembrane transport activity of anion transporters inspired by the structure of the tambjamine natural products. We have shown that lipophilicity is correlated to this activity, which is tuned by varying the lipophilicity of these compounds, being optimal for a log P = 4.2. This information was used to design new derivatives with optimal activity. These findings may be helpful in the analysis of structure–activity relationships of other ionophoric compounds.

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