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## COMMUNICATION

## Tambjamine alkaloids and related synthetic analogs: efficient transmembrane anion transporters $\ddagger\ddagger$

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The tambjamine alkaloids and related synthetic analogs are potent transmembrane anion tranporters promoting bicarbonate/ chloride exchange in model phospholipid liposomes and discharging pH gradients in living cells.

Facilitated anion transport through lipid bilayers is an emerging topic in bioorganic and supramolecular chemistry.<sup>1</sup> Simple anion transporters could be biologically active and have potential for the treatment of conditions derived from the abnormal function of natural anion transport mechanisms (channelopathies) such as cystic fibrosis.<sup>2</sup> In this regard, development of molecules capable of promoting efficient translocation of chloride and bicarbonate across lipid bilayers at low concentrations is an important goal.<sup>3</sup> Examples of naturally occurring molecules capable of promoting anion transport across lipid bilayers are rare. The tripyrrolic metabolite prodigiosin stands out as the best studied natural anion carrier, being able to promote both HCl and anion exchange.<sup>4</sup> This ionophoric activity has been linked to the biological activity of prodiginines.<sup>5</sup> Several research groups have studied synthetic analogs of prodigiosin, nevertheless the synthesis of these compounds is not straightforward and these modifications have failed to improve the properties of the natural derivatives.<sup>6</sup> The tambjamines are a class of natural products characterized by a 4-methoxy-2,2'-bipyrrolenamine structure.<sup>7</sup> They have evident structural relationships with the prodiginines and, like these compounds, tambjamines have shown intriguing biological activities including anticancer and antimicrobial properties.<sup>8</sup> The report of the total synthesis of tambjamines by Banwell and colleagues,<sup>9</sup> and the identification of new natural derivatives have led to further studies concerning the biological activity



Fig. 1 Tambjamine derivatives 1-4 and synthetic analogs 5, 6.

of these compounds.<sup>10</sup> Surprisingly, the ionophoric activity of these compounds has not been studied in detail.<sup>11</sup> Herein we report a detailed study of the transmembrane anion transport properties of tambjamine derivatives.

Compounds 1–6 were synthesized in high yield by acid catalyzed condensation of 4-methoxy-2,2'-bipyrrole aldehyde and different amines (Fig. 1) (see ESI‡ for details).<sup>9</sup> Compounds 1–4 are naturally occurring secondary metabolites for which biological activity has been reported in different studies.<sup>8,10</sup> Compounds 5 and 6 represent novel synthetic analogs with aromatic groups as amine substituents. The solid state structure of hydrochloride salts of compounds 2 and 6 was determined by X-ray diffraction (Fig. 2). Both compounds displayed an essentially flat bipyrrolenamine core with the chloride anion interacting with the tambjamine moiety through hydrogen bonds.



Fig. 2 Representation of the X-ray structure of compounds 2 (left) and 6 (right).

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The main difference between both structures arose from the relative orientation of one of the pyrrole rings. Whereas in 6 the three NH groups are found facing the chloride anion in 2 one of the pyrrole rings is rotated  $180^{\circ}$  and engaged in a hydrogen bond with a crystallization water molecule.

The spectroscopic data of these compounds also support a strong hydrogen bond interaction with the chloride anion in solution. Thus the chemical shifts of the N–H groups of compounds **1–6** (as hydrochloride salts) were found at very low field ( $\delta = 14-10$  ppm). These chemical shifts were significantly higher than those found for the corresponding hydroperchlorate salts. Titration experiments of hydroperchlorate salts of the compounds with TBAC1 in DMSO resulted in downfield shifts of the N–H signals up to one equivalent. Further addition of chloride produced no change, implying a strong 1:1 complexation.

The anion transport properties of these molecules were studied using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) vesicles (see ESI<sup>‡</sup> for details). Briefly, liposomes loaded with NaCl were suspended in an isotonic, chloride free external medium. Chloride release promoted by 1-6 was monitored over time using a chloride selective electrode. At the end of the experiment the vesicles were lysed by the addition of detergent, being the final chloride reading used as 100% release of chloride. In order to compare the ionophoric activity, initial transport rates expressed as % of chloride efflux per second were determined from the slope of the ISE assays.<sup>12</sup> The results are summarized in Table 1 and a representative graphic depicted in Fig. 3. Different assays were carried out to determine both the activity and mechanism of transport. When the vesicles were suspended in an external medium composed of sodium sulfate addition of 1-6 (1  $\mu$ M, 0.2% molar carrier to lipid) resulted in almost no chloride efflux (Table 1, column a). Sulfate is a highly hydrophilic anion  $(\Delta G_{\rm hyd} = -1081 \text{ kJ mol}^{-1})^{13}$  and it is very difficult to extract it through a lipid bilayer. Therefore, chloride efflux under these conditions should be the result of a formal HCl co-transport. On the other hand when an external pulse of NaHCO<sub>3</sub> was added to the sulfate containing external medium, chloride release was switched on, indicating the exchange of bicarbonate

**Table 1** Transport activities ( $\% s^{-1}$ ) of compounds 1–6

Compound	а	b	с	d	e
1	0.02	0.20	0.28	0.01	0.12
2	0.01	0.07	0.06	0.03	0.04
3	0.03	0.24	0.18	n.d.	0.14
4	0.03	0.51	0.34	n.d.	0.38
5	0.03	0.41	0.44	0.02	0.30
6	0.03	0.61	0.52	0.08	0.66
Blank	0.00	0.01	0.00	0.00	0.00

Column a: Vesicles loaded with 489 mM NaCl dispersed in 162 mM  $Na_2SO_4$  (pH 7.2). Column b: Vesicles loaded with 489 mM NaCl dispersed in 162 mM  $Na_2SO_4$  (pH 7.2, 20) upon addition of a NaHCO<sub>3</sub> pulse to make the extravesicular bicarbonate concentration 40 mM. Column c: Vesicles loaded with 489 mM NaCl dispersed in 489 mM NaNO<sub>3</sub> (pH 7.2). Column d: Vesicles loaded with 489 mM NaCl (pH 5.3) dispersed in 162 mM  $Na_2SO_4$  (pH 7.2). Column e: Vesicles loaded with 489 mM NaCl (pH 5.3) dispersed in 162 mM  $Na_2SO_4$  (pH 7.2). Column e: Vesicles loaded with 489 mM NaCl (pH 5.3) dispersed in 162 mM  $Na_2SO_4$  (pH 7.2) upon addition of a NaHCO<sub>3</sub> pulse to make the extravesicular bicarbonate concentration 40 mM.



Fig. 3 Chloride efflux promoted by 1–6 (1  $\mu$ M, 0.2% mol carrier to lipid concentration) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl buffered at pH 7.2 with 20 mM phosphate dispersed in 162 mM Na<sub>2</sub>SO<sub>4</sub> buffered at pH 7.2 upon addition of a NaHCO<sub>3</sub> pulse to make the extravesicular bicarbonate concentration 40 mM. Each trace represents the average of three trials.

and chloride (Table 1, column b).<sup>2</sup> Being monoanionic, bicarbonate  $(\Delta G_{hyd} = -335 \text{ kJ mol}^{-1})$  is easier to extract through the bilayer. To further prove the influence of the external medium in the anion translocation efficiency, experiments using sodium nitrate as external medium were carried out (Table 1, column c). Similar transport rates to those observed when bicarbonate is the external anion were obtained using ten-fold lower carrier concentrations (0.1  $\mu$ M, 0.02% molar carrier to lipid), as expected from the relative higher lipophilicity of nitrate ( $\Delta G_{hyd} = -300 \text{ kJ mol}^{-1}$ ).

We also carried out experiments in which a pH gradient was present, obtaining similar results to those described in the absence of pH gradient (Table 1, column d, e).

All these results strongly supported anion exchange as the main mechanism for the ionophoric activity of these metabolites. It should be noted that previously ionophoric activity of compound **4** was assumed to be the result of a HCl symport activity.<sup>11</sup> Since co-transport of HCl involves deprotonation of the host as opposite to anion exchange and tambjamines are likely to remain protonated at physiological pH (p $K_a$  of tambjamines is around 10),<sup>14</sup> we reasoned that this is the cause for the low efficiency of these carriers as HCl symporters compared to their activity as anion exchangers.

Significant differences in transport efficiencies were observed in the chloride efflux experiments. The most active carrier in all the assays was the novel derivative 6. A linear relationship between ionophoric activity and carrier concentration was found for this compound and significant chloride efflux was detected at carrier loadings as low as 125 nM, 0.025% molar equivalents with respect to lipids. The level of activity of this synthetic anionophore is within the range of that found for natural ionophore prodigiosin (0.32% s<sup>-1</sup> for 6 vs.  $0.36\% \text{ s}^{-1}$  for prodigiosin using  $0.25 \,\mu\text{M}$ , 0.05% molar carrier to lipid loadings (see Fig. S41, ESI<sup>‡</sup>)). On the other hand 2, tambjamine E, was found to be the less active compound in all the assays. At the concentration level screened, this compound displayed very low ionophoric activity. The lower lipophilicity of 2 compared to the other derivatives examined could be responsible for this result.



**Fig. 4** Acridine orange staining of small cell lung cancer line (GLC4) cells after exposure for 1 h to compounds **1**, **4** and **6** (8  $\mu$ M). (a) Untreated cells (control). (b) Cells treated with **4**. (c) Cells treated with compound **6**. (d) Cells treated with **2**.

The *in vitro* ionophoric activity of compounds 1-6 on small cell lung cancer line (GLC4) cells was studied using vital staining with acridine orange (AO). This cell permeable dye exhibits a characteristic orange fluorescence emission in acidic compartments such as lysosomes as a result of its protonation and accumulation in such organelles, whereas it emits green fluorescence at higher pH.<sup>15</sup> When GLC4 cells were stained with AO, the nuclei and the cytoplasm showed green fluorescence, while granular orange fluorescence was observed in the cytoplasm (Fig. 4a), suggesting that the orange fluorescence is due to acidified lysosomes. Cells were treated with 8 µM of compounds 1-6 and the changes in the fluorescence monitored over 1 h. Representative results are depicted in Fig. 4. Cells treated with compounds 4 and 6, the most active carriers identified in the liposome experiments, showed a complete disappearance of orange emission (Fig. 4b and c). Similar results were obtained with 1 and 5. On the other hand, cells treated with tambjamine E 2, a poor ionophore, showed no changes (Fig. 4d). These results correlate well with the activity observed in the liposome assays. Active ionophores induce an increase in the lysosomal pH whereas the inactive 2 is not able to do so. Based on the results obtained in the liposome assays, it might be supposed that facilitated influx of bicarbonate to the interior of the lysosomes could be responsible for the increase of the internal pH although other mechanisms cannot be ruled out.

The results described demonstrate that tambjamine alkaloids are natural anionophores and their structure is an excellent motif to develop new anion transporters. The straightforward synthesis of unnatural analogs of the tambjamine family makes these compounds promising candidates to identify new anion transporters with potential biological applications. Efforts aimed at understanding the structure–activity relationships between the ion transport and biological activity of these compounds are now underway in our laboratories.

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## Notes and references

 (a) A. P. Davis, D. N. Sheppard and B. D. Smith, *Chem. Soc. Rev.*, 2007, **36**, 348; (b) G. W. Gokel and N. Barkey, *New J. Chem.*, 2009, **33**, 947; (c) J. T. Davis, O. A. Okunola and R. Quesada, *Chem. Soc. Rev.*, 2010, **39**, 3843; (d) P. A. Gale, *Acc. Chem. Res.*, 2011, **44**, 216; for recent examples see: (e) S. Hussain, P. R. Brotherhood, L. W. Judd and A. P. Davis, J. Am. Chem. Soc., 2011, 133, 1614; (f) R. E. Dawson, A. Hennig,
D. P. Weimann, D. Emery, V. Ravikumar, J. Montenegro,
T. Takeuchi, S. Gabutti, M. Mayor, J. Mareda, C. A. Schalley and S. Matile, Nat. Chem., 2010, 2, 533; (g) A. Hennig, L. Fischer,
G. Guichard and S. Matile, J. Am. Chem. Soc., 2009, 131, 16889; (h) X. Li, B. Shen, X.-Q. Yao and D. Yang, J. Am. Chem. Soc., 2009, 131, 13676.

- 2 F. M. Ashcroft, *Ion Channels and Disease*, Academic Press, San Diego, 2000.
- 3 (a) J. T. Davis, P. A. Gale, O. A. Okunola, P. Prados, J. C. Iglesias-Sanchez, T. Torroba and R. Quesada, Nat. Chem., 2009, 1, 138;
  (b) P. A. Gale, C. Tong, C. J. E. Haynes, O. Adeosun, D. E. Gross, E. Karnas, E. M. Sedenberg, R. Quesada and J. L. Sessler, J. Am. Chem. Soc., 2010, 132, 3240; (c) W. A. Harrell, M. L. Bergmeyer, P. Y. Zavalij and J. T. Davis, Chem. Commun., 2010, 46, 3950; (d) N. Busschaert, P. A. Gale, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis and W. A. Harrell Jr., Chem. Commun., 2010, 46, 6252;
  (e) N. J. Andrews, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis, W. A. Gale, Chem. Sci., 2011, 2, 256.
- 4 (a) J. T. Davis, *Top. Heterocycl. Chem.*, 2010, **25**, 145; (b) J. L. Seganish and J. T. Davis, *Chem. Commun.*, 2005, 5781.
- 5 (a) A. Fürstner, Angew. Chem., Int. Ed., 2003, 42, 308; (b) N. R. Williamson, P. C. Fineran, F. J. Leeper and G. P. C. Salmond, Nat. Rev. Microbiol., 2006, 4, 887; (c) N. R. Williamson, P. C. Fineran, T. Gristwood, S. R. Chawrai, F. J. Leeper and G. P. C. Salmond, Future Microbiol., 2007, 2, 605; (d) M. S. Melvin, M. W. Calcutt, R. E. Noftle and R. A. Manderville, Chem. Res. Toxicol., 2002, 15, 742; (e) R. Pérez-Tomás, B. Montaner, E. Llagostera and V. Soto-Cerrato, Biochem. Pharmacol., 2003, 66, 1447.
- 6 (a) J. L. Sessler, L. R. Eller, W.-S. Cho, S. Nicolaou, A. Aguilar, J. T. Lee, V. M. Lynch and D. J. Magda, *Angew. Chem., Int. Ed.*, 2005, **44**, 5989; (b) R. I. Sáez Díaz, J. Regourd, P. V. Santacroce, J. T. Davis, D. L. Jakeman and A. Thompson, *Chem. Commun.*, 2007, 2701; (c) J. Regourd, A. Al-Sheikh Ali and A. Thompson, *J. Med. Chem.*, 2007, **50**, 1528; (d) R. I. Sáez Díaz, S. M. Bennett and A. Thompson, *ChemMedChem*, 2009, **4**, 742.
- 7 (a) B. Carté and D. J. Faulkner, J. Org. Chem., 1983, 48, 2314;
  (b) A. J. Blackman and C. P. Li, Aust. J. Chem., 1994, 47, 1625;
  (c) R. A. Davis, A. R. Carroll and R. J. Quinn, Aust. J. Chem., 2001, 54, 355.
- 8 (a) B. C. Cavalcanti, H. V. N. Junior, M. H. R. Seleghim, R. G. S. Berlinck, G. M. A. Cunha, M. O. Moraes and C. Pessoa, *Chem.-Biol. Interact.*, 2008, **174**, 155; (b) K. Kojiri, S. Nakajima and H. Suzuki, *J. Antibiot.*, 1993, **46**, 1894.
- 9 D. M. Pinkerton, M. G. Banwell and A. C. Willis, *Org. Lett.*, 2007, 9, 5127.
- (a) M. Carbone, C. Irace, F. Costagliola, F. Castelluccio, G. Villani, G. Calado, V. Padula, G. Cimino, J. L. Cervera, R. Santamaria and M. Gavagnin, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2668; (b) L. N. Aldrich, S. L. Stoops, B. C. Crews, L. J. Marnett and C. W. Lindsley, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5207; (c) D. M. Pinkerton, M. G. Banwell, M. J. Garson, N. Kumar, M. O. de Moraes, B. C. Cavalcanti, F. W. A. Barros and C. Pessoa, *Chem. Biodiversity*, 2010, **7**, 1311; (d) F. Ballestriero, T. Thomas, C. Burke, S. Egan and S. Kjelleberg, *Appl. Environ. Microbiol.*, 2010, **76**, 5710.
- 11 K. Tanigaki, T. Sato, Y. Tanaka, T. Ochi, A. Nishikawa, K. Nagai, H. Kawashima and S. Ohkuma, *FEBS Lett.*, 2002, 524, 37.
- 12 B. A. McNally, A. V. Koulov, T. N. Lambert, B. D. Smith, J. B. Joos, A. L. Sisson, J. P. Clare, V. Sgarlata, L. W. Judd, G. Magro and A. P. Davis, *Chem.-Eur. J.*, 2008, **14**, 9599.
- 13 E. Leontidis, Curr. Opin. Colloid Interface Sci., 2002, 7, 81.
- 14 M. S. Melvin, D. C. Ferguson, N. Lindquist and R. A. Manderville, J. Org. Chem., 1999, 64, 6861.
- 15 A. C. Allison and M. R. Young, In *Lysosomes in Biology and Pathology*, ed. J. T. Dingle and H. B. Fell, North Holland Publishing, Amsterdam, 1969, vol. 2, pp. 600–628.