Antibiotiques et résistances

Le problème de la résistance aux antibiotiques est complexe, et le développement de nouveaux antibiotiques est constamment sous la pression de l'émergence de nouvelles résistances. Les bactéries Gram – sont les plus difficiles à éradiquer du fait de leur membrane externe, qui sert de barrière filtrant les molécules potentiellement toxiques, dont les antibiotiques. La membrane externe (« outer membrane » = OM) est composé de lipopolysaccharides (LPS) sur la couche externe et de phospholipides sur la couche interne. Plusieurs piste sont explorées pour passer cette barrière : utiliser des agents qui déstabilisent les LPS directement (aminoglycosides (AG), EDTA) ou qui interfèrent avec leur mise en place.

Le problème de l'utilisation des AGs est leur nombreux effets secondaires, dus à un manque de spécificité de leur action. On explore ici une classe d'AG synthétiques, moins toxiques pour les mitochondries et cellules humaines.

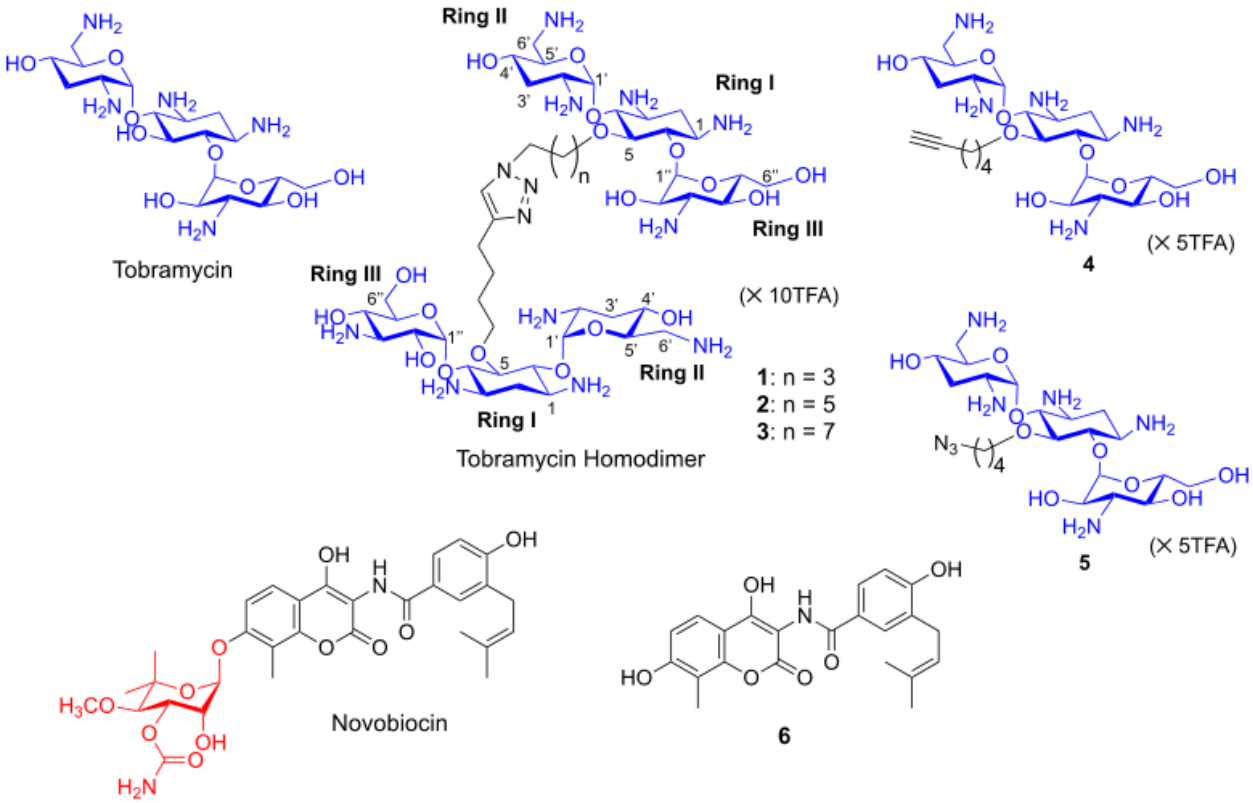

Figure 1. Structures of newly synthesized and reference compounds. Compounds **1**–**3** are tobramycin homodimers conjugated at the C-5 position of tobramycin with different tether lengths, compounds **4** and **5** are fragments of lead structure **1**, and compound **6** is an aglycone derivative of novobiocin.

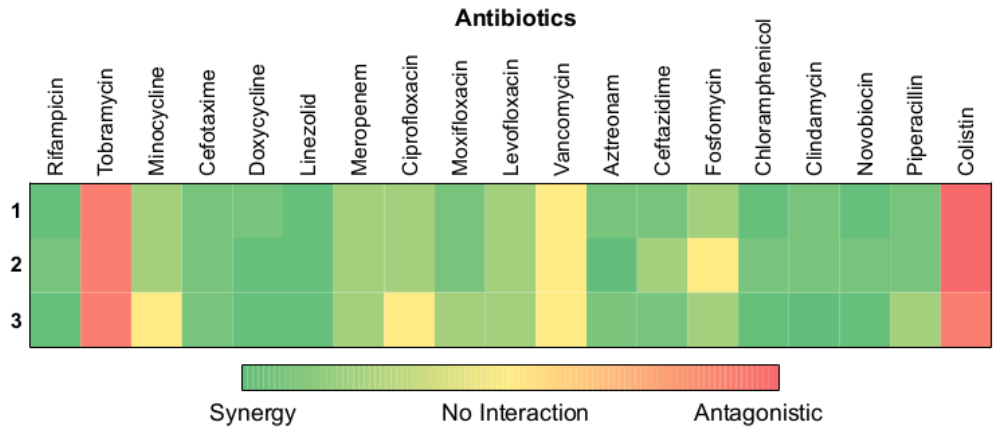

Figure 2. Interactions of compounds **1**–**3** (at ≤ 7.1 μM) with different antibiotics against *P. aeruginosa* PAO1. FICI ≤ 0.5 = Green (synergistic); FICI > 0.5 but < 1 = Yellow (no interaction); FICI > 4 = Red (antagonistic)

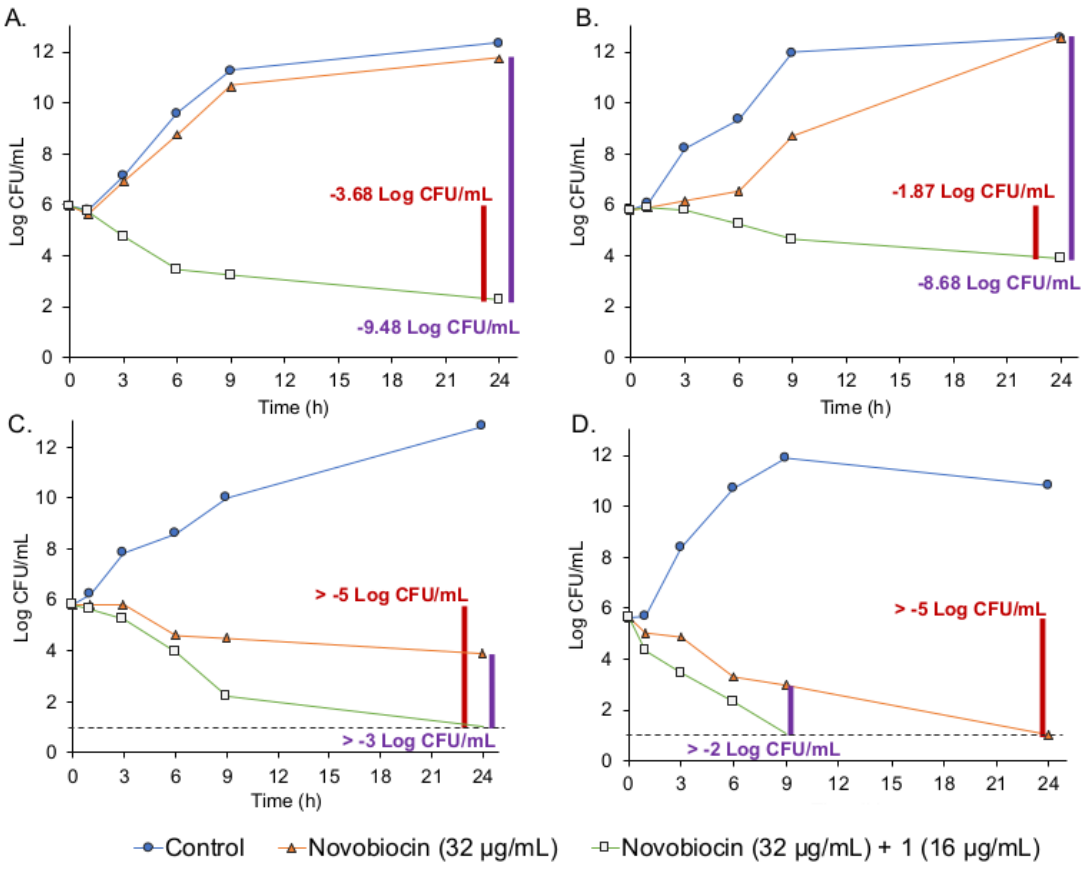

Figure 3. Time-kill synergy graphs. The activities of novobiocin (32 μg/ml) in combination with compound **1** (16 μg/ml, i.e. 7.1 μM) against (A) *P. aeruginosa* PAO1, (B) *K. pneumoniae* 116381, (C) *A. baumannii* ATCC 17978, (D) *E. coli* ATCC 25922. Red bars and numbers indicate differences in bacterial concentrations between the starting inoculum and drug combination at 24 h. Purple bars and numbers indicate differences in bacterial concentrations between the combination and the most active single agent at 24 h (9 h for *E. coli*). The dashed lines represent the lower limit of detection. Each data point represents an average of three independent determinations.

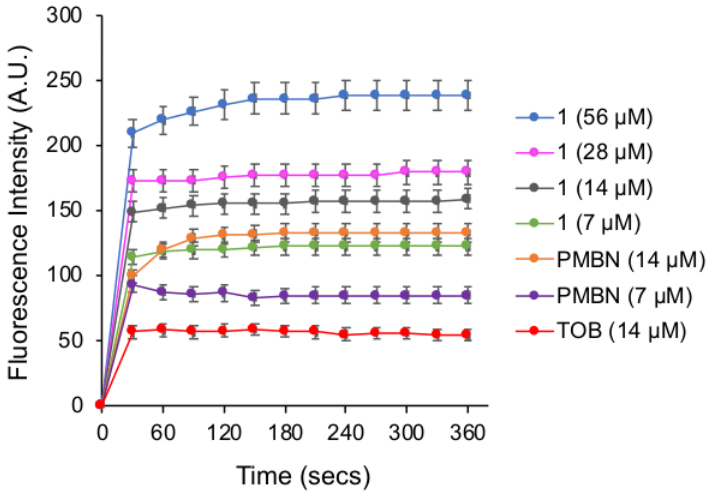

Figure 4. Outer membrane permeabilization by compound **1**, polymyxin B nonapeptide (PMBN) and tobramycin (TOB) was determined by measuring the accumulation of 1-*N*-phenylnaphthylamine (NPN) in *P. aeruginosa* PAO1 cells. Each data point is an average of four independent determinations ± SD.

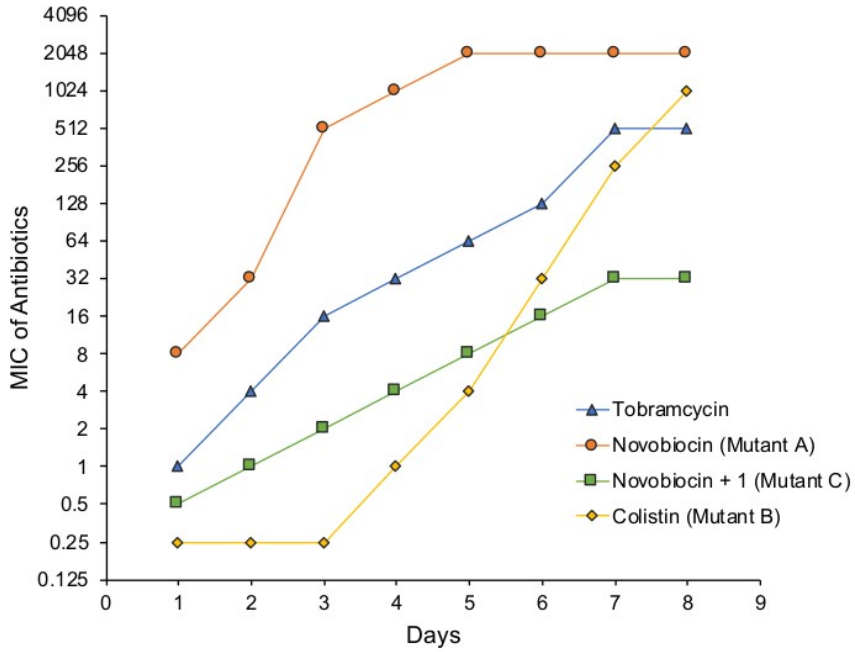

Figure 5. Emergence of resistance study. Resistance acquisition during serial passaging of *A. baumannii* ATCC 17978 in the presence of sub-MIC levels of antibiotics. For combination study, compound **1** was kept constant at a concentration of 7.1 µM throughout the experiment.

MIC = Minimum inhibitory concentration

The design of tobramycin homodimers 1–3 was guided by previous SAR. Amphiphilic tobramycins with lipophilic groups at the 5-OH of deoxystreptamine have been shown to lose ribosomal activities but retain the ability to permeabilize the OM. Dimerization of ribosome-targeting antibiotics has also been shown to result in poor inhibitors of in vitro protein translation. Hence, to prepare non-ribosomal amphiphilic-like tobramycin homodimers with potentially broad-spectrum OM permeabilizing properties, we dimerized two fragments of short-chain amphiphilic tobramycins ligated at the 4,6-disubstituted 2-deoxystreptamine via a copper(1)-catalyzed azide-alkyne cycloaddition reaction.1–3. Analogs with different tether length were synthesized to investigate the optimal spatial separation between the two domains while compounds 4 and 5 were prepared to study the SAR of the lead compound 1.

Tobramycin by itself is not synergistic with these antibiotics. The antagonistic relationship between compounds 1–3 and tobramycin or colistin (FICI > 4) is consistent with observed antagonism between tobramycin and colistin at high concentrations. This is perhaps due to competition for LPS binding by both polybasic molecules. The lack of potentiation of vancomycin is consistent with other OM permeabilizing agents such as PMBN and pentamidine, where synergy is generally more pronounced with large hydrophobic molecules (e.g. rifampicin) than with large hydrophilic molecules (e.g. vancomycin). Compound 1 is the most potent and least toxic of the three, hence, it was used for further studies.

But → Perdre l’activité toxique pour les cellules mais rendre les OM perméables (amphiphiles, se mettent dans la membrane?)