ANTIBACTERIAL ACTIVITY OF 5-ACYLAMINOTHIAZOLE DERIVATIVES, SYNTHETIC DRUGS RELATED TO β-LACTAM ANTIBIOTICS

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Newly synthesized 5-acylaminothiazolium salts and one 5-acylaminothiazolidine, considering their chemical structure and reactivity, have been proposed as potential inhibitors of bacterial serine DD-peptidases. A moderate antibiotic activity with (5-phenylacetylamino-3-thiazolio)acetate and (5-phenylacetylaminothiazolidin-3-yl)acetic acid was observed on *Staphylococcus aureus* ATCC 25923. The methyl- and *tert*-butyl esters of the thiazolium salt have shown lower MIC values. Moreover, when introduced into an exponential growth phase culture of *S. aureus*, the three active thiazolium salts induced a partial lysis indicating an impairing of the bacterial cell wall biosynthesis. The observed time-dependent binding of the best compound to the PBPs of *S. aureus* was too slow and occurred at too high concentrations to account for its MIC value. Consequently, the antibiotic activity of the thiazolium salts on the *S. aureus* cells seems not to be satisfactorily explained by a penicillin-like interaction with the PBPs.

β-Lactam antibiotics are characterized by a high and very specific antibacterial activity. They inactivate characteristic bacterial enzymes, *i.e.*, the active serine DD-peptidases, by forming a very stable acyl enzyme adduct with the serine hydroxyl group[1].

Those enzymes represent a large family of proteins including the membrane-bound DD-peptidases, which are the targets of β-lactam action, and various soluble or membrane-bound DD-carboxypeptidases of unknown physiological function[1].

The major mechanism of resistance developed by bacteria is the production of β-lactamases, generally serine enzymes, which specifically and efficiently hydrolyze the β-lactam amide bond[1].

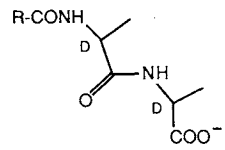
A major goal of the present research in this area of antibacterial chemotherapy is to prepare DD-peptidases inactivators, which might escape hydrolysis by β-lactamases, or to obtain inhibitors which might act on both types of bacterial penicillin-recognizing enzymes, namely DD-peptidases and β-lactamases.

For that purpose, we have synthesized new classes of heterocyclic compounds which might interact with the same bacterial targets, but according to a mechanism different from that which leads to the formation of an acyl enzyme (PIROTTE, 1989, thesis, unpublished).

Other investigators had explored the replacement of the β-lactam nucleus of classical antibiotics by another three-, four- or five-membered ring having a modified electrophilic center (*i.e.*, cyclobutanones[2], epoxides and oxaziridines[3]). Although they had conserved some structural analogy with the natural peptide substrate of the bacterial enzyme (**1**) or with common β-lactam compounds such as penicillins (**2**), none of these three classes of compounds have shown significant antibiotic activities.

However, the naturally occurring lactivicin (**3**)[4,5], some related structures[6] and some pyrazolidinones (**4**)[7,8] recently reported, are five-membered ring-containing analogues of β-lactam antibiotics possessing a very interesting antimicrobial activity.

Fig. 1. Structure of the natural substrate of DD-peptidases (**1**) (a D-Ala-D-Ala-terminated oligopeptide) compared to those of various antimicrobial compounds ((**2**) penicillins, (**3**) lactivicin and (**4**) pyrazolidinones) and derivatives of 5-aminothiazole ((**5**) 5-acylaminothiazolium salts and (**6**) 5-acylaminothiazolidines).



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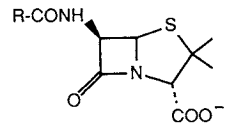

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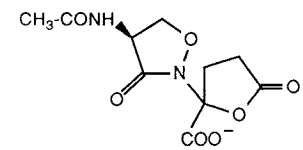

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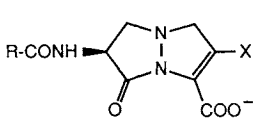

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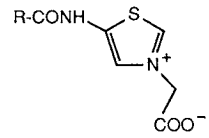

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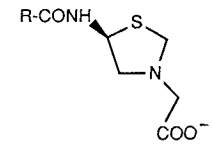

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We now present that, among the different classes of compounds that have been developed in our laboratory, derivatives of 5-acylaminothiazolium salts (**5**) and 5-acylaminothiazolidine (**6**) have shown some antibacterial activity.

Thiazolium salts bearing the aminoacyl side chain of classical β-lactam antibiotics (*i.e.*, benzylpenicillin and cefotaxime) were selected among other cationic heterocycles because of their known high reactivity toward nucleophilic addition[9].

Thus their interaction with a well-positioned nucleophilic element of the enzymatic cavity might lead to the formation of a non conventional covalent adduct and, as a result, to a new inhibition mechanism.

The present work tries to determine if the moderate antibacterial activity of these original compounds is the result of their interaction with the penicillin targets.

Materials and Methods

Chemistry

General Methods

MP's were determined in a capillary tube and are uncorrected. IR spectra were obtained using a Perkin-Elmer 297 spectrometer. ^{1}H NMR spectra were recorded on a Bruker 80 MHz (AW80) spectrometer. Flash chromatography was carried out on Silica gel 60 Merck (230 ~ 400 mesh ASTM). Elemental analyses were performed on a Heraeus Micro-U apparatus.

(5-Phenylacetylamino-3-thiazolio)acetate $2\frac{1}{2}$ hydrate (**7**), 3-methoxycarbonylmethyl-5-phenylacetylaminothiazolium bromide (**8**), 3-*tert*-butoxycarbonylmethyl-5-phenylacetylaminothiazolium bromide (**9**) and racemic (5-phenylacetylaminothiazolidin-3-yl)acetic acid (**10**) were obtained as described elsewhere[9]. {5-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]-3-thiazolio}acetate (**11**) was obtained as follows.

5-[(*Z*)-2-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]thiazole (**12**)

To a stirred solution of 5-aminothiazole[10] (1.0 g), (*Z*)-2-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid[11] (4.43 g) and 4-dimethylaminopyridine (0.06 g) in dry CH_2Cl_2 (50 ml) at 0°C was added dropwise a solution of dicyclohexylcarbodiimide (2.07 g) in dry CH_2Cl_2 (25 ml). After 5 hours at room temperature, the suspension was diluted with ethyl acetate (200 ml) and kept for one night at 4°C.

The insoluble material was filtered and the filtrate was washed with 0.5 N HCl (50 ml), water (150 ml × 2), aqueous 2% (w/v) $NaHCO_3$ (150 ml) and water (150 ml × 2). The separated organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with ethyl acetate - petroleum ether 50~75°C (6:4) as an eluent to give **12** (3.14 g, 65%), mp 170~173°C; ^1H NMR (DMSO-d_6, HMDS) δ 3.70 (3H, s, OCH_3), 6.75 (1H, s, 2-aminothiazole-H_5), 7.20 (15H, m, Ph × 3), 7.44 (1H, s, 5-aminothiazole-H_4), 8.48 (1H, s, 5-aminothiazole-H_2), 8.63 (1H, s, N*H*(Ph)$_3$).

Anal Calcd for $C_{28}H_{23}N_5O_2S_2$: C 63.98, H 4.41, N 13.32.
Found: C 63.54, H 4.89, N 13.11.

5-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]thiazole (**13**)

A solution of **12** (2.0 g) in methanol (50 ml) and 0.5 N HCl (50 ml) was refluxed for 5 minutes. The suspension so obtained was cooled and concentrated to 40 ml. Triphenylcarbinol was filtered off. The filtrate was neutralized with $NaHCO_3$ and concentrated to dryness. The residue was extracted with absolute ethanol and filtered. The filtrate was concentrated and the residue was extracted with ethyl acetate (60 ml). The insoluble material was filtered off and the filtrate was mixed with petroleum ether 40~60°C (60 ml) to give precipitation of crystalline (**13**) (0.75 g, 70%), mp 179~182°C; ^1H NMR (DMSO-d_6, HMDS) δ 3.78 (3H, s, OCH_3), 6.80 (1H, s, 2-aminothiazole-H_5), 7.10 (2H, br s, NH_2), 7.56 (1H, s, 5-aminothiazole-H_4), 8.54 (1H, s, 5-aminothiazole-H_2), 11.90 (1H, br s, CONH).

Anal Calcd for $C_9H_9N_5O_2S_2$: C 38.15, H 3.20, N 24.72.
Found: C 38.11, H 3.32, N 24.65.

{5-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]-3-thiazolio}acetate (**11**)

A solution of **13** (0.5 g) and bromoacetic acid (0.5 g) in nitromethane (7.5 ml) and DMF (2.5 ml) was heated at 70°C for 6 hours. The suspension was cooled and diluted with ether (50 ml). The precipitate was filtered, washed with ether and then purified by silica gel chromatography with $CHCl_3$ - MeOH (2:8) as an eluent to give **11** (0.36 g, 60%), mp 215°C; ^1H NMR (DMSO-d_6, HMDS) δ 3.60 (3H, s, OCH_3), 4.60 (2H, s, CH_2), 6.43 (1H, s, 2-aminothiazole-H_5), 6.90 (2H, s, NH_2), 7.40 (1H, s, 5-aminothiazole-H_4), 8.78 (1H, s, 5-aminothiazole-H_2). Ether (30 ml) was added to a solution of **11** (0.04 g) in 0.5 N HCl (0.3 ml) and ethanol (2.7 ml) to give crystalline 5-[2-(2-aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]-3-carboxymethylthiazolium chloride hydrochloride monohydrate, which was filtered, washed with ether and dried, mp 105°C; ^1H NMR (DMSO-d_6, HMDS) δ 3.85 (3H, s, OCH_3), 5.45 (2H, s, CH_2), 7.05 (1H, s, 2-aminothiazole-H_5), 8.33 (1H, s, 5-aminothiazole-H_4), 9.83 (1H, s, 5-aminothiazole-H_2).

Anal Calcd for $C_{11}H_{12}N_5O_4S_2Cl \cdot HCl \cdot H_2O$: C 30.56, H 3.50, N 16.20.
Found: C 30.97, H 3.80, N 16.05.

Bacterial Strains

The selected bacterial strains were *Streptococcus pyogenes* CIP 54.5 (ATCC 8668), *Staphylococcus aureus* CIP 76.25 (ATCC 25923), *Proteus vulgaris* P18, *Pseudomonas aeruginosa* CIP 76.110 (ATCC 27853), *Klebsiella pneumoniae* MIR A12, *Escherichia coli* CIP 76.24 (ATCC 25922), *Serratia marcescens* CIP 53.89 (ATCC 4003) and *Salmonella typhimurium* CIP 60.62 (DEMEREC LT2 wild). All the strains were in the collection of the Liège Department of Microbiology and were formerly obtained from the Institut Pasteur except *P. vulgaris* P18 and *K. pneumoniae* MIR A12 which were generously given by Dr. A. ROUSSET (Beaune, France) and Dr. P. CANEPARI (Verona, Italy), respectively.

Bacterial Growth

All the strains were inoculated from slants (kept at 4 °C) and grown at 37°C in Mueller-Hinton (MH) medium (Difco) except *S. pyogenes* which was grown in brain heart broth (Difco) added with 10% inactivated horse serum (Gibco).

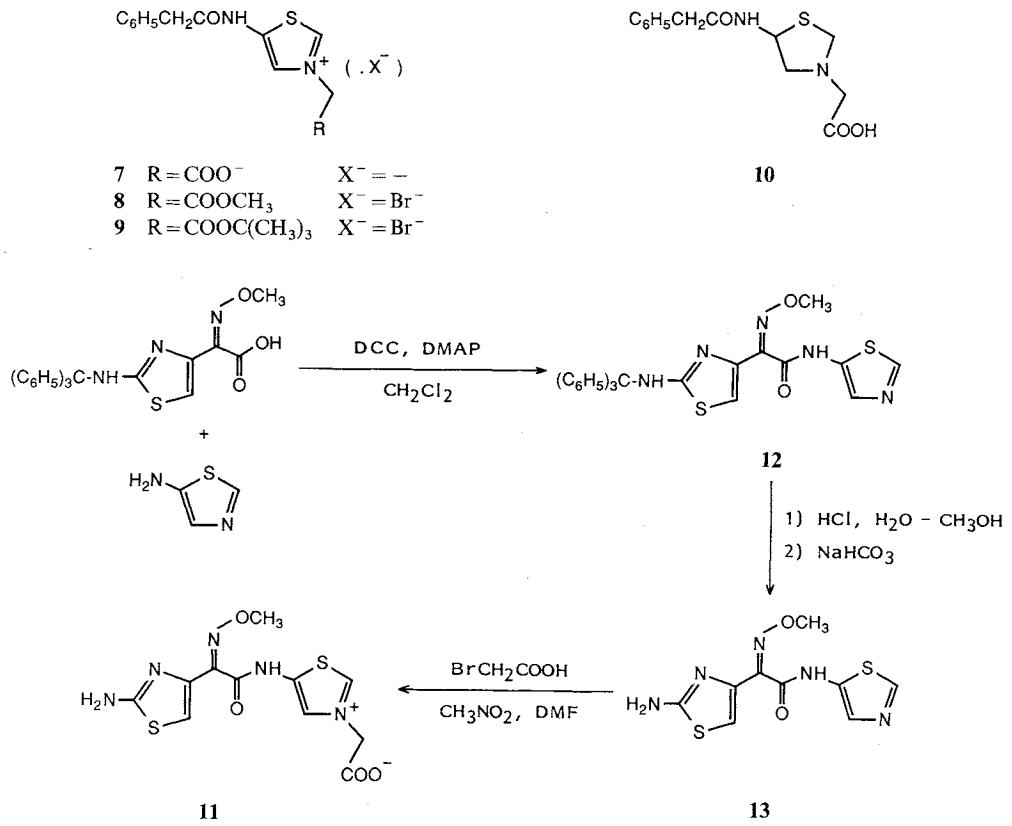
MIC Determination in Liquid Medium

The experimental procedure used was an adaptation of described techniques[12].

S. aureus Growth Curves and MIC Determination at Different pHs

Buffered MH media were prepared by mixing one part of sterile 0.4 M sodium phosphate buffer to

Fig. 2. Structure of the new synthetic compounds tested for their antibacterial activity and synthetic pathway to compound **11**.



three parts of 1.33-fold more concentrated sterile MH medium (final pH of 6.28, 7.10 and 7.83, respectively). Growth at 37°C of *S. aureus* was followed by recording A_{550} values in the three buffered media and compared with that of a normal non-buffered medium (pH 7.50).

MIC value of **7** on *S. aureus* grown in MH medium at the three pH values was determined as above.

Neutralized and sterile 10 mM solutions of **7**, **8**, **9** and **10** were prepared and diluted (1 mM and 2 mM final concentration) in *S. aureus* cultures ($A_{550}=0.1$) growing in non buffered MH broth (see above). Absorbance at 550 nm was measured every 15 minutes and corrected to account for the dilution observed when the drug solutions were added (10 to 20% of the final culture volume).

Interaction with PBPs of Permeabilized *S. aureus* Cells

(a) Cell Permeabilization: The crude permeabilized *S. aureus* cells preparations were obtained as described by HALEGOUA *et al.*[13].

(b) Protein Concentration Determination: The protein content of the " crude membrane preparation" was estimated according to LOWRY *et al.*[14] and modified as described by COYETTE *et al.*[15].

(c) Drug Interaction with *S. aureus* PBPs: permeabilized cells (400 μg proteins/16 μl) were incubated 10 minutes at 37°C with three concentrations of the drug (0.1, 1.0 and 10 mM, respectively except for **10**, 0.073, 0.73 and 7.3 mM) tested in 60 μl of 10 mM $MgCl_2$, 30 mM phosphate buffer pH 7.5. Then, they were incubated another 10 minutes at 37°C in presence of 83 μM benzyl[^{14}C]penicillin (54 Ci/mol, Radiochemical Center Amersham UK). The suspension was denatured by adding 4 μl of 20% stock solution of SDS and heating at 100°C for 90 seconds. After 15 minutes centrifugation (Eppendorf centrifuge), 50 μl of supernatant were mixed with 50 μl of the denaturing mixture reported by SUZUKI *et al.*[16] and kept at 0°C until electrophoresis was performed. Control samples were performed as described above by replacing the drug solution by phosphate buffer pH 7.5 (see above). A control sample of *Streptomyces* R61

DD-peptidase was obtained as follows: a mixture of 2 μl of 20 μM R61 enzyme with 20 μl water and 3 μl of 5 mM benzyl[^{14}C]penicillin was incubated 30 minutes at 37°C and then added with 15 μl of 0.1 M benzylpenicillin. A 10-μl aliquot of this solution was then mixed with 10 μl of denaturing mixture (see above) and kept at 0°C until electrophoresis.

The SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the technique of LAEMMLI & FAVRE modified by SUZUKI *et al.*[16]. A 10%-polyacrylamide gel with 0.17% bisacrylamide was prepared in a LKB model 2001 vertical apparatus using two 1.5 mm spaced glass plates of 16 × 18 cm. Electrophoresis was conducted during 16 hours at a constant 20 mA amperage and a variable voltage (90 to 300 V). After classical fixation and staining (Coomassie Blue), the gel was prepared to obtain a fluorography according to the method reported by LASKEY[17]. The dry gel was placed in contact with a preflashed Kodak Xomat AR5 radiographic film and kept 3 weeks at −70°C before photographic revelation.

A second experiment was performed under the same conditions with compound **9** which was incubated at various concentrations (1, 5 and 10 mM) for 5, 10 or 40 minutes at 37°C with the permeabilized cells preparation before addition of radioactive benzylpenicillin.

Inactivation of DD-Peptidases

The DD-carboxypeptidases were purified as described by FRÈRE *et al.*[18] and kept under the following conditions; for *Streptomyces* R61 (R61 enzyme), 50 μM EDTA in 10 mM Tris-HCl buffer, pH 8.0; for *Actinomadura* R39 (R39 enzyme), 5 mM $MgCl_2$ in 10 mM Tris-HCl buffer, pH 7.7. The enzyme (0.09 μg for R39 and 0.5 μg for R61) was incubated at 37°C with various concentrations of the tested compound in a total volume of 15 μl for a 5 minutes period. To the samples, 60 nmol of substrate (Ac_2-L-Lys-D-Ala-D-Ala) in 30 μl of the adequate buffer were added. After 10 minutes incubation at 37°C, the D-alanine released was estimated as described by FRÈRE *et al.*[18].

β-Lactamases

The β-lactamases of *Bacillus licheniformis* 749C, *Enterobacter cloacae* P99 and *Bacillus cereus* 5/B/6 were purified as described respectively by MATAGNE *et al.*[19], ROSS[20] and THATCHER[21], and kept in 10 mM sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl and 0.1 mg/ml of bovine serum albumin. In the case of the Zn^{++} containing *B. cereus* enzyme the buffer was supplemented with 1 mM $ZnCl_2$.

The enzyme (*B. licheniformis*: 2.5 ng; *E. cloacae*: 0.5 ng; *B. cereus*: 25 ng) was incubated at 37°C in a total volume of 15 μl with various concentrations of the tested compounds. After 10 minutes, 350 μl of a 100 μM solution of nitrocefin in 50 mM sodium phosphate buffer, pH 7.0, were added and the hydrolysis of nitrocefin was monitored at 482 nm. Under those conditions, inactivation and inhibition of the enzymes could easily be visualized[22].

Results

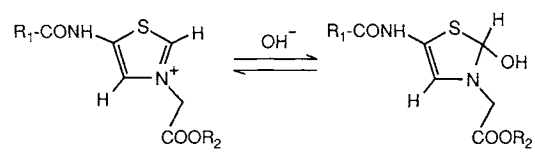
Determination of the MIC Values

The phenylacetamide derivatives (**7** and **10**), but not the cefotaxime analog (**11**), exhibited a moderate antibiotic activity on *S. aureus* and no significant activity against the Gram-negative ones (see Table 1). This result led us to examine the effect of the methyl (**8**) and *tert*-butyl (**9**) esters of the thiazolium salt (**7**)

Table 1. MIC values (mM) on Gram-positive strains.

Compound tested	Bacterial strain	
	Staphylococcus aureus ATCC 25923	*Streptococcus pyogenes* ATCC 8668
7	0.30 (96 μg/ml)	>1
8	0.20 (74 μg/ml)	1 (370 μg/ml)
9	0.08 (33 μg/ml)	0.80 (330 μg/ml)
10	0.15 (42 μg/ml)	>1
11	>1	>1

Fig. 3. Interaction between 5-acylaminothiazolium salts (**14**) and the hydroxide ion in aqueous alkaline solution giving pseudobases (**15**).



14 **15**

since it is well-known that esterification of the carboxylic function of common β-lactam antibiotics generally strongly decreases their antibacterial activity[23]. Compounds **8** and **9** surprisingly exhibited an increased antibacterial activity against the two Gram-positive strains and no activity against the Gram-negatives (see Table 1).

In addition, this increase seemed to be directly correlated to the lipophilicity of the 5-phenylacetylaminothiazolium salts.

It has been previously demonstrated that 5-acylaminothiazolium salts (**14**) may exist in aqueous solution in equilibrium with their pseudobase (**15**). The latter results from the nucleophilic addition of the hydroxide ion in the two-position of the thiazolium ring[9] (Fig. 3). Simple thiazolium salts without an aminoacyl side chain in the five-position behave differently by forming a ring-opened enethiolate in alkaline solutions[24].

Consequently, such an equilibrium is pH dependent, and an apparent pK value may be measured. This value reflects the pH for which the concentrations of the starting form **14** and the addition product (**15**) are equal in aqueous solution. The apparent pK values for the three thiazolium salts (**7**, **8** and **9**) were 7.85, 7.41 and 7.44, respectively[9]. One can predict that at pH 7.50, compound **7** exists as a mixture of its unchanged form ($\sim$70%) and its pseudobase form ($\sim$30%). Moreover, after prolonged conservation in alkaline solution, pseudobase (**15**) decomposes, most probably into one or more ring-opened by-products[9]. In order to verify if the active form of **7** on *S. aureus* growth was the starting thiazolium salt structure or its modified structure in slightly alkaline solution, we examined the influence of the pH on the MICvalue of **7**.

A preliminary experiment indicated that growth curves of *S. aureus* in MH medium at three different pHs (6.28, 7.10 and 7.83) were practically superimposable to that obtained in non-buffered medium (pH 7.50) (results not shown).

The MIC values of **7** on *S. aureus* at pH 7.10 and 7.83 were 0.7 mM and 0.6 mM, respectively. There was no growth inhibition at pH 6.28 up to a 1 mM concentration. At this pH, as predicted by the apparent pK value, the starting form **14** clearly predominates in solution. The pH increase promotes the pseudobase (**15**) or ring-opened by-product formation. Consequently, we tentatively concluded that in solution the active species on *S. aureus* could not be the thiazolium form **14**. The higher activity of the esters was partly explained by the lower proportion of their unchanged form at pH 7.50 as expected from their lower apparent pK values.

Bacteriolytic Effect

When introduced into a bacterial culture at concentrations higher than their MIC value, penicillins induce bacteriolysis. This effect would be an indirect result of the inhibition of the membrane-bound DD-peptidases involved in bacterial cell wall biosynthesis. It was suggested that this inhibition would stimulate endogenous hydrolases which would lyse the cells[25]. Observation of cell lysis with a new inhibitor is an encouraging indication for a possible inhibition mechanism similar to that of penicillins but may not constitute an absolute evidence for the interaction of that inhibitor with the penicillin targets.

Compounds **7**, **8**, **9** and **10** were added (1 and 2 mM final concentration) in an exponential growth phase culture of *S. aureus* (pH 7.50).

Compounds **8** and **9** (at 1 mM) and compound **7** (only at 2 mM) inhibited bacterial growth and induced a partial lysis of the culture, as demonstrated by the decrease of the A_{550} value. A typical result is given

Fig. 4. Effect of (5-phenylacetylamino-3-thiazolio)-acetate (7) on *Staphylococcus aureus* growth in Mueller-Hinton liquid medium.

□ Control, ■ treated culture: the arrow indicates the time (75 minutes) of compound **7** addition (final concentration: 2 mM).

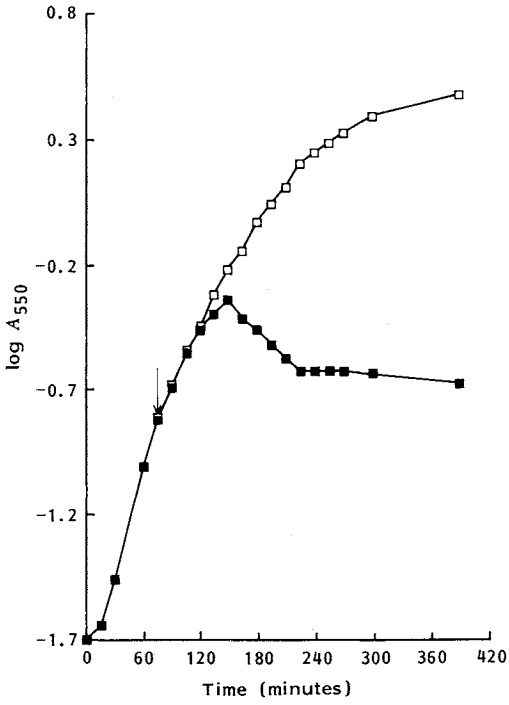
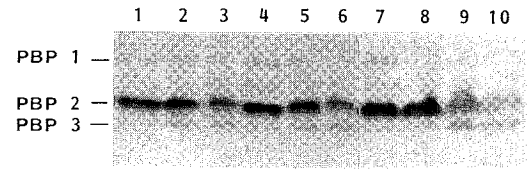


Fig. 5. Time dependent binding of compound **9** on *Staphylococcus aureus* PBPs.

1, 2, 3: 5-minute incubation with a 1, 5 and 10 mM concentration, respectively, 4, 5, 6: 10-minute incubation with a 1, 5 and 10 mM concentration, respectively, 7: control sample without inactivator, 8, 9, 10: 40-minute incubation with a 1, 5 and 10 mM concentration, respectively.



on Fig. 4.

Optical microscopy examination of the treated cultures after 300 minutes showed the presence, on one hand, of empty and lysed cells (ghosts) and, on the other hand, of intact abnormally enlarged cells. Such morphological modifications have already been described for *S. aureus* cells treated with β-lactam antibiotics at concentrations near the MIC value[26,27].

The presence of intact swollen cells explained the plateau noted on the growth curve after partial lysis of the cultures (Fig. 4).

We came to the conclusion that the bacterial cell wall biosynthesis was affected by 5-phenylacetylaminothiazolium derivatives, or most probably by a modified structure existing in slightly alkaline medium. A competition test with radioactive benzylpenicillin on *S. aureus* membrane-bound proteins could demonstrate if these derivatives form a long-lived covalent bond with PBPs.

Interaction with *S. aureus* PBPs

The affinity of the four active compounds **7**, **8**, **9** and **10** for the *S. aureus* PBPs was evaluated according to the classical competition procedure performed on a "crude membrane preparation" in presence of radioactive benzylpenicillin. A preliminary assay was conducted in which permeabilized cells samples (400 μg total protein) were incubated 10 minutes at 37°C in the presence of each of the four active compounds and submitted to an SDS-PAGE followed by a fluorography as described in Materials and Methods. Coomassie Blue staining of the gel prior fluorography indicated that the high inactivator concentrations used did not induce any disturbance in protein patterns. Inhibition of penicillin fixation was only apparent with the most active compound **9** (its pseudobase or a ring-opened derivative at pH 7.50) at a 10-mM concentration corresponding to more than 100 times its MIC value on *S. aureus*.

A second experiment was performed in which the effect of various concentrations of the most active compound **9** was examined after various incubation time with the membrane preparation. Fig. 5 shows that, after a 40-minute incubation, a partial inhibition of benzylpenicillin binding to PBP 2 was obvious

with a 5-mM concentration of **9**.

Interaction with DD-Peptidases and β-Lactamases

No inactivation or inhibition of DD-peptidases was observed in the presence of compounds **7**, **8**, **9**, **10** and **11** at concentrations ranging from 0.01 to 1 mM. Incubation of the β-lactamases with the same compounds in the same range of concentrations did not show any inhibition of these enzymes.

Discussion

The moderate antistaphylococcal activity of (5-phenylacetylamino-3-thiazolio)acetate (**7**) and its structural analogy with benzylpenicillin allowed us to suppose that its enzymatic target could be a membrane-bound serine DD-peptidase.

Compound **11** however, containing the cefotaxime aminoacyl side chain, did not exhibit any antibiotic activity on the different selected Gram-positive and Gram-negative strains.

The higher antibiotic activity on Gram-positive bacteria of the methyl (**8**) and *tert*-butyl (**9**) esters was surprising in the context of a hypothetical inhibitory activity on DD-peptidases. In fact, for most DD-peptidases inhibitors, a strong decrease of the antibiotic activity is observed after esterification of the carboxylic function[2,3].

The MIC value of **7** on *S. aureus* at different pHs indicates that the active form of the 5-acylaminothiazolium salt in solution was not the salt form but a modified structure (pseudobase or ring-opened derivative) resulting from the nucleophilic addition of the hydroxide ion on the two-position of the ring. Further experiments will be necessary to determine the exact structure of the active species on *S. aureus*.

However, observation of a partial bacteriolytic effect and important morphological alterations of the residual intact cells with the 5-phenylacetylaminothiazolium derivatives appears to be in good agreement with the inhibition of at least one step of the bacterial cell wall biosynthesis and more specifically that catalysed by membrane-bound DD-peptidases.

The competition test on *S. aureus* PBPs between radioactive benzylpenicillin and the four active compounds including the 5-phenylacetylaminothiazolidine derivative (**10**) indicates that their antibiotic effect is not the consequence of a rapid and stable covalent fixation to the enzymes, since concentrations inhibiting benzyl[^{14}C]penicillin binding were significantly above their MIC values.

A second competition test performed with the most active compound **9** indicated that, although inhibition was only observed at high concentrations, it clearly appeared to increase with time. That time-dependent fixation was however much too slow and occurred at too high concentrations to account for the MIC value and probably excludes a penicillin-like interaction for the lethal effect of compound **9** on the *S. aureus* cells.

The different compounds tested on various purified DD-peptidases and β-lactamases, do not cause any inhibition at concentrations up to 1 mM. The two selected soluble DD-peptidases catalyse both hydrolysis and transpeptidation reactions. Although they are not the lethal targets of β-lactams in the organisms which produce them, they have been successfully utilized as models in the elucidation of the interactions between β-lactams and the sensitive enzymes. They are generally inactivated by compounds which also inactivate the membrane-bound DD-transpeptidases[1]. If they are considered as sufficiently representative of all bacterial PBPs, we must then conclude that the bacterial target of the 5-phenylacetylaminothiazolium salts is most probably different from that of penicillin unless they interact specifically with the *S. aureus* PBPs according to a new mechanism, distinct from that of β-lactam compounds. Moreover, it remains theoretically possible that some molecules might not be recognized by the model enzymes and nevertheless react irreversibly with the serine residue of some DD-transpeptidases. Before any definitive conclusion could be drawn on the antibacterial effect of these compounds, a careful examination of other related structures should be done in the hope of observing an increase of the antibacterial activity and an enlargement of their antibiotic spectrum.

In addition, it would be interesting to perform the purification of the active isomer of the racemic

thiazolidine derivative in order to determine the configuration of the molecule active on *S. aureus*. Such a result should determine future researches for more powerful optically active analogues.

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