Supporting Information

N10-C11 Imine is essential for Gram-negative antibacterial activity of broad spectrum pyrrolobenzodiazepines

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Purity determination of synthesized final compounds

The level of purity of the compounds for biological testing has been evaluated through LC-MS analysis, using two different gradient methods, reported hereafter. LC-MS analyses were performed on a Waters Alliance 2695 system (from Waters), with elution in gradient. HPLC grade solvents were used as mobile phase while a Monolithic C18 50 X 4.60 mm column (from Phenomenex) was used as stationary phase. UV detection was performed using a Waters 2996 photo array detector (from Waters). Injection volume has been set to 10 µL. The compounds have been dissolved in a mixture of H_2O/ACN (50/50, v/v) or DMSO/ACN (50/50, v/v) accordingly to the solubility. The area of the peak corresponding to the compound has been automatically determined by the software included in the LC-MS system. The eventual presence of solvent UV trace has been subtracted to the total in order to determine the percentage of purity.

LC-MS methods:

Method A: flow 0.5 mL/min

A) water + 0.1 % formic acid

B) acetonitrile + 0.1% formic acid

Time (min)	**0**	**3**	**3.5**	**4.5**	**5**
A (%)	95	10	5	5	95
B (%)	5	90	95	95	5

Method B: flow 1 mL/min

A) water + 0.1 % formic acid

B) acetonitrile + 0.1% formic acid

Time (min)	**0**	**2**	**5**	**6**	**7.5**	**9**	**10**
A (%)	95	95	50	50	5	95	95
B (%)	5	5	50	50	95	5	5

Compound	**5 minutes method**	**10 minutes method**

	Retention time (min.)	Purity	Retention time (min.)	Purity
7a	3.12	≥98%	5.95	≥98%
8a	3.30	≥98%	6.40	≥98%

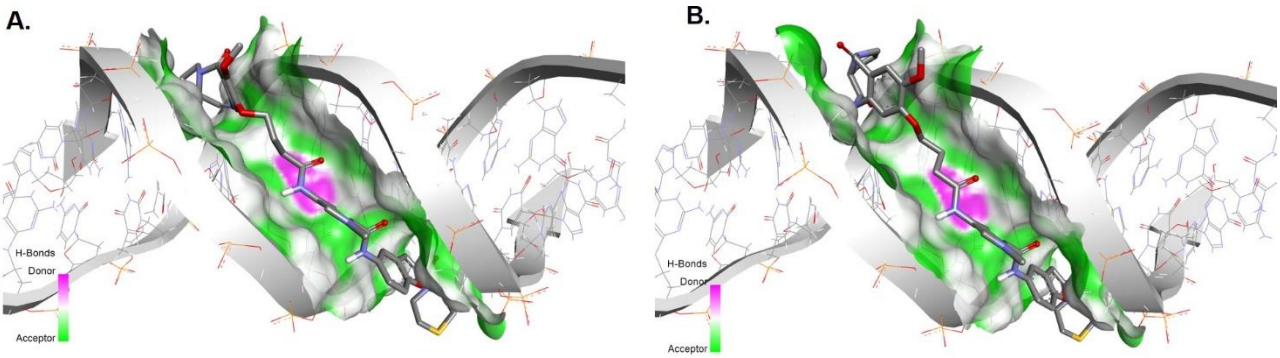

Figure S1: DNA binding of compounds 7 (A) and 7a (B) within the DNA minor groove of Sequence-2.