

Clinical potential of the β -lactamase inhibitor

NXL104 (formerly AVE1330)

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NXL104 compound

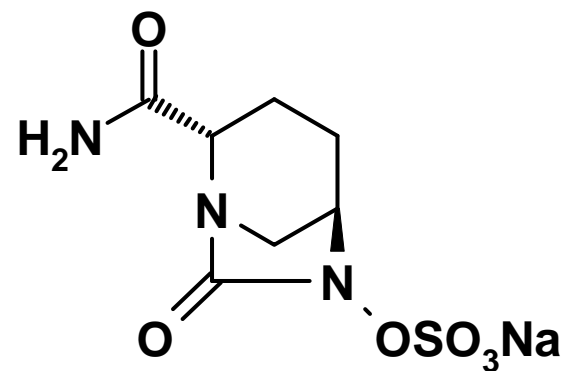
Formerly AVE1330A

Synthesis :

- 9 step enantioselective process

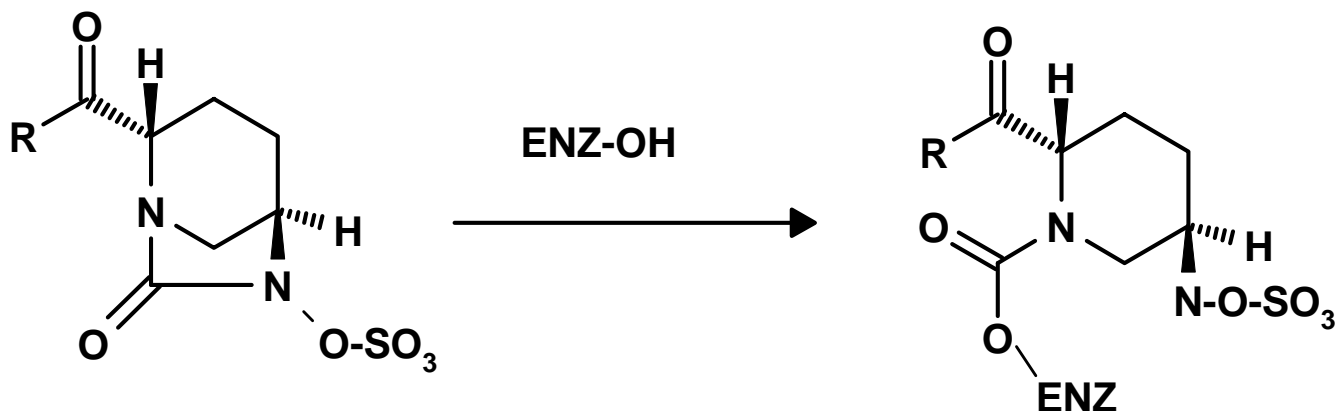
Physicochemistry :

- sodium salt
- soluble compound



NXL104 (active enantiomer)
(trans-7-oxo-6-(sulfoxy)-1,6-diazabicyclo[3.2.1]octan-2-carboxamide sodium salt)

Determination of 3D structure for P99 class C and TEM-1 class A β -lactamases complexed with an NXL104 close analogue.

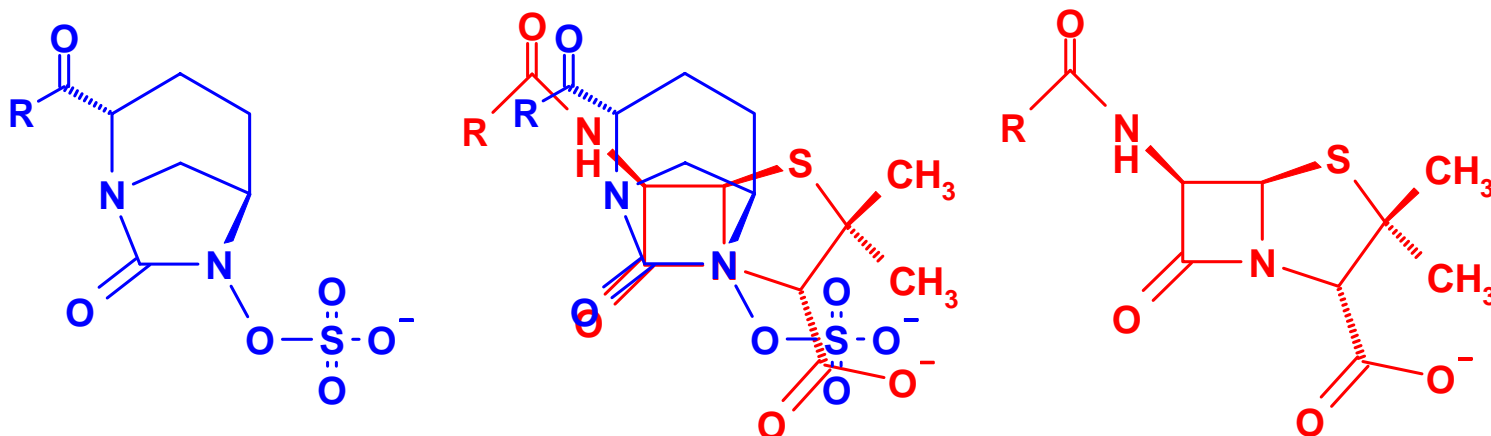


Novel mechanism of inhibition giving rise to the **formation of a very stable carbamoyl linkage in the acyl enzyme complex.**

NXL104 – Binding model

Binding model built

- Based on the 3D structure of the acylenzyme complex
- To account for the SAR of the series



Superimposition with penicillin

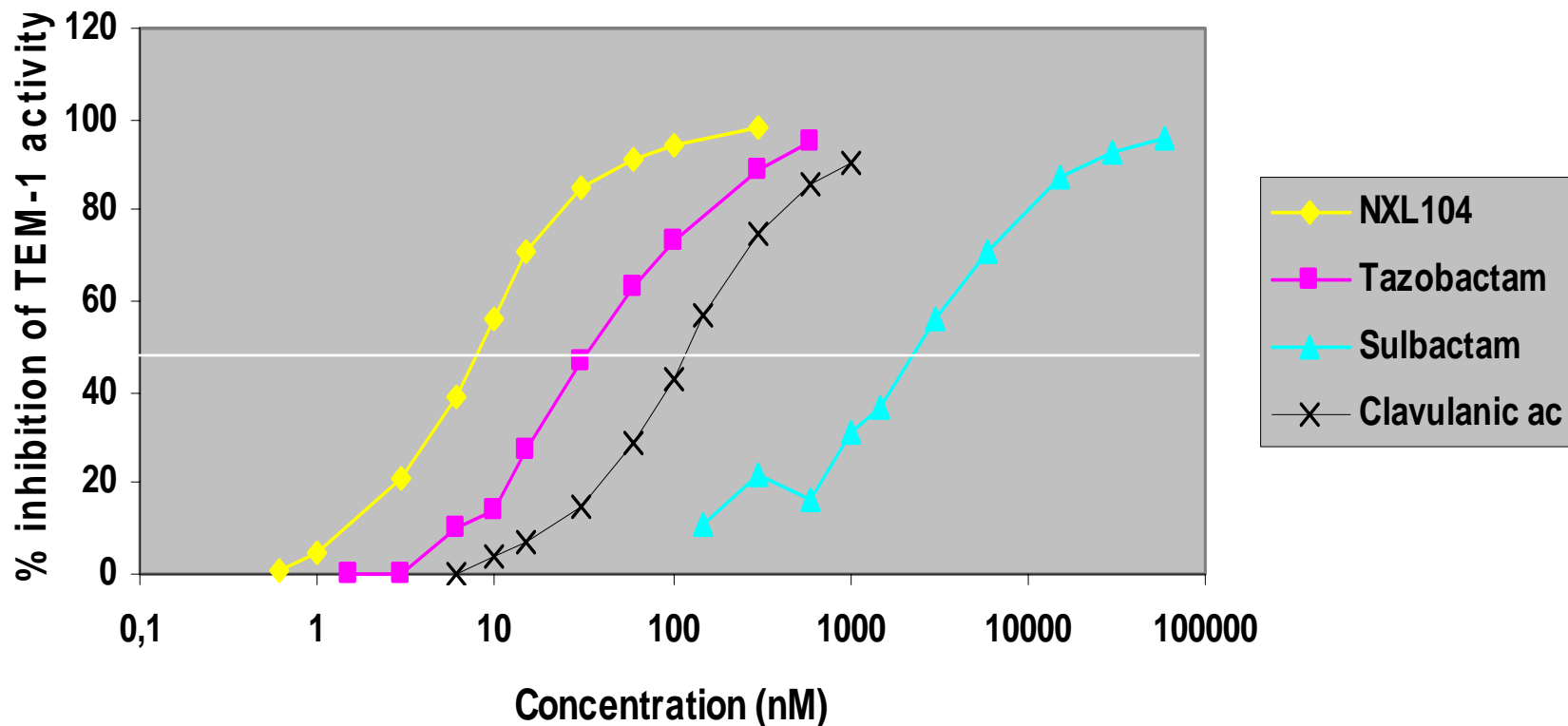
- The ester chain mimics the penicillin side chain
- The sulfate group is superimposed on to the penicillin carboxylate group

NXL104

Inhibition of β -lactamases

NXL104 - Class A TEM-1 inhibition

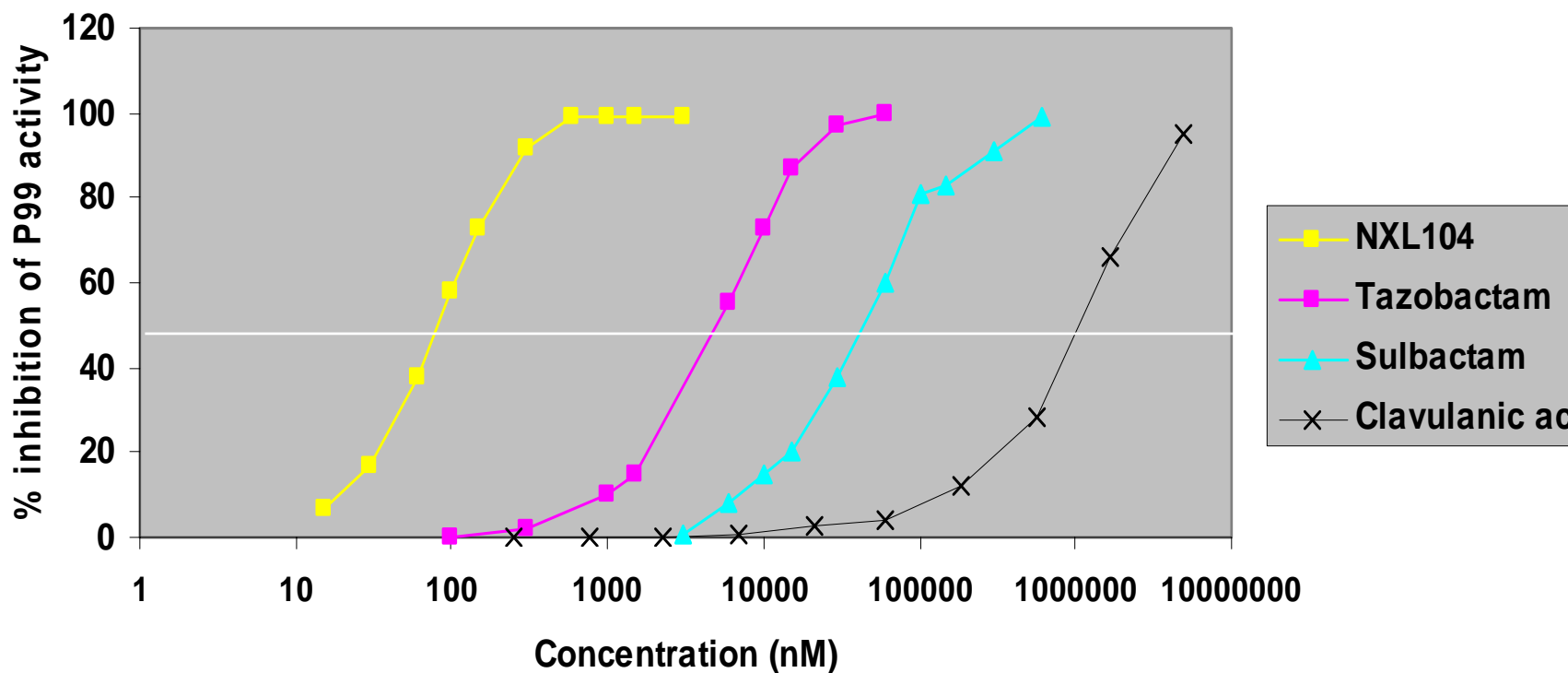
Measurement of β -lactamase activity Purified TEM-1 enzyme 1 nM in 50 mM phosphate pH 7.0, 2% glycerol, 0.1 mg/ml BSA buffer. 5 mn preincubation of enzyme + inhibitor, 37°C. Addition of substrate (nitrocefin, 100 μ M) to start the reaction. OD measurement at 485 nm. Data processing using GraFit software.



TEM-1	NXL104	Tazobactam	Sulbactam	Clavulanic acid
IC ₅₀ (nM)	8	40	2300	130

NXL104 - Class C P99 inhibition

Measurement of β -lactamase activity Purified enzyme (*E. cloacae* P99) 0.42 nM in 50 mM phosphate pH 7.0, 2% glycerol, 0.1 mg/ml BSA buffer. 5 mn preincubation of enzyme + inhibitor, 37°C. Addition of substrate (nitrocefin, 100 μ M) to start the reaction. OD measurement at 485 nm. Data processing using GraFit software.



P99	NXL104	Tazobactam	Sulbactam	Clavulanic acid
IC ₅₀ (nM)	80	5000	42000	10 ⁶

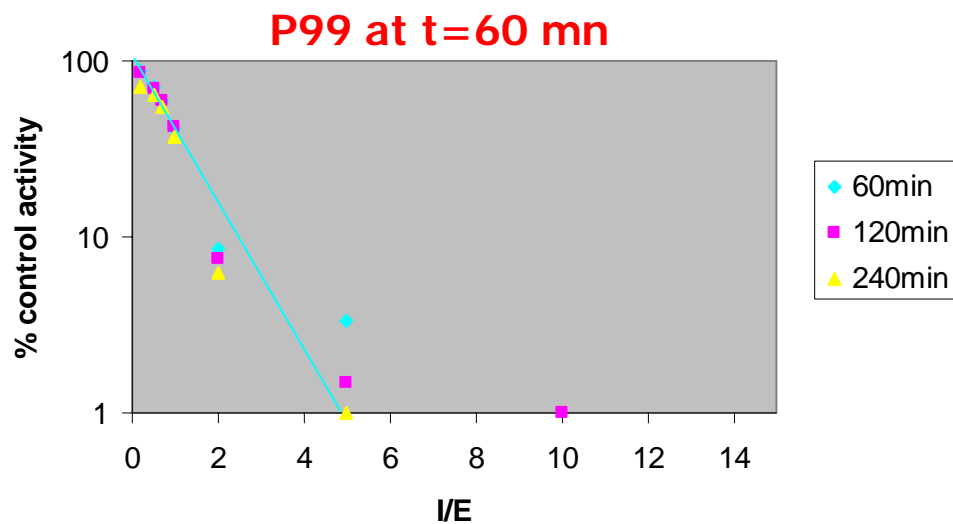
NXL104 – Inhibition of class C β -lactamases

Class C β -lactamases	IC ₅₀ (nM)	
	NXL104	TAZOBACTAM
<i>E. cloacae</i> P99 AmpC	190	2157
<i>E. aerogenes</i> 293 AmpC	552	7519
<i>E. aerogenes</i> 298 AmpC	254	5405
<i>P. aeruginosa</i> 34 AmpC	437	2132
<i>P. aeruginosa</i> CF17 AmpC	402	2840

NXL104 - Turnover number

T_n : number of inhibitor molecules required to inactivate one enzyme molecule

Kinetics of inactivation is performed at various inhibitor/enzyme molar ratios. Residual β-lactamase activity is measured with 400 μM nitrocefin. The minimal period of time taken to obtain maximal inhibition is defined ; at this time, the T_n value is deduced from the extrapolated value for 99% inhibition from the plot of residual activity vs inhibitor/enzyme ratios

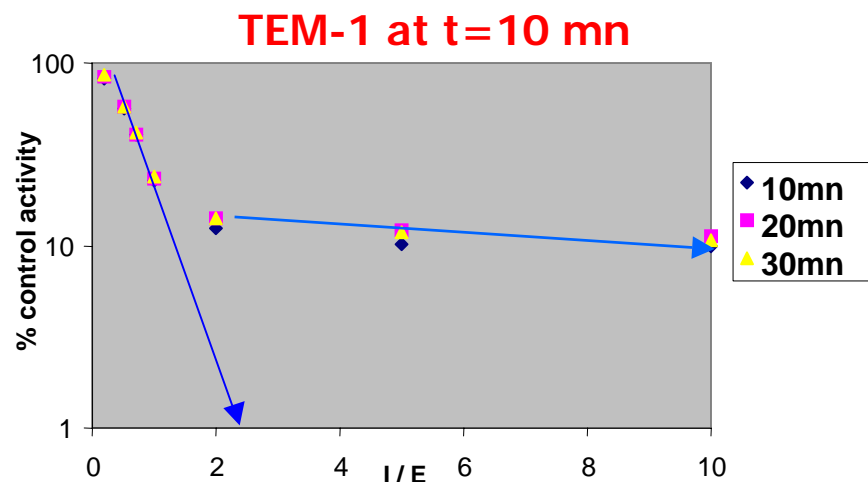


NXL104

T_n = 5

(Tazo

T_n = 55)



NXL104

T_{n1} = 2

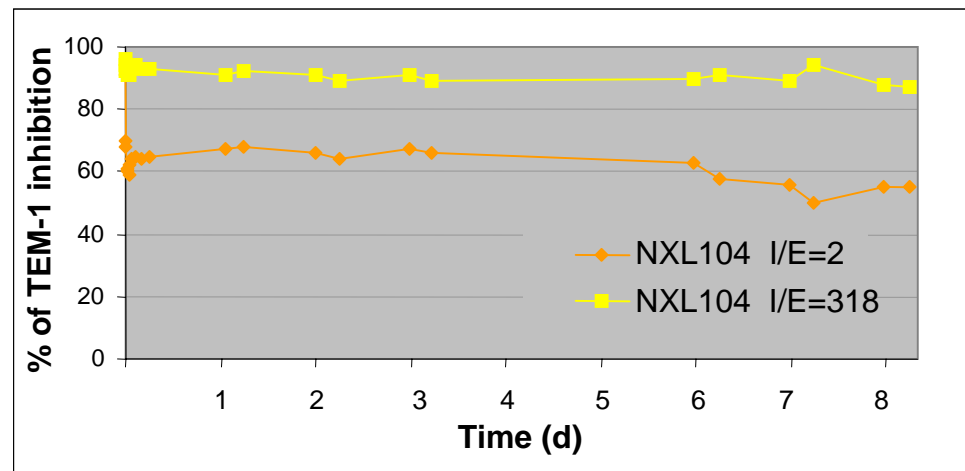
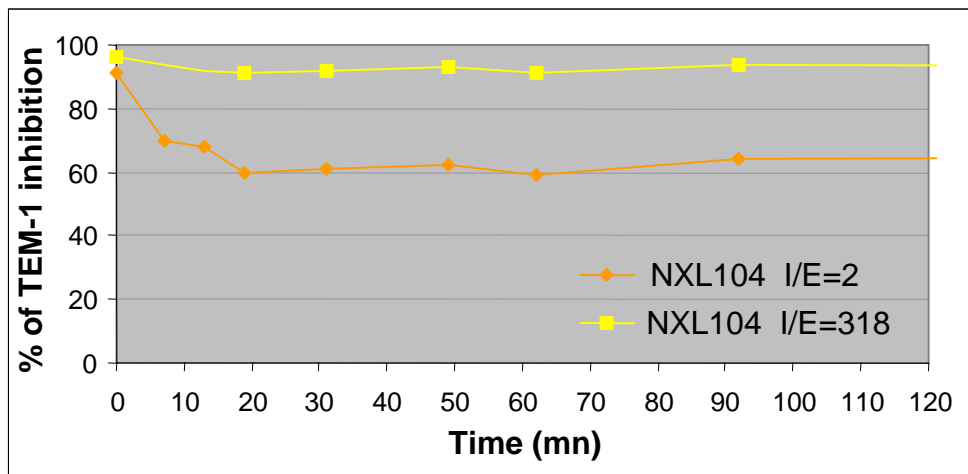
T_{n2} = 318

(CLA

T_{n1} = 214

T_{n2} >> 500)

Deacylation of TEM-1 / NXL104



Enzyme is saturated with **NXL104** inhibitor in the conditions of Tn determination

- concentration : **I/E = 318** or **I/E = 2**
- time of incubation : **10 mn**

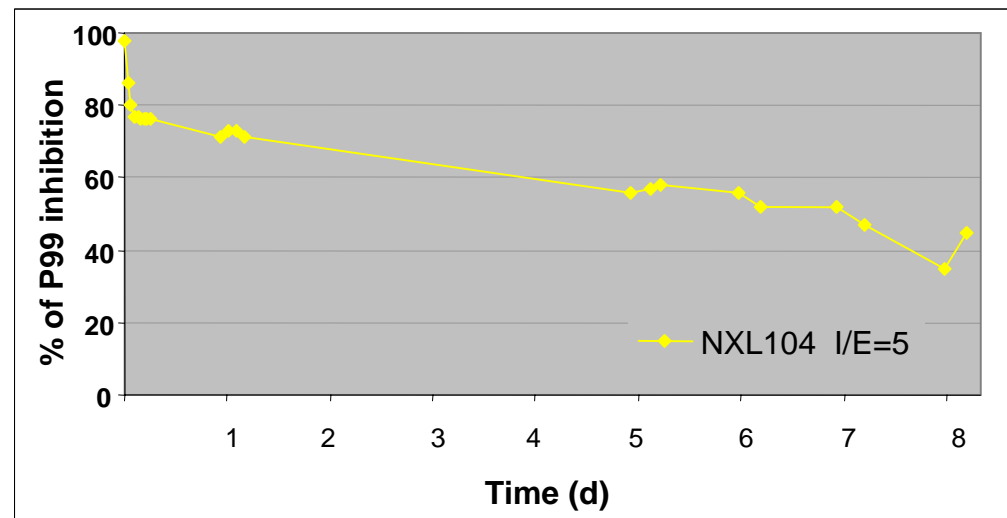
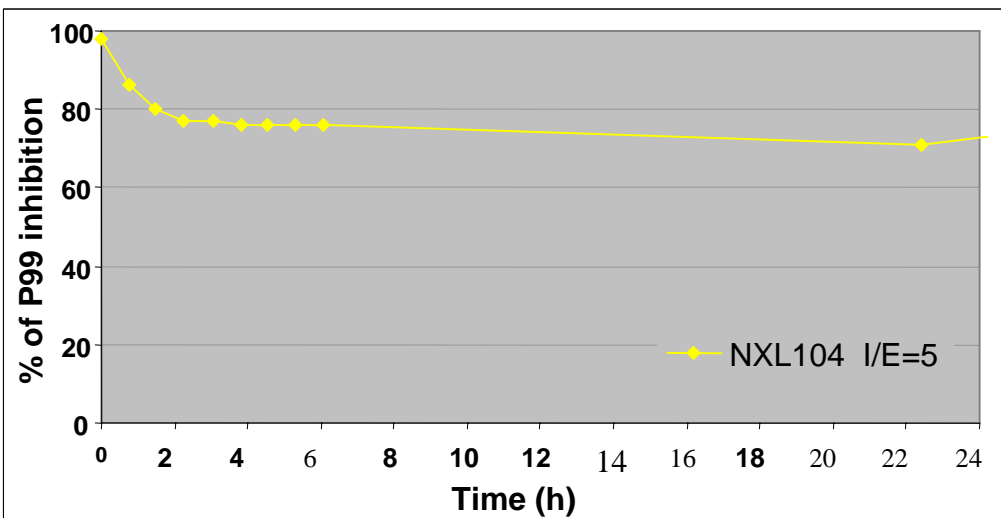
Free inhibitor is removed by gel filtration. Residual activity is measured at different times, at 37°C.

Recovery of TEM-1 β -lactamase activity:

I/E = 2 ~ 30% after 7 mn
 ~ 50% after 7 days

I/E = 318 < 10% after 7 days

Deacylation of P99 / NXL104



Enzyme is saturated with **NXL104** inhibitor in the conditions of Tn determination

- concentration : **I/E = 5**
- time of incubation : **60 mn**

Free inhibitor is removed by gel filtration. Residual activity is measured at different times, at 37°C.

Recovery of P99 β -lactamase activity:
~ 50% at Day 7

Summary of NXL104 activity against purified β -lactamases

- Broad spectrum activity - active on Class A and C β -lactamases
- Lower turnover number than β -lactam based inhibitors
- More stable covalent intermediate \Rightarrow longer half-life of the covalent intermediate

		NXL104	Tazobactam	Sulbactam	Clavulanic ac
IC ₅₀ (nM)	TEM-1	8	40	2300	130
	P99	80	5000	42000	100,000
Tn	TEM-1	2 / 318	nd	nd	214
	P99	5	55	nd	nd
Deacylation t _{1/2}	TEM-1	~ 7 d at Tn 2	nd	nd	~ 7 mn
	P99	~ 7 d	~ 290 mn	nd	nd

NXL104

Antibacterial activity

NXL104 *in vitro* antibacterial activity

➤ No antibacterial activity alone on most species

Only marginal activity on a few *E. coli* isolates (MIC \geq 8 μ g/ml)

➤ PBP inhibition

<i>E. coli</i> PBP	IC ₅₀ (μ M)
PBP1a	>100
PBP1b	>100
PBP2	3.7
PBP3	>100
PBP4	>100
PBP5	>100
PBP6	>100

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NXL104 *in vitro* activity against class A producing strains

Strain			Ceftazidime			Ceftriaxone			Cefpodoxime		
			0	NXL104	Clav	0	NXL104	Clav	0	NXL104	Clav
+ inhibitor 4 µg/ml											
E coli	250BE1	SHV4	32	0.25	0.25	2	0.03	0.03	32	0.5	0.5
E coli	250IP7	SHV5	32	0.5	0.5	2	0.06	0.06	32	0.5	0.5
E coli	250BE5	TEM3	32	0.5	0.5	4	0.06	0.06	>32	1	0.5
E coli	250CF2	TEM5	32	0.25	0.12	2	0.03	0.03	32	0.12	0.25
E coli	250SJ1	TEM7	32	0.03	0.03	0.12	≤0.015	≤0.015	8	0.03	0.12
E coli	250KB3	CTXM3 like	>32	1	1	>32	1	1	>32	2	4
K. pneumoniae	283IP13	SHV4	>32	0.5	0.5	4	0.12	0.12	>32	0.5	0.5
K. pneumoniae	283CF5	SHV5	>32	1	1	0.5	0.25	0.5	4	1	1
K. pneumoniae	283IP98	TEM4	16	0.25	0.25	2	0.06	0.06	16	0.12	0.12
K. pneumoniae	283IP1	TEM21	16	0.12	0.12	4	≤0.015	≤0.015	>32	0.12	0.25
K. pneumoniae	283KB2	CTXM19	>32	2	4	32	0.25	0.25	>32	0.5	0.5

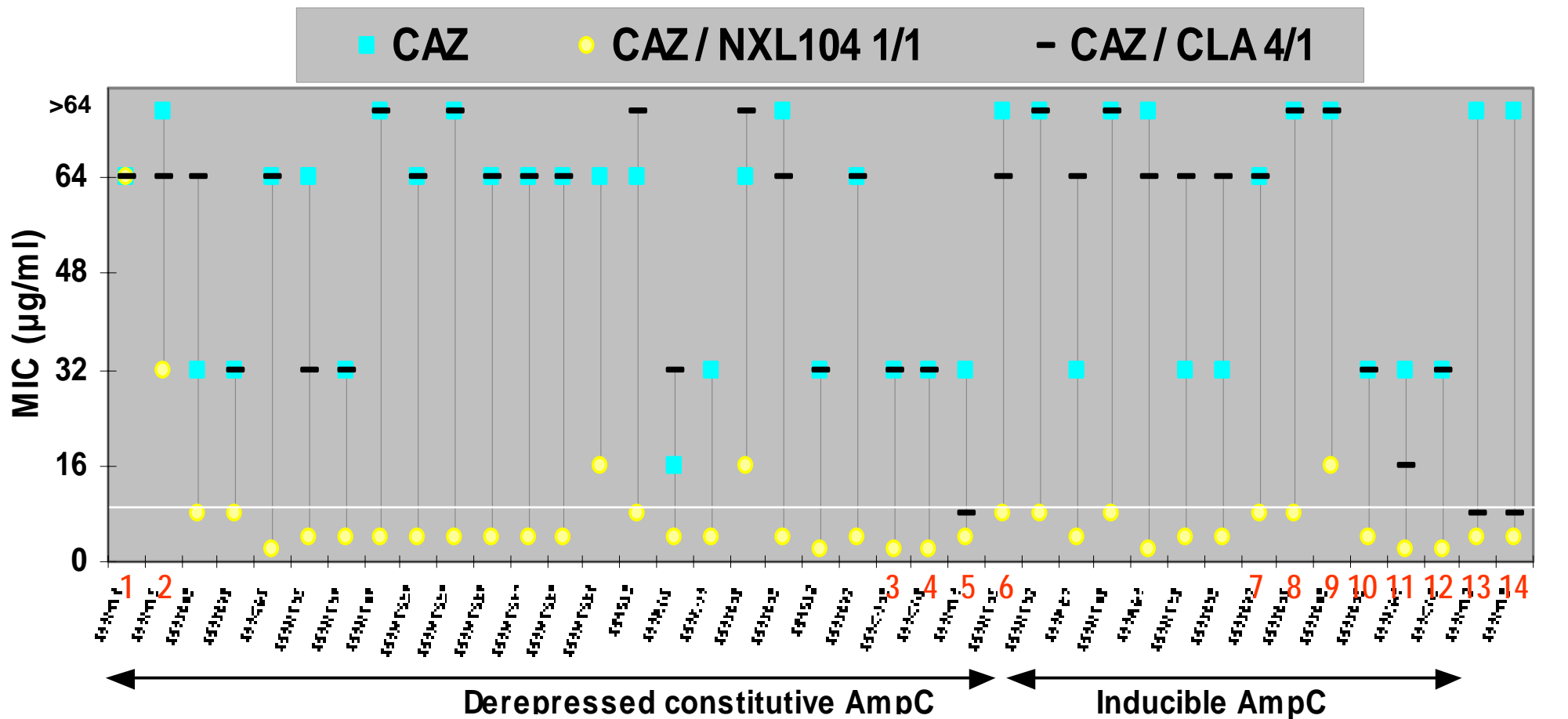
When combined to Ceftazidime, Ceftriaxone or Cefpodoxime, NXL104 activity against class A producing strains is similar to Clavulanic Acid activity

NXL104 *in vitro* activity against class C producing strains

Strain			Ceftazidime			Ceftriaxone			Cefpodoxime		
			0	NXL104	Clav	0	NXL104	Clav	0	NXL104	Clav
	+ inhibitor 4 µg/ml										
E. coli	250KB8	ACC1	>32	4	>32	16	0.25	16	>32	2	>32
K. pneumoniae	283KB4	DHA2	>32	0.5	>32	4	0.06	8	>32	0.5	>32
C. freundii	261CO3	AmpC	16	0.5	>32	16	0.12	32	>32	0.5	>32
C. freundii	261GR6	AmpC	>32	1	>32	32	0.25	32	>32	0.5	>32
E. cloacae	293CO20	AmpC	>32	2	>32	>32	0.5	>32	>32	8	>32

When combined to Ceftazidime, Ceftriaxone, or Cefpodoxime, NXL104 restores the antibacterial activity against class C producing strains

NXL104 activity against *P. aeruginosa* strains



Additional β-lactamases:

1 TEM24+VIM2

2 nd

3 CARB2+OXA10

4 nd

5 nd

6 SHV2a

7 CARB2+OXA10

8 CARB2

9 CARB2+OXA10

10 CARB2

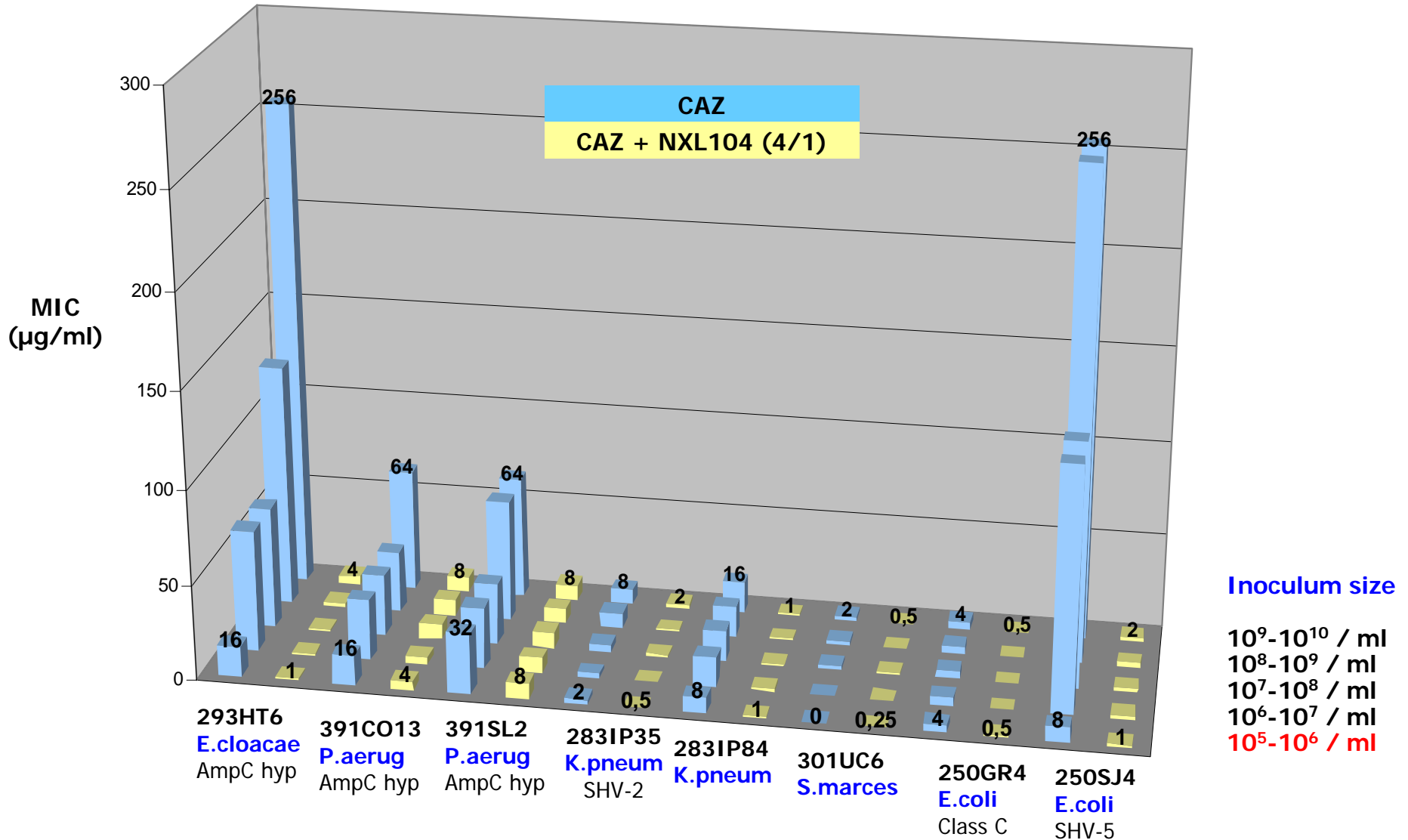
11 GES2

12 nd

13 PER1+OXA10

14 VEB1+OXA10

NXL104 - Inoculum size effect



NXL104 *in vitro* antibacterial activity

Antibacterial activity of NXL104/ β -lactam combinations

- Cephalosporin activity restored against resistant Enterobacteriaceae Class A and C producers :

E. coli, *Klebsiella spp.*, *Salmonella spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Proteus spp.*, *M. morgani.*, *M. catarrhalis*

- Association with NXL104 shown effective for a variety of cephalosporins:

Ceftazidime, Ceftriaxone, Cefpodoxime

- Synergy with CAZ against CAZ-R *P. aeruginosa*^A for most Class A and C β -lactamase producers

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in vivo antibacterial activity of NXL104 / cephalosporin combinations

Mouse septicemia model : in progress

➤ **Strains used**

class A ESBL and class C producers

K. pneumoniae, E. cloacae, E. coli, C. freundii

- **Preliminary results show efficacy of NXL104 when used in combination with various cephalosporins**

NXL104

ADME / PK

NXL104 - ADME

- **Low plasma protein binding :**
 - **10% in mouse plasma and 17% in human plasma for 100 µg/ml of NXL104**
- **Preliminary *in vitro* metabolic profile**
 - **S9 liver fractions: mouse 100% , human 92%**
 - **Recombinant human CYP isoforms (1A2, 2A6, 2E1, 3A4, 2C9, 2C19, 2D6) >93 %**
 - **Rat and mouse liver slices : no metabolite detected**
- **Inhibition of human CYP isoforms**
 - **1A2, 3A4, 2C9, 2C19 IC₅₀ > 50 µM**
 - **Inhibition of CYP 2D6 IC₅₀ = 2µM**
- **No significant induction of CYPs 3A and 1A in primary rat hepatocytes**



High *in vitro* metabolic stability
Low potential for polymorphic metabolism
Low risk for major drug interactions

NXL104 preliminary PK parameters in mice

Preliminary PK parameters following i.v. and oral administration of NXL104 (racemate) to the mouse at a dose level of 10 mg/kg.

i.v. route

Oral route

AUC_{0-∞} (h.ng/ml)	Cl_T (l/h/kg)	Vdss (l/kg)	Terminal T_{1/2} (h)	Cp max (ng/ml)	T max (h)	AUC_{0-∞} (h.ng/ml)	Terminal T_{1/2} (h)
5365	1.9	0.7	1.1	1375	0.25	1874	3.1
2500	4	0.31	0.06	1240	0.17	680	0.3

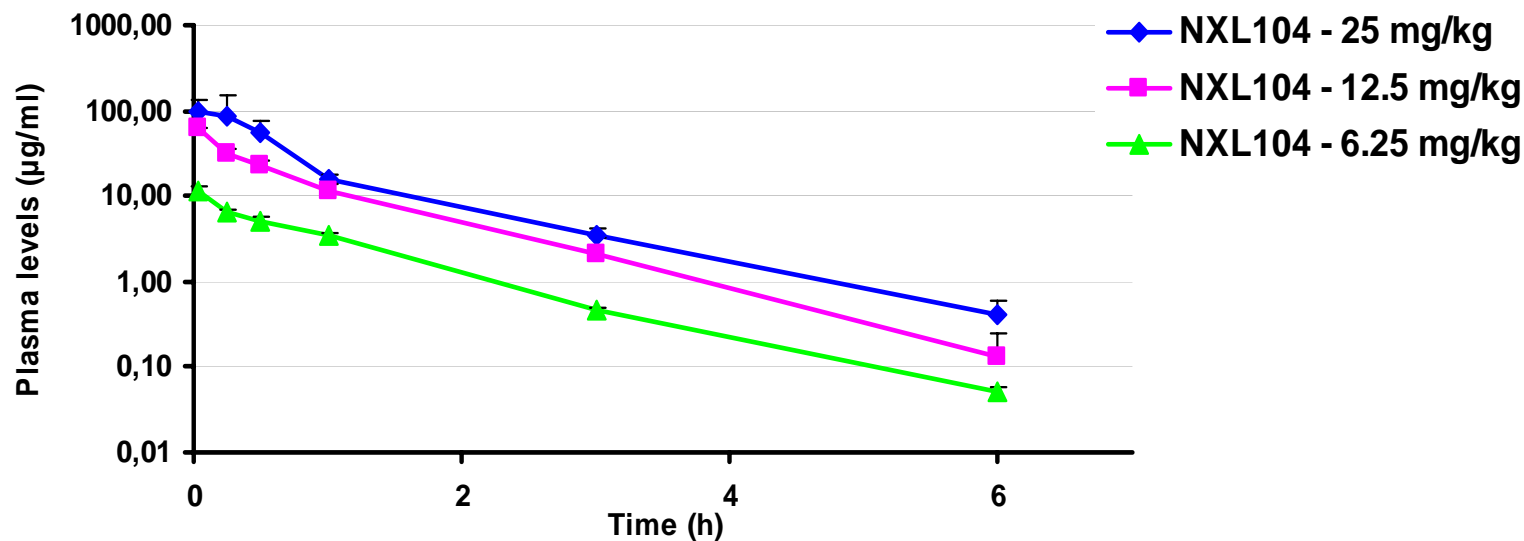
F=35%

For tazobactam, extrapolated at 10 mg/kg from J.A.C. (1993), 31, suppl A, 39-60

For clavulanic acid, extrapolated at 10 mg/kg from Drugs Exptl. Clin. Res.(1981), VII, 263-267

NXL104 - PK parameters in dog

Mean plasma concentration time curves i.v. administration of 6.25, 12.5 or 25 mg/kg of NXL104



NXL104 (mg/kg)	AUC 0-∞ (h.ng/ml)	Cl _T (l/h/kg)	Vd _{ss} (l/kg)	Half life T _{1/2} (h)
6.25	9.2 ± 0.6	0.7 ± 0.1	0.8 ± 0.1	1.0 ± 0.3
12.5	38.5 ± 4.9	0.3 ± 0.1	0.3	0.7 ± 0.1
25	69.4 ± 21.7	0.4 ± 0.1	0.4 ± 0.2	1.1 ± 0.3

NXL104

TOXICOLOGY

NXL104 - Toxicity

➤ Genetic toxicity

- *in vitro* Unscheduled DNA Synthesis assay (UDS) in rat liver cells : NXL104 racemate negative up to 2 mg/ml (max conc tested)
- *in vitro* Micronucleus Test in L5178Y mouse lymphoma cells with and without metabolic activation : negative up to 1 mg/ml (max conc tested)

➤ Cardiovascular:

- hERG channel assay: $IC_{50} > 300 \mu M$

➤ i.v. 7-day daily dosing in rats

- 1500 mg/kg : maximum tolerated dose / poor local tolerance
- 500 mg/kg : minor effects on body weight, food consumption and red cell parameters
- 167 mg/kg : NOAEL (No Observed Adverse Event Limit)



**No safety issue for NXL104
at this stage**

NXL104 - Conclusions

Medical need : increasing β -lactamase resistance is expected to become a major concern, primarily in the hospital

Cephalosporin use in the clinic is mainly compromised by the spread of Class A and Class C β -lactamase mediated resistance

Strengths of a cephalosporin / NXL104 combination :

- broad spectrum activity (ESBLs, AmpC)
- active on β -lactam resistant Gram-negatives, particularly on difficult to treat infections caused by *E. cloacae*, *C. freundii*, *K. pneumoniae*
- potentially active against some CAZ-R *P. aeruginosa*

**Clinical development of NXL104 to bring
a solution to β -lactamase-mediated resistance
in Gram-negative bacteria in the hospital**