**Roseophilin-inspired derivatives as transmembrane anion carriers**

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**Roseophilin-inspired derivatives as transmembrane anion carriers**

**Abstract:** Roseophilin is an alkaloid structurally related to the prodiginine family. The intriguing pharmacological properties of these derivatives prompted us to prepare synthetic compounds **1**-**3** inspired by its structure and to explore their transmembrane anion transport activity. The inclusion of a methoxyfuran heterocycle in the structure impacts the anionophoric activity of the compounds as a result of the reduced hydrogen-bonding ability and electrostatic repulsions between the oxygen of the furan ring and the anions. The position of the furan in these derivatives compounds was also found crucial for determining their anion transport activity. Overall, replacement of the characteristic methoxypyrrole moiety of prodiginines and tambjamines by the methoxyfuran found in roseophilin is detrimental for their ability as anion carriers, suggesting that the biological activity of roseophilin is likely not related to its potential activity as anion carrier. Compound **2**, bearing a furan ring attached to a dipyrromethene moiety, was found to be the most active anion carrier.

Keywords: supramolecular chemistry, ionophores, alkaloids, roseophilin, vesicles, anion transport

# 1. Introduction

In recent years, development of small molecules capable of promoting the transmembrane transport of anions, anionophores, has attracted significant attention [1-3]. Using core supramolecular concepts, it is possible to apply rational design to produce this type of molecules [4-5]. Biological activities of these compounds and the prospects of developing future drugs based on this mechanism of action has fuelled the interest in this area [6-8]. The alteration of cellular homeostasis could induce cellular toxicity and stress resulting in anticancer or antimicrobial activity [9-13]. Replacing the activity of faulty natural transport proteins could also be an interesting therapeutic approach to conditions such as cystic fibrosis related to this problem [14-16]. In addition to purely synthetic anionophores, natural products represent an important inspiration for the design of these compounds. In particular, prodiginine and tambjamine alkaloids represent examples of biologically active anionophores (Figure 1) [17-18]. These compounds are characterised by a methoxybipyrrole moiety and their intriguing pharmacological properties are linked to their activity as anionophores [19]. Roseophilin represents another group of natural alkaloids related to the prodiginine family (Figure 1) [20]. This product was first isolated from a culture broth of *Streptomices*. Its structure combines a strained macrocycle motif joined to an extended conjugated heterocycle core, responsible of the intense red colour of the compound. Although this product shares with their relatives the azafulvene structure, the methoxypyrrole ring of the prodiginines’ backbone is replaced by a methoxyfuran moiety in roseophilin skeleton. The main biological application of roseophilin is related to its cytotoxicity against some cancer cell lines (human erythroid leukemia and human epidermoid carcinoma) [21-22]. Its mechanism of action is still unknown, although it seems to be not associated to DNA damage under oxidative conditions [23]. Despite the efforts to find an effective total synthesis of this natural product and the different projects to study its biological activity, there are no previous reports about the syntheses of natural product-mimetics of roseophilin and their transmembrane anion transport properties have never been tested either. In this work we decided to prepare derivatives inspired in the structure of roseophilin and to explore their anionophoric properties.

Figure 1. Natural alkaloids

# 2. Materials and methods

## 2.1 General experimental details

All reactions involving air-sensitive compounds were carried out under a nitrogen atmosphere. Oxygen was evacuated and purged with nitrogen, and deoxygenation processes were carried out by bubbling with a continuous nitrogen flow. Starting materials and solvents were obtained from commercial suppliers and used without further purification. TLC analysis was performed on aluminium-backed plates coated with silica gel 60 with F254 indicator or plastic TLC plates coated with aluminium oxide 60 neutral with F254 indicator; the plates were visualized under 254 or 366 nm light. Flash column chromatography was carried out in silica gel 60, 230−350 mesh ASTM; or aluminium oxide 90 activated, neutral, -60 mesh powder, Brockman grade I, 58 Å. NMR spectra were recorded on a Varian Mercury 300 MHz or Bruker Avance III HD 300 MHz. Chemical shifts for 1H and 13C NMR are reported in parts per million (ppm), using the residual solvent peak as reference, and 13C NMR spectra were recorded using broad band proton decoupling. 1H NMR coupling constants are reported in Hz and splitting pattern abbreviations are: s = singlet, t = triplet, dd = doublet of doublets, br = broad, m = multiplet. Multiplicities in 13C NMR (CH3, CH2, CH and quaternary carbons as Cq) were determined by DEPT 135 experiments. High resolution mass spectra (HRMS) were recorded in an Agilent 6545 Q-TOF spectrometer using +ESI and the results are reported as *m/z*.

## 2.2 Synthesis of compounds

Compounds **7** and **9** were prepared according to previously reported synthetic procedures and showed identical spectroscopic properties to those reported therein [24-26].

### Synthesis of (Z)-2-((3-Methoxy-5-(1H-pyrrol-2-yl)furan-2-yl)methylene)-3,5-dimethyl-2H-pyrrol-1-ium chloride(**1**)

2,4-dimethyl-pyrrole (0.11 mL, 1.00 mmol, 2.0 equiv.) was added to a deoxygenated solution of aldehyde **4** (0.10 g, 0.50 mmol, 1.0 equiv.) in methanol (5 mL) under nitrogen. Then, HCl in methanol (0.5 M, 1.97 mL, 1.0 mmol, 2.0 equiv.) was added dropwise to the mixture. The reaction was stirred under nitrogen atmosphere at room temperature for 5 hours. After this time, the crude was concentrated to dryness. Then, the residue was purified by precipitation in a mixture of dichloromethane and diethyl ether. The resulting dark-green precipitate was filtered off in a plate to give the prodiginine-like product **1** (0.11 g, 70%). **1H NMR** (300 MHz, CDCl3) δ (ppm) 14.26 (1H, br s), 12.72 (1H, br s), 7.45-7.40 (1H, m), 7.02-6.97 (1H, m), 6.92 (1H, s), 6.44 (1H, br s), 6.42-6.35 (1H, m), 6.14-6.11 (1H, m), 4.11 (3H, s), 2.79 (3H, s), 2.30 (3H, s); **13C NMR** (75 MHz, CDCl3) δ (ppm) 168.3 (Cq), 164.0 (Cq), 154.9 (Cq), 142.9 (Cq), 136.5 (Cq), 130.1 (CH), 126.2 (Cq), 120.9 (Cq), 118.9 (CH), 117.3 (CH), 113.1 (CH), 111.0 (CH), 95.0 (CH), 60.1 (CH3), 15.4 (CH3), 12.1 (CH3); **HRMS (ESI)** *m/z*: calcd. for C16H17N2O2Na [M+Na]+ 292.1188, found 292.1154; **m.p.** 184-186 oC.

### Synthesis of (Z)-2-((5-(furan-2-yl)-3-methoxy-1H-pyrrol-2-yl)methylene)-3,5-dimethyl-2H-pyrrol-1-ium chloride(**2**)

In a Schlenk flask a solution of aldehyde **7** (0.30 g, 1.57 mmol, 1.0 equiv.) in degassed methanol (25 mL) was deoxygenated with several vacuum/nitrogen cycles. Then, 2,4-dimethylpyrrole (0.32 mL, 3.14 mmol, 2.0 equiv.) was added to the system with a syringe through a septum and, subsequently, HCl in methanol (0.5 M, 6.30 mL, 3.14 mmol, 2.0 equiv.) was added to the mixture dropwise. The resulting orange solution was stirred under nitrogen at room temperature for 16 hours. After this time, methanol was removed by rotary evaporation and the crude was recrystallized in a mixture of dichloromethane and hexane (1:1, *v/v*). The resulting crystalline purple solid was filtrated *in vacuo* and washed with hexane to afford the prodiginine-like product **2** as a dark purple solid (0.34 g, 70%). **1H NMR** (400 MHz, CDCl3) δ (ppm) 13.78 (1H, br s), 13.23 (1H, br s), 8.41 (1H, d, *J* = 3.6 Hz), 7.55 (1H, d, *J* = 1.4 Hz), 7.15 (1H, s), 6.60 (1H, dd, *J* = 3.7, 1.7 Hz), 6.24 (1H, d, *J* = 1.9 Hz), 6.07 (1H, s), 3.99 (3H, s), 2.59 (3H, s), 2.29 (3H, s); **13C NMR** (100 MHz, CDCl3) δ (ppm) 165.3 (Cq), 152.9 (Cq), 145.7 (CH), 145.2 (Cq), 144.0 (Cq), 143.8 (Cq), 126.4 (Cq), 119.5 (Cq), 117.5 (CH), 116.7 (CH), 116.3 (CH), 113.8 (CH), 93.3 (CH), 58.9 (CH3), 14.4 (CH3), 12.1 (CH3); **HRMS (ESI)** *m/z*: calcd. for C16H18N2O2 [M+H]+ 270.1368, found 270.1320; **m.p.** 180-182 oC (dec).

### Synthesis of (E)-N-((3-Methoxy-5-(1H-pyrrol-2-yl)furan-2-yl)methylene)cyclohexanaminium chloride (**3**)

In a round-bottom flask, cyclohexylamine (178 μL, 1.60 mmol, 2.0 equiv.) was added to a solution of aldehyde **4** (0.15 g, 0.80 mmol, 1.0 equiv.) in chloroform (15 mL). Then, acetic acid (100 µL) was added and the reaction mixture was stirred at 65 oC until the starting material was consumed. Once the reaction had finished, the solvent was evaporated under reduced pressure and the crude was washed with 1 M HCl. The organic fractions were combined and dried over anhydrous Na2SO4. After evaporating to dryness, tambjamine **3** was isolated as a black solid (0.20 g, 82%). **1H NMR** (300 MHz, CDCl3) δ (ppm) 12.41 (1H, br s), 11.58 (1H, br s), 7.49 (1H, d, *J* = 14.7 Hz), 7.20-7.12 (1H, m), 6.82-6.75 (1H, m), 6.35-6.30 (1H, m), 6.27-6.20 (1H, m), 4.00 (3H, s), 3.57-3.34 (1H, m), 2.09-2.00 (2H, m), 2.20-1.54 (7H, m), 1.35-1.15 (5H, m); **13C NMR** (75 MHz, CDCl3) δ (ppm) 166.5 (Cq), 160.2 (Cq), 137.7 (CH), 127.9 (Cq), 126.6 (CH), 121.2 (Cq), 114.5 (CH), 111.6 (CH), 93.0 (CH), 60.7 (CH3), 59.8 (CH), 32.9 (CH2), 24.9 (CH2), 24.7 (CH2); **HRMS (ESI)** *m/z*: calcd. for C16H21N2O2 [M]+· 273.1598, found 273.1596; **m.p.** 87-89 oC.

### Synthesis of 3-methoxy-5-(1H-pyrrol-2-yl)-furan-2-carbaldehyde (**4**)

Intermediate **5** (1.25 g, 4.20 mmol, 1.0 equiv.) was dissolved in tetrahydrofuran (6 mL) and a suspension of lithium hydroxide (1.03 g, 42.0 mmol, 10.0 equiv.) in methanol (6 mL) was added dropwise under nitrogen. The mixture was stirred at room temperature for one hour. After this time, the solvent was removed and the resulting solid was re-dissolved in chloroform and this solution washed with water. The organic phase was dried over anhydrous Na2SO4 and concentrated to dryness. The residue was purified by column chromatography in silica gel using a mixture of hexane and ethyl acetate (1:1, *v/v*) as eluent to afford **4** as a dark green solid (0.65 g, 80%). **1H NMR** (300 MHz, CDCl3) δ (ppm) 9.44 (1H, s), 6.93 (1H, br s), 6.69 (1H, br s), 6.33 (1H, s), 6.30 (1H, br s), 3.96 (3H, s); **13C NMR** (75 MHz, CDCl3) δ (ppm) 171.3 (CH), 162.4 (Cq), 153.9 (Cq), 135.6 (Cq), 122.1 (CH), 121.9 (Cq), 110.9 (CH), 110.9 (CH), 93.6 (CH), 59.1 (CH3); **HRMS (ESI)** *m/z*: calcd. for C10H10NO3 [M+H]+ 192.0661; found 192.0657; **m.p.** 135-137 oC.

### Synthesis of 5-(N-tert-butoxycarbonyl-pyrrol-2-yl)-3-methoxy-furan-2-carbaldehyde (**5**)

In a Schlenk flask compound **6** (0.46 g, 2.30 mmol, 1.0 equiv.), *N*-*tert*-butoxycarbonyl -pyrrole-2-boronic acid (0.53 g, 2.50 mmol, 1.1 equiv.) and Pd(PPh3)4 (0.26 g, 0.23 mmol, 0.1 equiv.) were subjected to three vacuum/nitrogen cycles. A deoxygenated mixture of 1,4-dioxane and water (25 mL, 9:1, *v/v*) was added to the Schlenk under an inert atmosphere, followed by Na2CO3 (0.72 g, 6.8 mmol, 3.0 equiv.). The mixture was heated at 110 oC until the total consumption of the starting material. Upon completion of the reaction, it was cooled, quenched with water and neutralised with HCl (38%) to pH 7. The crude was extracted with ethyl acetate and the combined organic phases were dried over anhydrous Na2SO4 and concentrated under vacuum. The residue was then purified by column chromatography in Al2O3, using a mixture of hexane and ethyl acetate (1:1, *v/v*) as eluent to yield **5** as a brown-green oil (0.45 g, 70%). **1H NMR** (300 MHz, Acetone-*d6*) δ (ppm) 9.57 (1H, s), 7.48 (1H, dd, *J* = 3.4, 1.7 Hz), 6.86 (1H, s), 6.71 (1H, dd, *J* = 3.4, 1.7 Hz), 6.33 (1H, t, *J* = 3.4 Hz), 4.02 (3H, s), 1.51 (9H, s); **13C NMR** (75 MHz, Acetone-*d6*) δ (ppm) 172.6 (CH), 152.3 (Cq), 149.2 (Cq), 137.3 (Cq), 126.1 (CH), 124.1 (Cq), 119.0 (CH), 112.0 (CH), 101.5 (CH), 85.5 (Cq), 59.7 (CH3), 27.7 (CH3).

### Synthesis of 5-bromo-3-methoxy-furan-2-carbaldehyde (**6**)

A solution of phosphorous oxybromide (8.43 g, 29.3 mmol, 2.5 equiv.) in chloroform (10 mL) was added to compound **7** (2.00 g, 11.6 mmol, 1.0 equiv.) in chloroform (4 mL) at 0 oC. The mixture was stirred during 10 min at 0 oC and then it was heated for 4 hours at 55 oC. Upon completion of the reaction, it was quenched with water and the organic layer was discarded. The aqueous layer was adjusted to pH 14 with a 2 M NaOH aqueous solution and extracted with ethyl acetate. The combined organic phases were dried over anhydrous Na2SO4 and evaporated to dryness. The residue was purified by column chromatography in silica gel using a mixture of ethyl acetate and hexane (2:1, *v/v*) as eluent to afford aldehyde **6** as an instable dark brown solid (0.80 g, 22%). **1H NMR** (300 MHz, CDCl3) δ (ppm) 9.34 (1H, s), 6.40 (1H, s), 3.95 (3H, s). Spectroscopic data match those previously reported for this compound [24].

### Synthesis of 5-(furan-2-yl)-3-methoxy-1H-pyrrole-2-carbaldehyde (**8**)

In a Schlenk flask precursor **9** (0.25 g, 0.97 mmol, 1.0 equiv.), *N*-Boc-pyrrole-2-boronic acid (0.12 g, 1.06 mmol, 1.1 equiv.) and Pd(PPh3)4 (0.11 g, 0.09 mmol, 0.1 equiv.) were subjected to three vacuum/nitrogen cycles. A deoxygenated mixture of 1,4-dioxane and water (5 mL, 9:1, *v/v*) was added to the Schlenk under an inert atmosphere, followed by Na2CO3 (0.31 g, 2.90 mmol, 3.0 equiv.). The mixture was heated at 110 oC until the total consumption of the starting material. Once the reaction was completed, it was cooled, quenched with water and neutralised with HCl (38%) to pH 7. The crude was extracted with ethyl acetate and the combined organic phases were dried over anhydrous Na2SO4 and concentrated under vacuum. Purification was carried out by column chromatography in silica gel using a mixture of hexane and ethyl acetate (2:1, *v/v*) as eluent to yield aldehyde **8** as a brown solid (0.11 g, 58%). **1H NMR** (300 MHz, CDCl3) δ (ppm) 10.51 (1H, br s), 9.52 (1H, s), 7.44 (1H, dd, *J* = 1.7, 0.7 Hz), 6.93 (1H, d, *J* = 3.4 Hz), 6.47 (1H, dd, *J* = 3.5, 1.8 Hz, 1H), 6.11 (1H, s), 3.89 (3H, s); **13C NMR** (75 MHz, CDCl3) δ (ppm) 174.5 (CH), 159.3 (Cq), 146.4 (Cq), 143.0 (CH), 131.0 (Cq), 119.0 (Cq), 112.2 (CH), 108.3 (CH), 92.2 (CH), 58.1 (CH3); **HRMS (ESI)** *m/z*: calcd. for C10H10NO3 [M+H]+ 192.0660, found 192.0658; **m.p.** 140-142 oC.

## 2.3 Transmembrane anion transport experiments

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Sigma-Aldrich) stock solutions (20 mg/mL = 26.32 mM) were prepared in chloroform and kept in the freezer. For preparing POPC vesicles, 3 mL of the POPC stock solution were added to a round-bottom flask. The solvent was evaporated using a rotary evaporator (25 oC) and lipids were dried overnight under high vacuum. On the next day, the lipid film was rehydrated with 1 mL of the NaCl internal solution corresponding to each assay. Then, the mixture was shaken in a vortex and subjected to seven freeze-thaw cycles (freeze by introducing the flask in a Dewar with liquid nitrogen, and then melting by introducing the flask into warm water). After this process the lipid suspension was extruded twenty-nine times through a 200 nm polycarbonate nucleopore membrane using a LiposoFast Basic extruder (Avestin, Inc.). For removing the non-encapsulated chloride, the lipid suspension was introduced in a dialysis membrane and the packet was dialysed against the required external solution (2 x 500 mL) for around 1.5 hours (45 min with each dialysis solution of 500 mL). Finally, the dialysed suspension of vesicles was placed into a 10-mL volumetric flask and made up with the corresponding external solution to obtain the vesicle stock suspension used in the assays (7.89 mM for 3 mL of POPC).

Chloride efflux experiments were performed using an Ion-Selective Electrode (ISE) [27]. To 5 mL of 0.5 mM POPC suspended in the corresponding external solution, an aliquot of the corresponding anionophore in DMSO was added to the experiment (the volume of DMSO was always less than 20 μL). The chloride efflux from the vesicles was monitored over time using a chloride-selective electrode. For NO3–/Cl–exchange experiments, the intravesicular solution contained 489 mM NaCl, 5 mM NaH2PO4, pH 7.2 (I. S. 500 mM), whereas the extravesicular solution was composed of 489 mM NaNO3, 5 mM NaH2PO4, pH 7.2 (I. S. 500 mM). In these assays, at t = 0 s the carrier was added to the experiment and its activity was recorded with the chloride-selective electrode. At the end of the experiment (t = 300 s), an aliquot of detergent was added to lyse the vesicles and release all the encapsulated chloride; this value was subsequently used to normalise the data. For HCO3–/Cl–exchange assays vesicles, which contained a solution of 451 mM NaCl, 20 mM NaH2PO4, pH 7.2 (I. S. 500 mM), were suspended in an extravesicular solution made of 150 mM Na2SO4, 20 mM NaH2PO4, pH 7.2 (I. S. 500 mM). In these experiments, at t = -10 s a pulse of a HCO3– solution (40 mM HCO3–, 150 mM Na2SO4, 20 mM NaH2PO4, pH 7.2) was added, followed by the compound (t = 0 s). Finally, at t = 300 s an aliquot of a detergent was added to the experiment to lyse the vesicles and release all the entrapped chloride.

# 3. Results and discussion

## Synthesis of compounds

Prodiginines are characterized by a dipyrrolyldipyrromethene structure, whereas in roseophilin the azafulvene moiety is replaced by a methoxyfuran ring (Figure 1). Aiming to evaluate the impact of this major structural change in the anion transport properties of this compound prompted us to design compound **1**. For comparison purposes, the furanyl-pyrrolo prodiginine **2** was also targeted. The synthesis of these compounds is presented in Scheme 1. The pyrrolyl-furan-2-carbaldehyde **4** precursor has been previously described by Challis [24]. We used a slightly modified procedure to prepare furan-2-carbaldehyde **6** using chloroform as solvent, in the presence of an excess of phosphorous oxybromide. A Suzuki-Miyaura coupling reaction with *N-tert-*butoxycarbonyl-2-pyrroleboronic acid, using Pd(PPh3)4 as catalyst and Na2CO3 as base, yielded compound **5**. Finally, base-promoted hydrolysis with lithium hydroxide was used to remove the Boc protecting group, affording pyrrolyl-furan aldehyde **4**. Condensation of **4** and2,4-dimethylpyrrole, using a hydrochloric acid solution as catalyst, provided roseophilin analogue **1** (Scheme 1a).Similarly, prodiginine-like derivative **2** was also prepared by condensation of aldehyde **8** and 2,4-dimethylpyrrole under similar conditions (Scheme 1b).

**Scheme 1.** Synthesis of target compounds **1**-**2**.

Suitable crystals for single-crystal X-ray diffraction analysis of compounds **1** and **2** as their hydrochloride salts were obtained, and the solid-state structures of these derivatives are shown in Figure 2.



**Figure 2**. Solid-state structures of compounds **1** (left) and **2** (right). Atoms: C, light gray; N, blue; O, red; Cl, dark green; H, white.

These solid-state structures confirm that both compounds are forming hydrogen bonding 1:1 complexes with the chloride anion. These derivatives show the expected planarity of the tris-heterocyclic system, reflecting their high conjugation. The tris-heterocycle skeleton of **1** displays a conformation where all heteroatoms from heterocycles are oriented toward the anion [28]. On the other hand, prodiginine **2** exhibits a conformation where the furan ring is flipped away to minimise the electrostatic repulsions of the furan oxygen atom with the anion, thus favouring the coordination of the latter. The NH groups of the pyrrole heterocycles interact, in both cases, with the chloride anion through hydrogen bonds. Moreover, in prodiginine **2** an additional hydrogen bond is formed between the CH group of the furan ring and the chloride anion.

Hydrogen bonds are quite directional and distances and angles can be used as a measurement of their strength, being values close to 180º associated to stronger hydrogen bonds. In the case of **1**, the values of the NH···Cl angles (167.9º and 153.6º) and the length of these non-covalent bonds (3.09 and 3.23 Å) are typical of moderately strong interactions [29-30]. The distance between chloride and the oxygen atom of the furan ring is 3.60 Å. The high aromatic conjugation of **1** is also reflected on the C(sp2)-C(sp2) distances. Thus, the length of the C-C bond connecting the pyrrole and furan is shorter than the typical values of the corresponding single bonds because it has a partial double bond character. NOESY NMR spectra in CDCl3 also supported this disposition as the preferred conformation for **1** in solution (See Figure S22). For compound **2**, the distances of both NH···Cl hydrogen bonds are similar (3.20 and 3.10 Å). Furthermore, the CH···Cl interaction displays a longer hydrogen bonding length (3.51 Å), which is in agreement with the lower strength of this interaction. Regarding the angles, the NH···Cl hydrogen bonds display values of 174.6º and 166.6º, while that of the CH···Cl hydrogen bond is 151.9º. In compound **2** the C-C bonds around the methine group connecting the two pyrrole systems show similar distances (around 1.37 Å) and an angle of 134.7º. As well as in **1**, the C(sp2)-C(sp2) distances in compound **2** are intermediate between the typical values of single and double bonds, which highlights the high aromatic conjugation of the system. All crystallographic data of **2** evidence that its structure is similar to those of the click-prodiginines reported recently by us [31].

**Scheme 2.** Synthesis of compound **3**.

The availability of compound **4** prompted us to explore the preparation of compounds resembling the structure of tambjamines (Scheme 2). Tambjamines are remarkably stable Schiff bases (imines) synthesised by condensation of an amine and a carbonyl compound under mild acidic conditions. Thus, reaction of **4** with cyclohexylamine using acetic acid as catalyst allowed to obtain compound **3** as its hydrochloride salt after washing the crude with a hydrochloric acid solution. It should be noted that when aromatic amines were employed no reaction was observed, precluding the synthesis of aromatic-substituted imines. This result could be explained by the lower nucleophilicity of aromatic amines compared to aliphatic amines and the low electrophilicity of the carbonyl carbon of this derivative.

## Anion transport experiments

Anion transport assays were carried out in 1‐palmitoyl‐2‐oleoyl‐*sn*‐glycero‐3‐phosphocholine (POPC) vesicles using a chloride-selective electrode. Full details on vesicles preparation and performance of the assays are described in the experimental section. The NO3–/Cl– exchange and the HCO3–/Cl– exchange assays were explored. Aliquots of DMSO solutions of the studied compounds were added to a suspension of the chloride-containing vesicles. Chloride efflux was monitored with a chloride-selective electrode and a detergent was added at the end of the experiments to lyse the vesicles and release all the encapsulated chloride, being this value used as 100% of chloride efflux. Performing the assays at various concentrations allows Hill analysis of the chloride efflux observed at 300 seconds and the calculation of the EC50 and Hill parameter *n*. EC50 represents the concentration needed to elicit 50% chloride release under the conditions explored. A representative example is shown in Figure 3.



**Figure 3**. Chloride efflux promoted by **1** at different concentrations in unilamellar POPC vesicles containing a NaCl solution (489 mM NaCl and 5 mM NaH2PO4, pH 7.2). Left: vesicles were suspended in an isotonic NaNO3 solution (489 mM NaNO3 and 5 mM NaH2PO4, pH 7.2). Right: vesicles were suspended in an isotonic Na2SO4/NaHCO3 solution (150 mM Na2SO4, 40 mM HCO3– and 20 mM NaH2PO4, pH 7.2). Each trace represents the average of at least three different experiments, performed with three batches of vesicles.

**Table 1.** Summary of the transport activity calculated for **1**-**3** in the different assays.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **EC50 (M)****NO3–/Cl–** | **EC50 (mol%)****NO3–/Cl–** | **Hill parameter, *n*****NO3–/Cl–** | **EC50 (M)****HCO3–/Cl–** | **EC50 (mol%)****HCO3–/Cl–** | **Hill parameter, *n*****HCO3–/Cl–** |
| **1** | 1.513 ± 0.166 | 0.303 ± 0.033 | 1.04 ± 0.10 | -a | -a | -a |
| **2** | 0.059 ± 0.002 | 0.0117 ± 0.0004 | 1.06 ± 0.04 | 4.335± 0.755 | 0.867 ± 0.151  | 0.84 ± 0.14 |
| **3** | -a | -a | -a | -a | -a | -a |

aThe low activity precluded the calculation of these parameters.

Compound **2** was found to be the most active in these assays, displaying a submicromolar EC50 value in the NO3–/Cl–exchange assay. This result is in agreement with the better stabilisation of the anion suggested by the solid-state structure of this compound (Figure 2), showing the involvement of the furan C-H in the hydrogen-bond cleft displayed by this derivative. Incorporation of the furan heterocycle as the B ring in roseophilin-inspired derivatives **1** and **3** is clearly deleterious for the transmembrane transport activity of these derivatives. When comparing the calculated EC50 for **1** and **2** a 25-fold increase is observed. A release of less than 50% chloride was observed for **3** even when explored at 1% under these conditions (see Figure S30). It should be noted that the parent prodiginine and tambjamine analogues of **1** and **3** are very active transmembrane transporters [32-33]. The marked drop in chloride efflux observed when the lipophilic nitrate is replaced by the more hydrophilic bicarbonate (and sulfate) is a result commonly observed in these experiments. It can be interpreted as the result of the differences in the ease of the anion to be extracted into the membrane. These results also support an exchange mechanism as the main anion transport mechanism and rule out detergent effects exerted by these compounds.

# 4. Conclusions

Inspired by the structure of the natural product roseophilin, compounds **1**-**3** were prepared and characterised. These derivatives include a furan ring in their structure and are structurally related to other alkaloids such as prodiginines and tambjamines. The anionophoric activity of these compounds was tested in model phospholipid vesicles using ISE-based assays. The results demonstrated that the substitution of a pyrrole ring by a furan heterocycle in these natural product mimetics is detrimental for the anionophoric activity of the compounds. The elimination of a hydrogen-bonding interaction with the anion, together with electrostatic repulsions between the oxygen of the furan ring of **1** and **3** and chloride, could explain these results. Moreover, a comparison between the anionophoric properties of prodiginines **1** and **2** showed that the position of the furan in these derivatives compounds was crucial for the anion transport activity, being compound **2** in which the furan ring is attached to the dipyrromethene moiety the most active derivative. These results suggest that, differently from prodiginines and tambjamines, the biological activity of roseophilin is likely not related to its potential activity as anion carrier.

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# Disclosure statement

No potential conflict of interest was reported by the author(s).

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# References

[1] Davis JT, Gale PA, Quesada R. Advances in anion transport and supramolecular medicinal chemistry. Chem Soc Rev. **2020**;49:6056–6086. DOI: 10.1039/C9CS00662A.

[2] Wu X, Gilchrist AM, Gale PA. Prospects and Challenges in Anion Recognition and Transport. Chem. **2020**;6: 1296–1309. DOI: 10.1016/j.chempr.2020.05.001.

[3] Busschaert N, Caltagirone C, Van Rossom W, Gale PA. Applications of Supramolecular Anion Recognition. Chem Rev. **2015**;115:8038–8155. DOI: 10.1021/acs.chemrev.5b00099.

[4] Bickerton LE, Johnson TG, Kerckhoffs A, Langton MJ. Supramolecular chemistry in lipid bilayer membranes Chem Sci. **2021**;12:11252–11274. DOI: 10.1039/D1SC03545B.

[5] Spooner MJ, Li H, Marques I, Costa PMR, Wu X, Howe ENW, Busschaert N, Moore SJ, Light ME, Sheppard DN, Félix V, Gale PA. Fluorinated synthetic anion carriers: experimental and computational insights into transmembrane chloride transport. Chem Sci. **2019**;10(7):1976–1985. DOI: 10.1039/C8SC05155K.

[6] Picci G, Marchesan S, Caltagirone C. Ion Channels and Transporters as Therapeutic Agents: From Biomolecules to Supramolecular Medicinal Chemistry. Biomedicines. **2022**;10(4):885–908. DOI: 10.3390/biomedicines10040885.

[7] Roy A, Talukdar P. Recent Advances in Bioactive Artificial Ionophores. Chem Bio Chem. **2021**;22:2925–2940. DOI: 10.1002/cbic.202100112.

[8] Akhtar N, Biswas O, Manna D. Biological applications of synthetic anion transporters. Chem Commun. **2020**;56:14137–14153. DOI: 10.1039/D0CC05489E.

[9] Akhtar N, Pradhan N, Saha A, Kumar V, Biswas O, Dey S, Shah M, Kumar S, Manna D. Tuning the solubility of ionophores: glutathione-mediated transport of chloride ions across hydrophobic membranes. Chem Commun. **2019**;55:8482–8485. DOI: 10.1039/C9CC04518J.

[10] Ko SK, Kim SK, Share A, Lynch VM, Park J, Namkung W, Van Rossom W, Busschaert N, Gale PA, Sessler JL, Shin I. Synthetic ion transporters can induce apoptosis by facilitating chloride anion transport into cells. Nat Chem. **2014**;6(10):885–892. DOI: 10.1038/nchem.2021.

[11] Rodilla L, Korrodi-Gregório AM, Hernando E, Manuel-Manresa P, Quesada R, Pérez-Tomás R, Soto-Cerrato V. Synthetic tambjamine analogues induce mitochondrial swelling and lysosomal dysfunction leading to autophagy blockade and necrotic cell death in lung cancer. Biochem Pharmacol. **2017**;126:23–33. DOI: 10.1016/j.bcp.2016.11.022.

[12] Park S-H, Park S-H, Howe ENW, Hyun JL, Chen L-J, Hwang I, Vargas-Zuñiga, G, Busschaert N, Gale PA, Sessler JL, Shin I. Determinants of Ion-Transporter Cancer Cell Death. Chem. **2019**;5: 2079−2098. DOI: 10.1016/j.chempr.2019.05.001.

[13] Carreira-Barral I, Rumbo C, Mielczarek M, Alonso-Carrillo D, Herran E, Pastor M, Del Pozo A, García-Valverde M, Quesada R. Small molecule anion transporters display in vitro antimicrobial activity against clinically relevant bacterial strains. Chem Commun. **2019**;55:10080−10083. DOI: 10.1039/C9CC04304G.

 [14] Cossu C, Fiore M, Baroni D, Capurro V, Caci E, García-Valverde M, Quesada R, Moran O. Anion-transport mechanism of a triazole-bearing derivative of prodigiosine: A candidate for cystic fibrosis therapy. Front Pharmacol. **2018**;9:852−865. DOI: 10.3389/fphar.2018.00852.

[15] Li H, Valkenier H, Thorne AG, Dias CM, Cooper JA, Kieffer M, Busschaert N, Gale PA, Sheppard DN, Davis AP. Anion carriers as potential treatments for cystic fibrosis: transport in cystic fibrosis cells, and additivity to channel-targeting drugs. Chem Sci. **2019**;10(42):9663−9672. DOI: 10.1039/C9SC04242C.

[16] Gianotti A.; Capurro V, Delpiano L, Mielczarek M, García-Valverde M, Carreira-Barral I, Ludovico A, Fiore M, Baroni D, Moran O, Quesada R, Caci E. Small Molecule Anion Carriers Correct Abnormal Airway Surface Liquid Properties in Cystic Fibrosis Airway Epithelia. Int J Mol Sci. **2020**;21:1488−1502. DOI: 10.3390/ijms21041488.

 [17] Fürstner A. Chemistry and Biology of Roseophilin and the Prodigiosin Alkaloids: A Survey of the Last 2500 Years. Angew Chem Int Ed. **2003**;42:3582−3603. DOI: 10.1002/anie.200300582.

[18] Pinkerton DM, Banwell MG, Willis AC. Total Syntheses of Tambjamines C, E, F, G, H, I and J, BE-18591, and a Related Alkaloid from the Marine Bacterium Pseudoalteromonas tunicate. Org Lett. **2007**;9:5127−5130. DOI: 10.1021/ol7024313.

[19] Sakai-Kawada FE, Ip CG, Hagiwara KA, Awaya JD. Biosynthesis and Bioactivity of Prodiginine Analogs in Marine Bacteria, Pseudoalteromonas: A Mini Review. Front Microbiol. **2019**;10:1715−1723. DOI: 10.3389/fmicb.2019.01715.

[20] Hayakawa Y, Kawakami K, Seto H, Furihata K. Structure of a new antibiotic, roseophilin. Tetrahedron Lett. **1992**;33:2701−2704. DOI: 10.1016/S0040-4039(00)79061-7.

[21] Zhang X, Zhang C, Wang X, Liang G. Mild Friedel–Crafts Reactions Enable a Robust Synthesis of Roseophilin. Org Lett. **2019**;21:3357−3360. DOI: 10.1021/acs.orglett.9b01100.

[22] Fürstner A, Weintritt H. Total Synthesis of Roseophilin. J Am Chem. Soc. **1998**;120:2817−2825. DOI: 10.1021/ja973846k.

[23] Fürstner A, Grabowski EJ. Studies on DNA Cleavage by Cytotoxic Pyrrole Alkaloids Reveal the Distinctly Different Behavior of Roseophilin and Prodigiosin Derivatives. ChemBioChem. **2001**;2:706−709. DOI: 10.1002/1439-7633(20010903)2:9<706::AID-CBIC706>3.0.CO;2-J.

[24] Haynes SW, Sydor PK, Stanley AE, Song L, Challis GL. Role and substrate specificity of the Streptomyces coelicolor RedH enzyme in undecylprodiginine biosynthesisChem Commun. **2008**;1865−1867. DOI: 10.1039/B801677A.

[25] Daïri K, Yao Y, Faley M, Tripathy S, Rioux E, Billot X, Rabouin D, Gonzalez G, Lavallée J-F, Attardo G. A Scalable Process for the Synthesis of the Bcl Inhibitor Obatoclax. Org Process Res Dev. **2007**;11:1051−1054. DOI: 10.1021/op7001613.

[26] Aldrich LN, Dawson ES, Lindsley CW. Evaluation of the Biosynthetic Proposal for the Synthesis of Marineosins A and B. Org Lett. **2010**;12:1048−1051. DOI: 10.1021/ol100034p.

[27] Jowett LA, Gale PA. Supramolecular methods: the chloride/nitrate transmembrane exchange assay, Supramolecular Chemistry. **2019**;31:5,297−312. DOI: 10.1080/10610278.2019.1574017.

[28] García-Valverde M, Alfonso I, Quiñonero D, et al. Conformational Analysis of a Model Synthetic Prodiginine. J Org Chem. **2012**;77:6538−6544. DOI: 10.1021/jo301008c.

[29] Jeffrey GA. An Introduction to Hydrogen Bonding. Oxford University Press, Oxford, UK; **1997**.

[30] Steiner T. The Hydrogen Bond in the Solid State. Angew Chem Int Ed. **2002**;41: 48–76. DOI: 10.1002/1521-3773(20020104)41:1<48::AID-ANIE48>3.0.CO;2-U

[31] Carreira-Barral I, Mielczarek M, Alonso-Carrillo D, Capurro V, Soto-Cerrato V, Pérez-Tomás R, Caci E, García-Valverde M, Quesada R. Chem Commun. **2020**;56:3218–3221. DOI: 10.1039/D0CC00643B

[32] Díaz de Greñu B, Iglesias Hernández P, Espona M, Quiñonero E, Light ME, Torroba T, Pérez-Tomás R, Quesada R. Synthetic Prodiginine Obatoclax (GX15-070) and Related Analogues: Anion Binding, Transmembrane Transport, and Cytotoxicity Properties. Chem Eur J. **2011**;17:14074–14083 DOI: 10.1002/chem.201101547

[33] Knight NJ, Hernando E, Haynes CJE, Buschaert N, Clarke HJ, Takimoto K, García-Valverde M, Frey JG, Quesada R, Gale PA. QSAR analysis of substituent effects on tambjamine anion transporters. Chem Sci. **2016;**7:1600–1608. DOI: 10.1039/c5sc03932k