ELECTRONIC SUPPORTING INFORMATION

Structure–Permeability Relationships of Vitamin-B-Based Quaternary Ammonium Salts for Oral Drug Development

Adriana Olejniczak[1], Mara G. Freire[2], Michał Niemczak[1]*

[1] Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, Poznan 61-131, Poland

[2] CICECO-Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal

*Corresponding author: Michał Niemczak, Poznan University of Technology, Berdychowo 4, Poznan 61-131, Poland; orcid.org/0000-0002-4364-8267; Phone: +48 616653681; Email: michal.niemczak@put.poznan.pl

1. Synthesis of 1-8, [NA][NIC] and [NA][PABA]

Initially, nicotinic acid (NIC) and *p*-aminobenzoic acid (PABA) were neutralized with stoichiometric amounts of potassium hydroxide in dehydrated methanol in a EasyMax 102 semi-automated system (Mettler Toledo, Switzerland) equipped with a pH-meter with calibrated glass electrode. Then, the solvent was evaporated using a rotary vacuum evaporator. The obtained potassium nicotinate ([K][NIC]) and potassium *p*-aminobenzoate ([K][PABA]) were dried in a vacuum oven at 25°C for 24 h. Appropriate *N*-alkylnicotinamide bromides were obtained *via* quaternization of nicotinamide (NA) with the corresponding alkyl bromide in prOH. Subsequently, the products were filtered off, washed and dried in a vacuum oven under reduced pressure (1–2 mbar) at 25°C for 24 h.

1.1. Preparation of *N*-alkylnicotinamide nicotinates (1–4)

0.01 mol of an appropriate *N*-alkylnicotinamide bromide (**P1**–**P4**) was dissolved in 10 mL of dehydrated methanol in round-bottomed flask equipped with a mechanical stirrer. Next, [K][NIC] dissolved in 10 mL of dehydrated methanol, was added with a 2% of molar excess (0.0102 mol) to perform the ion exchange reaction. The reaction mixture was stirred at 40 °C for 15 min and then cooled to 0 °C. As a result of anion exchange, a sediment of potassium bromide precipitated from the post-reaction mixture. Subsequently, the inorganic salt was filtered off and the solvent was evaporated from the filtrate. The obtained products were additionally purified by the addition of a small portion (10–15 mL) of acetone, which enabled to isolate the residues of inorganic impurities and excess reactant through vacuum filtration. After evaporation of acetone, the obtained products were dried in a vacuum oven under reduced pressure (1–2 mbar) at 25°C for 24 h.

1.2. Preparation of *N*-alkylnicotinamide *p*-aminobenzoates (5–8)

0.01 mol of an appropriate *N*-alkylnicotinamide bromide was dissolved in 10 mL of dehydrated methanol in round-bottomed flask equipped with a mechanical stirrer. Next, [K][PABA] dissolved in 10 mL of methanol, was added with a 2% of molar excess (0.0102 mol) to perform the ion exchange reaction. The reaction mixture was stirred at 40 °C for 15 min and then cooled to 0 °C. As a result of anion exchange, a sediment of potassium bromide precipitated from the post-reaction mixture. Subsequently, the inorganic salt was filtered off and the solvent was evaporated from the filtrate. The obtained products were additionally purified by the addition of mixture of hot acetone and methanol (20-25 mL) in proportion 20:1 followed by vacuum filtration. This action allowed to isolate the desired product, which precipitated from cooling

filtrate and was separated by vacuum filtration. Finally, the obtained compounds were dried in a vacuum oven under reduced pressure (1–2 mbar) at 25°C for 24 h.

1.3. Preparation of [NA][NIC] and [NA][PABA]

The preparation of nicotinamide (NA) and nicotinic acid (NIC) mixture was accomplished by mixing the appropriate amount of nicotinamide (0.01 mol) with the equimolar amount of nicotinic acid in 25 mL of methanol, and stirred for 2 h at room temperature. The remaining solvent was then removed using a rotary evaporator. The same methodology was applied for synthesis of nicotinamide (NA) and *p*-aminobenzoic acid (PABA). Finally, **[NA][NIC]** and **[NA][PABA]** were dried in a vacuum oven under reduced pressure (1–2 mbar) at 25°C for 24 h.

2. Determination of purity - LC/MS/TOF analysis

The purity of products was analyzed in 3 replicates using liquid chromatography/time-of-flight/mass spectrometry (LC/MS/TOF). The analysis was performed using a 1290 Infinity UHPLC system coupled with a 6546 Quadrupole Time-of-Flight Mass Spectrometer with a Dual AJS ESI source (Agilent Technologies, Santa Clara). Chromatographic separation was performed using a ZORBAX RRHD Eclipse Plus C18 column 2.1 x 50 mm, 1.8 µm (Agilent Technologies, Santa Clara), which was maintained at a temperature of 35°C. The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile). The injection volume was 3 µL. Compound detection was carried out in positive electrospray ionization mode using the Dual Agilent Jet Stream source. Parameters were set as follows: drying gas temperature, 320°C; drying gas flow, 10 L/min; nebulizer pressure: 45 psi, sheath gas temp: 350°C, sheath gas flow: 12 L/min, capillary entrance voltage 4000 V, nozzle voltage 0 V. Stock solutions of each compound were prepared by accurately weighing an appropriate amount of substance and dissolving it in water, followed by dilution to obtain the desired mass spectrometric response.

Table S1. Analysis of purity of the obtained products

Compound	Alkyl chain (R)	LC/MS/TOF Analysis (%)
1	C_6H_{13}	99.2
2	C_8H_{17}	99.5
3	$C_{10}H_{21}$	99.4
4	$C_{12}H_{25}$	98.6
5	C_6H_{13}	98.5
6	C_8H_{17}	99.4
7	$C_{10}H_{21}$	99.3
8	$C_{12}H_{25}$	99.2
[NA][NIC]	---	99.7
[NA][PABA]	---	99.4

Table S2. *In silico* calculations regarding key pharmacokinetic parameters of QASs **1-8**, **[NA][NIC]**, **[NA][PABA]** and DFC-Na.

Compound	Alkyl chain (R)	Caco2 permeability (Papp in 10^{-6} cm/s)	Intestinal absorption (%)	Fraction unbound	Water solubility (mg/mL)
1	C_6H_{13}	1.55	59.0	0.469	66.6
2	C_8H_{17}	20.89	61.1	0.379	31.1
3	$C_{10}H_{21}$	20.89	62.6	0.293	17.8
4	$C_{12}H_{25}$	20.89	64.1	0.218	5.0
5	C_6H_{13}	1.29	57.2	0.373	33.4
6	C_8H_{17}	3.02	58.6	0.287	15.5
7	$C_{10}H_{21}$	3.02	60.0	0.209	8.8
8	$C_{12}H_{25}$	3.02	61.5	0.145	2.5
[NA][NIC]	---	1.10	53.6	0.434	40.8
[NA][PABA]	---	0.90	53.6	0.335	2.39
DFC-Na	---	16.98	77.0	0.101	0.01

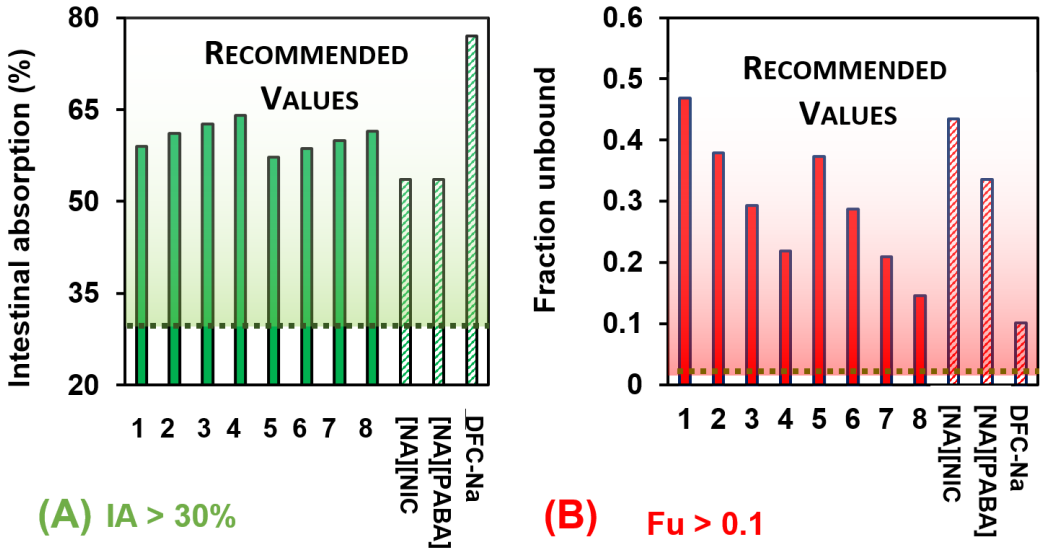

Figure S1. Calculated values of absorption in the human intestine (**A**) and unbound fraction (**B**) for the studied compounds and DFC-Na. Colored zones indicate the minimum threshold for a candidate to be considered as a potentially effective drug.

Table S3. The permeability coefficients determined after 6 hours for tested QASs **1-8**, **[NA][NIC]**, **[NA][PABA]** and DFC-Na.

Compound	Alkyl chain (R)	Permeability coefficient (P_{app} in 10^{-6} cm/s)
1	C_6H_{13}	5.46± 0.19
2	C_8H_{17}	4.66±0.16
3	$C_{10}H_{21}$	5.20±0.05
4	$C_{12}H_{25}$	5.82±0.01
5	C_6H_{13}	6.05±0.15
6	C_8H_{17}	4.21±0.10
7	$C_{10}H_{21}$	4.57±0.08
8	$C_{12}H_{25}$	5.23±0.01
[NA][NIC]	---	5.80±0.08
[NA][PABA]	---	6.02±0.10
DFC-Na	---	5.91±0.10

Table S4. The permeation percentage determined after 0.5, 1, 6 and 16 hours for **1**–**4**, **[NA][NIC]** and DFC-Na.

Compound	Alkyl chain (R)	Permeation percentage (%)			
		Time (h)			
		0.5	1	6	16
1	C_6H_{13}	7.8±0.2 a*	16.3±0.1 a	21.6±0.7 a	21.9±0.4 a
2	C_8H_{17}	8.6±0.2 a	15.5±0.1 a	18.8±0.4 a	22.6±0.2 ab
3	$C_{10}H_{21}$	9.3±0.1 a	16.0±0.3 a	20.7±0.6 a	22.8±0.1 ab
4	$C_{12}H_{25}$	13.0±0.3 b	18.4±0.2 b	22.3±0.4 ab	22.9±0.4 ab
[NA][NIC]	---	13.8±0.3 b	17.8±0.7 ab	22.8±0.3 b	23.2±0.2 b
DFC-Na	---	10.5±0.6 ab	17.4±0.5 ab	23.2±0.3 b	25.4±0.6 c

*The same letters mean there is no statistically significant difference.

Table S5. Statistical analysis comparing differences between specific time intervals for **1**–**4**, **[NA][NIC]** and DFC-Na.

Compound	Time (h)		
	0.5 *vs* 1	**1 *vs* 6**	**6 *vs* 16**
1	***	**	SI
2	***	**	SI
3	***	**	SI
4	***	**	SI
[NA][NIC]	***	**	SI
DFC-Na	***	**	*

*p < 0.05, **p < 0.01, ***p < 0.001, SI – statistically insignificant

Table S6. The permeation percentage determined after 0.5, 1, 6 and 16 hours for **5**–**8**, **[NA][PABA]** and DFC-Na.

Compound	Alkyl chain (R)	Permeation percentage (%)			
		Time (h)			
		0.5	1	6	16
5	C_6H_{13}	16.4±0.6 a*	20.7±0.6 a	23.7±0.5 a	28.6±0.2 a
6	C_8H_{17}	14.8±0.4 b	16.1±0.0 ab	18.0±0.7 a	23.7±0.4 a
7	$C_{10}H_{21}$	14.5±0.5 b	14.5±0.3 ab	18.5±0.9 b	22.4±0.1 b
8	$C_{12}H_{25}$	16.3±0.6 bc	17.4±0.3 ab	20.4±0.3 c	21.3±0.1 b
[NA][PABA]	---	17.5±0.9 bc	22.2±0.2 bc	23.6±0.4 c	27.6±0.3 c
DCF-Na	---	10.5±0.6 b	17.4±0.5 b	23.2±0.3 c	25.39±0.6 c

*The same letters mean there is no statistically significant difference.

Table S7. Statistical analysis comparing differences between specific time intervals for **1**–**4**, **[NA][NIC]** and DFC-Na.

Compound	Time (h)		
	0.5 *vs* 1	**1 *vs* 6**	**6 *vs* 16**
5	**	**	***
6	*	**	***
7	SI	**	***
8	SI	**	*
[NA][PABA]	**	*	***
DCF-Na	***	**	*

*p < 0.05, **p < 0.01, ***p < 0.001, SI – statistically insignificant

Table S8. Fitting of permeation data to Higuchi and first-order kinetic models and corresponding permeation parameters (v_0 and Q_p) for QASs **1-8**, **[NA][NIC]**, **[NA][PABA]** and DFC-Na.

Comp.	Higuchi model $Q = k \cdot t^{1/2}$		First-order kinetics model $\ln(1-Q/Q_{max}) = -k \cdot t$		Initial rate v_0 ($\%\cdot h^{-1}$)	Plateou Q_p (%)
	y= a·x+b	**R^2**	**y= a·x+b**	**R^2**		
1	y = 3.5584x + 9.6450	0.6665	y = 0.7042x + 0.2315	0.9772	**17.1**	**21.7**
2	y = 3.5642x + 9.1008	0.8200	y = 0.331x - 0.3497	0.5737	**14.0**	**20.7**
3	y = 3.5196x + 10.0025	0.7890	y = 0.3504x + 0.3775	0.8803	**13.5**	**21.7**
4	y = 2.5495x + 13.9318	0.7237	y = 0.5379x + 0.5231	0.9162	**10.8**	**22.6**
5	y = 3.2803x + 15.6573	0.9288	y = 0.1354x + 0.9558	0.8338	**8.6**	**26.2**
6	y = 2.5783x + 13.1239	0.9858	y = 0.2127x + 0.5719	0.6307	**2.6**	**21.3**
7	y = 2.3364x + 12.6724	0.9798	y = 0.1797x + 0.5886	0.5613	**1.6**	**20.0**
8	y = 1.5014x + 15.7759	0.9122	y = 0.1715x + 0.6116	0.5209	**2.2**	**20.8**
[NA][NIC]	y = 2.6252x + 14.0482	0.7814	y = 0.6152x + 0.4533	0.9579	**8.2**	**23.0**
[NA][PABA]	y = 2.5850x + 17.3441	0.8825	y = 0.2235x + 0.7042	0.5502	**8.5**	**25.6**
DFC-Na	y = 3.9765x + 10.9918	0.8121	y = 0.3586x + 0.3578	0.9022	**13.8**	**24.3**

Q = permeation percentage (%), Q_{max} = permeation percentage (%) after 16h; k = permeation constant;
$v_0 = (Q_{1h}-Q_{0.5h})(t_{1h}-t_{0.5h})$; $Qp = (Q_{6h}+Q_{16h})/2$

Table S9. The surface tension and surface pressure for tested compounds.

Compound	Alkyl chain (R)	Surface tension ($mN\cdot m^{-1}$)	Surface pressure ($mN\cdot m^{-1}$)
1	C_6H_{13}	62.4 + 0.2	9.2
2	C_8H_{17}	57.9 + 0.1	13.6
3	$C_{10}H_{21}$	45.2 + 0.1	26.4
4	$C_{12}H_{25}$	32.6 + 0.1	39.0
5	C_6H_{13}	70.4 + 0.2	1.2
6	C_8H_{17}	67.2 + 0.1	4.4
7	$C_{10}H_{21}$	57.8 + 0.1	13.8
8	$C_{12}H_{25}$	39.4 + 0.1	32.2
PBS	---	71.6 + 0.8	-
Water	---	72.7 + 0.1	-