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ACTIVITY OF NEW PENEMS AGAINST DEFINED MRSA STRAINS

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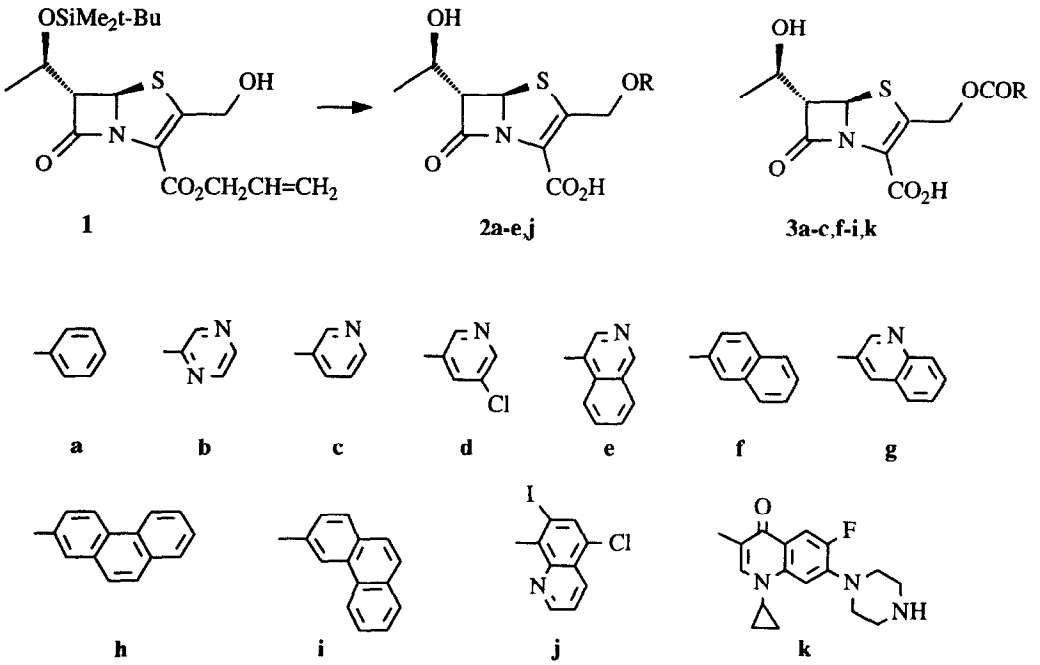
Abstract. The activity of penems against methicillin-resistant *Staphylococcus aureus* (MRSA) was determined on strains representative of the four expression classes of resistance. Two families of compounds, characterized by a C2-(hetero)aromatic moiety connected by an ether (CH_2O) or ester (CH_2OCO) link, displayed superior activity *in vitro*.

The incidence of staphylococcal infections in the hospital environment is a worldwide phenomenon of great concern. A study from an US ospital participating in a National Nosocomial Infections Surveillance System revealed that while the share of nosocomial infections caused by methicillin-sensitive pathogens have remained constant during 1986-1990, infections with methicillin-resistant *Staphylococcus aureus* (MRSA) have increased over 30 times within those four years.[1] Methicillin resistance is characterized by several peculiar features that contribute to make staphylococcal infections an increasingly serious clinical problem. First, the level of methicillin resistance varies widely among strains, ranging from few micrograms to several milligrams per milliliter. Moreover, several subpopulations of bacteria with varying degrees of resistance can be distinguished in a bacterial culture, and sometimes the frequency of highly resistant cells can be as low as 10^{-8}-10^{-9}. This so called heterogeneous form of resistance, due to different expression for each cell of the resistant phenotype, is present in most clinical isolates of MRSA. In a recent investigation of the phenotypic expression of methicillin resistance by population analysis,[2] MRSA strains have been divided into four arbitrary expression classes, each representing a stable and reproducible profile of the distribution of resistance within the bacterial population. The majority of bacterial cells in each class have methicillin MICs of 1.53-3.12, 6.25-25, 50-200, and >200 μg/mL respectively for class 1, 2, 3 and 4. The first three classes include heterogeneous strains in which the proportion of highly resistant cells is increasing from class 1 to class 3. Class 4 isolates are homogeneous, all cells of the culture having the same high MIC value. Methicillin resistance is mainly due to the production of an extra penicillin-binding protein (PBP 2A), characterized by a very low level of affinity for β-lactams, including methicillin[3]. Therefore β-lactam antibiotics are clinically not recommended as anti-MRSA agents in the clinical use, despite their superior safety profile. Nonetheless new β-lactams, including penems, are recurrently proposed as potential drugs against MRSA.

In 1988, the synthesis and antimicrobial activity of the 2-phenoxymethylpenem (**2a**) was simultaneously reported by Ciba-Geigy[4] and our group.[5] The striking difference in activity against MRSA strains included in the two screening panels (MRSA A331, 0.02 μg/mL; MRSA 2101, >50 μg/mL) is illustrative of the equivocal results obtained by standard assay procedures. The guidelines for the antibiotic susceptibility testing on MRSA suggest to use inocula of 10^4 cfu/spot on tryptic soy agar (TSA) supplemented with 2% NaCl and an incubation temperature of 30°C to enhance the phenotypic expression of methicillin resistance.[6] With such a low inoculum, however, the small proportion of highly resistant cells could be overlooked with the risk to overestimate the antibacterial activity. In the present study the penem FCE 22101[7] (ritipenem, RITI), carbapenems imipenem (IPM) and meropenem (MPM), the quinolone ciprofloxacin (CPX) and methicillin (METH) were used as reference compounds to investigate the potential activity of **2a** and new penem molecules against MRSA (Table 1). Activity against three clinical isolates has been determined according to standard procedures, while MICs for strains representative of the four expression classes of methicillin resistance have been evaluated in undiluted cultures, in order to determine the activity against the majority of the bacterial population.



Scheme 1. Structure and general synthesis of MRSA-active penems (**2**,**3**)

Table 1. Activity[a] of penems and reference compounds[b] against MRSA (clinical strains[c] and representatives of the four expression classes[d] of resistance)

Compound	Clinical strains	Class 1	Class 2	Class 3	Class 4
2a	0.15	0.39	50	100	50
2b	0.38	0.19	0.39	>100	100
2c	1.24	0.19	0.78	100	100
2d	0.09	0.09	0.78	50	25
2j	0.19	0.09	0.78	25	6.25
2e	0.03	0.19	3.12	12.5	6.25
3a	0.35	0.09	0.39	50	25
3b	0.69	0.19	0.39	>100	100
3c	0.18	0.09	0.19	100	50
3f	0.09	0.09	0.39	6.25	12.5
3g	0.06	0.09	0.19	12.5	6.25
3h	0.045	0.022	0.09	6.25	3.12
3i	0.09	≤0.011	≤0.011	6.25	6.25
3k	0.50	0.39	1.56	50	6.25
METH	100	3.12	6.25	>100	>100
RITI	0.39	0.19	0.39	100	100
IPM	1.56	0.09	0.19	25	25
MPM	12.5	0.19	1.56	25	100
CPX	0.19	0.39	0.78	25	6.25

([a]) Minimal inhibitory concentrations (MICs), μg/mL.
([b]) METH = methicillin; RITI = ritipenem (FCE 22101); IPM = imipenem; MPM = meropenem; CPX = ciprofloxacin.
([c]) Geometric mean of three strains (belonging to class 3 and 4 on the basis of population profile), determined on TSA + 2% NaCl with inocula of 10^4cfu/spot, according to NCCLS indications [5].
([d]) Representative strains selected for each expression class of resistance were:
Class 1, *Staphylococcus aureus* CDC1; Class 2, *S. a.* SN 7; Class 3, *S. a.* SN 43; Class 4, *S. a.* COL. MICs were determined on TSA + 2% NaCl with inocula of 10^7 cfu/spot.

In a preliminary investigation, the structural features characterizing **2a** (aromatic group linked to the C2 atom of the penem ring by an ethereal bridge, CH_2O) were found important to confer anti-MRSA activity (data not shown). Thus, activity against MRSA (but not against methicillin-susceptible strains) was lost in the corresponding 2-phenylpenem (deletion of the ethereal link), 2-cyclohexyloxymethylpenem (saturation of the aromatic group), and 2-benzyloxymethylpenem (insertion of an additional methylene in the link).

Following these results, several other penems differing in the aromatic group and the link connecting that group to the penem ring system were screened. Two important families of MRSA-active penems were selected for in-depth evaluation,[8] the aryl (or hetero-aryl) ethers (**2**) and the aryl (or hetero-aryl) esters (**3**). The Mitsunobu-Volante condensation between the parent penem carbinol (**1**) (protected as the *tert*-butyldimethylsilyl ether at the C8 hydroxyl and as the allyl ester at the C3 carboxy group) and the pertinent phenols (ROH) or carboxylic acids (RCO_2H) was used in the synthesis of most of the products described herein[9] (Scheme 1). The synthesis and general antimicrobial properties of two "dual-action" penems included in this study, the iodochlorhydroxyquin and ciprofloxacin conjugates (**2j**, **3k**), have been detailed elsewhere.[10]

The activity of representative compounds of the two penem families, ethers (**2**) and esters (**3**), is reported in Table 1. Based on the activity against clinical strains determined at standard inoculum size, all new penems can be considered comparable (quite often superior) to the reference compounds. Similar results are obtained also against class 1 MRSA, in which the great majority of the cells have MICs very close to the MIC for a typical susceptible strain, despite the higher inoculum used to perform the test. Differences can rather been seen comparing the MIC values for class 2 MRSA. The phenoxymethylpenem (**2a**), lead compound of the ether family, showed only marginal activity. Similarly, the activity of several other penems within this family (for simplicity not included in this report) was confined to class 1,[8] but the heteroaryl ethers (**2b-e**, **2j**) and all of the esters (**3**) were active also against class 2. Among the latter compounds, the phenanthrenyl ester **3i** was one order of magnitude more active than the best β-lactam reference, imipenem. The MRSA strains of class 3 and 4 are much more difficult to treat, but significant levels of activity (MICs 3.12-6.25 μg/mL) were reached with the phenanthrenyl esters (**3h**, **3i**). Interestingly, against class 4 the same level of activity was found also for the 5-chloro-3-pyridyl ether (**2e**) and the "dual action" penems (**2j**, **3k**). For the latter two compounds, activity was inherent in the whole molecule, as demonstrated in a dedicated study.[10]

The distribution of resistance to methicillin and four new penems (**2d**, **3a**, **3g**, **3k**) within MRSA cultures (selected as representatives of each expression class) has been evaluated by population analysis (Figure 1). In the first three classes, the pattern of distribution of resistance within the bacterial population, determined with methicillin (full squares), is more or less maintained upon exposure to the penems, although curves are shifted towards the left side of the graph, indicating a markedly superior intrinsic activity (particularly for **3k**). The bacterial population of class 4, homogeneous towards methicillin, becomes heterogeneous towards the penems tested, which sterilize the culture at concentrations between 25 and 100 μg/mL.

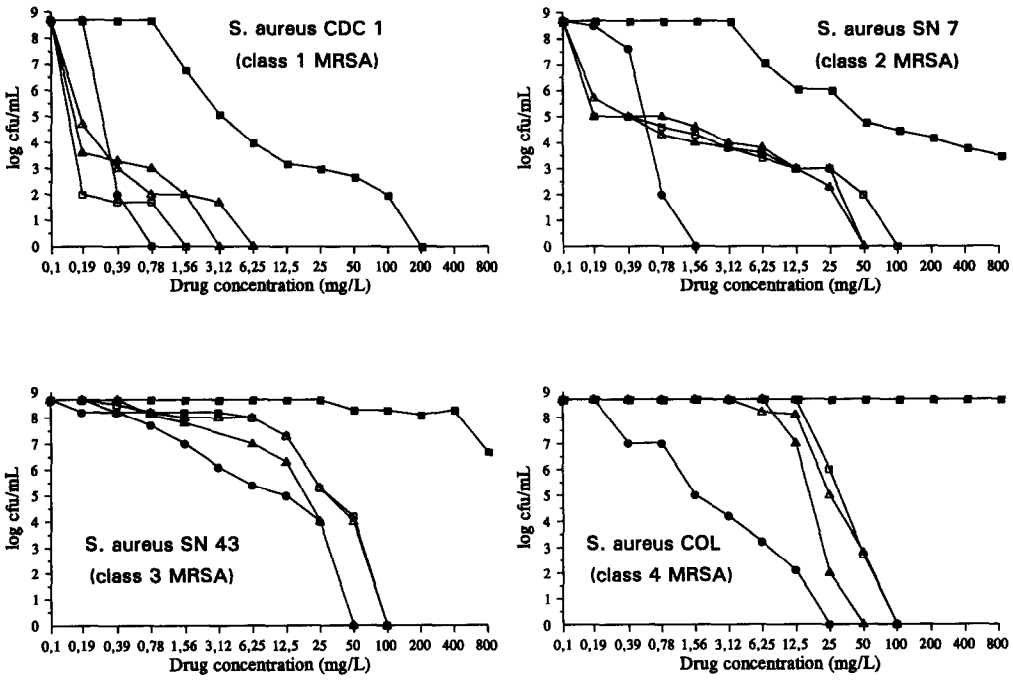


Figure 1. Distribution of susceptibility in the population of the four expression classes of MRSA. (■) Methicillin; (□) **2d**; (▲) **3a**; (△) **3g**; (•) **3k**.

In conclusion, the antibacterial activity of penems against MRSA has been evaluated according to a recommended protocol, including introduction of strains representative of the four expression classes of resistance used at high inocula to determine the susceptibility of the majority of cells in each culture. Based on structure-activity data obtained by these methods, penems characterized at C2 by the presence of a (hetero)aromatic moiety, particularly a bi- or poly-cyclic one, linked by an ether (CH_2O) or ester (CH_2OCO) bridge, are superior β-lactam antibiotics. It must be added, however, that the high lipophilicity of the penems investigated in the present work confers prohibitive levels of affinity for plasma proteins, precluding the attainment of useful *in vivo* efficacy. The challenge of ongoing research is whether hydrophilic heads properly inserted in selected structures will not diminish activity against all of the four expression classes of methicillin resistant strains.

Acknowledgements

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