10.1071/CH19479_AC

Australian Journal of Chemistry 2020, 73(4), 344-351

Supplementary Material

High Cell Permeability Does Not Predict Oral Bioavailability for Analogues of Cyclic Heptapeptide Sanguinamide A

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COMPOUND 1

UPLC R_t = 5.74 min (0-100% B in 6.00 min). HRMS-TOF (m/z): [M + H]+ calcd for $C_{40}H_{58}N_7O_6S^+$, 764.4164; found, 764.4166.

COMPOUND 2

UPLC R_t = 5.91 min (0-100% B in 6.00 min). HRMS-TOF (m/z): [M + H]+ calcd for $C_{41}H_{60}N_7O_6S^+$, 778.4320; found, 778.4323.

COMPOUND 3

UPLC R_t = 4.20 min (Method A), HRMS-TOF (m/z): [M + H]+ calcd for $C_{40}H_{58}N_7O_6S^+$, 764.4164; found, 764.4164.

COMPOUND 4

UPLC R_t = 3.87 min (0-100% B in 6.00 min). HRMS-TOF (m/z): [M + H]+ calcd for $C_{41}H_{60}N_7O_6S^+$, 778.4320; found, 778.4320.

COMPOUND 5

UPLC R_t = 4.26 min (Method A), HRMS-TOF (m/z): [M + H]+ calcd for $C_{41}H_{60}N_7O_6S^+$, 764.4164; found, 764.4166.

COMPOUND 6

UPLC R_t = 4.36 min (Method A), HRMS-TOF (m/z): [M + Na]+ calcd for $C_{41}H_{59}N_7O_6SNa^+$, 800.4140; found, 800.4133.

Table S1 – Assignment of δ ^{1}H (ppm), multiplicity and J (Hz) for **1** and **2**

Residue	Atom	**Compound 1**	Residue	Atom	**Compound 2**
Ile1	NH	8.14 d (7.8)	Ile1	NH	8.24 d (9.2)
	αCH	5.09 dd (6.0, 7.3)		αCH	5.33 dd (9.3, 4.5)
	βCH_2	1.73 m		βCH_2	2.00 m
	$\gamma 1CH_2$	1.29 m, 1.13 m		$\gamma 1CH_2$	1.39 m, 1.24 m
	$\gamma 2CH_3$	0.85 t (7.5)		$\gamma 2CH_3$	0.93 m
	δCH_3	0.73 d (6.8)		δCH_3	0.92 m
Thz	CH (3)	8.26 s	Thz	CH (3)	8.28 s
Ala 2	NH	8.04 d (6.4)	tBuGly 2	NH	7.84 d (9.4)
	αCH	4.57 dt (6.4, 6.8)		αCH	5.15 d (9.4)
	βCH_3	1.26 d (6.4)		βCH_3	0.96 s
Cha 3	NH	8.28 d(2.5)	Phe 3	NCH_3	3.25 s
	αCH	3.93 m		αCH	4.55 m
	βCH_2	1.47 m, 1.36 m		βCH_2	3.27 m, 3.01 m
	γC	1.16 m		δCH_2	7.26-7.22 m
	δCH_2	1.13-0.75 m		εCH_2	7.33-7.29 m
	εCH_2	1.13-0.75 m		ζCH	7.26-7.24 m
	ζCH	1.13-0.75 m	Pro 4	N	
Pro 4	N			αCH	3.7 d (7.1)
	αCH	4.22 d (8.1)		βCH_2	1.99 m, 0.55 m
	βCH_2	2.05 m		γCH_2	1.58 m, 1.28 m
	γCH_2	1.86 m		δCH_2	3.4 m, 3.0 m
	δCH_2	3.44 m, 3.32 m	Ile 5	NH	9.14 d (8.7)
Ile 5	NH	9.15 d (8.1)		αCH	4.22 m
	αCH	4.35 t (9.0)		βCH_2	1.97 m
	βCH_2	2.19 m		$\gamma 1CH_2$	1.38 m, 1.08 m
	$\gamma 1CH_2$	1.63 m, 1.13 m		$\gamma 2CH_3$	0.89 d (6.8)
	$\gamma 2CH_3$	0.85 m		δCH_3	0.72 t (7.4)
	δCH_3	0.96 d (6.9)	Pro 6	N	
Pro 6	N			αCH	4.2 m
	αCH	4.45 d (7.9)		βCH_2	2.04 m, 1.77 m
	βCH_2	1.64 m		γCH_2	2.04 m, 1.77 m
	γCH_2	1.99 m		δCH_2	3.8 m, 3.5 dd (15.0, 7.8)
	δCH_2	3.88 m, 3.67 t (8.7)		βCH_2	2.04 m, 1.77 m
				γCH_2	2.04 m, 1.77 m
				δCH_2	3.8 m, 3.5 dd (15.0, 7.8)

Table S2 – Assignment of δ ^{1}H (ppm), multiplicity and J (Hz) for **4** and **3**

Residue	Atom	**Compound 4**	Residue	Atom	**Compound 3**
Ile 1	NH	8.27 d (9.6)	Ile1	NH	8.57 d (7.7)
	αCH	5.32 dd (9.6, 3.6)		αCH	5.00 t (7.5)
	βCH_2	2.06 m		βCH_2	1.70 m
	$\gamma 1CH_2$	1.36 m		$\gamma 1CH_2$	1.43 m, 1.06 m
	$\gamma 2CH_3$	0.93 m		$\gamma 2CH_3$	0.70 d (6.7)
	δCH_3	0.94 t (6.7)		δCH_3	0.84 t (7.3)
Thz	CH (3)	8.33	Thz	CH (3)	8.26 s
Ile 2	NH	7.81 d (9.7)	Ile 2	NH	7.86 d (8.3)
	αCH	4.88 t (9.5)		αCH	4.54 m
	βCH_3	1.83 m		βCH	1.72 m
	$\gamma 1CH_2$	1.38 m, 1.05 m		$\gamma 1CH_3$	1.19 m
	$\gamma 2CH_3$	0.84 d (6.6)		$\gamma 2CH_3$	0.95 d (6.7)
	δCH_3	0.81 t (7.4)		δCH_2	0.91 t (7.4)
Phe 3	NCH_3	3.16 s	Phe 3	NN	9.09 d (1.7)
	αCH	4.31 m		αCH	4.17 ddd (12.7, 5.1, 2.1)
	βCH_2	3.30 m, 2.96 t (11.6)		βCH_2	3.06 dd (12.7, 5.3), 2.80 t (12.1)
	δCH_2	7.24-7.20 m		δCH_2	7.34-7.29 m
	εCH_2	7.34-7.30 m		εCH_2	7.29-7.25 m
	ζCH	7.29-7.25 m		ζCH	7.24-7.22 m
Pro 4	N		Pro 4	N	
	αCH	3.46 d (7.4)		αCH	3.47 d (7.4)
	βCH_2	1.88 dd (11.7, 6.5)		βCH_2	1.90 m,
	γCH_2	1.55 m, 1.27 m		γCH_2	1.56 m, 1.24 m
	δCH_2	3.32 m, 3.09 t (10.1)		δCH_2	3.31 m, 3.10 m
Ile 5	NH	9.36 d (8.3)	Ile 5	NH	9.24 d (8.3)
	αCH	4.10 t (9.6)		αCH	4.26 t (8.6)
	βCH_2	1.97 m,		βCH_2	2.20 m
	$\gamma 1CH_2$	1.47 m, 1.03 m		$\gamma 1CH_2$	1.31 m
	$\gamma 2CH_3$	0.92 m		$\gamma 2CH_3$	0.95 d (6.7)
	δCH_3	0.78 t (7.4)		δCH_3	0.79 t (7.4)
Pro 6	N		Pro 6	N	
	αCH	4.29 m		αCH	5.52 t (8.3)
	βCH_2	2.06 m, 1.77 m		βCH_2	2.38 m, 1.93 m
	γCH_2	2.00 m, 1.76 m		γCH_2	1.72 m, 1.64 m
	δCH_2	3.91 m, 3.54 m		δCH_2	3.68 m, 3.62 m

Table S3 – Assignment of ^{1}H-NMR signals in DMSO-d_6 at 298 K: δ (ppm), multiplicity and J (Hz) for **5** and **6**

Residue	Atom	**5**	Residue	Atom	**6**
Ile1	NH	8.24 d (9.7)	Ile1	NH	8.4 d (7.6)
	αCH	5.26 dd (9.7, 3.3)		αCH	5.11 m
	βCH_2	2.06 m		βCH_2	1.60 m
	$\gamma 1CH_2$	1.48 m,		$\gamma 1CH_2$	1.40 m
	$\gamma 2CH_3$	0.90-0.85 m		$\gamma 2CH_3$	0.90 d (6.2)
	δCH_3	0.90-0.85 m		δCH_3	0.84 m
Thz	CH (3)	8.27 s	Thz	CH (3)	8.26 s
Leu 2	NH	7.92 d (7.6)	Leu 2	NH	8.02 d (8.8)
	αCH	4.63 m		αCH	5.08 t (6.8)
	βCH	1.62 m, 1.48 m		βCH	1.76 m
	γH	1.36 m		γCH	1.43 m
	$\delta 1CH_3$	0.95-0.90 m		δCH_2	0.97 d (6.0)
	$\delta 2CH_2$	0.90-0.85 m	Phe 3	NMe	3.17 s
Phe 3	NH	8.97 d (0.8)		αCH	4.69 dd (8.7, 7.6)
	αCH	3.88 dd (11.4, 4.4)		βCH_2	3.26 dd (13.4, 7.3), 3.07 m
	βCH_2	2.97 dd (12.3, 4.9), 2.8 t (12.1)		δCH_2	7.30-7.22 m
	δCH_2	7.34-7.30 m		εCH_2	7.30-7.22 m
	εCH_2	7.30-7.26 m		ζCH	7.30-7.22 m
	ζCH	7.16 d (7.4)	Pro 4	N	
Pro 4	N			αCH	3.8 d (7.4)
	αCH	3.12 d (8.0)		βCH_2	1.96 m
	βCH_2	1.87 m, 0.82 m		γCH_2	1.60 m, 1.43 m
	γCH_2	1.61 m,		δCH_2	3.43 m, 3.03 m
	δCH_2	3.26 m, 3.18 m	Ile 5	NH	9.07 d (8.4)
Ile 5	NH	9.31 d (8.1)		αCH	4.37 t (8.1)
	αCH	4.06 dd (10.4, 8.5)		βCH_2	2.07 m
	βCH_2	2.00 m,		$\gamma 1CH_2$	1.25 m
	$\gamma 1CH_2$	1.04 m		$\gamma 2CH_3$	0.94 d (6.8)
	$\gamma 2CH_3$	0.90-0.85 m		δCH_3	0.84 m
	δCH_3	0.78 t (7.3)	Pro 6	N	
Pro 6	N			αCH	4.52 dd (8.2, 1.8)
	αCH	4.40 t (7.1)		βCH_2	2.38 m, 1.96 m
	βCH_2	2.06 m, 2.00 m		γCH_2	1.70 m
	γCH_2	1.79 m, 1.72 m		δCH_2	3.71 m, 3.64 m
	δCH_2	3.93 m, 3.54 m			

EXPERIMENTAL PROCEDURES

UPLC

UPLC analysis was performed by measuring light absorption at wavelength 200-600 nm on a Shimadzu UHPLC system (LC-30AD, SIL-30AC, CBM-20A, SPD-M20A, CTO-20A) using solvent mixtures of 0.1 % trifluoroacetic acid in water (buffer A) and 0.1 % trifluoroacetic acid in acetonitrile (MeCN)/H_2O (9/1) (buffer B) with a flow rate of 0.6 ml/min on a Eclipse Plus C18 column (2.1 µm x 100 mm).

Method A: 0-100% B in 6.00 min. **Method B:** 50-100% B in 6.00 min.

MOLECULAR ION UPLC-MS ANALYSIS

UPLC-MS analysis was done measuring light absorption at wavelengths 200-400 nm and full scan mass/charge ratio analysis from m/z 300 to 1200 on Shimadzu UHPLC system (LC-30AD, LC-30AC, SPD-M20A) connected to a LCMS-2020 single quadrupole mass spectrometer using gradient mixtures of 0.1 % formic acid in water (buffer A) and 0.1 % formic acid in H_2O/MeCN (9/1) (buffer B) with a flow rate of 0.6 ml/min on a Shim-pack XR-ODS III column (1.6 µm x 75 mm).

HPLC ANALYSIS

Analytical RP-HPLC were measured on Phenomenex Luna 5 µm C18 column (250 x 4.60 mm) using gradient mixtures of 0.1 % trifluoroacetic acid in water (buffer A) and 0.1 % trifluoroacetic acid in MeCN/H_2O (9/1) (buffer B) with flow rate of 1 ml/min eluting with 20 % B to 100% B in 15 min. Method A: 0-100% B in 6.00 min. Method B: 50-100% B in 6.00 min.

MOLECULAR ION HPLC-MS ANALYSIS

Molecular ion mass spectroscopy was performed on a QSTAR pulsar (ESI QqTOF mass spectrometer, ABSCIEX, Canada). Chromatography was carried out on a C18 Phenomenex column (5 µm, 2.1 × 50mm) using a linear gradient (2-98% Buffer B in 15 minutes, flow rate 0.3 mL/min). For detection of molecular ions (H^+; i.e. exact peptide mass [M+H]+) and sodium adducts (Na^+; i.e.

exact peptide mass [M+Na]+) the column was eluted with 0.1 % formic acid in water (buffer A) and 0.1 % formic acid MeCN/H_2O (9/1) (buffer B). Where detection of molecular ions or sodium adducts were interfered by back ground signals (plasma proteins) ammonium adducts (NH_4^+; i.e. exact peptide mass M+18.03, [M+NH_4]+) were formed and analysed eluting the column with 0.1 % formic acid in water containing 2 mM ammonium formate (buffer A) and 0.1 % formic acid in MeCN/H_2O (9/1) containing 2 mM ammonium formate.

PRODUCT ION MS/MS QUANTITATIVE ANALYSIS

Molecular fragmentation was carried out using a product ion MS/MS experiment in positive ion detection mode on a QSTAR pulsar (ESI QqTOF mass spectrometer, ABSCIEX, Canada). Chromatography was performed on a C18 Phenomenex column (5 µm, 2.1 × 50mm) using a linear gradient (2-98% Buffer B in 15 minutes, flow rate 0.3 mL/min).

MOLECULAR ION HIGH-RESOLUTION MASS SPECTROMETRY

High-resolution mass spectrometry (HRMS) was performed on a Bruker TOF mass spectrometer by direct infusion of compounds in acetonitrile, using sodium formate clusters as an internal standard.