Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

2,6-Bis(benzimidazol-2-yl)pyridine as a potent transmembrane anion transporter

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ARTICLE INFO

Article history:
Received 9 February 2016
Revised 28 March 2016
Accepted 31 March 2016
Available online xxxx

Keywords:
Anionophore
Benzimidazole
Anionophoric activity
Ion selectivity

ABSTRACT

2,6-Bis(benzimidazol-2-yl)pyridine was shown to exhibit potent anionophoric activity via a process of both Cl^-/NO_3^- antiport and H^+/Cl^- symport. This is in sharp contrast to the finding that its corresponding *N*-methylated analog exhibited negligible activity and reveals the importance of the imidazolyl-N*H* fragments in the anion-transport process.

Anions play a central role in biology and transmembrane anion transport across a cell membrane is crucial for maintaining cellular functions.[1] Uncontrolled transmembrane transport of anions may lead to serious disorders, notably among which is cystic fibrosis that originates from the defects in natural chloride ion channels.[2] Therefore, during the past decades, increasing interest has been attracted in identifying small-molecule organic compounds that are capable of efficiently mediating the transport of anions, in particular chloride across lipid bilayer membranes.[3] By replacing the missing anionophoric activity of defective anion channels, it is thought that these compounds have high potentials as chemotherapeutic agents for channelopathies and cancers.[4] To date, various non-peptidic structures have been reported to exhibit promising anion transporting properties.[3]

Albeit successful to date, some of anion-transporting molecules have potential disadvantages. In particular, their molecular weights and lipophilicity may be too high to be drug-like.[5] Accordingly, it is highly challenging to develop small-molecule organic compounds that have high anionophoric activity and meanwhile fall within rules of thumb, such as Lipinski's rule of five.[6] To this end, imidazoles and benzimidazoles are an attractive class of molecular scaffolds for the creation of anion transporters,[7] because of their biocompatibility and ability to form complexes with anions, in particular through hydrogen bonding.[7b,8] While the imidazole ring affords a wealth of biophysical-related applications, it is reported that incorporation of (benz)imidazolyl or (benz)imidazolium groups into the structures of transport carriers enhances the anion affinity and thereby transport activity. For example, Schmitzer et al. have reported a series of imidazolium-based anion transporters,[3c,7b–h] some of which exhibit potent antibacterial activity.[7d]

In the work reported herein, we sought to identify drug-like anion transporters containing benzimidazolyl groups. As such, we reason that 2,6-bis(benzimidazol-2-yl)pyridine (Bimpy, Fig. 1), because of its appropriate lipophilicity ($c\log P = 4.6$)[9] and the ability of the two benzimidazolyl groups to cooperatively act on bound anions most probably through hydrogen bonding (vide infra),[10] would act as a potent anion transporter.[11] To evaluate the role of the imidazolyl N*H* fragments in the ion-transporting process, Bimpy was methylated to afford 2,6-bis(*N*-methylbenzimidazol-2-yl)pyridine (Me_2bimpy).[12] Herein we describe the detailed investigation into the anionophoric activity of Bimpy and Me_2bimpy by means of pyranine and lucigenin assays.

As discussed in literature, a precondition for anionophoric activity of a transporter is its ability to bind the anions that are to be transported.[13] Therefore, the potential of Bimpy as a receptor for halides was studied virtually. It is clear from Figure 2 that Bimpy is able to form complexes with halides through hydrogen-bonding interaction,[10] whereas no such interaction was observed between Me_2bimpy and halides (Fig. S2). To verify this, we measured the association constants of Bimpy and Me_2bimpy with halides and nitrate by means of spectrophometric titrations (Figs. S3 and S4 and Table 1). The results indicate that Bimpy exhibits up to 22-fold higher binding constants than Me_2bimpy.

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http://dx.doi.org/10.1016/j.bmcl.2016.03.114
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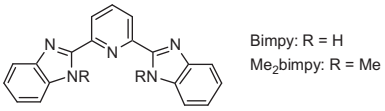

Figure 1. Structures of Bimpy and Me_2bimpy.

The ionophoric activity of Bimpy and Me_2bimpy was firstly confirmed by examining their ability to eliminate a pH differential across liposomal membranes derived from egg-yolk L-α-phosphatidylcholine (EYPC).[14] For this purpose, a pH sensitive dye, pyranine is loaded within large unilamellar vesicles (100 nm diameter, extrusion) and used as a fluorescence-responsive reporter of pH changes within the vesicle interior. The data are shown in Figures 3 and S5 and indicate that Bimpy is active in efficiently inducing pH discharge, whereas Me_2bimpy exhibits negligible activity.

Because pH discharge experiments cannot provide clear clues for clarifying the transporting mechanism of action,[15] we directly detected the transport of chloride across EYPC bilayer membranes by using a conventional fluorescence method based on a chloride-sensitive fluorescent dye, lucigenin.[16] It is known that the fluorescence of lucigenin is quantitatively quenched by chloride and insensitive to nitrate, phosphate and sulfate. Thus, any change in the fluorescence of lucigenin is evidence for the transport of chloride.[17] The data indicate that Bimpy is capable of promoting chloride influx into the EYPC vesicles (Fig. 4a), whereas no chloride transport was observed with Me_2bimpy (data not shown). Interestingly, the chloride transport by Bimpy was significantly suppressed when the internal nitrate was replaced with highly hydrophilic sulfate (Fig. 4b), implying that Cl^-/NO_3^- antiport is predominant in the overall transport process.[18] It should be noted that H^+/Cl^- symport cannot be excluded as the chloride influx was unaffected when sulfate was present in both internal and external vesicles under the condition of which Cl^-/SO_4^{2-} antiport is not favored (Fig. S6).

Table 1
Association constants (K_a's, M^{-1}) of Bimpy and Me_2bimpy with tetrabutylammonium salts[a]

Anion	K_a		
	Bimpy	Me_2bimpy	RA [b]
Cl^-	$(8.71 \pm 3.44) \times 10^3$	$(4.00 \pm 1.08) \times 10^2$	22
Br^-	$(3.04 \pm 0.36) \times 10^3$	$(5.03 \pm 1.65) \times 10^2$	6.0
I^-	$(1.57 \pm 0.06) \times 10^3$	$(7.07 \pm 5.23) \times 10^2$	2.2
NO_3^-	$(4.90 \pm 1.19) \times 10^3$	$(7.23 \pm 3.32) \times 10^2$	6.8

[a] Measured by means of spectrophotometric titrations in a mixture of acetonitrile and water (1:1, v/v) at room temperature.
[b] RA denotes the relative affinity of Bimpy relative to Me_2bimpy.

To gain further insight into the probable mechanism of action and the ion selectivity, we carried out pH discharge experiments with chloride salts of alkali metal ions (i.e., Li^+, Na^+, K^+, Rb^+ and Cs^+) and sodium salts of different anions (i.e., NO_3^-, Cl^-, Br^- and I^-), respectively.[19] It is clear from Figure 5 that the discharge activity is essentially independent of alkali metal ions, excluding the alkali metals in the ion permeation process. In contrast, the pH discharge activity varies in the order of $I^- \approx NO_3^- > Br^- > Cl^-$. These observations imply that the pH gradient decay correlates with OH^-/Cl^- antiport and H^+/Cl^- symport.

In addition, preliminary study indicates that Bimpy displays much lower pH discharge activity across lipid membranes derived from POPC with 30% cholesterol (Fig. 6), indicative of a mobile carrier mechanism.[20]

Taken together, the above-mentioned observations reveal the importance of the imidazolyl-N*H* fragments in the ion-transporting process, and suggest that Bimpy mediates both Cl^-/NO_3^- antiport and H^+/Cl^- symport. This may be due to the ability of Bimpy to bind anions to form complexes of appropriate lipophilicity that are able to diffuse within the membranes. This was evidenced from the above-discussed spectrophotometric titrations as well as the

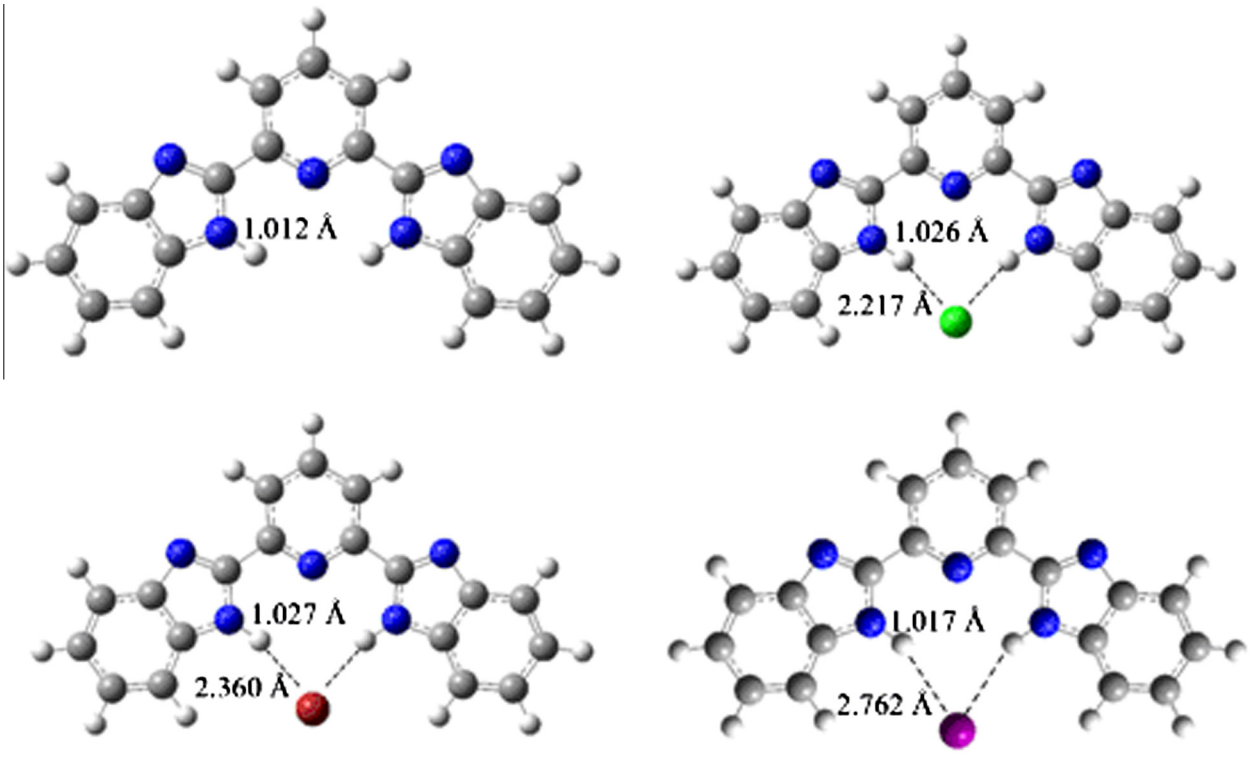

Figure 2. Complexes of Bimpy with Cl^-, Br^- and I^-. Calculations were performed at the M06-2x/6-311++G**/SMD (water) level of theory.